

Research Article

Effect of Carbon Source Variations on Growth, Physiological Stress, and Saponin Levels of *Talinum paniculatum* Gaertn. Adventitious Roots

Nindi Novia Erin¹, Arif Yachya², Alfinda Novi Kristanti³, Djarot Sugiarto⁴, Yosephine Sri Wulan Manuhara^{1*}

1)Biology Department, Faculty of Science and Technology, Universitas Airlangga

2)Biology Department, Faculty of Math and Science, Adi Buana PGRI University

3)Chemistry Department, Faculty of Science and Technology, Universitas Airlangga

4)Chemistry Department, Faculty of Science and Data Analytics, Institut Teknologi Sepuluh Nopember

* Corresponding author, email: yosephine-s-w-m@fst.unair.ac.id

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ABSTRACT

Monosaccharide and disaccharide as carbon sources can affect the production of secondary metabolites. The study aims to determine the effect of variations in carbon sources on growth, physiological stress, and saponin levels of the adventitious roots of *Talinum paniculatum* Gaertn. Adventitious roots are subculture in liquid MS medium treated with various sugars: 3% sucrose, 3% glucose, 3% fructose, 3% lactose, 3% maltose, 3% dextrose, sucrose + fructose (1.5% + 1.5%), sucrose + glucose (1.5% + 1.5%), glucose + fructose (1.5% + 1.5%), sucrose + dextrose (1.5% + 1.5%) for 6 weeks. The results of this study show that the 3% fructose treatment produces the highest fresh and dry biomass, which are 1.30 g and 0.23 g compared to the control. The morphology of adventitious roots in the treatment of carbon source variation is not different from the control treatment. The highest MDA (malondialdehyde) levels are found in the sucrose + fructose treatment (1.5% + 1.5%). Meanwhile, the highest proline levels are found in the 3% maltose treatment. Saponin levels analyzed using thin layer chromatography show the data in the form of color intensity and stain area based on ImageJ software analysis. The 3% fructose treatment shows the highest color intensity and stain area compared to the control. Variations in carbon sources affect physiological stress, biomass, and saponin levels of adventitious roots of *T. paniculatum*, but do not effect on root morphology.

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INTRODUCTION

Talinum paniculatum Gaertn. (java ginseng) is a family of Portulacaceae. *T. paniculatum* is widely used as a substitute for Korean ginseng which is still imported because it is relatively cheap, easy to obtain, and easy to cultivate (Widiyani 2006). The roots of *T. paniculatum* were known to have androgenic potential (Winarni 2009) and sperm viability potential (Rahmi & Widayarsi 2011). Saponins can be used as anti-inflammatory drugs, have androgenic effects, as well as can induce cells through cell receptors (Zuo et al. 2009) and increase the body's resistance to disease (Hu et al. 2003). In its natural habitat, java ginseng root grows slowly. Getting the root in the form of bulbs

takes approximately two to three years, so efforts are needed to obtain bioactive compounds of java ginseng root through in vitro culture techniques. Enhancement of saponin accumulation in adventitious roots culture of java ginseng have been done in various treatment, for instance by elicitation (Faizal et al. 2019), sucrose and potassium nitrate treatment (Manuhara et al. 2015), but the production of saponin compounds that influenced by the carbon source (sugar) not done yet. However, plant cells and culture media do not have autotrophic capabilities. Thus, it requires an external carbon source for metabolism.

Sugar has been known as a compound that can be utilized by plants as a carbon source that can affect metabolism, development, growth, and gene expression. It is either monosaccharide or disaccharide sugar that can act as the carbon source (Wang & Weathers 2007). The culture of plant cells, tissues, or organs usually requires the introduction of a carbon source into the culture medium (George 1993). Among the many available carbon sources, sucrose is the main one (Fuentes et al. 2005). However, it can lead to hypoxia and cell ethanol accumulation due to its rapid metabolism (Scott 1995). In some cases, sucrose is completely or partially replaced by another carbon source (George 1993). Many studies report the use of other carbon sources such as maltose (Percival & Fraser 2005); glucose (Sami et al. 2016); fructose (Uozumi et al. 1991); glucose + fructose (1:1), sucrose + fructose (1:1), sucrose + glucose (1:1) (Praveen & Murthy 2012).

Based on the ideas that have been described, this study is conducted to provide a variety of carbon sources, including sucrose, lactose, maltose, glucose, fructose, dextrose, sucrose + fructose, sucrose + glucose, sucrose + dextrose, and glucose + fructose to determine the effect on morphology, physiological stress, biomass production, and levels of saponin compounds in adventitious roots of *T. paniculatum*. The changes in malondialdehyde (MDA) and proline levels as markers for cellular oxidative stress were also evaluated. The results of this study can be used as a basis for the development of java ginseng adventitious root culture to increase biomass and saponin compounds on a larger scale.

MATERIALS AND METHODS

Material Preparation

The *T. paniculatum* plant used in this study is obtained from the Bratang flower market, Surabaya, and has been identified by Indonesian Institute of Sciences, Purwodadi Botanical Garden, Pasuruan, East Java, Indonesia. The materials used in this study include materials for making media Murashige and Skoog, *Indole Butyric Acid* (IBA) 2 mg/L, sucrose (Gulaku), glucose (Glucolin), lactose (Grdane Lactose 200), maltose (Merck kGaA), fructose (Krystar 300), dextrose (R&W), agar compaction, 0.5 mL *anisaldehyde*, 10 mL glacial acetic acid, 5 mL concentrated sulfuric acid, 2-propanol, distilled water, 4.5 mL H₂SO₄ 1%, aqueous solution *Trichloro Acetic Acid* (TCA) 0.5 mL, *Trichloro Acetic Acid* (TCA) + *Thiobarbizuric Acid* (TBA) 0.5 mL,

Sulfosalicylic Acid (SAS) 4.5 mL, Ninhydrin 1 mL, Toluene 1.5 mL, Folin-Ciocalteu reagent, and Na₂CO₃.

Adventitious roots were induced in MS solid medium supplemented with IBA 2 mg/L, sucrose 3% and agar 7 g/L. After four weeks cultured adventitious roots were sub cultured in MS liquid medium supplemented with IBA 2 mg/L and various carbon source; there are 3% sucrose (control) (Murashige & Skoog 1962), 3% glucose, 3% fructose, 3% lactose, 3% maltose, 3% dextrose, sucrose + fructose (1.5% + 1.5%), sucrose + glucose (1.5% + 1.5%), glucose + fructose (1.5% + 1.5%), sucrose + dextrose (1.5% + 1.5%); and were cultured for 6 weeks. Treatments were replicated three times respectively based on Federer formula.

Morphological Observations

Adventitious roots that are six weeks old in liquid culture that have been mentioned above were harvested. Their morphology was observed using a stereomicroscope at 30x magnification. The length of the root branch and its diameter was measured using an inverted microscope. The results of morphological observations are presented in images. Meanwhile, the measurements are presented in the form of an average, which is then analysed quantitatively.

Malondialdehyde (MDA) and Proline Tests

The MDA test is conducted based on the method by Peixoto et al. (1999) with a few modifications. In the MDA test, a fresh sample of 0.5 g is required, which is mixed with 4.5 mL of 1% sulphuric acid solution. Afterward, it is centrifuged, and the supernatant is taken as much as 0.5 mL. The supernatant is mixed with 0.5 mL TCA solution and centrifuged again. The supernatant is mixed with 2 mL of TCA + TBA solution and heated in a 100°C water bath for 60 minutes. The next one is cooled in ice cubes for 30 minutes, then centrifuged again and taken about 200 µL on a microplate for spectrophotometry at a wavelength of 532 nm. The proline test is conducted based on the method by (Bates et al. 1973) with slight modifications. In the proline test, a fresh sample of 0.5 g is mixed with 4.5 mL of SAS solution and centrifuged. In addition, take 0.5 mL of the supernatant and mix with 1 mL of ninhydrin, and then heat it in a 100°C water bath for 60 minutes. Then, cooled in ice cubes for 30 minutes, added with 1.5 mL toluene, and vortexed for 1 minute. Finally, about 200 µL is taken on a microplate for spectrophotometry at a wavelength of 520 nm.

Biomass Measurement

Adventitious roots that are six weeks old in liquid culture in each treatment are harvested and weighed using an analytical balance to determine their fresh weight, followed by heating in the oven at a temperature of around 60°C for ± five days. Then it was weighed using an analytical balance to determine the dry weight. The measurement results are presented in the form of

an average and then analysed quantitatively. The number of samples used in this study was three replicates. The growth index was calculated using the following formula:

$$\text{Growth index} = \frac{(\text{final fresh weight}) - (\text{initial fresh weight})}{\text{initial fresh weight}}$$

Saponin Extraction

To extract the saponins, the adventitious root of *T. paniculatum* is weighed 0.02 g dry, grounded using a mortar to become a powder, given 5 mL of 96% ethanol, and left for 24 hours. That was followed by heating for 45 minutes in a water bath at 80°C. The sample is filtered using filter paper to separate the precipitate and liquid. The obtained liquid is tested using thin-layer chromatography (three replicates).

Thin Layer Chromatography Test

T. paniculatum adventitious root extract samples are spotted on 20 µL silica gel GF₂₅₄ TLC plates of each treatment. To compare the presence of saponins in the sample, a standard saponin solution is made, which is also spotted on the TLC plate. Afterward, elution is carried out using a vessel containing a solution of 2-propanol: water (14: 3) (Yachya 2012). Moreover, it is sprayed on the surface of the TLC plate using the *anisaldehyde-Sulfuric Acid* solution and then dried in an oven at a temperature of 100-110°C for 7-10 minutes (Stahl 1985). Finally, the researchers observed the appearance of saponin stains was observed on the TLC plate. The saponin was determined by the green color. When spraying with a stain viewer, if a dark green (Manuhara et al. 2015), purple or red-purple color appears (Marliana et al. 2005) then the extract contains saponins.

RESULTS

The Effect of Carbon Source Variations on Adventitious Root Biomass

Adventitious roots are obtained from the induction of leaf explants of *T. paniculatum* on solid *Murashige and Skoog* (MS) media plus 2 mg/L IBA for four weeks, then sub cultured in a 250 mL Erlenmeyer for six weeks culture with the addition of various carbon sources, result shown in Figure 1.

The yield of adventitious root culture (Figure 1) is weighed to determine the fresh and dry biomass. The averages of the fresh and dry biomass are summarized and presented in Table 1. Plant propagation by in vitro culture is also shown by the growth index obtained from fresh biomass using the growth index formula (IP), which is the difference between fresh biomass at harvest and fresh biomass inoculated. Afterward, it is divided by fresh biomass inoculated. Among all treatments, the highest IP is obtained in adventitious roots with 3% fructose treatment, which is 0.30. The IP value of sucrose + dextrose (1.5% + 1.5%) treatment is lower, i.e. 0.01 compared to sucrose alone. The mean biomass and growth index of *T. paniculatum* adventitious roots are shown in Table 1.

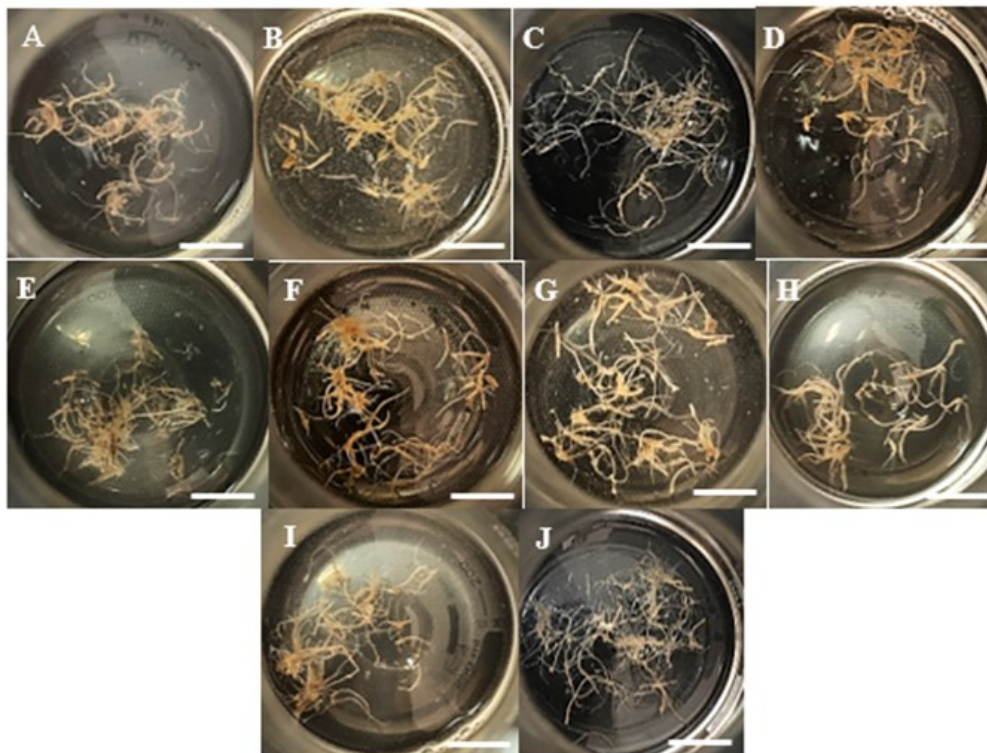


Figure 1. Adventitious roots of *T. paniculatum* cultured for 6 weeks in liquid MS medium with various carbon sources treatment. **A.** 3% sucrose; **B.** Lactose 3%; **C.** Maltose 3%; **D.** 3% glucose; **E.** Dextrose 3%; **F.** Fructose 3%; **G.** Sucrose+Fructose (1.5%+1.5%); **H.** Sucrose+Glucose (1.5%+1.5%); **I.** Sucrose+Dextrose 1.5%+1.5%; **J.** Glucose+Fructose (1.5%+1.5%). Bars = 1 cm.

Table 1. Average biomass and growth index of *T. paniculatum* adventitious roots at 6 weeks of culture age in various treatments of carbon sources (n=3).

Treatment	Initial inoculum fresh biomass (g)	Fresh biomass (g)	Dry biomass (g)	Growth Index (IP)
Sucrose 3% (control)	1	1.06 ± 0,02	0.07 ± 0.00	0.06 ± 0,02
Lactose 3%	1	1.21 ± 0,10	0.14 ± 0.07	0.21 ± 0.03
Maltose 3%	1	1.24 ± 0,16	0.16 ± 0.19	0.24 ± 0.16
Glucose 3%	1	1.05 ± 0,00	0.05 ± 0.03	0.05 ± 0.00
Dextrose 3%	1	1.14 ± 0,01	0.06 ± 0.01	0.14 ± 0.01
Fructose 3%	1	1.30 ± 0,07*	0.23 ± 0.12*	0.30 ± 0.07*
Sucrose+Fructose (1.5% + 1.5%)	1.	1.02 ± 0,01	0.05 ± 0.00	0.02 ± 0.01
Sucrose+Glucose (1.5% + 1.5%)	1	1.03 ± 0,02	0.03 ± 0.01	0.03 ± 0.02
Sucrose+Dextrose (1.5% + 1.5%)	1	1,01 ± 0,00	0.02 ± 0.00	0.01 ± 0.00
Glucose+Fructose (1.5% + 1.5%)	1	1,25 ± 0,09	0.20 ± 0.16	0.25 ± 0.09

*highest yield of fresh biomass and dry biomass

The Effect of Carbon Source Variations on Adventitious Root Morphology

The results of the observation of the morphology of the adventitious roots of *T. paniculatum* using a stereo microscope with a magnification of 30x are shown in Figure 2. The adventitious roots cultured in the treatment media of various carbon sources show the growth of root branches and root hairs as in control. In addition, this study also measures the root branch length and

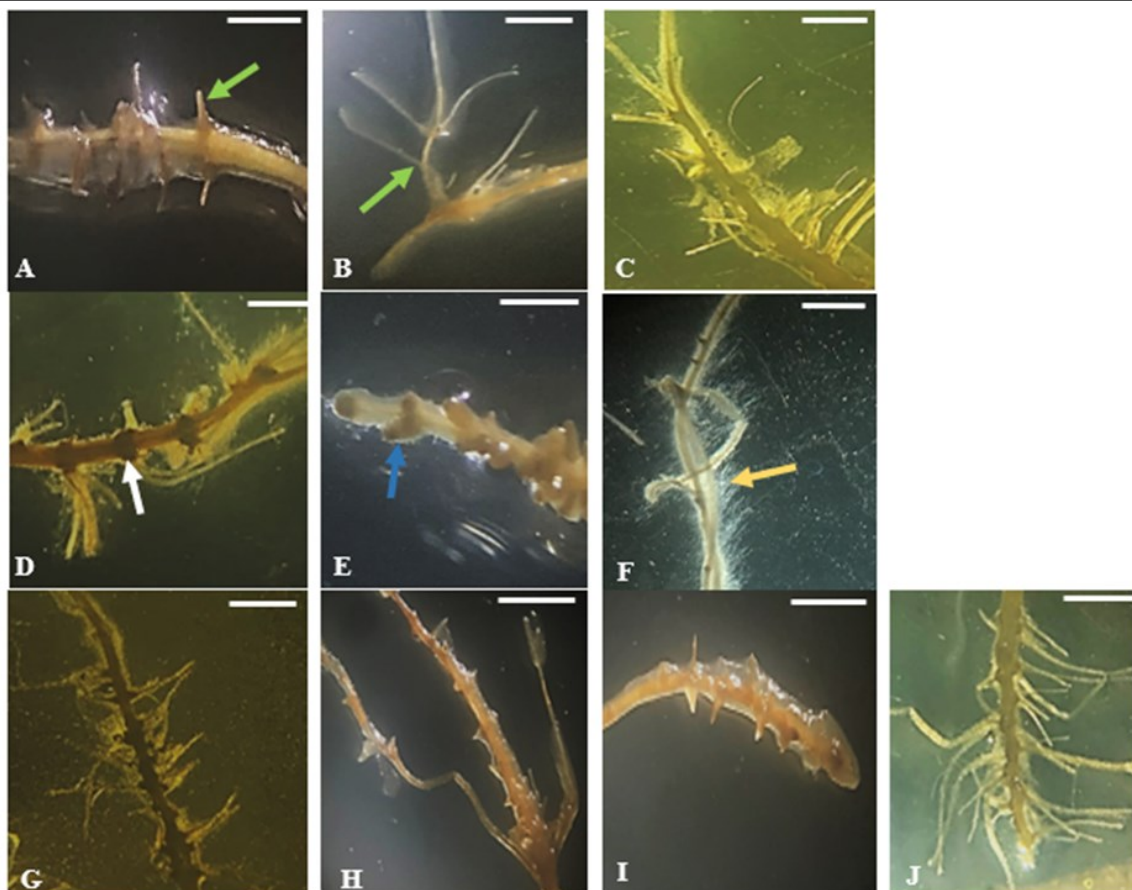


Figure 2. Morphology of adventitious roots of *T. paniculatum* cultured for six weeks in liquid MS medium with various carbon sources treatment. **A.** 3% sucrose; **B.** Lactose 3%; **C.** Maltose 3%; **D.** 3% glucose; **E.** Dextrose 3%; **F.** Fructose 3%; **G.** Sucrose+Fructose (1.5%+1.5%); **H.** Sucrose+Glucose (1.5%+1.5%); **I.** Sucrose+Dextrose (1.5%+1.5%); **J.** Glucose+Fructose (1.5%+1.5%). Observed with a 30x magnification stereo microscope. Bars = 1mm. Green arrow = root branching; white arrow = root initiation; blue arrow = root initiation starts to grow; yellow arrow = root hair.

root diameter using an inverted microscope. Measurements are conducted with 3 repetitions so that the average root branch length and root diameter average are obtained, as presented in Table 2.

Table 2. Average branch length and diameter of adventitious roots of *T. paniculatum* cultured for 6 weeks in liquid MS medium with various carbon source treatments (n=3).

Treatment	Root Branch Length (mm)	Root Diameter (mm)
Sucrose 3% (control)	0.89 ± 0.34	0.76 ± 0.06
Lactose 3%	2.63 ± 0.29	0.70 ± 0.13
Maltose 3%	1.39 ± 0.61	0.72 ± 0.05
Glucose 3%	1.80 ± 0.16	1.53 ± 0.05*
Dextrose 3%	1.14 ± 0.25	0.68 ± 0.28
Fructose 3%	2.94 ± 0.17	0.56 ± 0.10
Sucrose+Fructose (1.5% + 1.5%)	1.97 ± 0.08	0.18 ± 0.06
Sucrose+Glucose (1.5% + 1.5%)	1.69 ± 1.15	0.73 ± 0.04
Sucrose+Dextrose (1.5% + 1.5%)	0.81 ± 0.18	1.28 ± 0.13
Glucosa+Fructose (1.5% + 1.5%)	3.86 ± 0.09*	0.41 ± 0.02

The Effect of Carbon Source Variations on MDA and Proline Levels of Adventitious Roots

MDA and proline test were performed to know whether the adventitious roots of *T. paniculatum* could do adaptation during cultured. The damage of cells membrane due to lipid peroxidation can be predicted through the accumulation of MDA (Baque et al. 2014). Since oxidative stress occurred in cells, they could activate defence mechanisms through increased activity of antioxidants (Esfandiari et al. 2007; Baque et al. 2013). The highest MDA was found in the treatment of sucrose and fructose combination with 1.5% concentration, respectively, whereas the highest proline was found in the treatment of maltose at 3%. The measurement results are presented in Table 3. It's indicated that adventitious roots have stress in medium supplemented with sucrose and fructose 1.5%. The combination of sucrose and fructose in the media will produce a high concentration of sugar in the media because some of the sucrose undergoes hydrolysis during autoclave sterilization into glucose and fructose molecules. High proline levels determine the physiological stress of roots on drought stress due to sugar stress. The sugar in the culture media causes hypertonic conditions in the root cells, so the cells shrink due to lack of water.

Table 3. MDA and proline levels of adventitious roots of *T. paniculatum* cultured for 6 weeks in a variety of carbon source treatment media were measured using a spectrophotometer.

Treatment	MDA level (nmol/0.5 g FW)	Proline level (µmol/0.5 g FW)
Sucrose 3% (control)	169.321	223.772
Lactose 3%	49.476	1033.621
Maltose 3%	48.672	1894.666*
Glucose 3%	60.737	512.339
Dextrose 3%	41.433	261.007
Fructose 3%	52.693	256.352
Sucrose + Fructose (1.5% + 1.5%)	183.799*	591.462
Sucrose + Glucose (1.5% + 1.5%)	42.237	247.044
Sucrose + Dextrose (1.5% + 1.5%)	41.433	1112.744
Glucose + Fructose (1.5% + 1.5%)	45.454	195.847

*the results of the highest MDA and proline levels

The Effect of Carbon Source Variations on Levels of Adventitious Root Saponin Compounds

Based on TLC test results (Figure 3), highest saponin levels were obtained at 3% dextrose treatment. This treatment produces saponin stains with large colour intensity and area. Afterward, it is analysed using ImageJ software to obtain results in the form of colour intensity and area of saponin stains as

shown in Figure 4. Based on ImageJ software, the highest saponin content was also found at 3% dextrose, which was shown by the largest area and colour intensity of *T. paniculatum* adventitious roots (Figure 4A and 4B).

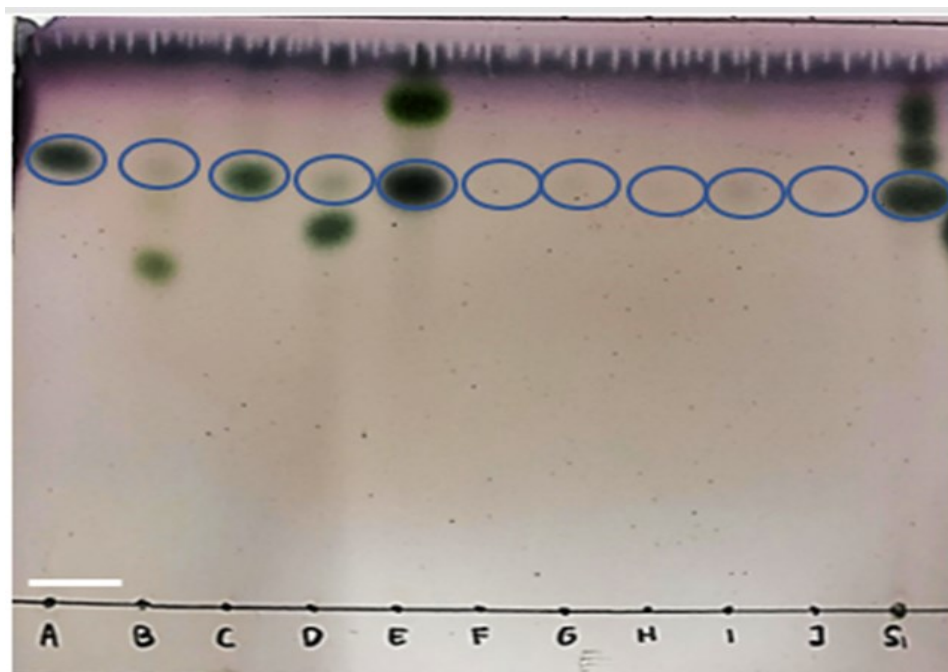


Figure 3. Saponin stains of adventitious roots of *T. paniculatum* on Thin Layer Chromatography of silica gel GF254 using 2-propanol:water (14:3) as eluent. **A.** 3% sucrose; **B.** Lactose 3%; **C.** Maltose 3%; **D.** 3% glucose; **E.** Dextrose 3%; **F.** Fructose 3%; **G.** Sucrose+Fructose (1.5%+1.5%); **H.** Sucrose+Glucose (1.5%+1.5%); **I.** Sucrose+Dextrose (1.5%+1.5%); **J.** Glucose+Fructose (1.5%+1.5%); **S1** = standard saponins. Bars = 1 cm.

DISCUSSION

The data in Table 1 shows that there is an increase in the fresh weight of the roots compared to the fresh weight of the initial inoculum. The increase in fresh biomass that occurred indicates adventitious root growth activity and is a result of the increase in root cell mass compared to the initial inoculum. The increase in biomass occurs due to the presence of sufficient energy sources in the liquid medium to support the need for adventitious roots to grow. The energy is in the form of sugar, which can be utilized by plants as a carbon source for metabolism, development, growth, and gene expression. It is either monosaccharide or disaccharide sugar that can act as a carbon source (Wang & Weathers 2007).

The results show that the highest fresh biomass is produced by 3% fructose treatment, which is 1.30 g compared to sucrose as a control. This is similar to the research of Uozumi et al. (1991), which stated that there is an increase in root hair biomass infected by *Agrobacterium rhizogenes* A4 in carrot culture with the administration of a fructose concentration of 30 g/L on day 48 of the culture period. For fed-batch culture, the average root hair growth rate increased to 0.8 g-dry biomass/L/day and 0.25 g-dry biomass/L/day for batch culture. According to Uozumi et al. (1991), if the plant cell is ready to

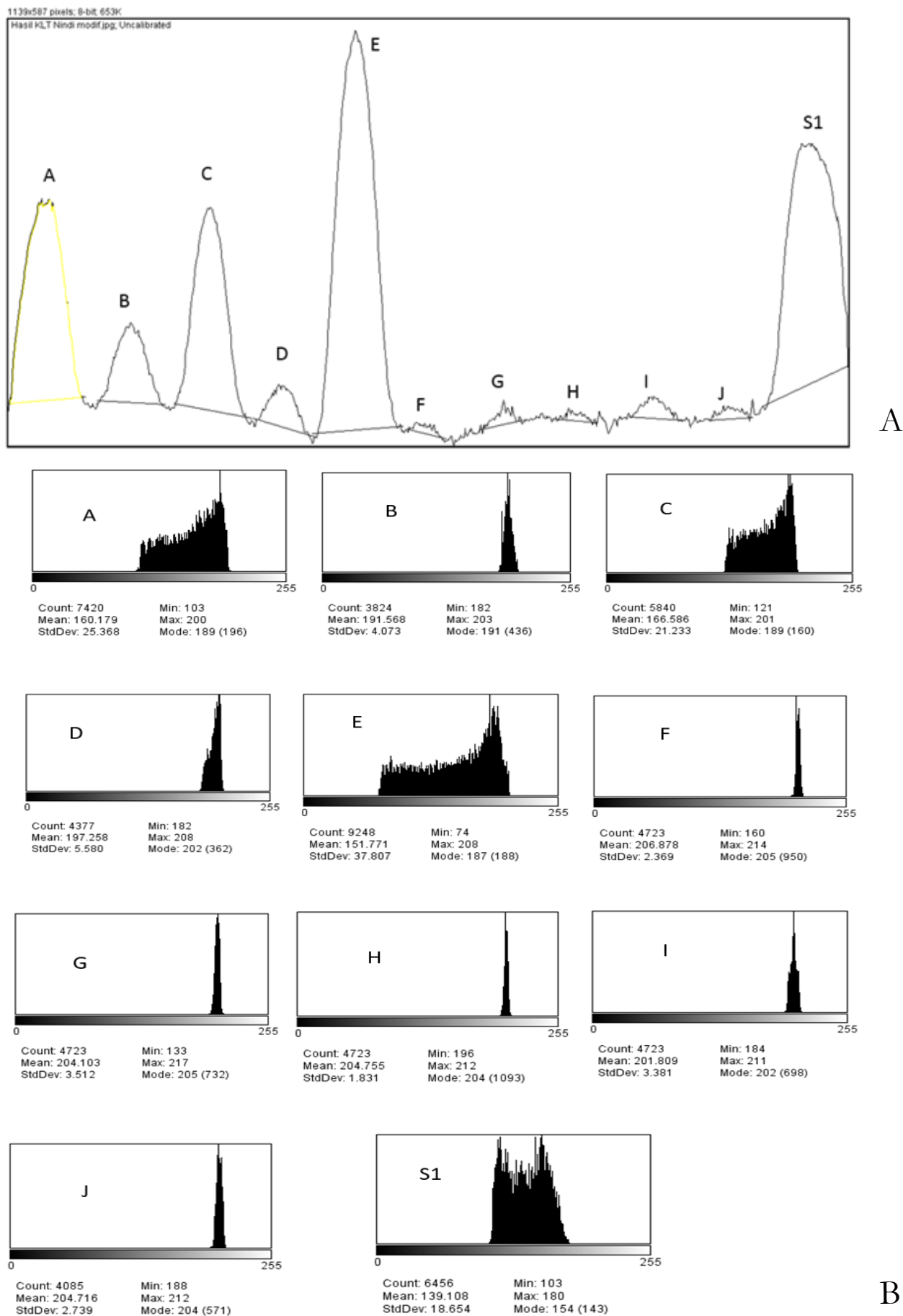


Figure 4. The results of the analysis of saponin levels using Image J software. (a) The size of the area; (b) color intensity of adventitious root saponins of *T. paniculatum* on Thin Layer Chromatography plate. **A.** 3% sucrose; **B.** Lactose 3%; **C.** Maltose 3%; **D.** 3% glucose; **E.** Dextrose 3%; **F.** Fructose 3%; **G.** Sucrose+Fructose (1.5%+1.5%); **H.** Sucrose+Glucose (1.5%+1.5%); **I.** Sucrose+Dextrose (1.5%+1.5%); **J.** Glucose+Fructose (1.5%+1.5%); (S1) standard saponins.

consume carbohydrate, as in the case of bacteria, the medium containing the monosaccharide fructose can achieve a higher cell mass. However, the researchers are unable to identify the factors that caused the increase in cell density completely. In this study, supplemented sucrose 3% as a control was not showed the maximum fresh weight biomass, even though in another research showed the maximum yield was achieved at 3% sucrose (Cui et al. 2014; Thanh et al. 2014). Effect of carbohydrate source on biomass accumulation also showed that treatment of sucrose 3% has the highest yield in cell suspension culture, adventitious roots and hairy roots of *Whitania somnifera* (Nagella & Murthy 2014). The yield of *T. paniculatum* adventitious root biomass in this study is low. This happens because the inoculum that is immersed continuously in the medium is prone to hyperhydricity. Hyperhydricity is the occurrence of tissue damage and improper shape changes in explants, such as symptoms of excess water in plant cells or tissues (Solim 2017).

The combination treatment of glucose and fructose (1.5% + 1.5%) resulted in the longest average root branch length of 3.86 mm compared to the sucrose treatment. This can be explained by the fact that glucose is the raw material for respiration and can be used as energy along with light in metabolism for the production of ATP and NADH so that growth can be accelerated (Yang et al. 2007). Administration of fructose increased the growth of *M. griffithii* significantly about 1-4 times after 25 days of culture compared to control. According to Yee (2015), if the two carbon sources were combined, it will result in rapid root branch growth. The largest average diameter produced by the addition of glucose is 1.53 mm (double the root diameter in the control treatment). This is because glucose molecules are active as raw materials for respiration and used as energy in primary plant metabolisms, such as cell enlargement and elongation to produce large root diameters. The results of this study are in accordance with the research by Yusuf et al. (2021), that the supplementation of 20 mM glucose can produce a larger leaf area per plant than the control. In the other study showed that was added of 5% sucrose increase biomass of adventitious root of *G. procumbens* (Noviyanti et al. 2017; Lestari et al. 2017).

MDA levels in this study indicate physiological stress of root cells, not acclimatization or a form of root adaptation to culture conditions. This research is in line with Ikhtimami (2012) research, which shown root hair achieves stationary growth (no increase root hair length) in the 4th week, as indicated with a horizontal line at weeks four to ten. Although the subculture treatments are different, the three treatments in this study show the same results in terms of achieving stationary growth. Different things are shown in the 2-week subculture treatment. In this study, adventitious roots of *T. paniculatum* are subcultured in a liquid medium for six weeks (Figure 2), which was in line with Ikhtimami's research.

The highest MDA levels are obtained in the sucrose + fructose treatment: 183.799 nmol/0.5 g fresh weight (Table 3). If the MDA level is high,

describe that the cell is experiencing oxidative stress, which affects the decrease in biomass. The results of this study can be explained that the combination of sucrose and fructose in the media will produce a high concentration of sugar in the media because some of the sucrose undergoes hydrolysis during autoclave sterilization into glucose and fructose molecules. In another study (adventitious roots culture of *Hypericum perforatum* L.) showed an increase of sucrose levels followed by an increase of MDA levels (Cui et al. 2014). Aeration was used in liquid culture by shaking, consequently, shear stresses occurred inside the culture caused cells damage. The damage of cells membrane due to lipid peroxidation can be predicted through the accumulation of MDA (Baque et al 2014). Since oxidative stress occurred in cells, they could activate defences mechanisms through increased activity of antioxidants (Esfandiari et al. 2007; Baque et al. 2013).

This study also measures proline levels to determine the physiological stress of roots on drought stress due to sugar stress. The sugar contains in the culture media causes hypertonic conditions in the root cells so that the cells shrink due to lack of water. The root cells produce proline so that the fluid inside and outside the cell is the same (isotonic). The highest proline content is obtained in the 3% maltose treatment. This is similar to the research of (Ibrahim & Abdellatif 2016), maltose and trehalose treatment significantly increase the concentration of soluble protein and proline compared to the control. According to Luo et al. (2010), externally applied maltose and trehalose accumulate rapidly, are transported by leaf and root tissues, as well as play an important role as osmoprotectants. Maltose has a hydrophilic group, and replacing water molecules with maltose can reduce damage caused by drought (Lerbret et al. 2005).

Based on the results of the TLC test, the highest levels of saponins were obtained in the 3% fructose treatment. This is similar to the results of Wang & Weathers (2007) that the production of artemisinin in a medium with fructose resulted in 2 times the levels of artemisinin compared to sucrose. However, when these sugars are combined, such as sucrose + glucose or fructose in seed culture, there is a decrease in artemisinin levels compared to sucrose alone. In this study, the TLC results were then analysed using ImageJ software, which showed the area of the stain and the intensity of the colour (Figure 4). The results of both show that the 3% fructose treatment produced the highest levels of saponins.

CONCLUSION

Based on the results of the research above, it can be concluded that the variation of carbon sources has an effect on biomass, MDA levels, proline levels, and saponin levels of adventitious roots of *T. paniculatum*. The adventitious root biomass obtained in the treatment of various carbon sources increases compared to the initial inoculum biomass. The levels of MDA, proline levels, and levels of adventitious root saponins produced in the treatment of various carbon sources are higher than the control. However, the morphology of ad-

ventitious roots in the treatment of variations in carbon sources does not differ from the morphology of roots in control, so that the provision of variations in carbon sources has no effect on the morphology of adventitious roots. The highest yield of root biomass and content of saponin compounds is produced by 3% fructose treatment. Hence, 3% fructose can be used as a substitute for sucrose for the liquid culture of adventitious roots of *T. paniculatum*.

AUTHORS CONTRIBUTION

Y.S.W.M designed the research and supervised all the process, A.Y and A.N.K collected and analyzed the data, D.S designed the research and supervised the data, and N.N.E analyzed the data and wrote the manuscript.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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