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OPTIMIZATION OF *Talinum paniculatum* Gaertn. ROOT INDUCTION AND THE EFFECT OF PHOSPHATE CONCENTRATIONS AND AMMONIUM:NITRATE RATIO ON BIOMASS OF ADVENTITIOUS ROOTS IN IN VITRO CULTURE

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ABSTRACT

Java ginseng (*Talinum paniculatum* Gaertn.) is a medicinal plant, the roots of which are commonly used in traditional medicine. In its natural habitat, the roots grow very slowly, requiring two to three years to produce 100 g of roots per plant. Plant tissue culture could therefore provide an alternative means of accelerating root growth. This research aimed to optimize root induction and determine the effect of phosphate (KH_2PO_4) concentration and the ratio between ammonium and nitrate (ammonium:nitrate) on the biomass of Java ginseng adventitious roots in in vitro culture. Stem and leaf were used as explants and various combinations and concentrations of IBA and BAP, kinetin, and TDZ were used as growth regulators. Leaf explants were grown in Murashige and Skoog (MS) media supplemented with IBA 2 mg/L and various concentrations of phosphate (170; 212.5; 255; 297.5; 340; 382.5; 425; 467.5; 510 mg/L) and various ammonium:nitrate ratios (21:19 mM as the control, 0:30 mM, 10:20 mM, 15:15 mM, 20:10 mM, 30:0 mM). Cultures were maintained for 6 weeks. The observed parameters were fresh weight, dry weight, the duration of root formation, and the number and length of adventitious roots. The data were analyzed using Analysis of Variance. The results showed that the concentration of phosphate and the ammonium:nitrate ratio significantly influenced the amount, length, fresh weight, and dry weight of Java ginseng adventitious root. The highest fresh weight (37.47 mg) and dry weight (5.53 mg) were achieved in the treatment of double phosphate concentration (KH_2PO_4 340 mg/L), while an ammonium:nitrate ratio of 10:20 mM was the optimum treatment to produce the highest biomass (fresh weight 73.6 mg and dry weight 8.2 mg).

Keywords: adventitious roots, ammonium, nitrate, phosphate, *Talinum paniculatum* Gaertn.

INTRODUCTION

Plants provide an incredible source of new medicinal product inventions for development. One of the medicinal plants often used by the community is Java ginseng (*Talinum paniculatum* Gaertn.) from the Portulacaceae family. Java ginseng is widely used as a substitute for Korean ginseng, which continues to be imported, because it is relatively cheap, easy to obtain, and easy to cultivate (Widiyani, 2006). The plant's chemical content comprises saponins, triterpenes, polyphenols, and essential oils (Komatsu, 1982). The root of the Java ginseng plant is the part that can be used as a medicinal ingredient. The most important and dominant component in the chemical content of Java ginseng root is saponins. *Talinum paniculatum* Gaertn. has very slow root growth in its natural habitat, requiring around two to three years to produce 100 g of roots per plant (Manuhara *et al*., 2015). Therefore, in vitro culture techniques offer potential as an alternative means of accelerating the root growth of this plant.

Root growth can be increased by manipulation in culture media with the addition of nutrients (Manuhara, 2014). As such, in this study, an increase in root biomass was achieved with the addition of phosphate and nitrogen sources. Phosphate has an important function in

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plant growth due to its role in transferring energy molecules such as ADP and ATP, NAD and NADP, as well as genetic information system compounds such as DNA and RNA (Barker and Pilbeam, 2007). In plant tissue culture, the concentration of phosphate in the medium can be a major factor influencing plant growth. According to Dormatey *et al.* (2021), phosphite supply in the Murashige and Skoog (MS) medium influenced root morphological characteristics and fresh biomass in five genotypes of potatoes. Furthermore, Pavlov *et al.* (2000) stated that *Lavandula vera* biomass and rosmarinic acid was maximally produced with the addition of a two-time phosphate concentration in MS media.

Apart from phosphate, nitrogen sources also affect cell growth and the formation of secondary metabolites. Nitrogen functions as a component of amino acids, proteins, and nucleic acids in plants (Wiedenhoeft, 2006). Ammonium (NH^{4+}) and nitrate (NO^{3-}) are used as the main sources of nutrition in plant cell and tissue culture (Zhang *et al.* 1996, in Kim *et al.* 2005). When both nitrogen sources were administered simultaneously, growth and yield were significantly increased compared to ammonium or nitrate alone (Zhang *et al.* 2011). Research by Yin *et al.* (2013) on *Pseudostellaria heterophylla* plants showed that the highest adventitious root biomass, namely 9.11 g fresh weight and 0.54 g dry weight, was obtained with an ammonium and nitrate ratio of 20:40. Furthermore, Panda *et al.* (1992) reported that the only received information on ammonium was used as not only the nitrogen source. Therefore, it is very important to determine the optimal ratio of ammonium to nitrate. No study has reported the effect of various concentrations of phosphate and the ammonium:nitrate ratio on the biomass production of Javanese ginseng plants. This study therefore aims to investigate their effect in culture media on the adventitious root biomass of Javanese ginseng.

MATERIALS AND METHODS

Adventitious Root Induction from Leaf and Stem Explants

The adventitious roots of Java ginseng were induced from stem and leaf explants grown in solid MS medium supplemented with 30 g/L sucrose, 6 g/L agar, and various combinations of IBA growth regulator 2 mg/L with three types of cytokinins, namely BAP (0.1, 0.3, and 0.5 mg/L), kinetin (0.1, 0.3, and 0.5 mg/L), and TDZ (0.1, 0.3, and 0.5 mg/L). The leaf samples (1 cm^2) were taken from the second and third leaves of the shoots from intact plant. All explants were immersed in detergent solution for 3 minutes and rinsed with water. The explant surfaces were sterilized by immersing them in 10% Clorox solution for 10 minutes. The explants were then washed 3 times using sterile distilled water. Following this, they were maintained in an incubation room with an average temperature of 25°C in dark conditions. After 28 days, the adventitious roots were harvested and the fresh weight, dry weight, number of roots, and duration of adventitious root formation were measured.

Phosphate Concentration Treatments

The explants used the second and third leaves from the shoots. Solid MS medium was supplemented with a combination of IBA 2 mg/L and TDZ 0.1 mg/L, agar 8 gr, and sucrose 30 gr/L. Different concentrations of KH_2PO_4 (0; 42.5; 85; 127.5; 170; 215.5; 255; 297.5; and 340 mg/L) were added to the medium. The leaf explants were grown in culture bottles containing the medium and incubated at 25°C in the dark for 6 weeks. After 6 weeks the fresh weight, dry weight, root formation time, number of roots, and root length were measured. Adventitious root growth was first recorded and used as the initial observational data for the first time that root growth occurred. After 6 weeks of cultivation, the number of roots, fresh weight, and dry weight were measured.

Ammonium: Nitrate Ratio Treatments

The explants used the second and third leaves from the shoots. The MS medium was supplemented with a combination of IBA 2 mg/L and TDZ 0.1 mg/L, agar 8 gr, and sucrose 30 gr/L. Different ammonium:nitrate ratios (21:19 mM as the control, 0:30 mM, 10:20 mM, 15:15 mM, 20:10 mM, and 30: 0 mM) were added to the medium. The leaf explants (1 cm^2)

were grown in culture bottles containing the medium and incubated at 25ºC in the dark for 6 weeks. After 6 weeks, the fresh weight, dry weight, root formation time, number of roots, and root length were measured. Adventitious root growth was first recorded and used as the initial observational data for the first time root growth occurred. After 6 weeks of cultivation, the number of roots, fresh weight, and dry weight were measured. Root fresh weight was measured after the roots had been rinsed with distilled water and drained. Dry weight was measured after drying in an oven at 50ºC for 5 days to obtain a constant dry weight.

Data Analysis

The data obtained, including the fresh weight, dry weight, number of adventitious roots, and length of adventitious roots, were analyzed using ANOVA (Analysis of Variance) at a significance level of 5%.

RESULTS AND DISCUSSION

Adventitious Root Induction

The reactions of *T. paniculatum* adventitious root induction to the addition of an IBA growth regulator with various types of cytokinins in the third week can be seen in Figure 1 (stem explants) and Figure 2 (leaf explants).

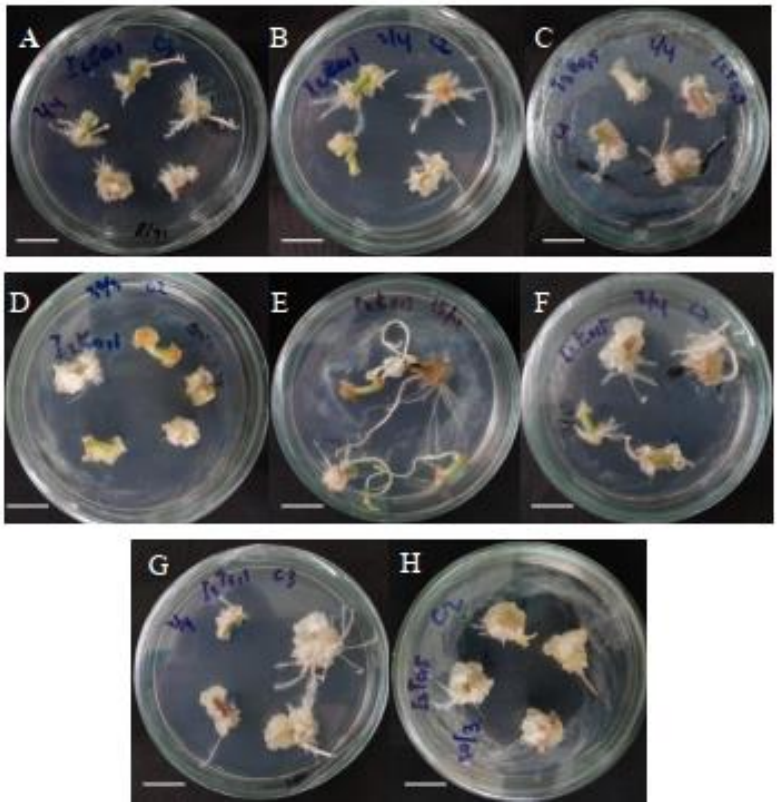

Figure 1 Root induction of *Talinum paniculatum* stem explants in various combinations of IBA and BA, kinetin, and TDZ during a 3-week culture. A) I2B0.1; B) I2B0.3; C) I2B0.5; D) I2K0.3; E) I2K0.5; F) I2T0.1; G) I2T0.3; H) I2T0.5. (bar scale = 1cm). I: IBA, B: BA, K: kinetin, T: TDZ. The number after the letter indicates the concentration of the growth regulator in mg/L

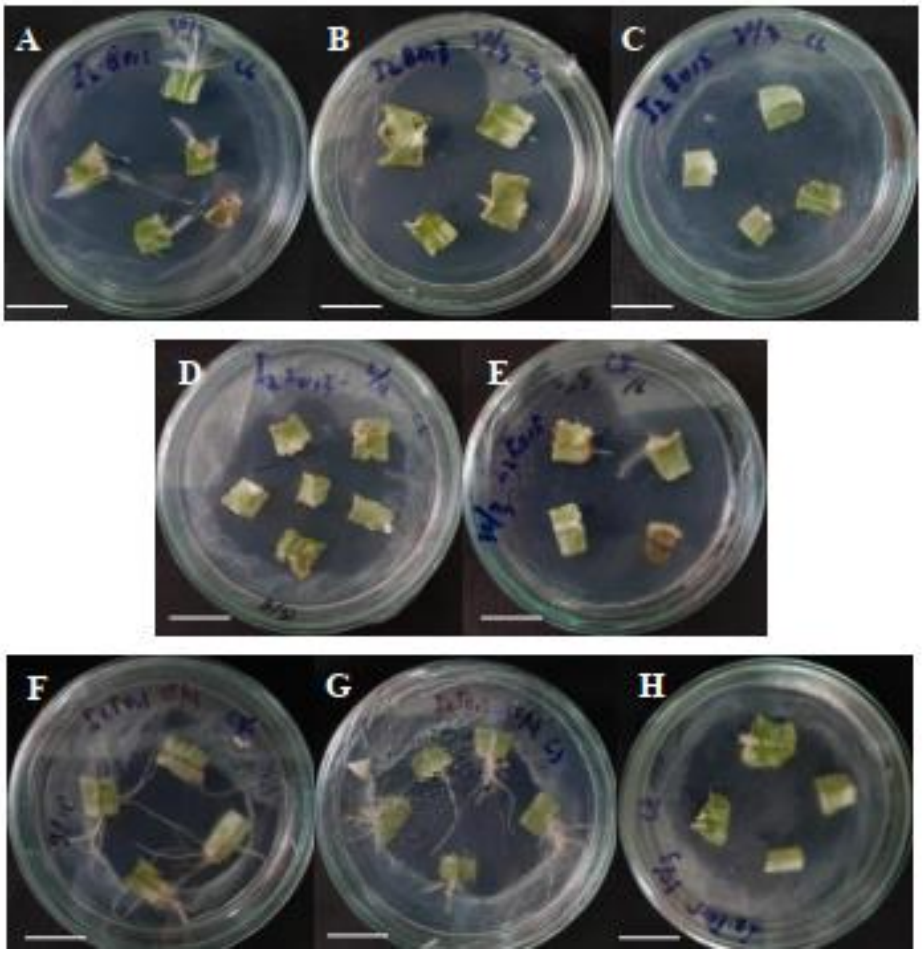

Figure 2 *T. paniculatum* adventitious root induction using leaf explants at the third week. A) I2B0.1; B) I2B0.3; C) I2B0.5; D) I2K0.3; E) I2K0.5; F) I2T0.1; G) I2T0.3; H) I2T0.5 (bar scale = 1cm)

The stem and leaf explants induced from various combinations of growth regulator IBA 2 mg/L with the different types of cytokinins (BAP, kinetin, and TDZ) at separate concentrations (0.1, 0.3, and 0.5 mg/L) produced varied responses, as seen in Tables 1 and 2.

Table 1 The average duration of root formation, number of roots, root length, fresh weight, and dry weight of *T. paniculatum* Gaertn. adventitious roots with various combinations of concentrations of IBA 2 mg / L and BAP, kinetin, and TDZ (0.1, 0.3, 0.5 mg / L) on stem explants

Treatment	Duration of root formation (days)	Number of roots	Root length (mm)	Fresh weight (mg)	Dry weight (mg)
I2B0.1	9.33 ± 4.04	8.33 ± 1.52	11.44 ± 5.58	2.86 ± 1.30	1.00 ± 0.20
I2B0.3	14.00 ± 0.00	4.00 ± 2.64	14.68 ± 5.77	4.10 ± 1.94	0.93 ± 0.28
I2B0.5	14.00 ± 0.00	1.33 ± 0.57	8.50 ± 2.50	1.23 ± 0.05	0.53 ± 0.28
I2K0.1	**4.67 ± 8.08**	1.67 ± 2.88	2.60 ± 4.50	0.12 ± 0.21	0.03 ± 0.05
I2K0.3	11.67 ± 4.04	**8.67 ± 6.11**	11.81 ± 2.50	**11.63 ± 9.60**	**6.67 ± 7.23**
I2K0.5	16.33 ± 4.04	4.00 ± 0.00	7.08 ± 1.52	4.67 ± 3.78	1.06 ± 0.90
I2T0.1	0	0	0	0	0
I2T0.3	16.33 ± 4.04	2.00 ± 1.00	**13.33 ± 3.78**	3.83 ± 2.41	1.43 ± 1.00
I2T0.5	21.00 ± 7.00	1.33 ± 0.57	8.83 ± 2.36	4.63 ± 1.05	1.63 ± 0.15

Table 2 The average duration of root formation, number of roots, root length, fresh weight, and dry weight of *T. paniculatum* Gaertn. adventitious roots with various combinations of concentrations of IBA 2 mg/L and BAP, kinetin, and TDZ (0.1, 0.3, 0.5 mg/L) on leaf explants

Treatment	Duration of root formation (days to)	Number of roots	Root length (mm)	Fresh weight (mg)	Dry weight (mg)
I2B0.1	16.33 ± 4.04	5.33 ± 1.15	13.3 ± 5.67	1.93 ± 1.04	1.16 ± 0.23
I2B0.3	21.00 ± 7.00	3.33 ± 3.21	10.9 ± 2.85	0.93 ± 0.23	0.10 ± 0.11
I2B0.5	**11.67 ± 10.69**	1.00 ± 1.00	3.33 ± 3.05	2.53 ± 2.96	0.83 ± 1.27
I2K0.1	0	0	0	0	0
I2K0.3	**11.67 ± 10.69**	2.00 ± 2.00	3.66 ± 3.32	0.63 ± 0.92	0.50 ± 0.86
I2K0.5	16.33 ± 4.04	2.00 ± 1.00	9.00 ± 5.29	2.40 ± 0.85	1.06 ± 0.90
I2T0.1	21.33 ± 9.01	**7.33 ± 1.15**	**21.33 ± 9.01**	4.53 ± 1.76	**1.33 ± 0.64**
I2T0.3	18.67 ± 8.08	4.33 ± 2.30	8.15 ± 2.58	3.23 ± 1.88	1.03 ± 0.95
I2T0.5	14.00 ± 0.00	1.67 ± 1.15	8.22 ± 5.17	**4.83 ± 2.43**	1.10 ± 0.90

The fastest mean root formation time was obtained from the induction of stem explants with the addition of a combination of growth regulator IBA 2 mg/L + kinetin 0.1 mg/L, namely in 4.67 days. Meanwhile, for the leaf explants, the fastest root formation time, at 11.67 days, was obtained by adding the combinations of growth regulator IBA 2 mg/L + kinetin 0.3 mg/L and IBA 2 mg/L + BAP 0.5 mg/L. Whereas the addition of a combination of growth regulator IBA 2 mg/L + TDZ 0.5 mg/L for stem explants and IBA 2 mg/L + TDZ 0.1 mg/L for leaf explants resulted in the longest mean times to root formation, namely 21 and 21.33 days, respectively.

Based on Table 1, the highest average fresh weight, dry weight, and number of roots for the stem explants were obtained in the IBA treatment of 2 mg/L + kinetin 0.3 mg/L. While the highest average root length of stem explants, namely 13.33 cm, was obtained in the IBA treatment of 2 mg/L + 0.3 mg/L TDZ. In Table 2, the greatest average root fresh weight was obtained from the results of leaf explant induction with the addition of a combination of growth regulator IBA 2 mg/L + TDZ 0.1 mg/L, namely 4.53 mg. Meanwhile, the highest dry weight, number of roots, and root length were obtained from the induction of leaf explants with the addition of a combination of growth regulator IBA 2 mg/L + TDZ 0.1 mg/L. The lowest average dry weight was obtained with the combination of IBA 2 mg/L + BAP 0.3 mg/L, namely 0.1 mg.

Adventitious roots can be induced from various explants including leaves, stems, roots, and various other factors such as auxins (Baque *et al.*, 2010). The selection of the types, concentrations, and combinations of growth regulators is very important. Based on previous research, adventitious roots have been successfully induced from Javan ginseng leaf explants on a solid MS medium with the addition of IBA 2 mg/L, which produced the highest root mass of 5.929 g (Solim *et al.* 2017). Meanwhile, Erin *et al.* (2020) reported that the treatment of IBA 2 mg/L + ethephon 1 mg/L produced the highest average number of roots, namely 7.33, compared to other treatments.

BAP and kinetin hormones are chemical compounds that are included in the cytokinin group and play a role in shoot growth. This time, however, they were combined with auxin IBA to stimulate adventitious root growth on *T. paniculatum* stem and leaf explants. In the research of Isda and Fatonah (2014), the highest number of roots was found at a BAP concentration of 0.5 mg/L + 1.0 mg/L NAA, namely 5.00 fruit on the explants of *Grammatophyllum scriptum* orchid shoots. However, in the results of this study on stem and leaf explants, the combination of IBA 2 mg/L + BAP 0.5 mg/L did not provide optimal results in all parameters of the observation. It is thus evident that the effect of these growth regulators depends on the type of plant and the dosage concentration of growth regulator combination that is suitable; as such, for *T. paniculatum* this is not the optimal concentration of auxin and cytokinin combination to produce the most roots. Apart from BAP, kinetin is also often combined with the auxin hormone in its use in vitro, as in the research of Mahadi *et al.* (2013) where the highest average number of dragon fruit explant roots was found in the $N_{0.4}$ K_4 treatment, namely 5.25 roots. However, the lowest average number of roots was also found in the $N_{0.4}$ K_4 treatment. This is presumably because high kinetin administration can produce stunted explant growth; Wahidah (2011) stated

that kinetin hormone can affect the process of plant development at low concentrations while inhibiting growth at high concentrations.

TDZ can play a role in stimulating endogenous cytokinin production. Therefore, TDZ can increase the action of other cytokinins, both exogenous and endogenous cytokinins (Guol *et al*., 2011). The administration of TDZ at a low concentration induces callus faster than at a high concentration; for callus regeneration, it is better to combine TDZ and NAA at low concentrations than TDZ alone (Oláh *et al*., 2003). This is corroborated by the findings of this study, where TDZ with a concentration of 0.1 mg/L induced the formation of a large number of adventitious roots to produce a large fresh weight and dry weight compared to concentrations of 0.3 mg/L and 0.5 mg/L. The formation of adventitious roots is a type of positive synergy between TDZ and IBA as the best adventitious root-forming hormone.

Effect of Phosphate Concentration (KH_2PO_4) on Adventitious Root Growth

The best treatment for inducing root growth (a combination of IBA 2 mg/L and TDZ 0.1 mg/L) was used to determine the effect of phosphate concentration and the ammonium:nitrate ratio on adventitious root growth. The average fresh weight, dry weight, root growth, number of roots, and adventitious root length of *T. paniculatum* in various phosphate concentration treatments of MS medium are listed in Table 3.

The highest average values for fresh weight, dry weight, and the number of roots were obtained for the P5 treatment (phosphate concentration 340 mg/L). Meanwhile, the fastest root growth rate of 7.3 days was achieved with the P7 treatment (phosphate concentration 425 mg/L), and the longest average root is shown for the P8 treatment (phosphate concentration 467.5 mg/L). The highest average fresh weight and dry weight values were identified for the P5 treatment. This is consistent with research conducted by Curtis *et al*. (1991), which stated that the growth of *Opium poppy* in cell suspension culture increased by 50% in media with two times the concentration of phosphate added. This occurred since phosphate plays an essential role in the transfer of energy for cell metabolism, the constituents of cell membranes, and nucleic acids. The lowest fresh weight was found on the media with the P9 treatment (510 mg/L) due to the very high phosphate concentration. The plant cells in these explants were stressed due to the very high salt concentration. The increase in the plant's dry weight is attributable to the nutrients that were absorbed by the root and the accretion of protoplasm due to the increase and size of the cell count (Khristyana *et al*. 2005).

The fastest average duration of root formation was 7.3 days (phosphate concentration 425 mg/L) based on the application of the control treatment (phosphate concentration 425 mg/L) for 8 days. In this study, the P9 treatment (phosphate concentration 510 mg/L) showed the longest average duration of root formation (11.6 days) because media with high phosphate concentrations can suppress growth (George *et al*. 2007). Phosphate can bind with calcium and other microelements to reduce the absorption of other elements below the maximum level (Buddh, 2014).

Table 3 Average fresh weight, dry weight, duration of root formation, number of roots, and root length of *T. paniculatum* adventitious roots in various phosphate concentrations of MS medium

Treatment code	KH_2PO_4 concentration (mg/L)	Fresh weight (mg)	Dry weight (mg)	Duration of root formation (days to)	Number of roots	Root length (cm)
P1	170	18.77 ± 9.96 ^{b}	3.23± 0.60 ^{b}	8.00 ± 2.64	3.67 ±2.52 ^{a}	2.56 ±1.76 ^{ab}
P2	212.5	2.67 ± 2.56 ^{a}	1.10 ± 0.60 ^{a}	9.00 ± 1.73	3.00 ± 1.00 ^{a}	1.79 ± 0.31 ^{a}
P3	255	8.63 ± 5.55 ^{a}	2.07 ± 0.81 ^{ab}	10.67 ± 1.15	1.33 ± 0.58 ^{a}	2.85 ±1.52 ^{ab}
P4	297.5	16.30± 5.03 ^{ab}	3.30 ± 0.09 ^{b}	8.33 ± 3.21	4.00 ±1.73 ^{a}	1.39±0.22 ^{a}
P5	340	**37.43 ± 5.09** ^{c}	**5.53 ± 1.03** ^{c}	7.67 ± 0.57	**5.33 ±0.58** ^{ab}	1.70 ±0.39 ^{a}
P6	382.5	8.8 ± 6.61 ^{ab}	1.77 ± 1.22 ^{ab}	11.33 ± 1.52	4.00 ± 1.00 ^{ab}	1.55 ± 0.96 ^{a}
P7	425	19.13 ±14.89 ^{b}	3.17 ± 1.56 ^{b}	**7.33 ± 0.57**	4.67 ± 1.15 ^{ab}	2.15 ± 0.46 ^{a}
P8	467.5	19.17 ± 9.03 ^{b}	3.27 ± 0.50 ^{b}	8.00 ± 1.73	1.67 ±0.58 ^{b}	**4.20 ±0.75** ^{b}
P9	510	2.30± 1.15 ^{a}	1.13 ± 0.67 ^{a}	11.67 ± 2.51	2.00 ±1.00 ^{ab}	1.56±0.66 ^{a}

Note: Numbers followed by different letters show real differences according to Duncan's test. The number in bold shows the highest value for each treatment parameter.

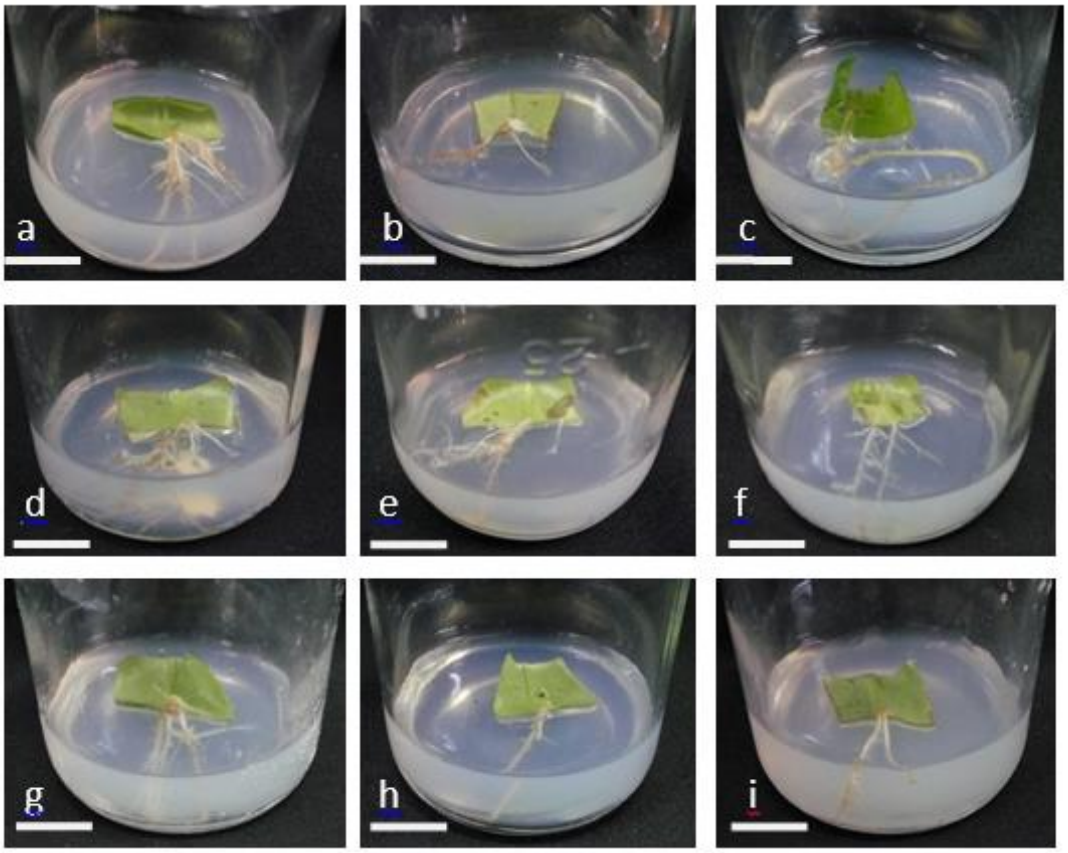

Figure 3 The number of adventitious roots formed from explants of Javanese ginseng leaves (*Talinum paniculatum* Gaertn.) after a 6-week incubation period, namely (a) P1 (KH_2PO_4 170 mg/L / control), (b) P2 (KH_2PO_4 212,5 mg/L), (c) P3 (KH_2PO_4 255 mg/L), (d) P4 (KH_2PO_4 297,5 mg/L), (e) P5 ($KH_2PO_4$340 mg/L), (f) P6 (KH_2PO_4 382,5 mg/L), (g) P7 ($KH_2PO_4$425 mg/L), (h) P8 (KH_2PO_4 467,5 mg/L), and (i) P9 (KH_2PO_4 510 mg/L) (bar scale = 1cm)

The number of roots produced in each explant is different (Figure 3). The highest mean number of roots was found in the explants treated with P5 (KH_2PO_4 340 mg/L). The treatment in this study was controlled by P1 (170 mg/L of KH_2PO_4). The number of roots stood at only 2.56; thus, while both were from the second and third leaves of the shoots, they were from different plants. Auxin plays a role in cell elongation, cell division, and adventitious root formation (George, 2007). Therefore, different endogenous auxins lead to differences in root formation, the number of adventitious roots, and the length of adventitious roots.

Effect of Ammonium:Nitrate Ratio on Adventitious Root Growth

The average fresh weight, dry weight, root growth, number of roots, and the adventitious root length of *T. paniculatum* in various ammonium:nitrate ratios on MS medium are listed in Table 4.

The highest average fresh weight, dry weight, and number of roots were obtained for the ammonium:nitrate ratio of 10:20. Meanwhile, the fastest average time to root formation was 10 days and the longest root length was 2.3 cm. However, the data showed no significant difference between all treatments, meaning the data did not affect the adventitious root biomass of Java ginseng.

Figure 4 contains pictures of 6 different ammonium:nitrate ratios, namely 21:19 mM as the control, and 0:30 mM, 10:20 mM, 15:15 mM, 20:10 mM, and 30: 0 mM for the adventitious roots of Java ginseng at 6 weeks.

Table 4 Average fresh weight, dry weight, duration of root formation, number of roots, and root length of *T. paniculatum* adventitious roots in various ammonium:nitrate ratios (21:19 mM, 0:30 mM, 10:20 mM, 15:15 mM, 20:10 mM, and 30:0 mM)

Ammonium:nitrate ratio	Fresh weight (mg)	Dry weight (mg)	Duration of root formation (days to)	Number of roots	Root length (cm)
A21 : N19 (normal MS)	47.9 ± 14.9^{a}	5.0 ± 1.7^{a}	10.2 ± 3.7^{a}	5.6 ± 5.3^{ab}	1.9 ± 0.8^{a}
A0 : N30	17.5 ± 9.5^{b}	2.1 ± 0.8^{b}	13.0 ± 4.3^{a}	3.0 ± 2.3^{a}	2.0 ± 0.9^{a}
A10 : N20	**73.6 ± 32.5^{a}**	**8.2 ± 3.2^{a}**	10.2 ± 3.0^{a}	**8.8 ± 2.9^{b}**	1.8 ± 0.6^{a}
A15 : N15	23.3 ± 17.6^{b}	3.4 ± 2.5^{b}	**10.0 ± 1.9^{a}**	7.0 ± 4.6^{ab}	1.4 ± 1.0^{a}
A20 : N10	19.6 ± 14.9^{b}	2.5 ± 1.5^{b}	11.0 ± 1.9^{a}	5.6 ± 4.7^{ab}	1.0 ± 0.3^{a}
A30 : N0	21.3 ± 9.6^{b}	3.2 ± 1.2^{b}	11.4 ± 3.4^{a}	5.8 ± 3.1^{ab}	**2.3 ± 1.0^{a}**

Note: Numbers followed by different letters show real differences according to Duncan's test. Numbers in bold show the highest value for each treatment parameter. A = Ammonium; N = Nitrate.

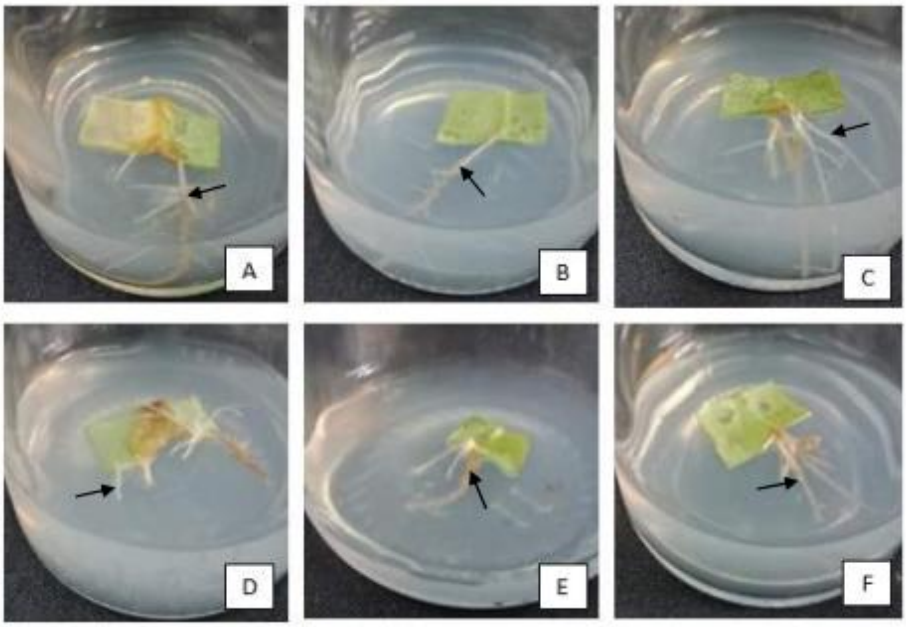

Figure 4 The adventitious roots (shown by arrows) of 6-week-old *T. paniculatum* were cultured in vitro with 6 types of treatment and ammonium:nitrate ratios, namely (a) 21:19 mM (control); (b) 0:30 mM; (c) 10:20 mM; (d) 15:15 mM; (e) 20:10 mM, and (f) 30: 0 mM

Adventitious roots (i.e., roots that form from non-root tissue) can arise in various tissue locations from groups of mature cells that renew their cell division activity (Taiz and Zeiger, 2003). In the treatment ratio ammonium:nitrate 10:20, NH_4Cl has good potential to replace NH_4NO_3 in control as a nitrogen source to increase adventitious root biomass production. This is because nitrogen is used for protein synthesis, both structural and enzymatic, and is thus needed for cell and organ growth, including for the production of plant biomass (Lawlor *et al.* 2001). In addition, the form and amount of nitrogen in the in vitro media have a significant effect on the rate of cell growth, differentiation, and cell totipotency (Kirkby and Mengel, 1987).

The average time to root formation in this study ranged from 10 to 13 days (Table 4). This aligns with the findings of research by Palestine (2008) on pule pandak (*Raufolvia serpentine*, L.) showing that the addition of IBA with a concentration of 2 to 4 mg/L can initiate root growth faster than other treatments, namely in 15 days.

The highest number of roots was found in the ammonium:nitrate ratio of 10:20, while the lowest number of roots was found in the ammonium:nitrate ratio of 0:30. Efforts to increase the number of roots include the addition of auxin growth regulators that can stimulate root induction. Wattimena (1988) explained that auxin is a plant hormone essential

for cell division and root formation. Root emergence is influenced by the number of roots and correlates with the absorption of nutrients present in the culture medium.

Several studies have shown that nitrogen compounds and the ratio between ammonium and nitrate can affect the differentiation, de-differentiation, growth, and development of explants, as well as organ formation (Preece 1995). The average root lengths of the treatments in this study are shown in Table 4; they range from 1 cm to 3 cm with no influence between one treatment and another. The increase in plant size reflects the increase in protoplasm that occurs due to the increase in cell size and number (Khristyana *et al.* 2005).

CONCLUSION

The combination of IBA 2 mg/L + kinetin 0.3 mg/L is the optimal concentration to produce the highest mean number of roots, fresh weight, and dry weight of *Talinum paniculatum* adventitious roots in stem explants. Meanwhile, for leaf explants, the best results were obtained with the combination of IBA 2 mg/L + Thidiazuron 0.1 mg/L. The highest fresh weight (37.47 mg) and dry weight (5.53 mg) were obtained in the phosphate concentration (KH_2PO_4) 340 mg/L treatment. Meanwhile, the ammonium:nitrate ratio of 10:20 was the best treatment to produce the highest biomass (fresh weight 73.6 mg and dry weight 8.2 mg).

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