

Research Article

Evaluation of Temperature Stress Under Different Hydroponic Systems on Growth and Saponin Content of *Talinum paniculatum* Gaertn. Cuttings

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ABSTRACT

Increases in the temperature of nutrient solutions have restricted the use of hydroponic cultivation in the tropics, predominantly due to plant stress. This study aimed to evaluate the effects of temperature stress under different hydroponic systems on the growth and saponin content of *Talinum paniculatum* cuttings. Three hydroponic systems, i.e., deep flow technique (DFT), nutrient film technique (NFT), and aeroponic, were tested. The temperature of the nutrient solution was set for each system, i.e., under ambient temperature (UAT) and with controlled temperature (WCT) at 26° C. The cultivation period was 60 days. The result showed peroxidation activity and proline accumulation for the adventitious roots of *T. paniculatum* cuttings with UAT and WCT, alongside various levels of plasma membrane damage. Levels of Malondialdehyde (MDA) and proline were analyzed by spectrophotometer. Membrane damage was analyzed with Evans blue dye. The results indicated that the levels of MDA and proline accumulation under the three hydroponic systems were higher for the WCT than for the UAT treatment. In contrast, vegetative growth was higher in UAT than in WCT. The saponin content of the adventitious root correlated with the MDA level. Saponin production was triggered by oxidative stress during cultivation, while the adventitious roots had a higher saponin content in all three hydroponic systems with the WCT treatment compared to the UAT treatment. Among the systems, aeroponic was superior for biomass and saponin. Root growth was promoted in the nutrient solution under ambient temperature whereas the production of saponins was stimulated under the controlled temperature. In the aeroponic system, root biomass values of 1.17 and 0.478 g dry weight were obtained under ambient and controlled temperatures, respectively. The total saponin contents differed slightly, namely 189.83 and 195.61 mg/g, respectively.

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INTRODUCTION

Indonesia, as a tropical country, is rich in medicinal plants. One such plant is *Talinum paniculatum* (Jacq.), which has the local name Ginseng Jawa. *Talinum paniculatum* roots are rich in saponins, which have medicinal properties. The root is known for its stamina enhancers and reproductive tonics such as Korean and Chinese ginseng. Traditional farming methods have been insufficient to satisfy market demand for *T. panicula-*

tum roots in terms of both quantity and quality. However, cultivation *in vitro* may offer a solution. Previous studies have reported success in cultivating adventitious roots of *T. paniculatum* in a balloon-type bubble bioreactor (BTBB) (Manuhara et al. 2015). Elicitation using methyl jasmonate and methyl salicylate has been found to increase saponin while reducing adventitious root weight (Ahmad & Anggita 2019). Slow growth, natural contamination by endogenous microbes, and low saponin content are among the problems posed by *T. paniculatum* adventitious root culture in BTBB, which to date have remained unresolved. The production of secondary metabolites from cell or organ culture in a bioreactor has been characterized by low productivity, high cost, and high process technology to control the culture parameters and create sterile conditions (Bourgaud et al. 2001). Furthermore, for large-scale production, the use of adventitious roots as inoculums has proven challenging with regard to transferring (Nguyen et al. 2003). Therefore, alternative *T. paniculatum* cultivation methods are required for root biomass production and saponin content. Many prior studies have demonstrated the positive impact of saponins on health (Sharma et al. 2023; Wang et al. 2023).

Recently, the potency of hydroponic cultures for secondary metabolites of medicinal plants has been investigated. Hydroponics is an alternative method of growing plants that are difficult to cultivate on land or in closed bioreactors with axenic conditions (Nguyen et al. 2003). There is no high technology or high cost involved in the construction of hydroponic systems or in controlling the parameters of culture. The hydroponic nutrient solution is sugar-free and requires no sterilization. These conditions result in inexpensive nutrient preparation and save energy. Nevertheless, the physical and chemical parameters of hydroponic culture can still be controlled to produce biomass with a high content of secondary metabolites (Giurgiu et al. 2014). The successful production of plant secondary metabolites using hydroponic techniques has been reported from various medicinal plants, including the production of flavonoid from *Acemella oleraceae* (Abeyasinghe et al. 2014), tropane alkaloid from *Datura innoxia*, and taxanes from *Taxus baccata* (Gontier et al. 2002).

To date, the hydroponic method has not been tested for *T. paniculatum* cuttings. High air temperatures act as a barrier to hydroponic cultivation in tropical regions. In the tropics, with average daily temperatures of around 30–32° C, plastic or metal containers are heated, thereby increasing the temperature of the nutrient solution in the system (Gur et al. 1972). Heat stress in plants can reduce their growth, number, and root mass (Huang et al. 2012) before impacting the growth of tissue above ground (Giri et al. 2017). It also triggers the accumulation of Reactive Oxygen Species (ROS), causing lipid peroxidation followed by membrane instability or damage (Taratima et al. 2022). The presence of lipid peroxidation products (such as MDA) and electrolyte leakage were identified as indicators of heat stress in rice (Taratima et al. 2022). Malondialdehyde (MDA) is widely used as a marker of free radical formation due to oxidative stress (El-Aal 2012). Excessive proline production in plants is also associated with membrane damage owing to its ability to stabilize cell membranes and prevent electrolyte leakage (Hayat et al. 2012).

In the tropics and countries and regions with high air temperatures, such as Indonesia, it is vital to investigate the appropriate type of hydroponic systems with or without controlled nutrient solution temperatures. Each system type has a specific pattern of nutrient delivery that affects the capacity of the nutrient solution to act as a buffer against temperature changes. This research aims to evaluate the effect of nutrient solution temperature as a consequence of the type of hydroponic system

on the stress, growth, and saponin content of *T. paniculatum* cuttings. The stress indicators comprise the MDA content, proline level, and the results of staining and analysis of absorbed Evans blue dye.

MATERIALS AND METHODS

Materials

Talinum paniculatum cuttings were cultivated in the greenhouse of the Biology Department, Faculty of Science and Technology, Airlangga University, Indonesia. The cultivation period lasted for 60 days, between September and November 2018. The cuttings were sourced from 1-year-old mother plants from the collection of the Biology Department. The cuttings were selected from the straight branch with a length of 10–12 cm from the tip and a diameter of 0.7–1 cm. A sterile blade was used to cut the chosen branch. The base of the cuttings was cut at a slant, at approximately a 45° angle. Each cutting was set in a net pot with circular foam as the holder and immediately placed in a planting hole within the hydroponic system to begin cultivation.

Methods

Preparation of nutrient solution

The hydroponic nutrient solution used Murashige and Skoog medium: NH_4NO_3 (1650 mg), KNO_3 (1900 mg), $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (440 mg), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (370 mg), KH_2PO_4 (170 mg), $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (27.8 mg), $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ (22.3 mg), $\text{ZnSO}_4 \cdot 4\text{H}_2\text{O}$ (8.6 mg), H_3BO_3 (6.2 mg), KI (8.3 mg), $\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$ (0.25 mg), $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (25 mg), $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ (25 mg) (Murashige & Skoog 1962). All materials were diluted in 1000 mL distilled water to make 100% MS. The acidity level of the nutrient solution was maintained within a pH range of 6.0–6.5 using HCl 37% or KOH 25% (Yachya et al. 2020).

Cultivation condition

This study used three types of hydroponic systems, i.e., the deep flow technique (DFT), nutrient film technique (NFT), and aeroponic. The three systems have different patterns of nutrient solution delivery. All systems (DFT, NFT, and aeroponic) were located in a greenhouse with similar environmental conditions, i.e., air temperature, humidity, and sunlight intensity. Each system had ten planting holes. In the aeroponic chamber, five micro sprayers (360° pattern) with a spraying capacity of 1 L/min were located below the planting holes. The volume of the nutrient solution in each system was 30 L. Circulation of the nutrient solution was conducted using a submersible pump with a maximum flow rate of 2400 L/h. Two temperature treatments were applied to the nutrient solutions in all systems, i.e., without controlled temperature or under ambient temperature (UAT) and with controlled temperature (WCT) or under a cooling treatment at 26° C using a chiller. The following nutrient solution strength levels were used during cultivation: 1st to 6th day of cultivation – 0% MS; 7th to 20th day of cultivation – 12.5% MS; 21st to 34th day of cultivation – 25% MS; 35th to 48th day of cultivation – 25%; 49th to 60th day of cultivation – 50% MS. The nutrient solution was replaced every two weeks. During cultivation, the air and nutrient solution temperatures were measured at midday. The acidity level of the nutrient solution was maintained within a pH range of 6.0–6.5. HCl 37% or KOH 25% solution was used to increase or reduce the pH of a nutrient solution if it moved outside the target pH range. The volume of the nutrient solution was also maintained using distilled water.

Measurement of MDA level

Reaction with thiobarbituric acid (TBA) was used to measure the MDA level (Zhang & Huang 2013). Fresh adventitious roots (500 mg) and 4.5 mL 1% sulfuric acid were homogenized using a mortar and pestle. The homogenate was centrifuged at 6000 rpm for 10 min. A volume of 0.5 mL supernatant was collected in a test tube and reacted with 0.5 mL 0.1% trichloroacetic acid (TCA) and 2 mL 0.5 % TBA. The test tube was heated in a water bath at 95° C for 30 min to induce a reaction. After this, the test tube was immediately cooled in ice for 30 min to stop the reaction. A red color will appear in the acid buffer as TBA reacts with MDA. The reaction mixture was then centrifuged at 6000 rpm for 5 min. The supernatant was collected for absorbance reading at λ 532 nm. The absorbance value was then converted to MDA using a regression equation of the standard curve. Construction of the standard curve used a serial concentration of tetra ethoxy propane (TEP): 0.625, 1.25, 2.5, 5, 10, 20, and 40 μ M. The regression equation was $y = 80.433x - 0.0392$ with a correlation coefficient $R^2 = 0.9999$. The MDA concentration (μ M) was plotted on the y -axis, while the x -axis showed the absorbance of the sample.

Measurement of proline level

This research also measured the level of proline and Evans blue uptake to determine damage in the root plasma membrane due to lipid peroxidation. The proline level in adventitious roots was measured spectrometrically (Bates et al. 1973). Fresh adventitious roots (500 mg) were ground using a mortar and pestle with 3% sulfosalicylic acid (4.5 mL) added to the mortar during preparation. The homogenate was centrifuged (6000 rpm) for 15 min or filtered with Whatman #2 filter paper. The supernatant (0.5 mL) was then placed in a test tube and reacted with ninhydrin reagent (1 mL) and glacial acetic acid (1 mL). The test tube was boiled in a water bath at 100° C for 1 h after which it was immediately soaked in ice water for 30 min to stop the reaction. Next, toluene (1.5 mL) was added to the test tube for extraction. The reaction mixture was shaken using a vortex for 1 min or until the chromophore appeared. The chromophore, which was one phase with toluene, was taken for absorbance reading at λ 520 nm. A regression equation of the standard curve was used to convert the absorbance value to proline. The construction of the standard curve used a serial concentration of proline: 1, 10, 50, 100, 150, 200, and 300 mM. The regression equation was $y = 465.43x - 8.3411$ with a correlation coefficient $R^2 = 0.9921$. The y -axis showed proline concentration (μ M), with the sample absorbance plotted on the x -axis.

Quantification of membrane damage

Evans blue dye was used to quantify the membrane damage in adventitious roots (Preethi et al. 2017). The tips of adventitious roots (weight 0.1 g and length 1 cm) were collected and then transferred to 2 mL Eppendorf tubes. Evans blue solution (2 mL) was added and the Eppendorf tubes were shaken using an orbital shaker at 50 rpm for 20 min. The roots were then washed using distilled water until no further color emerged. Next, a selected root was drained for observation and documentation under a transmitted light microscope. This was followed by quantification of the membrane damage. Evans blue dye, which was absorbed by the root tissue, was extracted using 1 % sodium dodecyl sulfate. Stained roots (0.1 g) and 1 mL SDS were homogenized using a mortar and pestle and then transferred to a centrifuge tube. Calcium dichloride 0.1 M pH 5.6 was added to the extract to produce an extract volume of 5

mL and then centrifuged at 6000 rpm for 10 min. The collected supernatant was taken for absorbance reading at λ 600 nm. The absorbance value was converted to Evans blue uptake using a regression equation of the standard curve. The standard curve was composed of a serial concentration of Evans blue solution: 100, 200, 300, 400, 600, 700, 800, 900, and 1000 ng/mL. The dissolution of Evans blue dye used 1 M CaCl₂ pH 5.6. The regression equation was $y = 1342x - 8.5442$ with a correlation coefficient $R^2 = 0.9982$. Evans blue concentration (μ M) was plotted on the y -axis, while the x -axis showed the sample absorbance.

Measurement of growth parameters

The growth parameters in this study, i.e., leaf area, plant height, and the fresh and dry weight of adventitious root, were measured at the end of the cultivation period. A scanner and Image J software were used to measure the leaf area. Plant height was measured manually using a ruler. The fresh and dry weights of adventitious root were measured using an analytical balance and moisture analyzer, respectively. Adventitious roots were separated from the stem using a blade and then blotted for 10 min on tissue paper to absorb excess water (Yu et al. 2005). The roots were then weighed using an analytical balance to obtain the fresh weight. Dry weight analysis was performed with a moisture analyzer (Mettler Toledo HB43) at 105°C for 20 min.

Measurement of saponin content

The extraction and analysis of saponin in adventitious roots was conducted based on Manuhara et al. 2015. The adventitious root (1 g dry weight) was first ground using a mortar and pestle. Absolute ethanol (10 mL) was then added and left overnight. The saponin content in the filtrate was measured using a Shimadzu HPLC with a 5 μ m (150 \times 14.6 mm) HRC ODS Shim pack column. The column temperature was 40° C with acetonitrile and water as the mobile phase. The saponin analysis results showed no repetitions due to sample limitations; therefore, statistical analysis was not carried out.

Data analysis

Data analysis was performed using the ANOVA test. If an F-value in ANOVA was significant, the next test was the Duncan or Games-Howell test with a significance level of 5%. The statistical tests were analyzed using IBM SPSS Statistics 23.

RESULTS AND DISCUSSION

Nutrient solution temperature

Figure 1 shows the daily temperature profile of the air and nutrient solutions in the three hydroponic systems with the UAT and WCT treatments during cultivation. The pattern of change in the nutrient solution temperature with the UAT treatment was relatively similar to that of the air temperature. In contrast, the nutrient solution temperature with the WCT treatment was categorically different from the air temperature. Due to the chiller, it remained relatively stable at 26°C. The average air temperature during cultivation was 36.22°C. This was a higher and significantly different temperature compared to the average nutrient solution temperature in the three hydroponic systems with the UAT and WCT treatments (Table 1).

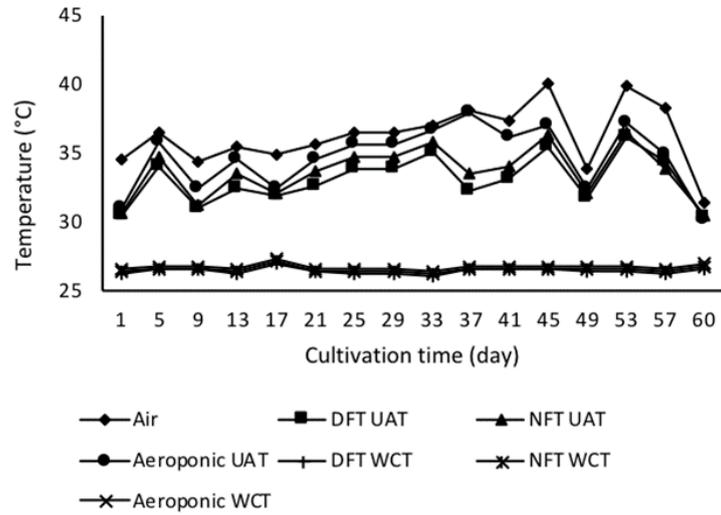


Figure 1. Profile of the air and nutrient solution temperatures of three hydroponic systems (DFT, NFT, and aeroponic) in two temperature treatments: under ambient temperature (UAT) and with controlled temperature (WCT) during 60 days of cultivation. Measurements were taken around midday, between 12:00 AM – 01:00 PM.

The similarity of the change patterns in the nutrient solution temperatures across the three hydroponic systems with the UAT treatment against the air temperature (Figure 1) indicates that the air temperature influenced the nutrient solution temperatures. However, different levels of influence on the nutrient solution temperature were evident (Table 1). The nutrient solution temperature in the DFT system was lower than in the NFT and aeroponic systems. In DFT, a high volume of nutrient solution was circulated via a pipe, which helped to stabilize the temperature of the solution (Park et al. 2001). In the NFT system, the nutrient solution flowed thinly inside a gully, which did not impede any temperature change (Van Os et al. 2008). Meanwhile, the nutrient solution temperature was highest in the aeroponic system and differed significantly from that of the DFT and NFT systems.

Table 1. Nutrient solution temperatures of three hydroponic systems under ambient temperature (UAT) treatment during 60 days of cultivation. Temperatures were measured around midday, between 12:00 AM – 01:00 PM.

Hydroponic System	Temperature (° C)
DFT	32.82 ± 1.7483 ^a
NFT	33.33 ± 1.8370 ^a
Aeroponic	34.56 ± 2.4083 ^b

Average values in the same column followed by different letters are significantly different, according to Duncan’s multiple comparison tests at $p < 0.05$.

In the aeroponic system, the nutrient solution was sprayed around the root zone for 20 seconds every 1 min. This method encouraged faster heating of the nutrient solution compared to the DFT and NFT systems (Table 1). The patterns of temperature change in the nutrient solutions among the three systems with the UAT treatment prove that the method of nutrient solution delivery affected the temperature of the solution during cultivation.

Oxidative stress and membrane damage on adventitious root

The MDA and proline levels, along with the Evans blue uptake and Evans blue staining images for adventitious roots in the three hydroponic

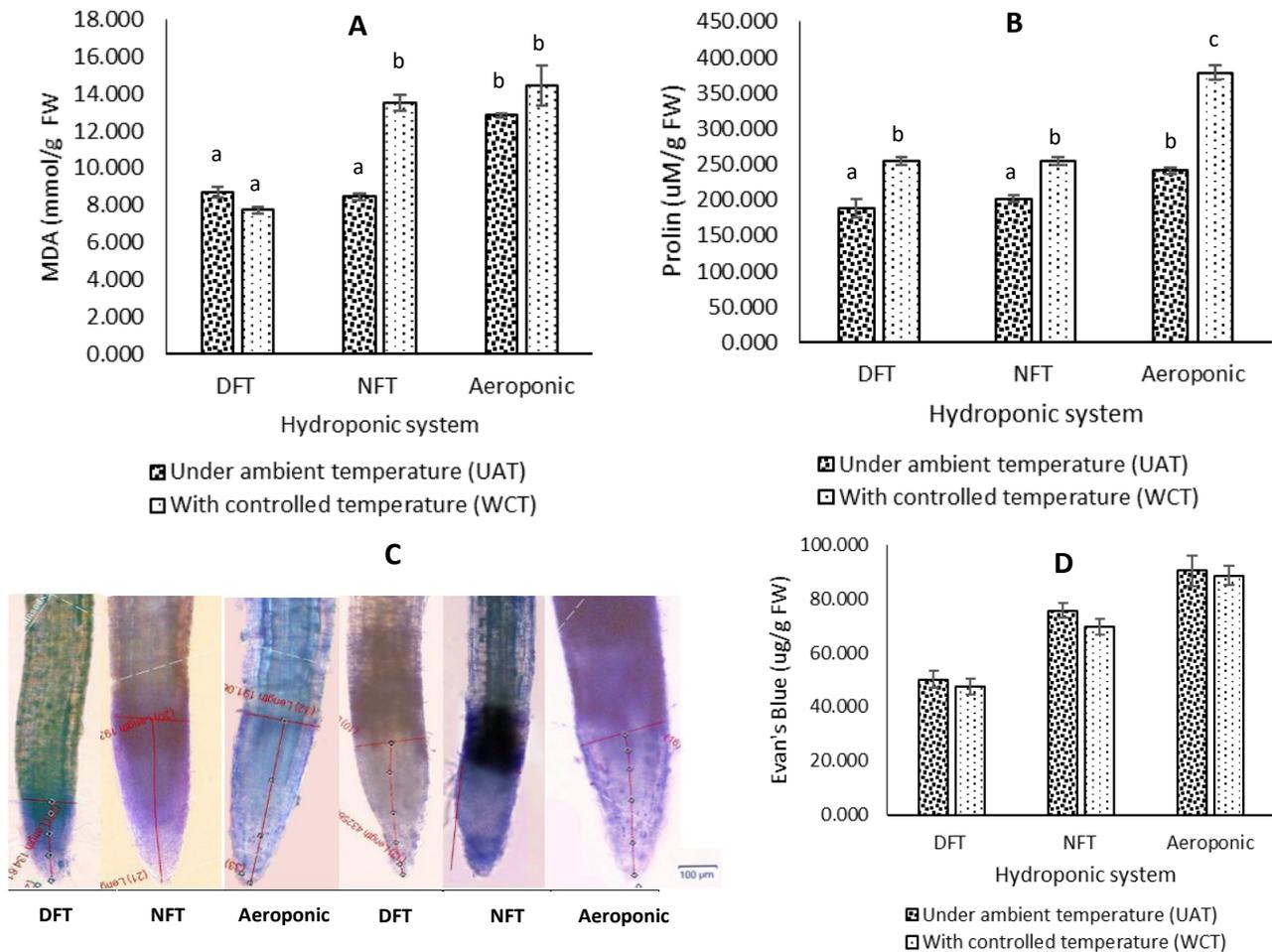


Figure 2. The content of MDA (A) and proline (B), the result of Evans blue staining (C), and the level of Evans blue uptake (D) in *T. paniculatum* adventitious roots after 60 days of cultivation in three hydroponic systems (DFT, NFT, and aeroponic) with two temperature treatments on nutrient solution: under ambient temperature (UAT) and with controlled temperature (WCT). The Games-Howell test at $p < 0.05$ was used to calculate the significant difference in MDA, while Duncan's test at $p < 0.05$ was used for proline and Evans blue.

systems with the UAT and WCT treatments were observed at 60 days of cultivation. The levels of MDA (Figure 2A) and proline (Figure 2B) in the three hydroponic systems were relatively higher with the WCT treatment compared to the UAT treatment. Relatively similar uptake levels of Evans blue were recorded for both the UAT and WCT treatments (Figure 2D). Among the three hydroponic systems in either group (UAT or WCT), the highest levels of MDA, proline, and Evans blue uptake were observed for adventitious roots in the aeroponic system. Within the aeroponic system, however, the MDA and proline levels (Figures 2A, 2B) of adventitious roots were higher for the WCT treatment compared to the UAT treatment. In contrast, there was no difference in the levels of Evans blue uptake between the UAT and WCT treatments in the aeroponic system (Figure 2D).

The lipid peroxidation analysis result (Figure 2A) indicated various levels of oxidation stress on *T. paniculatum* adventitious root. Accumulation of MDA, which is a by-product of lipid peroxidation, correlates with plant stress levels (Wang et al. 2015; Chen & Zhang 2016). With the UAT treatment, the aeroponic system had the highest MDA level, which was also significantly different from that observed with DFT and NFT, while no significant difference was found between the latter two systems (DFT and NFT). The result of the MDA analysis in Figure 2A was in line with the average nutrient solution temperature of the three hydro-

ponic systems with UAT treatments (Table 1). This implies that oxidative stress on the adventitious roots in the UAT treatment was linked to the change in the nutrient solution temperature during the cultivation period. The increase in the nutrient solution temperature to 32.83–34.56°C (Table 1) in the three hydroponic systems with the UAT treatment may have proven relatively hot to the adventitious roots of *T. paniculatum* cuttings, thereby triggering heat stress. For the WCT treatment, the lowest MDA level was recorded for the DFT system and was very similar to the respective levels for the DFT and NFT systems with the UAT treatment. This result indicated that the adventitious roots of *T. paniculatum* cultivated using DFT with the WCT treatment also underwent abiotic stress, particularly cold stress. Meanwhile, the MDA levels for NFT and aeroponics with the WCT treatment were relatively similar and higher than for both NFT and DFT with the UAT treatment. These results indicated that the adventitious roots of *T. paniculatum* cultivated using NFT and DFT with the WCT treatment underwent more abiotic stress than when using NFT and DFT with the UAT treatment.

In addition to cold stress, the adventitious roots in both the NFT and aeroponic systems with the WCT treatment underwent heat stress. In both the NFT and aeroponic systems, the adventitious roots did not sit entirely within the nutrient solution. In the NFT system, the lower roots, which were exposed to the flow of nutrient solution, were in a relatively cold condition (26°C), which may have triggered cold stress. The upper roots, which were exposed to the air, were in a relatively hot condition (36.22°C), which could have triggered heat stress. Among the various types of abiotic stress, cold is one of the most significant barriers to growth and also influences the genetics, physiology, and biochemical responses in a plant (Soydam Aydin et al. 2013). Cold stress will increase protein transcription and the activity of various enzymes that break down ROS in plants (Suzuki & Mittler 2006). Accumulation of hydrogen peroxide, which was also followed by lipid peroxidation, was observed in the leaves of red lettuce (*Lactuca sativa*) when plants were cultivated in the DFT system with cooling treatment in the root zone (Sakamoto & Suzuki 2015). Similar to the NFT system, adventitious roots in the aeroponic system were subjected periodically to both temperature conditions, i.e., cold and heat. When the pump was turned on, the cold nutrient solution was sprayed over all parts of the adventitious roots for 20 seconds. When the pump was subsequently turned off for 1 min, all parts of the adventitious roots were exposed to the air and were in a relatively hot condition. Finally, adventitious roots in both the NFT and aeroponic systems displayed overactivity of lipid peroxidation compared to DFT.

The accumulation of MDA in the adventitious roots of the *T. paniculatum* cuttings was followed by an accumulation of proline (Figure 2B). This indicated that proline accumulation was a response by the *T. paniculatum* cuttings to lipid peroxidation activity. The accumulation was triggered by temperature stresses (heat or cold stress) as proline accumulates when plants are subjected to various kinds of abiotic stress (Anwar Hossain et al. 2014). Previous studies have reported that proline concentration usually increases when plants experience abiotic stress (Yue et al. 2019). A rise in proline level during cold and heat stresses was reported for two chrysanthemum species and *Petunia* seedlings (Cheng et al. 2014; Yue et al. 2019). The trend for proline (Figure 2B) was relatively similar to that of MDA (Figure 2A). This indicated that *T. paniculatum* cuttings were able to maintain oxidative homeostasis by producing proline to reduce the MDA level since proline is among the non-enzymatic antioxidants that act as ROS scavengers (Hayat et al. 2012). Proline's ability to reduce

MDA has been reported for several plants, including chickpea (RSG 44) (Singh et al. 2010), melon (Yan et al. 2011), and barley (Reza et al. 2006). Proline also plays a role as a stabilizer of cellular and membrane structures to prevent electrolyte leakage (Hayat et al. 2012). The instability of the plasma membrane is due to lipid peroxidation, which can alter the composition, formation, structure, and dynamics of lipid membranes (Gaschler & Stockwell 2017). In contrast, it has also been reported that proline can protect the integrity of the callus membrane of *Solanum nigrum* treated with cadmium stress (Xu et al. 2012). Therefore, proline accumulation in all root tip samples was an indirect indicator of the instability of the plasma membrane in the adventitious roots of *T. paniculatum* cuttings.

It was possible to confirm disorder or damage in the plasma membrane of adventitious roots in the UAT and WCT treatments using an Evans blue assay. The results of the Evans blue staining (Figure 2C) showed that all samples were positive, thus confirming that the plasma membranes of the adventitious roots cultivated in the three hydroponic systems in conditions of UAT and WCT did not function normally or had damage. Normal plasma membranes are impermeable to Evans blue dye, while damaged plasma membranes are permeable and absorb Evans blue dye, staining the cells (Roy et al. 2019). Evans blue dye can only pass through cell membranes that lose their semi-permeability, or in which the plasma membranes do not function normally (Gaff & Okong'o-Ogola 1971). The accumulation of Evans blue in cells correlates with membrane damage (Preethi et al. 2017). The level of damage to the plasma membrane determines the volume of Evans blue that enters the cell to bind to many proteins. According to Alves et al. (2018), Evans blue can bind to many proteins and is not metabolized. Finally, the absorbance of extracted Evans blue rises with increasing membrane damage (Roy et al. 2019). Additionally, adventitious root cells that react positively with Evans blue may also have viability disorder. Loss of plasma membrane integrity affects cell viability, and Evans blue staining could determine this disorder (Tamás et al. 2006; Vemanna et al. 2017). Meanwhile, the uptake of Evans blue (Figure 2D) can determine the extent of plasma membrane damage and cell viability disorder in each treatment. The level of Evans blue uptake showed a similar trend to that of both MDA and proline. These results confirmed that lipid peroxidation activity caused membrane damage and viability disorders. Therefore, Evans blue dye is used to determine the presence of dead cells and damage in the plasma membrane, especially at the lipid bilayer (Tistama et al. 2012; Vemanna et al. 2017). Plasma membrane damage or cell viability disorder occurred in all of the adventitious roots cultivated in the three hydroponic systems with UAT and WCT treatments, although the *T. paniculatum* cuttings remained alive throughout the cultivation period.

Growth of cuttings

Table 2 and Figure 3 show the growth profiles of the *T. paniculatum* cuttings at the end of the cultivation period. All growth parameters (leaf area, plant height, fresh root, and dry weight) in the three hydroponic systems with the UAT treatment were higher compared to the same systems with the WCT treatment. In terms of each group (UAT or WCT), the highest to lowest growth parameter levels were obtained, respectively, by the aeroponic, NFT, and DFT systems. However, the growth level of the *T. paniculatum* cuttings in the aeroponic system with UAT treatment was significantly different and higher than the other systems with the UAT and WCT treatments.

Table 2. Growth level of *T. paniculatum* cuttings after 60 days of cultivation in three hydroponic systems (DFT, NFT, and aeroponic) with two temperature treatments on the nutrient solution: under ambient temperature (UAT) and with controlled temperature (WCT).

Hydroponic system - temperature treatment	Leaf area (cm ²)	Plant height (cm)	Adventitious root	
			Fresh weight (g)	Dry weight (g)
DFT – UAT	1714.91±41.81 ^d	70.00±2.00 ^c	2.49±0.17 ^a	0.24±0.02 ^a
NFT – UAT	2391.49±41.66 ^e	68.67±3.22 ^c	3.59±0.24 ^b	0.36±0.02 ^b
Aeroponic –UAT	2984.43±30.02 ^f	80.33±0.587 ^d	15.19±0.29 ^d	1.17±0.08 ^d
DFT – WCT	451.68±30.75 ^a	42.33±2.52 ^a	2.45±0.24 ^a	0.24±0.03 ^a
NFT – WCT	583.85±46.39 ^b	53.67±1.53 ^b	2.21±0.15 ^a	0.21±0.01 ^a
Aeroponic – WCT	1028.78±49.02 ^c	67.00±2.65 ^c	6.89±0.09 ^c	0.48±0.02 ^c

Average values in the same column followed by different letters are significantly different, according to Duncan’s multiple comparison tests at $p < 0.05$.

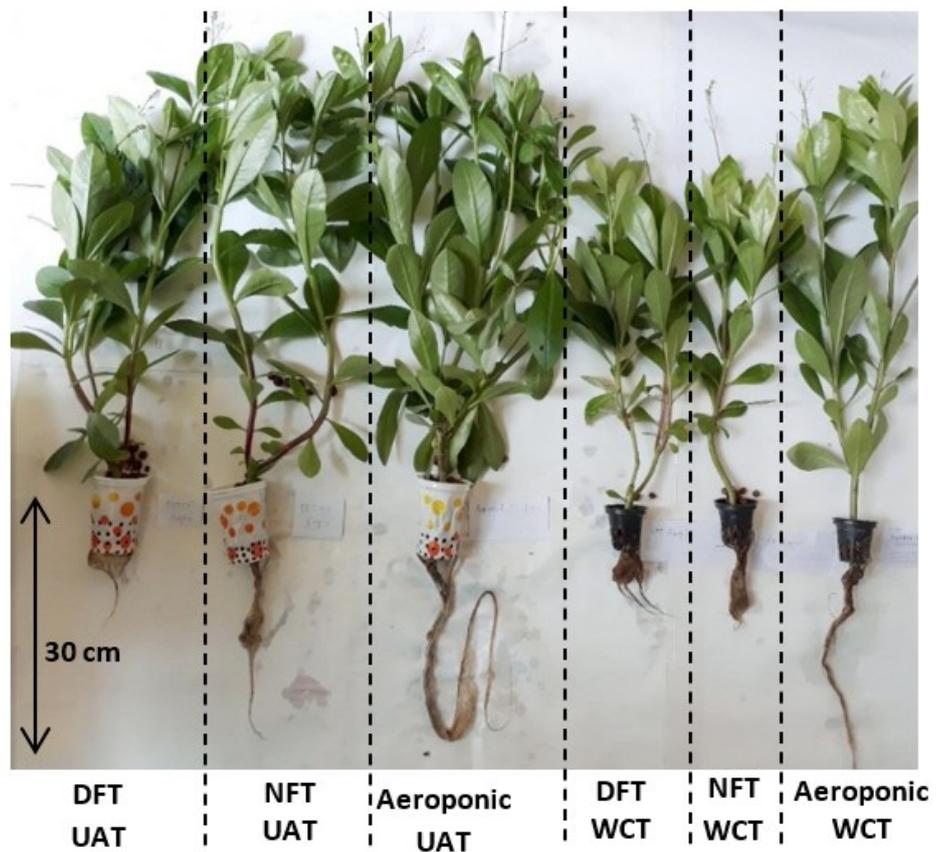


Figure 3. Morphology of *T. paniculatum* cuttings after 60 days of cultivation in three hydroponic systems (DFT, NFT, and aeroponic) with two temperature treatments on the nutrient solution: under ambient temperature (UAT) and with controlled temperature (WCT).

Among the three hydroponic systems with either UAT or WCT, while the aeroponic system produced the highest level of *T. paniculatum* cutting growth, the adventitious roots grown in this system also showed the highest levels of MDA (Figure 2A) and plasma membrane damage (Figure 2D). This indicated that oxidative stress and plasma membrane damage did not affect the vegetative growth of *T. paniculatum* cuttings in this study. Wang et al. (2022) reported different results, namely that damage to the plasma membrane of *Cinnamomum camphora* inhibited nutrient absorption and reduced biomass. In general, temperature stress can damage the plasma membrane and disrupt nutrient absorption, which is followed by a reduction in the growth, number, and

mass of roots and tissue above the ground (Huang et al. 2012; Giri et al. 2017; Wang et al. 2022). This can be caused by a level of oxidative stress or MDA (Figure 2A) below the tolerance limit, or by improving the adaptability through proline accumulation (Figure 2B). It was assumed that the nutrient solution temperature and oxygen availability played an essential role in the vegetative growth of *T. paniculatum* cuttings. Water temperature is another important growth factor that can affect plant development in hydroponic systems (Nxawe et al. 2010).

The cold nutrient solution in the WCT treatment could reduce the absorption of nutrients by the roots, while a warm solution in the UAT treatment could increase nutrient absorption (Morgan et al. 1980; Calatayud et al. 2008). Therefore, the increase in the three growth parameters in the three hydroponic systems was higher with the UAT than with the WCT treatment. The effect of nutrient solution temperature on nutrient uptake has also been demonstrated in various commercial plants. The rise in the nutrient solution temperature was found to accelerate the growth and increase the crop yields of *Cucumis sativus* (Daskalaki & Buggage 1998), *Simmondsia chinensis* (Reyes et al. 1977), and pine (Vapaavuori et al. 1992). In addition to the temperature of the nutrient solution, the availability of oxygen at the root zone area can support the vegetative growth of *T. paniculatum* cuttings. In the DFT system, all parts of the adventitious roots were immersed in the nutrient solution. However, due to a lack of oxygen, this position was unfavorable for the root. In the NFT system, only the lower parts of the adventitious roots were placed within the nutrient solution, while in the aeroponic system, all adventitious parts roots were in the air. Adventitious roots that were not fully immersed in the nutrient solution benefited from an increase in oxygen and gas exchange within all root structures. This condition is beneficial for growth and has been scientifically proven to increase yields by 10 times compared to conventional planting yields (Roberto 2003). Therefore, the result of this study showed that *T. paniculatum* cuttings cultivated in the aeroponic system and UAT showed the best results in terms of vegetative growth. A similar result was found for *Anemopsis californica* cultivated for eight months in an aeroponic system, namely increased production of its medicinal root, whereas NFT was found to reduce root production (Hayden 2006).

Saponin content of adventitious roots

The adventitious roots cultivated for 60 days in the three hydroponic systems with WCT treatment showed a higher saponin content than roots grown in the three systems with UAT treatment (Table 3). Similar to the growth level, for either group (UAT or WCT), the aeroponic, NFT, and DFT systems showed the highest to lowest saponin contents, respectively. Relatively similar saponin contents were obtained in the aeroponic system with both the UAT and WCT treatments. In comparison, *T. paniculatum* cuttings cultivated conventionally in soil for 120 days showed a lower saponin content than those cultivated in the three hydroponic systems.

Generally, secondary metabolites are produced under stress conditions (Dixon 2001). This study found that saponin production (Table 3) was aligned with the MDA level (Figure 2A). This result implies that the *T. paniculatum* cuttings cultivated in the three hydroponic systems with UAT and WCT treatments produced saponin under stress conditions. Figure 2A and Table 3 show that the high saponin content corresponded with a high level of MDA in adventitious roots in the aeroponic system with the UAT and WCT treatments. While in general, the production of

Table 3. Saponin content of the adventitious roots of *T. paniculatum* cuttings cultivated in three different hydroponic systems with two temperature treatments on the nutrient solution for 60 days and conventionally in soil for 120 days.

Cultivation method	Saponin content (mg/g DW)	
	Under ambient temperature (UAT)	With controlled temperature (WCT)
Hydroponic – DFT	131.76	126.46
Hydroponic – NFT	135.05	177.15
Aeroponic	189.83	195.61
Conventional (Soil)	56.61	

secondary metabolites in plants is not associated with cell growth (Malik et al. 2013), this study found otherwise. Figure 2A and Table 2 indicate that MDA levels did not affect vegetative growth. However, in this study, vegetative growth aligned with saponin production. This phenomenon was beneficial because under stress conditions, the cells, especially root cells, could grow and produce biomass and saponin. *Talinum paniculatum* root with a high saponin content will have a higher commercial value as both the pharmaceutical industry and local citizens will use it as a substitute for Korean or Chinese ginseng root.

The hydroponic cultivation of *T. paniculatum* cuttings showed greater potential in terms of obtaining root biomass with a high saponin content compared to conventional cultivation using soil as a medium. *Talinum paniculatum* cuttings cultivated conventionally for four months produce an adventitious root biomass of 0.014 g (DW) with saponin levels of 56.6 mg/g (DW). In this study, the best harvest was obtained by cultivating *T. paniculatum* cuttings for two months in an aeroponic system with UAT treatment. Compared with conventional methods, the use of an aeroponic system reduced the cultivation period by two months, produced a 30-fold increase in weight (1.175 g DW), and increased the saponin content by 3.5 times (195.61 mg/g DW). Finally, in the tropics, an aeroponic system with the nutrient solution under ambient temperature was identified as the appropriate system in which to cultivate *T. paniculatum* cuttings. These results are consistent with the previous assertion that aeroponic systems are most effective for growing medicinal and aromatic herbal plants in a controlled environment, where the plants and roots are harvested (Hayden 2006).

CONCLUSIONS

T. paniculatum cuttings were successfully cultivated in the tropics using three hydroponic systems, i.e., DFT, NFT, and aeroponic. Differences in the nutrient solution flow patterns in the three test systems affected the temperature of the nutrient solutions, which in turn promoted different levels of stress that affected the accumulation of biomass and saponins of *T. paniculatum* adventitious roots.

AUTHOR CONTRIBUTION

A.Y and Y.S.W.M conceptualized the research study. A.Y and A.N.K designed the research methodology. A.Y performed the research and analyzed the data. A.Y, Y.S.W.M, and A.N.K wrote the manuscript. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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