3BIO: *Journal of Biological Science, Technology and Management*
Volume 1., Issue 1 (2019): 1 - 6

Uniconazole increases starch content in duckweed (*Lemna aequinoctialis* Welw.)

Ahmad Faizal* and Rahman Tri Putra

School of Life Sciences and Technology, Institut Teknologi Bandung, Bandung 40132, Indonesia

*Correspondence author Ahmad Faizal; E-mail address: afaizal@sith.itb.ac.id

Abstract

Uniconazole has been used to improve starch production in plants through the regulation of endogenous hormone levels. Here, we reported the effect of this compound on the starch accumulation in a duckweed plant (*Lemna aequinoctialis*). *L. aequinoctialis* was grown in Hoagland medium supplemented with different uniconazole concentrations: 400, 800, and 1600 mg/L. The results showed that treatment with 800 mg/L uniconazole significantly increased plant growth rate, doubling time and its chlorophyll content. Consequently, the starch content also increased by 1.5 fold upon treatment with 800 mg/L uniconazole. We, therefore, concluded that uniconazole treatment offers an effective means to enhance the production of starch in *L. aequinoctialis*.

Keywords: duckweed, *Lemna aequinoctialis*, starch content, uniconazole

1. Introduction

Fossil fuel became the main source of energy in the world and its consumption increases along with increasing of the human population. However, this energy source has been predicted to deplete in the future [1,2]. Therefore, it is necessary to explore alternative renewable energy source such as bioethanol, which offers more sustainable and environmentally friendly.

Bioethanol is produced from sugar, starch and cellulosic fermentation [3,4]. On the other hand, the raw materials for bioethanol production are mostly from food or feed crops such as cassava, sweet potato, sugarcane, buckwheat, sago, palm sugar and nipa palm [5]. In Indonesia as other countries, this has stimulated a competition between food staples and development of bioethanol industry [6]. Therefore, the development of a non-staple source of bioenergy in Indonesia is required to answer this problem.

Lemna aequinoctialis, one of the duckweed plants, has recently drawn many attentions due to its potential for bioethanol production. In Indonesia, this tiny light green free-floating plant grows on freshwaters such as rice fields and lakes and generally used as feed for feedstock. Duckweed plants doubles its mass within 16-24 h with potential growth up to 4 ton dry weight/ha/day or about 80 ton dry weight /ha/year. Interestingly, this duckweed could accumulate starch from 3-75% of its dry weight depends on the growing conditions such as pH, the concentration of phosphate and nutrition availability [7]. These features should be further improved to optimize the production of bioethanol from a non-staple source.

As reported on other duckweeds, the starch accumulation in *L. aequinoctialis* can be improved through alteration of several processes such as starch formation, starch storage, and starch degradation. There are some factors that could increase the starch accumulation on duckweed, such as nutrition conditions, pH, and growth regulator addition [2]. The phosphorus deficiency can trigger starch accumulation on duckweed [8]. Abscisic acid was also also reported to increase starch accumulation in other duckweeds through inhibition of the plant growth [9].

Uniconazole is a plant growth retardant that has been used to increase plant biomass dry weight increased carotenoid and chlorophyll contents, increased drought tolerance and regulates endogenous hormones [10]. In addition, treatment with uniconazole has been reported to increase sucrose content in winter rape [11] and starch accumulation on *Landoltia punctata* [12]. Taken together, it will be interesting to evaluate the effect of uniconazole treatment on starch accumulation in *L. aequinoctialis.*

2. Material and Methods

2.1 Plant Material

Two grams of *L. aequinoctialis* were grown in 18 cm x 18 cm plastic container filled with 750 mL of 1/6 strength Hoagland medium. Plants were incubated at 30 °C in a 16/8 h light/dark period under 7000 lux of the light intensity (TL Philips® Cool White 36 W lamp). Subsequently, starch accumulation was measured 10 days later.

2.2 Uniconazole Treatments

Uniconazole was purchased from Embellish Leaf, China. This compound was dissolved in 10% methanol to have final concentration as follows: 400 mg/L, 800 mg/L, and 1600 mg/L. To evaluate the effect of uniconazole, each 5 mL of uniconazole from different concentration was sprayed evenly on the plant surface. Control plants were sprayed with 5 mL of water containing 10% methanol.

2.3 Plant Growth Observation

Plant growth was observed by measuring leaf surface area for every 12 h. The leaf surface data was then plotted into growth curve. Plant growth rate calculated with the following equation:

$$\mu = (\ln x_t - \ln x_0)/t \quad (1)$$

μ = growth rate (day^{-1})
x_t = leaf area on t (cm^2)
t = time (day)
x_0 = initial leaf area (cm^2)

2.4 Chlorophyll Content

Chlorophyll content was measured according to Winterman and de Mots (1965) method [13]. One gram of plant was extracted with 96% ethanol and filtered through Whatman filter paper. The filtrate was transferred into 100 mL volumetric flask and diluted with 96% ethanol until 100 mL volume. Chlorophyll content was determined by measuring the absorbance of plant extracts with a spectrophotometer at 649 nm and 665 nm. Chlorophyll calculated using these equations:

$$\text{Chlorophyll a} = 13.7\ A_{665} - 5.76\ A_{649} \quad (2)$$

$$\text{Chlorophyll b} = 25.8\ A_{649} - 7.60\ A_{665} \quad (3)$$

$$\text{Total Chlorophyll} = 20.0\ A_{649} + 6.10\ A_{665} \quad (4)$$

Where,

A_{665} = Absorbance on 665nm
A_{649} = Absorbance on 649nm

2.5 Plant Extraction and Starch Analysis

Fresh plants were grounded and extracted with 96% ethanol, after which the extracts were dried using a rotary evaporator. Starch was then extracted by adding two times 50 mL hot water to obtain a total volume of 100 mL. Lugol reagent was added to the chilled extract with ratio 1:9 followed by absorbance measurement with a spectrophotometer at 580 nm. Plant starch content was calculated by plotting the absorbance data with the starch standard curve. Subsequently, starch content was defined as the amount of starch that contained in every dry weight of the plant. Starch content calculated using the equation:

$$\text{Starch content} = m_{starch}/DW \quad (5)$$

m_{starch} = mass of starch (g)
DW = plant dry weight (g)

2.6 Nitrate Measurement

Nitrate medium was measured according to Indonesia National Standard procedure (SNI 01-3554-2006). A 5 mL of medium was diluted 10 times and then added with 1 mL of 1N HCl. The absorbance of each sample was measured

by using spectrophotometer at 220 nm and 275 nm. The difference between two absorbances was then plotted on the nitrate standard curve.

2.7 Statistical Analysis

Data were analyzed using one-way ANOVA with 95% confidence using SPSS version 19 followed by Duncan Multiple Range Test at $P < 0.05$.

3. Results

3.1 *L. aequinoctialis* Growth

Plant growth was monitored based on the leaf surface area measurements. Our experiment showed that the cultivation of young *L. aequinoctialis* resulted in exponential growth (Figure 1). It is clearly shown that there were no significant differences on the growth curve of *L. aequinoctialis* treated with different uniconazole concentrations.

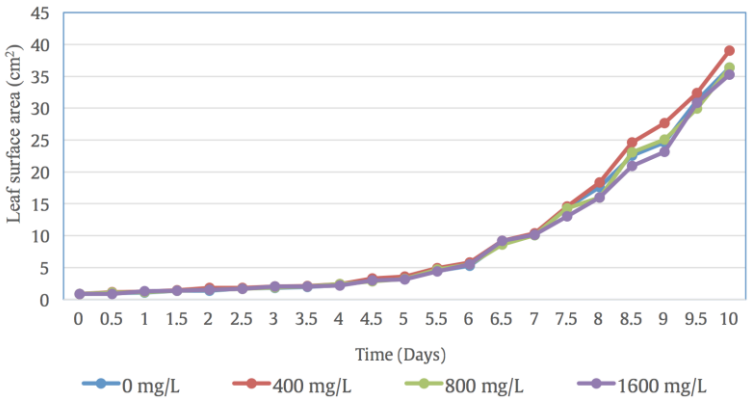

Figure 1 Sigmoid curve of *L. aequinoctalis* based on leaf surface area.

Additionally, we also measured plant fresh weight to monitor plant growth. Figure 2 shows that 400 mg/L and 800 mg/L uniconazole significantly increased *L. aequinoctalis* fresh weight. As depicted in Table 1, treatment with 800 mg/L of uniconazole induced significant increment in growth rate and doubling time of *L. aequinoctalis* by 0,257 ± 0,006 g/day and 2,710 ± 0,074 days, respectively. To correlate the plant growth with nutrient absorption, we measured initial and final nitrat concentration in the medium (Table 2)

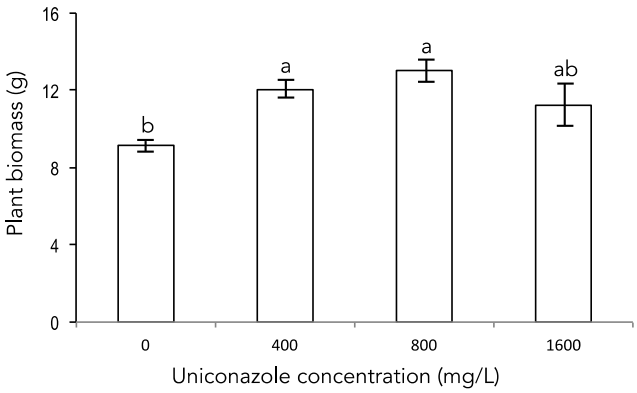

Figure 2 Fresh weight of *L. aequinoctalis*. Different letters above each column indicate significant differences between the mean values at $P < 0.05$

Table 1. Growth rate and doubling time.

Uniconazole Concentration (mg/L)	Specific growth rate of biomass (g/day)	Doubling time (day)
0	0.225 ± 0.001^{b}	3.086 ± 0.012^{b}
400	0.248 ± 0.006^{ab}	2.792 ± 0.085^{ab}
800	0.257 ± 0.006^{a}	2.710 ± 0.074^{a}
1600	0.243 ± 0.010^{ab}	2.854 ± 0.147^{ab}

Different letters indicate significant differences between mean values at P<0,05 within each group.

Table 2. Initial and final nitrate concentration on the medium.

Uniconazole concentration (mg/L)	Nitrate Consumption (ppm)
0	46.844 ± 1.103^{b}
400	51.894 ± 1.477^{a}
800	48.755 ± 1.283^{ab}
1600	37.877 ± 1.134^{c}

Different letters indicate significant differences between nitrate consumption (P<0,05)

3.2 Chlorophyll Content in *L. aequinoctalis*

Chlorophyll measurement showed that 800 mg/L uniconazole significantly increased chlorophyll a and total chlorophyll content among other treatments. However, different concentrations of uniconazole have no effect on chlorophyll b content (Figure 3).

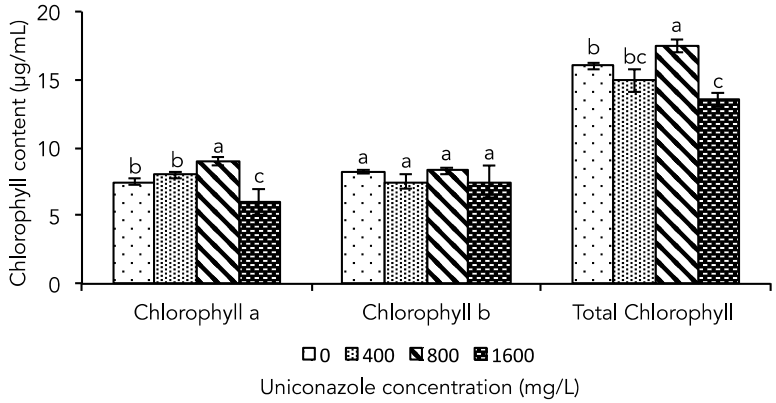

Figure 3 Chlorophyll content of *L. aequinoctalis* upon treated with different concentrations of uniconazole. Different character indicates a significant difference (P< 0.05) within the same measurements.

3.3 Starch Content in *L. aequinoctalis*

Starch is one of the highest metabolites that yielded by *L. aequinoctialis*. The result shows that the highest starch content (9.1 ± 0.7%) was obtained on *L. aequinoctialis* treated with 800 mg/L uniconazole. This treatment increased the starch content up to 1.5 fold compare to controls (Figure 4).

4. Discussion

Previous study reported that the high concentrations of uniconazole reduce leaf area on *Mirabilis jalapa* but not in the low concentrations [14]. Similar study also reported that uniconazole treatment reduced leaf size in *Vinca rocea* [15]. Furthermore, a lower concentration of uniconazole treatment on *Epipremnum aureum* increased its leaf size [16]. Here, we assume that the same concentrations of uniconazole applied in the previous studies were not sufficient to induce the similar effect in *L. aequinoctialis*. Apparently, the effect of uniconazole is species specific as well as other plant regulators. More importantly, uniconazole plays a role in cell division process by regulating plant endogenous hormones [17].

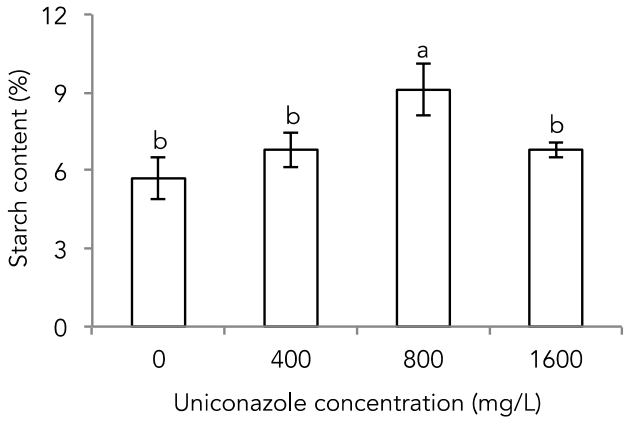

Figure 4 Starch content of *L. aequinoctalis* upon treated with different concentrations of uniconazole. Different character indicates significant difference ($P< 0.05$).

Generally, the increased plant biomass is correlated with their nutrient absorption. Indeed, a significant difference on nitrate absorption was observed on *L. aequinoctalis* treated by 400 mg/L of uniconazole, yet no difference on those treated with 800 mg/L uniconazole. On the other hand, nitrate absorption was decreased upon treatment with 1600 mg/L uniconazole. The increased nitrate consumption leads to increased fresh weight on the concentration of 400 mg/L uniconazole. On the concentration of 800 mg/L uniconazole, the increased fresh weight may due to another factor interfering with photosynthesis process and regulation of plant endogenous hormones [12].

The increased nitrogen absorption was also in accordance to the plant chlorophyll content as nitrogen required for biosynthesis of chlorophyll. Similar application of uniconazole increased the total chlorophyll content on another duckweed plant (*L. punctata*) due to up-regulation of key enzymes in chlorophyll biosynthesis [12]. The increased chlorophyll also could be affected by delayed chlorophyll degradation as reported in *Brassica napus* [11].

The increased starch also corroborated with total chlorophyll content that affected photosynthesis process. The increasing chlorophyll content will increase the photosynthetic rate that would lead to starch accumulation [12]. Chlorophyll acts as light energy receptor in antenna system. Light energy that absorbed by chlorophyll is emitted as an electron in electron transfer chain on light reactions. The final process of photosynthesis produces glyceraldehyde-3-phospate, which are the sugar skeleton and can be accumulated further as starch. It was also reported that uniconazole treatment increased starch accumulation in *L. punctata* by up-regulating the expression of key enzymes of starch biosynthesis [8].

Conclusion

Uniconazole treatment on the concentration 800 mg/L increases starch productivity by 1.5 fold, specific growth rate, doubling time and chlorophyll content on *L. aequinoctalis*.

References

[1] Chu, S., Cui, Y. and Liu, N. The path towards sustainable energy. Nature Materials. 2016; **16**: 16-22. DOI: 10.1038/nmat4834.

[2] Kaur, M., Kumar, M., Sachdeva, S. and Puri, S.K. Aquatic weeds as the next generation feedstock for sustainable bioenergy production. Bioresource Technology. 2018; **251**: 390-402. DOI: 10.1016/j.biortech.2017.11.082.

[3] Balat, M. (2011). Production of bioethanol from lignocellulosic materials via the biochemical pathway: A review. Energy Conversion and Management. 2011; **52**: 858-875. DOI: 10.1016/j.enconman.2010.08.013.

[4] Saha, K., Maharana, A., Sikder, J., Chakraborty, S., Curcio, S. and Drioli, E. Continuous production of bioethanol from sugarcane bagasse and downstream purification using membrane integrated bioreactor. Catalysis Today. 2017; In Press. DOI: 10.1016/j.cattod.2017.11.031

[5] Favaro, L., Cagnin, L., Basaglia, M., Pizzocchero, V., van Zyl, W.H. and Casella, S. Production of bioethanol from multiple waste streams of rice milling. Bioresource Technology. 2017; **244**: 151-159. DOI: 10.1016/j.biortech.2017.07.108.

[6] Mulyani, A. and Las, I. Potensi sumber daya lahan dan optimalisasi pengembangan komoditas penghasil bioenergi di Indonesia. Jurnal Litbang Pertanian. 2008; **27**: 31-41.

[7] Cui, W. and Cheng, J.J. Growing duckweed for biofuel production: a review. Plant Biology. 2015; **17**: 16-23. DOI: 10.1111/plb.12216.

[8] Zhao, Z., Shi, H.-j., Wang, M.-l., Cui, L., Zhao, H. and Zhao, Y. Effect of nitrogen and phosphorus deficiency on transcriptional regulation of genes encoding key enzymes of starch metabolism in duckweed (*Landoltia punctata*). Plant Physiology and Biochemistry. 2015; **86**: 72-81. DOI: 10.1016/j.plaphy.2014.11.007.

[9] Cui, W., Xu, J., J. Cheng, J. and M. Stomp, A. Starch Aacumulation in duckweed for bioethanol production. Biological Engineering Transactions. 2011; **3**: 187-197. DOI: 10.13031/2013.37123.

[10] Rademacher, W. Plant growth regulators: Backgrounds and uses in plant production. Journal of Plant Growth Regulation. 2015; **34**: 845-872. DOI: 10.1007/s00344-015-9541-6.

[11] Zhou, W. and Leul, M. Uniconazole-induced alleviation of freezing injury in relation to changes in hormonal balance, enzyme activities and lipid peroxidation in winter rape. Plant Growth Regulation. 1998; **26**: 41-47. DOI: 10.1023/A:1006004921265.

[12] Liu, Y., Fang, Y., Huang, M., Jin, Y., Sun, J., Tao, X., Zhang, G., He, K., Zhao, Y., and Zhao, H. Uniconazole-induced starch accumulation in the bioenergy crop duckweed (*Landoltia punctata*) I: transcriptome analysis of the effects of uniconazole on chlorophyll and endogenous hormone biosynthesis. Biotechnology for Biofuels. 2015; **8**: 57-68. DOI: 10.1186/s13068-015-0246-7.

[13] Wintermans, J.E.G. and De Mots, A. Spectrophotometric characteristics of chlorophyll a and b and their phaeophytins in ethanol. Biochimica et Biophysica Acta. 1965; **109**: 448-453. DOI: 10.1016/0926-6585(65)90170-6.

[14] Abdel-Maksoud, B., Ali, Y.A., Swedan, E.A. and El-Kinany, R.G. Effect of uniconazole concentration and its application method on growth, flowering and carbohydrate content of *Mirabilis jalapa*, L. plants. Journal of Agriculture and Environmental Sciences. 2010; **11**: 52-79.

[15] Choi, S.-H., Kang, J.-S., Choi, Y.-W., Lee, Y.-J., Park, Y,-H., Kim, M,-R., Son, B,-G., Kim, H,-K., Kim, H.-Y., Oh, W., Sim, H,-B., Lim, K,-B., and Kim, J,-K. Effect of diniconazole on growth and flowering of *Vinca rocea* and *Salvia splendis*. Korean Journal of Life Science. 2011; **21(7)**: 1004-1008.

[16] Wang, Y.-T. and Gregg, L.L. Chemical regulators affect growth, postproduction performance, and propagation of golden pothos. HortScience. 1994; **29(3)**: 183-185.

[17] Grossmann, K. (1992) Plant growth retardants: Their mode of action and benefit for physiological research. In Progress in Plant Growth Regulation. In: Proceedings of the 14th International Conference on Plant Growth Substances; 21–26 July, 1991, Amsterdam: Springer Netherlands, Dordrecht; 1992. pp. 788-797.

Article history: Received April 12, 2018; Revised June 15, 2018; Accepted Oct 2, 2018