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Chlorophyll and Carotenoid Level Comparisons of Pigeon Orchid (*Dendrobium crumenatum*) in Water and Light Stress Treatment

Dea Evani Amelia[1,3,a)], Febri Yuda Kurniawan[2,3,b)]
[1]Department of Tropical Biology, Faculty of Biology, Universitas Gadjah Mada.Teknika Selatan street, Sekip Utara, Bulaksumur, Depok, Sleman 55281, D. I. Yogyakarta, Indonesia. Telp (0274) 580839
[2]Study program of Biotechnology, Graduate School, Universitas Gadjah Mada. Teknika Utara street, Pogung, Sinduadi, Mlati, Sleman 55281, D. I. Yogyakarta, Indonesia. Telp (0274) 564305
[3]Biology Orchid Study Club (BiOSC), Faculty of Biology, Universitas Gadjah Mada.Teknika Selatan street, Sekip Utara, Bulaksumur, Depok, Sleman 55281, D. I. Yogyakarta, Indonesia. Telp (0274) 580839
e-mail: [a)]deaevani99@mail.ugm.ac.id, [b)]febriyuda14@gmail.com

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ABSTRACT

Environmental conditions in which plants grow are always changing, which when exceeding the tolerance limit, will result in stress. Water and light stress affect the pigment content of photosynthesis such as chlorophyll and carotenoids. *Dendrobium crumenatum* is one of orchid species that is judged to be resistant to various types of environmental conditions, so it can grow well in any environtment. This research aims to determine the comparison of chlorophyll and carotenoid levels of *D. crumenatum* from Bantul lowland population, Special Region of Yogyakarta which is given water and light stress treatment. The methods used are extraction with alcohol solvent (96%) and absorbtion level measurements with spectrophotometry at wavelengths 470, 645, and 663 nm. The highest chlorophyll a level contained in the leaves of *D. crumenatum* which is given water stress treatment, highest chlorophyll b level in control plant, highest total chlorophyll in the leaves which is given water stress treatment, and highest carotenoid level found in the leaves which is given light stress treatment .

Key Word: carotenoid, chlorophyll, *Dendrobium crumenatum*, light stress, water stress

INTRODUCTION

Orchids are flowering plants that are classified as ornamental plants because of their unique characteristics. There are 5000 species of orchids in Indonesia, 16.14% species from Java spread in Yogyakarta region (Setiaji *et al*., 2018).

The pigeon orchid (*Dendrobium crumenatum*) is an epiphyte orchid with white pigeon like flower buds. This orchid is judged to be resistant to various environmental conditions such as drought, high light intensity, and temperature (Metusala, 2011). This is because the pigeon orchid has longer roots and air roots that support keiki. Keiki is a seedling resulted from orchid asexual reproduction (Sofiyanti, 2014). Therefore, this species is easily grown naturally and widely found in tropical areas such as Malay Peninsula, Indonesia, Papua New Guinea, and Solomon Islands (Metusala, 2011).

According to Campbell (2003), environmental conditions that adversely affect the process of growth, reproduction, and survival of plants are called stress. Environmental stress may be an abiotic or biotic factor. For example, abiotic factors consisted of light, water, temperature, and

soil nutrient, meanwhile biotic factor consisted of herbivores, parasites or pathogens, and predators (Ai & Banyo, 2011).

Drought is one of the plant stress associated with water availability as an abiotic factor and can disrupt plant growth (Liu *et al*., 2012). Water is the main factor in the process of plant physiology, one of them is the opening and closing of stomata (Ai & Banyo, 2011). When plants suffer from severe water shortages, stomata will close and inhibit the photosynthesis process that leads to carbohydrate deposit reduction for survival (Mahajan & Tuteja, 2005; Liu *et al.*, 2012).

Beside water, light also participated in photosynthesis process through photon energy that will be captured by chlorophyll. Light intensity directly affects plants morphology (Asadi *et al*., 1997). Usually, plants that are accustomed to low light intensity have thin and wide leaf shape due to the reduction of palisade layers and mesophil cells (Taiz & Zeiger, 2002).

Chlorophyll is the main pigment found in chloroplast, and located inside tilakoid (Thorpe, 1984; Campbell *et al.*, 2003). Chlorophyll can accommodate light that absorbed by other pigments through photosynthesis, so that chlorophyll is called photosynthetic reaction center pigment (Ai & Banyo, 2011).

High-level plants have two kinds of chlorophyll: chlorophyll a that dark green coloured and chlorophyll b that light green coloured (Dwidjoseputro, 1980). Chlorophyll a and chlorophyll b can absorb light at most in the red part (600-700 nm), and at least absorb green light (500-600 nm) (Ai & Banyo, 2011).

A carotenoid is one of the leaf pigments that absorbs blue light with wavelength 450-475 nm or those that can't be absorbed by chlorophyll (Kurniawan *et al*., 2010; Kojo, 2004). Carotenoid together with chlorophyll b, will absorbing energy and forwarded to chlorophyll a to be used in the light reaction process that consisted from photosystem I and II, so does chlorophyll b (Ai & Banyo, 2011).

Water and light affect towards plants growth, because they play an important role in the photosynthesis process. So, if plants experienced water and light stress will certainly affect the growth paramters of plants, one of which is photosynthetic pigment levels, such as chlorophyll and carotenoids (Ai & Banyo, 2011; Asadi *et al*, 1997). In this research, we will doing chlorophyll and carotenoid levels measurement from pigeon orchid (*Dendrobium crumenatum*) from Bantul lowland, Special Region of Yogyakarta which is given water and light stress.

MATERIAL AND METHODS

Materials

Tools that used such as scissors, funnel, erlenmeyer, beaker, mortar, semi-analytical scale, cuvet, spectrophotometer, drip pipette, measuring pipette, propipette, reaction tube, and flakon.

Materials that used such as filter paper, tissue paper, aquades, alcohol 96%, and the leaves of pigeon orchid age ± 1 year with plant's height ± 7 cm from Bantul lowland which has been given water and light stress treatment. Water stress treatment means the plant remain exposed to sunlight (not sheltered) but not watered for ± 7 days. Light stress treatment means the plant always watered at 07.00 a.m. with volume ± 300 mL for ± 7 days but always sheltered (50% shade by tree canopy). Control means plant always watered at 07.00 a.m. with volume ± 300 mL for ± 7 days and exposed to sunlight 100% (not sheltered).

Methods

Chlorophyll and carotenoid levels measurement

Pigeon orchid leaves which are same from the control plant, water stress treatment, and light stress treatment picked and weighed, each 0.5 gram. The leaves are the third leaf from the peak, green to dark green colored, and ± 3 cm in length. The leaves cutted into smaller pieces and added 10 mL of alcohol 96%, then mashed using mortar. After that, the solution filtered with

filter paper and inserted into flakon. The solution was taken 1 mL using measuring pipette and diluted with alcohol 96% into 10^{-1}. The diluted solution was inserted into cuvet to be measured for its absorption with spectrophotometer at wavelength 470, 645, and 663 nm. According to Wellburn (1994) in Pompelli *et al*. (2012), chlorophyll and carotenoid levels can be calculated using formula:

Chlorophyll a (Chl a): $12{,}72 \times \text{Abs } 663 - 2{,}59 \times \text{Abs } 645$ (1)
Chlorophyll b (Chl b): $22{,}9 \times \text{Abs } 645 - 4{,}67 \times \text{Abs } 663$(2)
Carotenoid: $((1000 \times \text{Abs } 470) - ((2{,}13 \times \text{Chl a}) - (97{,}64 \times \text{Chl b})) \div 209$ (3)

The results of chlorophyll and carotenoid levels then converted to µg/L using formula:

Result x 10 x 1 (4)

Data analysis

Measurement data analyzed quantitatively using software Microsoft Excel, then presented in table and graphic form.

RESULT AND DISCUSSION

Table 1. Absorption values with light wavelength variation in samples

Treatment	Wavelength		
	470 nm	645 nm	663 nm
Control	0.32	0.107	0.228
Water stress	0.725	0.224	0.51
Light stress	0.839	0.196	0.442

In table 1, it can be known that there are differences in absorbtion of all three treatments. The highest absorption value at wavelength 470 nm is 0.839 in light stress treatment. This is due to 470 nm is the wavelength that can be captured by carotenoids (Kurniawan *et al*., 2010; Kojo, 2004), so that chlorophyll a and b levels have not been detected. The highest absorption values at wavelength 645 nm and 663 nm are 0.224 and 0.51 in water stress treatment.

Table 2. Chlorophyll a, b, total, and carotenoid levels in control, water stress, and light stress treatment's leaves

Treatment	Pigment levels (µg/L)			
	Chl. a	Chl. b	Total Chl.	Caroten -oid
Control	52.653	27.711	80.364	17.140
Water stress	59.272	27.479	86.751	21.247
Light stress	51.322	24.243	75.565	28.295

The calculation of chloropyll a, b, total and carotenoid showed that there are differences in each treatment. Chlorophyll a level in water stress plant's leaves is higher than control plant's leaves, i.e. 59.272 µg/L. Otherwise, chlorophyll a level in light stress treatment plant's leaves is lower than control plant's leaves at 51.322 µg/L. This is due to chlorophyll a formation was very affected by light, different from chlorophyll b formation that relatively didn't affected by light (Lawlor, 1987). So that in water stress treatment, the chlorophyll a level is still high because the plant's leaves didn't sheltered.

Chlorophyll b level in plant's leaves with water and light stress are lower than control plant's leaves, i.e. 27.479 and 24.243 µg/L. According to Hendriyani & Setiari (2009), lack of water availabilty will inhibit the synthesis of chlorophyll in leaves due to reduced photosynthetic rate and increased temperature and transpiration that causes chlorophyll disintegrate. This also applied to chlorophyll b content in water and light stress. Without water and only relying on light will reduce chlorophyll b level, likewise without light and only relying on water.

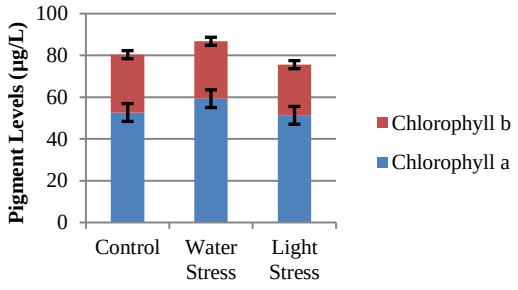

Figure 1. Chlorophyll a, b, and total in control, water stress, and light stress plant's leaves

Overall, chlorophyll a level is higher than chlorophyll b (Figure 1). This is due to chlorophyll a composed 75% from total chlorophyll, also this pigment have functionally as phytochrome in photosynthesis. Chlorophyll b is synthesized from chlorophyll a so that its amount lower than chlorophyll a (Pratama & Laily, 2015).

Total chlorophyll level in water stress plant's leaves is highest than control and light stress plant's leaves (Figure 1). As explained before, this is associated with chlorophyll a production that was very affected if the plant is not sheltered (Lawlor, 1987). So, chlorophyll a content coupled with chlorophyll b in water stress plant's leaves will produce higher total chlorophyll content.

Sheltered leaves (light stress treatment) chlorophyll is not active because it still in protochlorophyll form. Protochlorophyll is the chlorophyll production's precursor and only reduced when it gets enough amount of light (Pandey & Sinha, 1979). Chlorophyll content in light stress plant's leaves is lower than water stress plant's leaves because its chlorophyll level stay the same although in the protochlorophyll form.

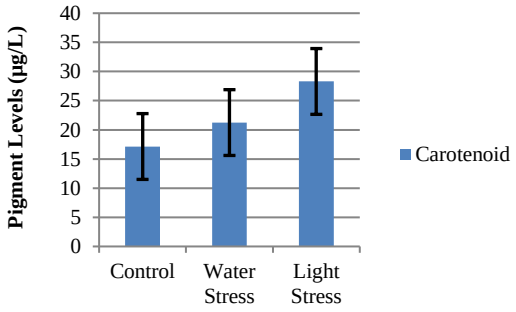

Figure 2. Carotenoid level in control, water stress, and light stress plant's leaves

Carotenoid level in light stress plant's leaves is highest than control and water stress plant's leaves at 28.295 µg/L (Figure 2). Though, light is one of important factors in carotenoid biosynthesis, because light participated in its enzyme activation (Johnson & An, 1991; Albrecht & Sandmann, 1994; Bramley, 2002). However, the result showed different instead. This is due to carotenoid level increased as an effort to improve light capturing in sheltered state (Bestari *et al.*, 2018). Moreover, low light intensity will reduce photo-oxydation process and carotenoid reduction rate (Pizarro & Stange, 2009).

CONCLUSION

Dendrobium crumenatum with water stress treatment has highest chlorophyll a level at 59.272 µg/L, highest chlorophyll b in control plant's leaves at 27.711 µg/L, highest total chlorophyll in water stress plant's leaves at 86.751 µg/L, and highest carotenoid level in light stress plant's leaves at 28.295 µg/L.

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