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The effect of IBA and Ethepon on growth and saponin content of *Talinum paniculatum* Gaertn. adventitious root *in vitro*

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ABSTRACT

Root of *Talinum paniculatum* is a raw material that is used as medicinal raw materials because it contains active compound of saponin which can increases the body's resistance to disease. Growth of *T. paniculatum* root in natural habitat was slow, so we want to improve production of *T. paniculatum* root through *in vitro* culture. This research aims to determine the effect of IBA and ethephon to growth and saponin content of adventitious root *T. paniculatum*. Leaf explants were planted in solid MS medium with combination of IBA 0; 1; 2; 3 mg/L and ethephon 0; 0, 1; 1 mg/L. Culture was incubated for 6 weeks. Data's root growth such as time of root formation, number of roots, length of root, fresh weight of root, and dry weight of root were analyzed using non parametric test (significance level 0.05). Data's saponin content such as stain area and thickness stain were analyzed semi quantitatively using Thin Layer Chromatography (TLC). The results showed that the treatment of IBA 1 mg/L + ethephon 0.1 mg/L produced adventitious roots with the fastest average time of day 7. The treatment of IBA 2 mg/L + ethephon 1 mg/L produced the highest average number of roots was 7.33 roots, the longest average length of root was 1.59 cm, also the highest average fresh weight and dry weight of root were 29.80 mg and 2.70 mg. The highest saponin content based on stain area on TLC plate respectively resulted by the treatment of IBA 1 mg/L + ethephon 1 mg/L was 0.38 cm^2/0.01 g dry weight and IBA 2 mg/L + ethephon 1 mg/L for thickness stain.

Key words : *Adventitious root, Ethephon, Indol Butiric Acid, Saponin, Talinum paniculatum Gaertn.*

Introduction

Most of Indonesian people used conventional medicine excessively, so the pharmaceutical industry as a provider of medicinal raw materials obtained that materials from import activities with value reaches was US$ 160 million per year, so there must be substitution from imported products to domestic products (Prastowo *et al.,* 2007). The meaning of domestic products are plants which can used as a raw material for medicine. According to Hidayat (2005), one of the most widely used medicinal plant is java ginseng (*Talinum paniculatum* Gaertn.). *T. paniculatum* has been known for its effectiveness as an inducer of spermatogenesis and sperm motility, anti-inflammatory, androgenic potential, and sperm viability (Saroni *et al.,* 1999).

Roots of *T. paniculatum* have chemical constituents such as saponins, flavonoids and tannins (Syamsuhidayat and Hutapea, 1991). Saponin is the main bioactive compound in *T. paniculatum*. Harborne (1987) described that saponins are triterpene and sterol glycosides namely triterpenoid and steroid groups that have been detected in plants

of more than 90 tribes. Saponins are reported to increase viability, motility, and number of spermatozoa. Saponins also act as anti-inflammatory agents, have androgenic potential, be able to induce cell differentiation through cell receptors (Zuo *et al.*, 2009), and increase the body's resistance to disease (Hu *et al.*, 2003). Root of java ginseng grew very slowly in their natural habitat. Further, it has known that saponins level of java ginseng roots of three months grew in natural habitat was lower than the java ginseng roots grew in vitro for 28 days (Manuhara *et al.*, 2015). To overcome that problems, it needs to cultivate of *T. paniculatum* through tissue culture technique. Tissue culture technique has several advantages, including be able to obtain new individual in large quantities and a relatively short time, can grow root from various plants, and be able to produce chemical compounds in a relatively short time. It is based on the totipotency of plant cells (Fowler, 1983). Tissue culture technique is important for increasing root availability, because it has known that saponin compounds are found in roots. Application of growth regulator in tissue culture has been stated by Muhallilin (2012) that the most effective type and concentration of auxin is IBA 2 mg/L. It can produce root with the fastest average time of roots formation, the highest average number of roots, and the longest average length of roots. Besides auxin, adding of other growth regulator like ethephon is also needed. Low concentration of ethephon during for in vitro phase can significantly increase the number, size and weight of microtuber potatoes compared to controls (Behzad, 2012). According to the research of Qiao *et al.* (2008), addition of 1 μM ethephon can produce the highest plant of *Saposhnikovia divaricata* after 4 weeks of culture compared to the treatment of ethephon 2, 3, 4, 5 μM. The aim of this research is to know how the effect of IBA and ethephon to growth and saponin content of *T. paniculatum* Gaertn. adventitious root in vitro.

Materials and Methods

Plant Material and Optimization Culture Conditions

The study was carried out in the plant physiology laboratory at Department of Biology, Faculty of Science and Technology, Airlangga University on December 2018 until March 2019. Roots were induced from leaf segments (1 cm^2) of *T. paniculatum* Gaertn. in solid MS (Murashige and Skoog's, 1962) medium supplemented with 30 g/L sucrose and 8 g/L agar (Manuhara *et al.*, 2014; Manuhara *et al.*, 2015). Leaf as explant were planted in solid MS medium with combination of IBA 0; 1; 2; 3 mg/L and ethephon 0; 0,1; 1 mg/L with 3 repetitions. Culture were kept on dark condition at (25 ± 3) °C temperature for 6 weeks.

Statistical Analysis

All experiments were set up in a completely randomized design and the data were collected from three replicates and mean values are tested using SPSS software (version 21.0). Data's root growth such as time of root formation, number of roots, length of root, fresh weight of root, and dry weight of root were analyzed using non parametric test (significance level 0.05).

Extraction and Analysis of Saponin

Dried of adventitious roots (0.01 g) were pounded and soaked in 5 mL ethanol for 24 hours, and then warmed in waterbath at 80 °C for 45 minutes to get saturated extract until the volume was obtained 1.5 mL. Extract and saponin standard were spotted on silica gel GF_{254} and eluted in propanol:water (14:3). The stain was detected by anisaldehide-sulfic acid (Merck) and dried in the oven at 110 °C for 7-10 minutes. The saponin standard (Calbiochem) will appear green color.

Results and Discussion

Effect of IBA and Ethephon on Growth of Adventitious Root

Adventitious root growth parameters were observed include time of root formation, number of roots, length of root, fresh weight of root, and dry weight of root. Based on the various concentration of IBA and ethephon, leaf explant of *T. paniculatum* produce varied responses. The results of observation of root growth parameters are presented in Table 1.

The results of this study indicated that IBA and ethephon gave significantly different effects between treatments to the time of root formation, number of root, length of root, fresh weight of root, and dry weight of root (Fig. 1). Based on the Table 1, the fastest average time of root formation was obtained from induction with IBA 1 mg/L + ethephon

0.1 mg/L at day 7. IBA are known to be able to induce roots from leaf explant of *T. paniculatum* faster than other types of auxin such as IAA and NAA. It was stated in Muhallilin's research (2012), IBA treatments showed the mean of time root formation at 7 to 7,8 days. Likewise, statement of ethephon in the research of Visser *et al.* (1996) that ethylene or ethephon treatment can grows adventitious root in some cutting of herbaceous plants.

Based on the Table 1 we know that the highest average number of roots of *T. paniculatum* in this study were produced by the treatment of IBA 2 mg/L + ethephon 1 mg/L, is 7.33 roots. According to Irwanto (2001), IBA has a little way of spreading. So, if IBA was given to roots, it will only stimulate in roots, so that it was tend to stimulate growth at the top of plant. Addition of ethephon formed a good synergy with IBA so that produced the highest average number of roots. It was explained by Dahnous *et al.* (1982) that ethephon stimulated the production of ethylene in plant tissues which can inhibit the movement of auxin so that auxin remain around the

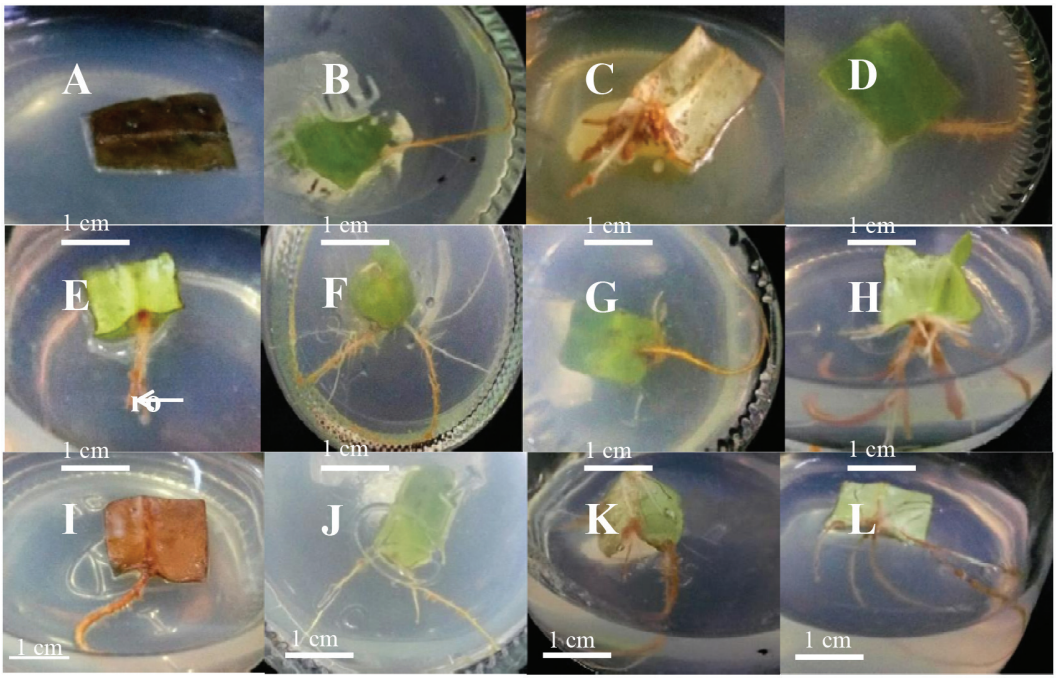

Fig. 1. Effect of IBA and ethephon on growth of *T. paniculatum* root from leaves explants for 6 weeks culture. (A) I_0E_0; (B) I_1E_0; (C) I_2E_0; (D) I_3E_0; (E) $I_0E_{0,1}$; (F) $I_1E_{0,1}$; (G) $I_2E_{0,1}$; (H) $I_3E_{0,1}$; (I) I_0E_1; (J) I_1E_1; (K) I_2E_1; (L) I_3E_1.

Table 1. Time of root formation, number of root, length of root, fresh weight of root, and dry weight of root of *T. paniculatum* Gaertn. in various treatments of IBA and ethephon (n = 3)

Treatments	Time of root formation (day)	Number of roots	Length of root (cm)	Fresh weight of root (mg)	Dry weight of root (mg)
I_0E_0	0a	0a	0a	0a	0a
I_1E_0	12.00 ± 3.21^{e}	2.33 ± 0.57^{c}	0.62 ± 0.10^{a}	1.03 ± 0.05^{c}	0.60 ± 0.10^{bc}
I_2E_0	10.33 ± 2.51^{cde}	4.00 ± 0.00^{d}	0.73 ± 0.17^{a}	2.30 ± 1.50^{bcde}	0.86 ± 0.28^{c}
I_3E_0	9.33 ± 1.52^{cde}	2.33 ± 0.57^{c}	1.06 ± 0.51^{ab}	1.96 ± 1.00^{cde}	1.13 ± 0.58^{cd}
I0E0,1	12.33 ± 5.77^{de}	1.33 ± 0.57^{bc}	1.15 ± 0.48^{ab}	3.20 ± 2.69^{cde}	0.70 ± 0.20^{bcd}
I1E0,1	7.00 ± 0.00^{b}	1.66 ± 1.15^{bc}	1.33 ± 0.70^{ab}	4.23 ± 1.96^{e}	1.20 ± 1.04^{bcdf}
I2E0,1	8.00 ± 1.00^{bcd}	2.60 ± 1.15^{cd}	1.30 ± 0.38^{ab}	2.96 ± 1.45^{de}	1.10 ± 0.55^{bcd}
I3E0,1	8.66 ± 1.52^{bcde}	5.33 ± 1.15^{de}	0.90 ± 0.39^{ab}	3.70 ± 3.30^{de}	1.43 ± 0.55^{d}
I_0E_1	10.00 ± 1.00^{de}	1.66 ± 0.57^{bc}	1.43 ± 0.37^{ab}	1.20 ± 0.55^{bcd}	0.50 ± 0.10^{b}
I_1E_1	7.33 ± 0.57^{bc}	1.00 ± 0.00^{b}	0.65 ± 0.18^{a}	0.70 ± 0.15^{b}	0.43 ± 0.11^{b}
I_2E_1	8.33 ± 2.30^{bcde}	7.33 ± 2.08^{e}	1.59 ± 0.18^{b}	29.80 ± 5.60^{g}	2.70 ± 0.34^{ef}
I_3E_1	12 ± 3.60^{de}	5.33 ± 3.51^{cde}	1.54 ± 0.47^{ab}	18.70 ± 1.53^{f}	2.56 ± 0.40^{e}

application site.

The longest average length of root was produced by IBA 2 mg/L + ethephon 1 mg/L is 1.59 cm. This is due the influence of ethephon. Low concentrations of ethephon can significantly increase the number, size and weight of microtuber potatoes compared to controls (Behzad, 2012). According to the research of Qiao *et al.* (2008), the addition of 1 µM ethephon can produce the highest plant of *Saposhnikovia divaricata* after 4 weeks of culture, which is 3.8 cm compared to 2 µM; 3 µM; 4 µM; 5 µM ethephon. The ability of IBA to initiate root extension was revealed in the research of Pandey *et al.* (2011) that plant of *Ginkgo biloba* L. which is treated with IBA 10 µM were able to induce roots with the longest average root length is 8.38 cm.

The highest average of fresh weight and dry weight of root were produced by IBA 2 mg/L + ethephon 1 mg/L. This treatment is able to increase fresh weight and dry weight significantly compared to other treatments. So that the addition of 1 mg/L ethephon in medium can increase root formation.

Saponin Content of Adventitious Root

The analysis of saponin with Thin Layer Chromatography test showed that differences in stain area and thickness stain (Fig. 2). Some treatment showed a thin green color on the spot. Among the treatments of ethephon, the treatment of IBA 2 mg/L + ethephon 1 mg/L has the thickest stain. However, the largest spot area was produced by IBA 1 mg/L + ethephon 1 mg/L (Table 2).

Application of IBA and ethephon was known to increase secondary metabolism in plants cultured in vitro. It was stated by Ironika (2012) that adventitious roots subcultured for 2 weeks in media added by IBA 2 mg/L had the highest saponin levels. The same thing about ethephon was stated by Cho (1987) that giving ethylene or ethephon into an air reactor of culture *C. arabica* increased the production of purine alkaloids.

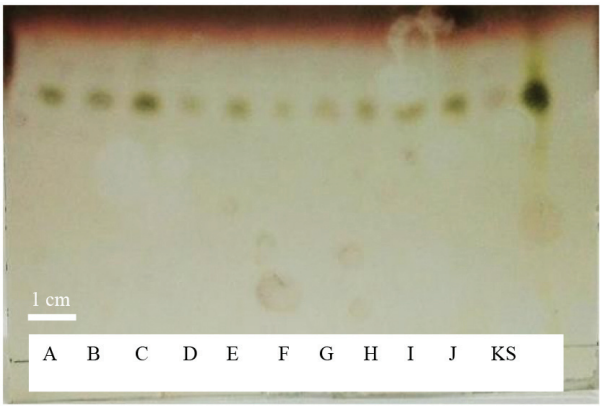

Fig. 2. Cromatogram of saponin on TLC silica gel GF_{254} used 2-propanol: water eluent (14:3), (A) I_1E_0; (B) I_2E_0; (C) I_3E_0; (D) $I_0E_{0,1}$; (E) $I_1E_{0,1}$; (F) $I_2E_{0,1}$; (G) $I_3E_{0,1}$; (H) I_0E_1; (I) I_1E_1; (J) I_2E_1; (K) I_3E_1; (S) saponin standard

Table 2. Saponin stain area of adventitious root of *Talinum paniculatum* Gaertn. in various treatments of IBA and ethephon

Treatments	Spot area (cm^2/0.01 g dry weight)
I_1E_0	0.19
I_2E_0	0.28
I_3E_0	0.28
I0E0,1	0.19
I1E0,1	0.28
I2E0,1	0.28
I3E0,1	0.28
I_0E_1	0.19
I_1E_1	0.38
I_2E_1	0.28
I_3E_1	0.28

Conclusion

Application of IBA and ethephon gave a significantly different effect at the time of root formation, number of roots, length of root, fresh weight of root, and dry weight of root of *T. paniculatum*. The treatment of IBA 1 mg/L + ethephon 0.1 mg/L produced adventitious roots with the fastest average time formation. The treatment of IBA 2 mg/L + ethephon 1 mg/L produced the highest average number of roots, the longest average length of roots, and the highest fresh weight and dry weight of roots. Application of IBA and ethephon also affected to adventitious root of *T. paniculatum*. The highest stain area was obtained by IBA 1 mg/L + ethephon 1 mg/L and the thickest stain was obtained by IBA 2 mg/L + ethephon 1 mg /L.

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