*Sys Rev Pharm 2020;11(7):507-514*
A multifaceted review journal in the field of pharmacy

# Impact of IBA and Ethephon Combination on Root Biomass Production of Javanese Ginseng (*Talinum paniculatum* Gaertn) Cuttings under Aeroponic System

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## ABSTRACT

*Talinum paniculatum* root is efficacious like Korean ginseng. Local pharmaceutical industries use this root to substitute Korean or Chinese ginseng root. The yield of traditional cultivation has not been fulfilled market demand. Cultivation using cuttings in aeroponic is potential to produce roots fastly. Hormones such as auxin and ethylene are internal factors that important to formation and growth of root cuttings. Therefore, this study aims to obtain the best combination of Indole-3-butyric acid (IBA) and ethephon for root biomass of *T. paniculatum* using cuttings in aeroponic. Various concentrations of IBA and ethephon were tested. The results showed root formation of *T. paniculatum* cuttings was depended auxin and ethylene. Both IBA and ethephon were affected and interacted significantly to emergence time, number, length, biomass weight and indole-3-acetic acid (IAA) content of roots. Application of both hormones improved IAA content and number of roots. Optimum concentration for number of roots was reached by combination of 4 $mgL^{-1}$ IBA and 0 $mgL^{-1}$ ethephon. High IBA showed inhibition to root. Low levels of IAA as results of ethephon treatment promoted the formation and growth of roots. Finally, a combination of 0 $mgL^{-1}$ IBA and 1 $mgl^{-1}$ ethephon was selected for producing roots biomass. This combination produced fresh weight of root biomass 1.5 times compared to control. Ethephon application is more profitable. However, its implications on the secondary metabolite content of *T. paniculatum* may need to be known for further research.

**Keywords:** indole-3-butyric acid, ethephon, ethylene, *Talinum paniculatum*, Javanese ginseng, aeroponic, cuttings

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## INTRODUCTION

Indonesia has high diversity of flora, one of them is *Talinum paniculatum*. This plant is a perennial herb that grows in the tropics and is believed to be effective in some countries (Winarni, 2007; Ngoc *et al*., 2018; Osathanunkul and Madesis, 2019). *Talinum paniculatum* has root morphotype and properties like ginseng, so that local people call it Javanese ginseng. (Liu *et al*., 2018). The root of this plant is efficacious as a tonic, aphrodisiac, and drug for several diseases, such as peneumotidis, diabetes, disorder on skin and digestion, cancer, liver, heart and herpes (Shimoda *et al*., 2001; Thanamool *et al*., 2013; Ngoc *et al*., 2018; Riyana *et al*., 2019). High antioxidants, such as ginsenosides, flavonoids, steroid saponins, phenol compounds, and tannins are contained in the roots of *T. paniculatum* (Manuhara *et al*., 2012; Vu *et al*., 2018; Riyana *et al*., 2019). These compounds are responsible for medicinal properties of *T. paniculatum*.

The price of *T. paniculatum* root is relatively cheaper than Korean and Chinese ginseng root. It provokes local pharmaceutical companies using *T. paniculatum* root for substituting both Korean and Chinese ginseng roots (Riyana *et al*., 2019). In addition, the average demand of ginseng roots as raw materials of traditional medicine in Indonesia is high, by 767.66 tons in 2002 (Seswita, 2010). It is known that *T. paniculatum* root grows slowly, which is 30-50 g per year in traditional culture using soil (Manuhara *et al*., 2015). Consequently, the demand for local pharmaceutical companies is not fully fulfilled. Meanwhile, the best yield of adventitious root culture of this plant in a bioreactor using liquid Murashige and Skoog (MS) medium supplemented with 2 ppm IBA was 1.588 $gL^{-1}$ (Manuhara *et al*., 2015). This cultivation technique is potential to improve, but contamination by endogenous microbes often occured during cultivation. Additionally, organ cultures such as adventitious roots are difficult to transfer by pipeline or to pump to the next cultivation vessel (Nguyen *et al*., 2013). A special skill and apparatus are also needed to inoculate this roots or other explants into large-scale bioreactor via inoculation port for reducing the risk of microbial contamination (Kawamura *et al*., 1996). Therefore, non-axenic cultivation in open bioreactor becomes a solution for plants that are difficult to cultivate in the field or in a closed bioreactor under axenic conditions (Nguyen *et al*., 2013). Aeroponics is an excellent open bioreactor for producing medicinal root biomass compared to other types of hydroponic systems (Hayden, 2006). It is known that cuttings in aeroponic is easy to apply for large scale production of medicinal plant (Lenora *et al*., 2012; Gontier *et al*., 2002).

Besides external factors, internal factors such as hormones on the formation and growth of adventitious root in cuttings are important to be evaluated early. The application of root-inducing hormones is generally applied in commercial propagation to induce roots quickly, long and uniformly in the cuttings (Costa *et al*., 2017; G. J. De Klerk *et al*., 1999). This study will evaluate the effect of the combination of indole-3-butyric acid (IBA) and ethephon on the formation and growth of adventitious roots of *T. paniculatum* cuttings in the aeroponic system. The accumulation of auxin and ethylene in submerged organs plays an important role in the production of adventitious roots (Steffens & Sauter, 2009). It is known that IBA as auxin source has a greater ability to trigger root formation than indole-3-acetic acid

(IAA) (G. J. De Klerk *et al*., 1999). Ethephon is used as a source of ethylene in this study, because this compound releases ethylene via autohydrolysis mechanism (Mori *et al*., 2011). This study aims to get the best combination concentration of IBA and ethephon for root biomass. The combination of IBA and ethephon is predicted to influence endogenous IAA content in the cuttings, and consequently affect to the emergence time, number, length and biomass weigh of roots.

## Materials and methods

### *Study area and cuttings preparation*

This study was conducted in the tissue culture laboratory and greenhouse of Biology Department, Faculty of Science and Technology, Airlangga University. Cultivation was carried out for 20 days. Collection of 6-month-old seedlings from the Department of Biology, Faculty of Science and Technology, Airlangga University as a donor plant. The cuttings were prepared by cutting 10-122 cm in length below branch tips using sterile blade. The cuttings had 4-5 leaves. Each leaf was trimmed using scissor up to half of leaf area. The end cut of the cuttings was re-cut at a slope of 45 ° before hormonal treatments.

### *Cultivation condition*

The nutrient solution used composition of MS (Murashige & Skoog, 1962) free carbon source. Dilution of MS elements using mountain water containing total dissolved solids 30-40 $mgL^{-1}$. The strength of nutrient solution agreed with the cuttings age, such as MS 0% on first week and MS 12,5% on second and third week. Growth chamber of aeroponic was built from white plastic bucket with lid, 0.45 m height and 0.30 m diameter. There were 4 holes (80 mm diameter) on the lid to place the mesh pot net basket. The cuttings were inserted in the middle of the basket and provided with clay pellets as growing medium and clone collar foam as a clamp. A immerse pump (such as flow max capacity 2400 $Lh^{-1}$) and 3 sprayers (such as 360° pattern) were positioned inside the chamber for spraying the basal part of the cuttings. Spray cycle was 20 seconds on per 60 seconds off routine. The volume and pH of nutrient solution was maintained at 30 L and 5.5-6.5, respectively.

### *Hormonal treatment*

Different IBA concentrations, such as 0, 1, 2 and 4 $mgL^{-1}$ were combined with different ethephon concentrations, such as 0, 0.01, 0.1 and 1 $mgL^{-1}$. Ethylene synthesis inhibitors, such as 100 µM silver tiosulphate (STS) and auxin transport inhibitors, such as 100 µM 2,3,5-triiodobenzoic acid (TIBA) were also applied. Hormones and inhibitors were applied at the beginning of cultivation by dissolving them into nutrient solution.

### *Measuremen of root growth*

Observations of emergence time and number of roots were conducted during cultivation. Roots length and weigh (fresh and dry weigh) were measured at the end of cultivation using caliper and analytical balance, respectively. The fresh (FW) and dry weight (DW) of roots were measured using analytical balance and moisture meter analyzer at 105 °C for 20 min, respectively. Root preparation before weighing according to Yu *et al.* (2005).

### *Measurement of IAA and IAA-like compounds*

At the end cultivation, one g of fresh adventitious root tips was collected the root was crushed using a mortar. 5 mL of distilled water was added gradually during grinding for IAA Extraction. The mixture was filtered with Whatman # 2 filter paper to collect the filtrate. 1 mL of filtrate was mixed with 2 mL of Salkowski reagent (150 mL of $H_3PO_4$, 250 mL of aquades and 7.5 mL of 0.5 M $FeCl_3.6H_2O$), then incubated under dark conditions at room temperature for 20 minutes. The presence of IAA was characterized by the appearance of pink in the test solution. The level of IAA was measured by a spectrophotometer at a wavelength of 530-535 nm (Gravel *et al*., 2007; Lwin *et al*., 2012).

### *Statistical analysis*

The data obtained were analyzed using the analysis of variance test and continued with the Duncan test with a significance level of 95%.

## Results

### *Impact of IBA and ethephon on adventitious root growth*

The mean of emergence time, number, length and biomass of roots on various combinations of both IBA and ethephon showed significant differences ($p<0.05$), and there was significant interaction between both hormones ($p<0.05$) (Table 1 and Figure 1). The treatment of IBA separately or together with ethephon showed a negative impact on rooting time compared to control. An increase in concentrations of both hormones showed an inverse trend with the time of root emergence. As a result, the best time for root emergence was achieved by a combination of 0 $mgL^{-1}$ IBA and 0.01 $mgL^{-1}$ ethephon on the $9^{th}$ day of cultivation. This result was faster than control ($10^{th}$ day cultivation), but between them was not significantly diferent ($p>0.05$). In contrast, the application of IBA and ethephon separately or together showed a positive impact on root number (Table 1). The highest root number was 85 roots in cuttings treated with 4 $mgL^{-1}$ IBA and 0 $mgL^{-1}$ ethephon. This number was 4 times the root number of controls. Conveserly, the root number decreased significantly in cuttings treated with 4 $mgL^{-1}$ IBA and 0.01 $mgL^{-1}$ ethephon. Furthermore, formation of roots was completely prevented by high combination of both hormones, such as 4 $mgL^{-1}$ IBA and 0.1 $mgL^{-1}$ ethephon, and 4 $mgL^{-1}$ IBA and 1 $mgL^{-1}$ ethephon 1, as a result, the cuttings were dead ( Figure 1D).

The next phase of root formation after root emergence was elongation or growth. This phase was closely related to the formation of biomass, because roots in growth. The cuttings treated with various concentration of IBA (such as 1, 2, 4 $mgL^{-1}$) without ethephon showed reduction on length and biomass of root compared to control (Table 1 and Figure 1). Similarly, the ethephon treatment separately (such as 0.01, 0.1, 1 $mgL^{-1}$) without IBA showed reduction on root length. The longest root was 290 mm, found in cuttings treated with 0 $mgL^{-1}$ IBA and 0.01 $mgL^{-1}$ ethephon. This result was not significantly different compared to control (29.5 mm). Suprisingly, the cuttings treated with ethephon separately showed positive impact on biomass. The highest biomass was 6 g FW and 0.37 g DW observed in cuttings treated with 0 IBA and 1 $mgL^{-1}$ ethephon. Obviously, the cuttings treated with a combination of IBA and ethephon tended to decrease in length and root biomass.

**Table 1.** Adventitious root growth of *T. paniculatum* cuttings for 20 days of cultivation in the aeroponic system with combination of IBA and ethephon in different concentrations.

| Hormone ($mgL^{-1}$) | | Root emergence time | | | Root number | | | Root length | | | Root weight (g) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| IBA | Ethephon | (Day) | | | | | | (mm) | | | Fresh weight | | | Dry weight | | |
| 0 | 0 (Control) | 10.25 | ± | 0.50 ab | 20.25 | ± | 1.26 a | 295.00 | ± | 19.14 e | 4.24 | ± | 0.07 f | 0.29 | ± | 0.00 g |
| | 0.01 | 09.75 | ± | 0.50 a | 23.00 | ± | 0.82 a | 290.00 | ± | 16.33 e | 4.69 | ± | 0.07 g | 0.32 | ± | 0.01 h |
| | 0.1 | 10.00 | ± | 0.82 a | 28.00 | ± | 0.82 b | 237.50 | ± | 15.00 d | 4.92 | ± | 0.16 h | 0.33 | ± | 0.02 h |
| | 1 | 11.00 | ± | 0.82 b | 31.00 | ± | 0.82 b | 240.50 | ± | 17.32 d | 6.06 | ± | 0.11 i | 0.37 | ± | 0.01 i |
| 1 | 0 | 12.75 | ± | 0.50 cd | 30.50 | ± | 1.73 b | 140.00 | ± | 24.45 bc | 1.49 | ± | 0.07 c | 0.12 | ± | 0.00 cd |
| | 0.01 | 12.75 | ± | 0.50 cd | 31.25 | ± | 2.99 b | 155.00 | ± | 17.32 c | 1.79 | ± | 0.07 d | 0.13 | ± | 0.00 de |
| | 0.1 | 13.50 | ± | 0.58 d | 34.75 | ± | 1.26 c | 152.50 | ± | 12.58 c | 2.16 | ± | 0.08 e | 0.18 | ± | 0.00 f |
| | 1 | 12.50 | ± | 0.58 cd | 40.75 | ± | 1.26 d | 125.00 | ± | 23.80 ab | 2.08 | ± | 0.08 e | 0.15 | ± | 0.01 d |
| 2 | 0 | 14.50 | ± | 0.58 e | 36.25 | ± | 2.87 c | 122.50 | ± | 20.62 ab | 1.25 | ± | 0.10 b | 0.08 | ± | 0.01 b |
| | 0.01 | 13.00 | ± | 0.82 cd | 39.75 | ± | 1.26 d | 102.50 | ± | 12.58 a | 1.38 | ± | 0.15 bc | 0.10 | ± | 0.01 c |
| | 0.1 | 12.00 | ± | 0.82 c | 62.75 | ± | 2.06 e | 100.00 | ± | 08.17 a | 1.28 | ± | 0.04 b | 0.13 | ± | 0.02 d |
| | 1 | 10.25 | ± | 0.50 ab | 73.50 | ± | 3.32 f | 100.50 | ± | 12.91 a | 0.82 | ± | 0.06 a | 0.05 | ± | 0.01 a |
| 4 | 0 | 12.75 | ± | 0.50 cd | 85.50 | ± | 4.20 g | 110.25 | ± | 09.57 a | 0.65 | ± | 0.15 a | 0.05 | ± | 0.01 a |
| | 0.01 | 12.75 | ± | 0.50 cd | 34.50 | ± | 5.26 bc | 0* | | | 0* | | | 0* | | |
| | 0.1 | 0* | | | 0* | | | 0* | | | 0* | | | 0* | | |
| | 1 | 0* | | | 0* | | | 0* | | | 0* | | | 0* | | |

Mean values in the same column followed by different superscripts represent a significant difference according to the Duncan test at $p<0.05$. (*) no roots formation.

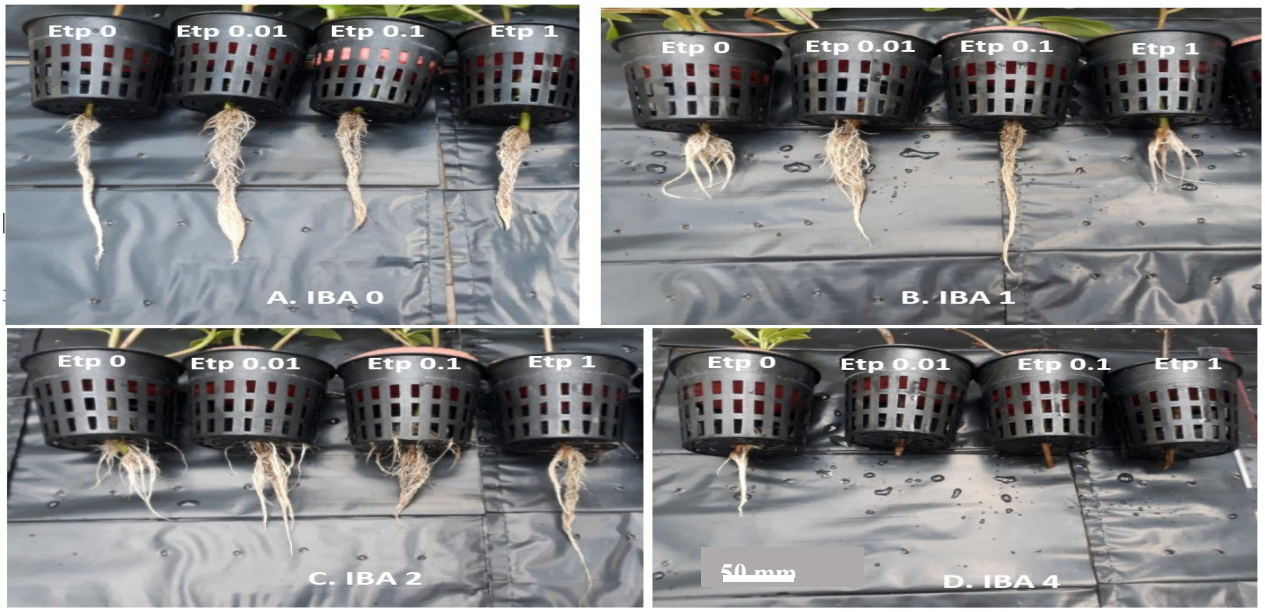


**Figure 1.** Morphologi of adventitious roots of T. paniculatum cuttings after 20 days of cultivation in the aeroponic system with combination of IBA and ethephon (Etp) in different concentrations ($mgL^{-1}$).

***Impact of auxin and ethylene inhibitors on adventitious root growth***

The role of auxin and ethylene in root formation of *T. paniculatum* cuttings in present study was approached by

| Hormonal inhibitor (µM) | | Root emergence time (Day) | | | Root number | | | Root length (mm) | | | Root weight (g) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| TIBA | STS | | | | | | | | | | Fresh | | | Dry | | |
| 100 | 0 | 12.5 | ± | 1.73 | 18.25 | ± | 0.96 | 155.70 | ± | 0.58 | 3.55 | ± | 0.07 | 0.30 | ± | 0.06 g |
| 0 | 100 | 0 | | | 0* | | | 0* | | | 0* | | | 0* | | |
| 100 | 100 | 0 | | | 0* | | | 0* | | | 0* | | | 0* | | |

TIBA and STS treatments. The number, length and weight of roots in cuttings treated with 100 µM TIBA (Table 2 and Figure 2A) were smaller compared to control (Table 1 and Figure 1A). In contrast, aplication 100 µM STS separately or together with 100 µM TIBA absolutely inhibited root formation. The cut end of the cuttings was hardening and dark brown. Failure in root formation is the main reason for cuttings to die in this treatment (Table 2 and Figure 2A-B).

**Table 2.** Adventitious root growth of *T. paniculatum* cuttings for 20 days of cultivation in the aeroponic system with auxin and ethylene inhibitors.

(*) no roots formation

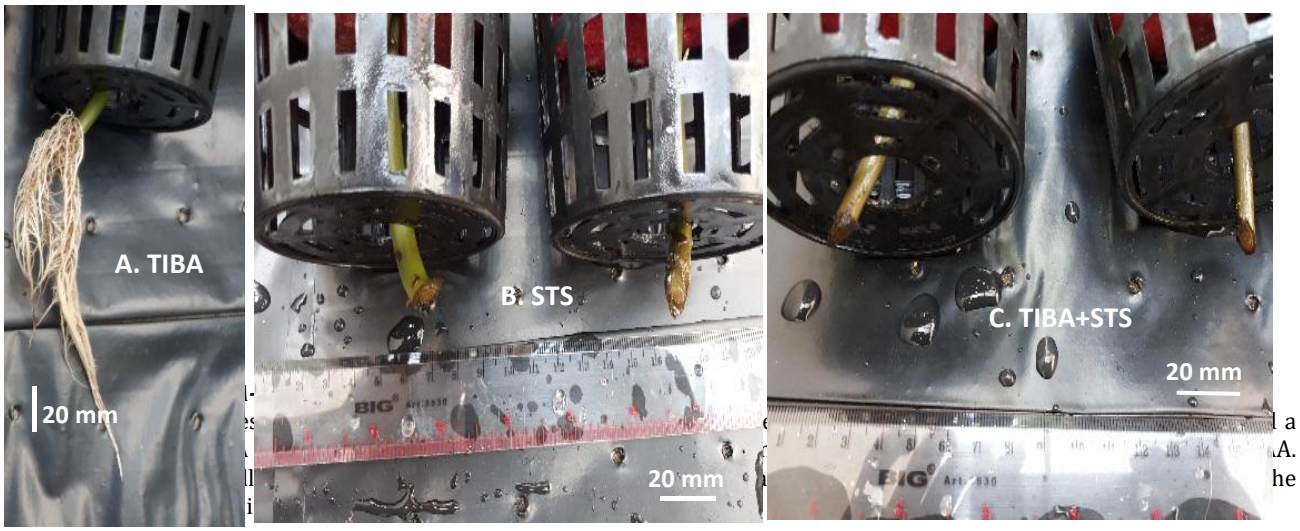


**Figure 2.** Impact of auxin and ethylene inhibitors, such as TIBA and STS, respectively on formation and growth of adventitious root of *T. paniculatum* cuttings after 20 days of cultivation in the aeroponic system.

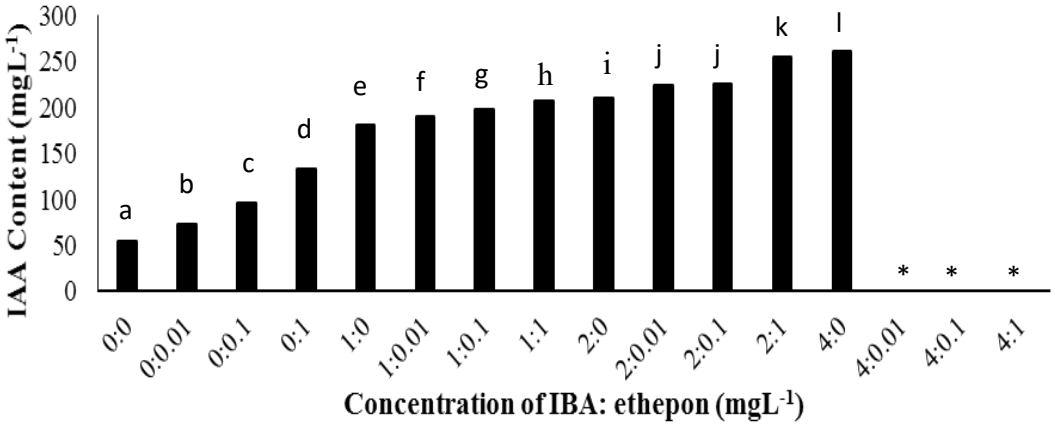


**Figure 3.** The IAA content of adventitious roots of *T. paniculatum* cuttings after 20 days of cultivation in the aeroponic system with combination of IBA and ethephon in different concentrations. Bars followed by different superscripts represent a significant difference according to the Duncan test at $p<0.05$. (*) no roots formation.

## Discussion

*Talinum paniculatum* is one of the spermatophyte species. Most species in this taxon can develop adventitious roots constitutively and, or inductively as response to environmental signals, such as mechanical damage, flooding, biotic stress, or as response to hormones during tissue culture (Bellini *et al*., 2014; Druege *et al*., 2016). The result at present study showed that root formation of *T. paniculatum* cuttings occurred inductively. It is indicated by control, which is the cuttings without

hormone treatment (Table 1, Figure 1A). Wound at the cut end and separate condition from the parent plant can be signals for rooting in this species. Wound can trigger expression of adventitious roots formation in some types of plant cells that did not appear before (Amalero *et al.*, 2003). Similarly, cutting at the bottom of the 8-day-old hypocotyl *Arabidopsis thaliana* seedling stimulated adventitious root formation 10-fold compared to controls (Sukumar *et al.*, 2013). However, root formation in cuttings cannot be separated from the role of phytohormones as an internal factor (Costa *et al.*, 2017). As an external factor, wound and separate conditions can provoke *T. paniculatum* cuttings to produce endogenous rooting hormones. The roots emergence on control indicates that the endogenous rooting hormones of this species enough for rooting process. The formation of adventitious roots in the area of cut end does not always require extra stimulus, such as exogenous hormone (Gonin *et al.*, 2019). This result was consistent with some species of tropical plants, such as *Shorea macrophylla* (Lo, 1985), *Nauclea diderrichii* (Leakey, 1990), *Miliciu excelsa* (Ofori *et al.*, 1996) and *Allanblackia floribunda* (Atangana *et al.*, 2006). However, in commercial agriculture, application of exogenous auxin is needed to get long and uniform roots quickly, and indole-3-butyric acid (IBA) is often used for this purpose (Costa *et al.*, 2017; G. J. De Klerk *et al.*, 1999).

Besides auxin, root formation also involves ethylene. Both hormones, such as auxin and ethylene are often reported as activators of adventitious root formation (Geiss *et al.*, 2018). The role of auxin and ethylene in root formation of *T. paniculatum* cuttings in present study was approached by TIBA and STS treatments. Sukumar *et al.* (2013) reported an increase of IAA flow in the cut area of *A. thaliana* hypocotyl. It is known that ethylene plays a role in stimulating the flow of IAA (Negi *et al.*, 2010). Inhibition of root emergence, number, length, and biomass weight of roots in cuttings treated with TIBA compared to control (Table 1, Figure 1A) proves the role of auxin is significant for rooting of *T. paniculatum* cuttings. However, there were formation and growth of roots in cuttings treated with TIBA although the auxin flow was disrupted, because TIBA has a weak inhibition to auxin flow (Katekar & Geissler, 1980). This result is consistent with Mignolli *et al.* (2017) and Reed *et al.* (1998), application of 20 uM TIBA reduced root branches of *A. thaliana* seedling aged 5 days old compared to control. This compound only interferes with activity of IAA and 2,4-D, because TIBA and IAA compete to occupy the same binding site (Jablanović & Noodén, 1974; Hertel *et al.*, 1973). TIBA moves in the same channel as the IAA and has the same transportation system as the IAA, such as polar transport (Widholm & Shaffer, 1971). Meanwhile, the importance of ethylene for rooting is proven by STS. Completely inhibition on rooting formation was shown by the cuttings treated with 100 µM STS. Application of 10 µM STS was reported to be effective in inhibiting the ethylene response in *A. thaliana* (Schaller and Binder, 2017). Finally, the results of the TIBA and STS treatments prove the root formation of *T. paniculatum* cuttings depends on the interaction of auxin and ethylene. These results are consistent with the results of the statistical test. The interaction of both IBA and ethephon was significant for emergence time, number, length, biomass weight, and IAA content of roots.

The impact of the combination treatment of IBA and ethephon was positive on IAA content (Figure 3). An increase of IAA content indicates that IBA and ethephon were successfully diffused into cells around the cut end of cuttings. The cells may convert IBA to IAA, because IBA is a precursor of IAA (Sauer *et al.*, 2013). Whereas ethylene plays a role to stimulate the conversion rate of IBA to IAA (Veloccia *et al.*, 2016). Variation of IAA contents in the present study depend on the concentration of IBA and ethephon. Consequently, this variation affects the phase of root formation, because the physiological formation of the root formation is associated with changes in auxin concentration (Heloir *et al.*, 1996; Costa *et al.*, 2017). Under normal conditions, the initial phase of root formation, such as induction and initiation require high auxin (Caboni *et al.*, 1997; De Klerk *et al.*, 1999). At this phase, auxin induces a high formation of root primordia (Pop *et al.*, 2011). The number of roots (Table 1) as a representative of root primordia, increased significantly in all cuttings treated with IBA and ethephon. This increase corresponded to the IAA content (Figure 3) as an impact of elevation in the concentration of IBA and ethephon. This result is consistent with Guan *et al.* (2019), the application of IAA on cuttings of *Tomato* sp increased the number of adventitious roots 4-fold compared to the control. However, every organism has an optimal point of treatment. The combination of 4 $mgL^{-1}$ IBA and 0 $mgL^{-1}$ ethephon was the optimal concentration for the induction of root primordia. In the next combination such as 4 $mgL^{-1}$ IBA and 0.01 $mgL^{-1}$ ethephon, there was a reduction in the number of roots. Moreover, in a combination of 4 $mgL^{-1}$IBA and 0.1 and 1 $mgL^{-1}$ ethephon, the root formation was completely prevented (Figure 1D) and the impact on the cuttings was death. Similarly, the decrease in survival percentage reported on *Litsea monopetala* cuttings treated with high IBA concentration, by 0.4 %, (Kumar *et al.*, 2011). Inhibition and completely prevention on root formation of *T. paniculatum* cuttings indicate the response of this species on auxin may decrease, because high-level auxin tends to be harmful rather than beneficial (Kumar *et al.*, 2011). The high hormone concentrations make the presence of hormones turn into poisons (Costa *et al.*, 2017) and further inhibit root formation (Edson *et al.*, 1991).

The next phase of root formation after the induction and initiation is the elongation or growth. At this phase, root primordia grow and emerge from epidermal tissue (Itoh *et al.*, 2005; Steffens *et al.*, 2012; Costa *et al.*, 2017). In the present study, the elongation phase was approached with some parameters, such as rooting time, length, and biomass weight of roots. Negative impact on root growth was showed by *T. paniculatum* cuttings treated with combinations of IBA and ethephon. An elevated IAA level (Figure 3) is responsible for this impact, because the root needs low auxin in the growth phase (G. J. De Klerk *et al.*, 1999). In contrast, the presence of high levels of auxin inhibits root growth (G. De Klerk *et al.*, 1990). High IAA as impact of elevation on concentration of IBA and ethephon in this study proved to inhibit rooting time, length, and biomass weight of *T. paniculatum* cuttings. The same effect was reported by Costa *et al.* (2017) on the cuttings of *Rosa* sp var Sonia. Different results were reported in several plants, such as *Robinia pseudoacacia* and *Grewia optiva* (Swamy *et al.*, 2002), *Tectona grandis* (Husen & Pal, 2007), and *Khaya anthotheca* and *K.ivorensis* (Opuni-Frimpong *et al.*, 2008) which demonstrated an increase of root length at various IBA concentrations. However, the

response of root formation to IBA applications varies between species (Kumar *et al*., 2011).

The combination treatment of 0 $mgL^{-1}$ IBA with various concentrations of ethephon (0.01, 0.1, 1 $mgL^{-1}$) promoted rooting time, length, and biomass weight of root of *T. paniculatum* cuttings compared to other combinations, because the contribution of ethephon to IAA levels is lower than IBA (Figure 3). These results are consistent with Guan *et al.* (2019) who reported the role of ethylene in supporting the root emergence of tomato cuttings. The combination of 0 $mgL^{-1}$ IBA and 0.01 $mgL^{-1}$ ethephon is the optimal combination for rooting time and length of the root. The combination of 0 $mgL^{-1}$ IBA and 1 $mgL^{-1}$ ethephon is the optimal combination for the biomass weight of root. The difference in optimal concentration of the combination of IBA and ethephon between the stages of root formation is a consequence of the difference in auxin requirements at each stage (G. J. De Klerk *et al*., 1999). The same results also were reported in other plants, such as micro-cutting *Malus* (G. De Klerk *et al*., 1990) and apple buds (G. J. De Klerk *et al*., 1999). Finally, the combination of 0 $mgL^{-1}$ IBA and 1 $mgL^{-1}$ ethephon was selected as the best combination for the production of adventitious root biomass from *T. paniculatum* cuttings which was the main objective of this study. The fresh weight of root biomass in this combination was 1.5 times compared to control. The rooting time and length of root in this combination were slightly different with a combination of 0 $mgL^{-1}$ IBA and 0.01 $mgL^{-1}$ ethephon. On the other hand, ethephon is cheaper than IBA. It can save production costs and therefore, suitable for large scale applications, but the effect on the secondary metabolite content of *T. paniculatum* may need to be investigated for further research.

## Conclusion

IBA and ethylene played an important role in root formation of *T. paniculatum* cuttings. Application both hormones improved IAA content and number of roots. High concentration IBA and ethephon reduced root growth which is indicated from the emergence time, length and biomass weight of root. The cuttings which treated with 0 mgL-1 IBA and various ethephon concentrations (0.01, 0.1 and 1mgL-1) had low IAA content. These combinations promoted formation and growth of root. Finally, this study suggests the application of 1 $mgL^{-1}$ ethephon for producing root biomass of medicinal plants such Javanese ginseng (*T. paniculatum*) using cuttings in an aeroponic system.

## Acknowledgement

This research was funded by the Educational Fund Management Institution (LPDP) through Domestic Scholarship Program for Indonesian Lecturers (BUDI-DN). This program is synergy between the Ministry of Research and Technology (Kemenristekdikti) with LPDP for improving the qualifications and competencies of Indonesian lecturers.

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