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RESEARCH ARTICLE

The Change of Chlorophyll Content and Chlorophyll Efficiency in *Epipremnum aureum* by Water and pH

Man Kyu Huh and Byeongryong Lee

ABSTRACT

***Epipremnum aureum* (pothos) is an herbaceous species and is originated to tropical or subtropical South East Asia and Solomon islands. This study investigated the effect of excessive moisture on chlorophyll content and photosynthesis efficiency on *E. aureum*. The chlorophyll a, b, and total (chlorophyll a + b) contents were measured by using spectrophotometer. Fluorescence analysis for chlorophyll efficiency was measured with the PAM Chlorophyll Fluorometer. The chlorophyll content increases when moisture increases to 30%, but gradually decreases when it exceeds 40%. When the soil moisture was 80%, the values of chlorophyll a and b were 0.317 mg/g and 0.126 mg/g, respectively. This decreased the chlorophyll a, b, and total contents by 38.1%, 46.6%, and 36.8%, respectively, compared to 30% in 80% moisture. The chlorophyll content was highest at pH 6.5 of the soil. The content for chlorophylls a, b, and total were 0.471, 0.219, and 0.446 mg/g at pH 6.5, respectively. The correlation coefficients were subjected of the chlorophyll efficiency as a function of moistures. The change of chlorophyll efficiency in the quenched state (Fv) was also increased at 30% of moisture and then decreased steeply. The maximal possible value for fluorescence (Fm) was varied from 4310 (40% moisture) to 4220 (80% moisture). The Fm was varied from 4098 (pH 4.0) to 4356 (pH 6.5). The maximum quantum yield of photosynthesis (Fv/Fm) was varied from 0.798 (30% moisture) to 0.810 (60% moisture). The slope factors of Fo, Fm, Fv, and Fv/Fm for chlorophyll efficient indicators were 0.938, -0.806, 0.013, and 0.846, respectively. Excess watering decreased chlorophyll a, chl b, and chl (a + b) contents, and the chl a/b ratio in the *E. aureum*. The growth of this species was not as sensitive to pH compared to moisture.**

Keywords: chlorophyll content, chlorophyll efficiency, *Epipremnum aureum*, soil pH.

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I. INTRODUCTION

Epipremnum aureum (pothos) is an important foliage plant and belongs to the family, Araceae. The species is an herbaceous species and is originated to tropical or subtropical South East Asia and Solomon islands in Indonesia (Huxley *et al*., 1994). *E. aureum* is synonymous with Golden pothos. Its decorative marbled leaves and ease of maintenance make it very popular as an indoor plant Thus, there are many improved varieties such as ivy arum and devil's ivy (Meshram & Srivastava, 2014). *E. aureum* has many advantage to grow at home such as tolerant of low light levels, beautiful evergreen foliage, air-cleansing qualities, and desirable vast principles (Sawada & Oyabu, 2008; Zhao *et al*., 2012; Zhao *et al*., 2013).

E. aureum is known that this species was first introduced to the United Sates in the 1920s and was considered the second-most popular foliage plant at home or garden from the 1950s to 1960s (Tian *et al*., 2018; Zhao *et al*., 2013).

Plants are continuously exposed to various abiotic stresses in nature. The coupling of plant growth and development with environmental signals determines the seasonality of growth, reproduction, and dormancy, ultimately fostering survival of the plant and the species a given environment. In particular, indoor plants do not have the help of nature, so it is difficult to raise them unless they are well managed.

E. aureum deprived of water to induce progressively increasing water stress were monitored for water status and primary yield factors including leaf growth (expansion and net photosynthesis) and retention (survival). Foliage plants such as *E. aureum* are usually watered once or twice a week and are sufficient. It dries up in the middle of every 1-2 weeks, allowing soil to dry out between watering. Some signs of overwatering include yellowing leaves and black stems, while under-watered plants will wilt, and their potting mix will dry out.

E. aureum thrives in a soil pH ranging from 6.1 to 6.8 on the scale. It is tolerant of a range of conditions, from neutral to slightly acidic.

Photosynthesis is the light-driven reaction that light energy, carbon dioxide, and water convert chemical compounds such as carbohydrates (Moore *et al*., 1995). Light is absorbed by pigments. Chlorophylls (Chl a and Chl b) are the primary pigments for photosynthesis and occurs in all photosynthetic organisms. Chlorophylls can be extracted from the protein into organic solvents (Marker, 1972). In this way, the concentration of chlorophyll within a leaf can be estimated (Cate & Perkins, 2003). Methods also exist to separate chlorophyll a and chlorophyll b.

The inability of plants to maintain enough water in their leaves causes stomata to close, thereby inhibiting photosynthesis. The lack of water is the most limiting factor controlling photosynthesis (Moore *et al*., 1995). Chlorophyll fluorescence is a non-invasive measurement of photosystem II (PSII) activity and is a commonly used technique in plant physiology (Murchie & Lawson). The sensitivity of PSII activity to abiotic and biotic factors has made this a key technique not only for understanding the photosynthetic mechanisms but also as a broader indicator of how plants respond to environmental change.

We analyzed the effects of chlorophyll content and chlorophyll fluorescence under degree of watering and soil pH on *E. aureum* (pothos).

II. Materials and Methods

A. Measurement of Chlorophylls

Each leaf of *E. aureum* was in row form. Growth of *E. aureum* was performed in a standard conditioned in pots in a controlled greenhouse on temperature, light, loam, and complete fertilizers. The various stress (degrees of water and pH) treated in a growth chamber in a green house. Obtained samples were 3.0 g in weight. Leaves were pulverized in liquid nitrogen (-70 ℃) or homogenized. 80% acetone solution with $NaCO_3$ added to the powders. Then the solutions were incubated for 24 hours at 25 ℃ in a dark. Samples were filtered through Whatman No. 2 filter paper (Advantec, Toyo, Japan). Samples were centrifuged for 5 minutes at 10,000 rpm at 4 ℃. The cylinder filter (Whatman, Merck, Germany) was used to decant the supernatant solution. 80% aqueous acetone add to the solutions for the re-extraction, shaking for two minutes, centrifuging, and removing the pellet. The mixture was properly diluted for the intensity of the optical absorption. The values are necessary to give a reading in the range of 0.2 to 0.8 absorbance units at wavelengths of 647 nm and 664.5 nm.

The contents of chlorophyll a and chlorophyll b were measured at λ = 664.5 and λ = 647, respectively.

The values of optical density (OD) obtained by the Microplate Reader (VersaMax, California, USA. Formula of Inskeep and Bloom (1985) for the calculation of the contents of chlorophyll were applied as fellow.

$$\text{Chlorophyll a} = 12.63\ A664.5 - 2.54\ A647$$

$$\text{Chlorophyll b} = 20.47\ A647 - 4.73\ A664.5$$

$$\text{Total Chlorophyll} = 17.95\ A647 - 7.90\ A664.5$$

Each experiment was repeated at least triplicate to get similar data. The correlation coefficients were subjected of the chlorophyll efficiency as a function of moistures. The degree of association was estimated the relationship of one variable (chlorophyll) with another (moisture) by expressing the one in terms of a linear function of the other, denoted by *r* (Sokal & Rohlf, 1969).

B. Measurement of Chlorophyll Fluorescence

Chlorophyll fluorescence is a non-invasive measurement and a key techniques of parameter in photosynthesis (Murchie & Lawson, 2013). Thus, we performed fluorescence analysis to measure photosynthetic parameters (Baker, 2008). Chlorophyll fluorescence was determined by the Imaging-PAM Chlorophyll Fluorometer-2500 (Heinz Walz GmbH, Effeltrich, Germany). By exposing plants to a very bright flash of light, i.e., a saturating light pulse, the reactive centers of the plants' photosystems become saturated according to the manufacturer's protocol.

The relationship between steady-state fluorescence yields (Ft) was calculated. Leaves of *E. aureum* were dark adapted for 20 min. This light is of intensity too low to induce electron transport through PSII but high enough to elicit a minimum value for chlorophyll fluorescence, termed Fo. On application of a saturation pulse, the dark-level fluorescence yield (Ft = Fo) and the maximum fluorescence yield (Fm) were determined. The maximal PSII quantum yield, Fv/Fm, and the quantum yields of regulated and nonregulated energy dissipation in PSII, Y (NPQ) and Y (NO) were imaged. Fv/Fm was calculated according to the equation: Fv/Fm = (Fm − Fo) Fm according to Kramer *et al*. (2004).

C. Measurement of Soil pH

There are many different ways of measuring the pH of soil. pH in soils can be provided various information for growth of plants. To obtain the effect of pH values from the natural soil, the pH of the soil was measured with a simple instrument (PIXTA, Siyasinnya, Japan) in the soil (Thompson *et al*., 2016). The potentiometer is calibrated with buffer solutions of known pH prior to the analysis of samples. This standard operating procedure (SOP) describes the measurement of pH (the ratio of hydrogen [H+] and hydroxyl [OH-] ion activities at a given temperature) of soils using a Cole-Palmer Digi-Sense® digital pH/millivolt/oxidation reduction potential (pH/mV/ORP) meter (Star A210, Thermo Fisher Scientific, Carlsbad, USA).

Fig. 1. Measurement of chlorophyll fluorescence of Epipremnum aureum by Chlorophyll Fluorometer.

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Fig. 2. Measurement of soil pH by portable pH meter of *Epipremnum aureum* growing in the pot.

III. Results

A. Chlorophyll Content

The chlorophyll content at the different moisture of the pots was investigated in *E. aureum*. The content of chlorophylls a and b in *E. aureum* were obtained by setting up the equations. These results of the experiment on *E. aureum* were shown in Table I. The change of chlorophyll a and b contents significantly depended on the moisture conditions. Chlorophyll content increases when moisture increases to 30%, but gradually decreases when it exceeds 40%. When the soil moisture was 80%, the values of chlorophyll a and b were 0.317 mg/g and 0.126 mg/g, respectively. The content of total chlorophyll a was 0.430 mg/g at 80% moisture. The content for chlorophylls a, b, and total were 0.512, 0.236, and 0.517 mg/g at 30% moisture, respectively. This decreased the chlorophyll a, b, and total contents by 38.1%, 46.6%, and 36.8%, respectively, compared to 30% in 80% moisture. There was a significant difference with regard to the contents of chlorophylls a and b, and total chlorophyll at the 5% level ($p<0.05$). The simple type of regression showed the relation between chlorophylls as a function of moistures. The slope factors for chlorophyll content (a, b, and total) were 0.898, 0.959, and 0.939, respectively.

The results of chlorophyll content for *E. aureum* on soil pH were shown in Table II. The content of chlorophylls was reduced in accordance with the acidification of soil as a whole. The chlorophyll content was highest at pH 6.5 of the soil. The content for chlorophylls a, b, and total were 0.471, 0.219, and 0.446 mg/g at pH 6.5, respectively. The chlorophyll a content was similar at pH 6.0 to 0.472 and at pH 6.5 to 0.471. It was slightly higher at pH 6.5, which is acidic, but pH 7.0, which is neutral, had low chlorophyll content. There was a significant difference with regard to the contents of chlorophylls a and b, and total chlorophyll at the 5% level ($p<0.05$). Chlorophyll content at pH 6.5 was slightly higher than pH 7.0, but there was no significant difference. The simple type of regression showed the relation between chlorophylls as a function of acidity. The slope factors for chlorophylls a, b, and total were 0.750, 0.535, and 0.729, respectively.

TABLE I: The Values of Chlorophyll Content on *Epipremnum aureum* by Different Moistures (unit: mg/g)

Moisture (%)	Chl-a	Chl-b	Total	Chl-a/Chl-b
20	0.506±0.040	0.227±0.004	0.496±0.034	2.227
30	0.512±0.043	0.236±0.006	0.517±0.039	2.168
40	0.484±0.058	0.205±0.005	0.463±0.041	2.359
50	0.460±0.030	0.188±0.011	0.412±0.026	2.451
60	0.437±0.015	0.167±0.009	0.368±0.036	2.617
70	0.354±0.039	0.150±0.013	0.349±0.048	2.359
80	0.317±0.051	0.126±0.012	0.327±0.031	2.506

TABLE 2: The Values of Chlorophyll Content on *Epipremnum aureum* by Different Soil pH (unit: mg/g)

pH	Chl-a	Chl-b	Total	Chl-a/Chl-b
4.5	0.393±0.023	0.166±0.026	0.352±0.059	2.368
5.0	0.416±0.014	0.178±0.041	0.381±0.051	2.332
5.5	0.447±0.017	0.201±0.033	0.428±0.043	2.224
6.0	0.472±0.010	0.212±0.032	0.437±0.033	2.229
6.5	0.471±0.006	0.219±0.037	0.446±0.030	2.148
7.0	0.458±0.012	0.194±0.029	0.430±0.019	2.358

B. Chlorophyll Efficiency

The current fluorescence yield for chlorophyll efficiency with moisture concentrations on *E. aureum* were shown in Fig. 3. The current fluorescence yield (Fo) decreased at 30% of moisture and then increased.

Whereas, the maximum fluorescence yield (Fm) was increased at 30% of moisture and then decreased (Fig. 4).

The change of chlorophyll efficiency in the quenched state (Fv) was also increased at 30% of moisture and then decreased steeply (Fig. 4).

The maximal PSII quantum yield, Fv/Fm was the highest at 30% moisture among seven moisture percentage stages (Fig. 6). The simple type of regression showed the relation between chlorophyll efficiency as a function of moistures. The slope factors of Fo, Fm, Fv, and Fv/Fm for chlorophyll efficient indicators were 0.938, -0.806, 0.013, and 0.846, respectively.

The results for chlorophyll efficiency with soil pH were shown in Table III. The Ft was increased to the decrease of pH in soil. The Ft value was the lowest at pH 5.5 (831.0). Fv/Fm was the highest at pH 6.5 (0.808). The simple type of regression showed the relation between chlorophyll efficiency as a function of pH. The slope factors of Ft, Fm, Fv, and Fv/Fm for soil pH were 0.421, -0.849, 0.814, and -0.596, respectively.

TABLE III: The Values of Chlorophyll Efficiency on *Epipremnum aureum* by different pH

pH	Ft	Fm	Fv	Fv/Fm
4.5	827±7.0	4098±15.9	3271±19.4	0.798±0.03
5.0	831±5.5	4190±32.0	3359±67.0	0.803±0.05
5.5	816±14.8	4193±28.7	3423±24.8	0.804±0.02
6.0	821±24.1	4262±11.5	3377±11.6	0.805±0.01
6.5	827±18.6	4356±48.8	3441±30.3	0.808±0.04
7.0	843±15.5	4263±64.6	3420±49.1	0.802±0.01

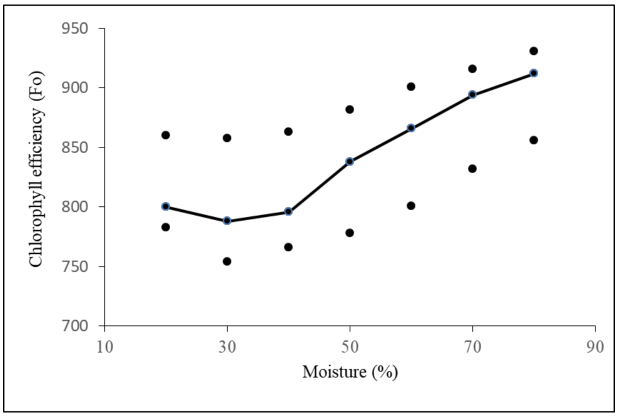

Fig. 3. The change of chlorophyll efficiency on *Epipremnum aureum* at different moisture (%). Fo: The current fluorescence yield.

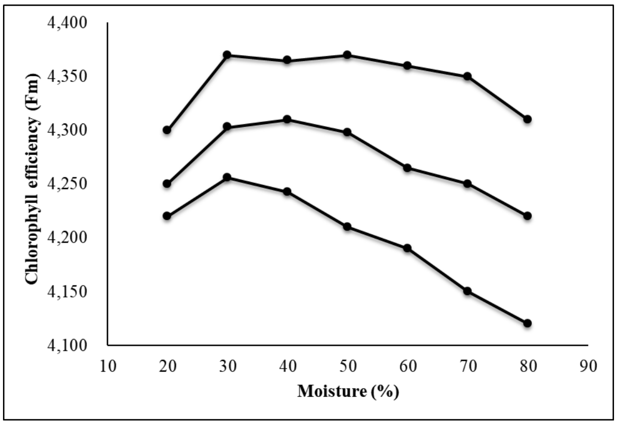

Fig. 4. The change of maximum fluorescence yield (Fm) on *Epipremnum aureum* at different moisture (%).

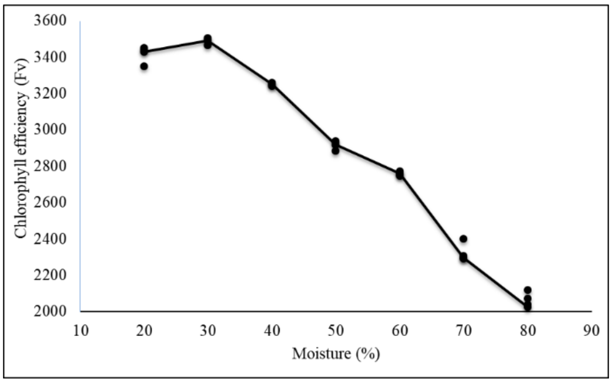

Fig. 5. The change of chlorophyll efficiency in the quenched state (Fv: the quenched state) on *Epipremnum aureum*.

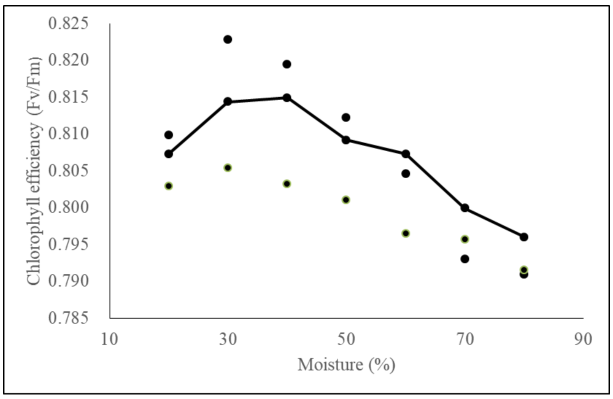

Fig. 6. The change of maximal PSII quantum yield of *Epipremnum aureum*. Fv/Fm: The maximal PSII quantum yield.

IV. Discussions

E. aureum (pothos) is a very easy to grow houseplant preferring medium light, well-lit but not direct sunlight. It is even able to survive for long periods in low light. Mist the leaves regularly to keep humidity levels up and let the well-drained potting medium dry out between watering. The moisture concentration on *E. aureum* recommended average 30% or 40% (Table I).

Although plants achieve growth through the absorption of water, there are reports that excessive moisture is a hindrance to plant growth (Fukao *et al*., 2019). If less water is sprayed on plants grown at home, roots and soil compete to absorb moisture from each other. On the other hand, giving too much water to a plant can damage its roots. Root loss occurs when excess water reduces oxygen in the soil. A plant cannot grow without healthy roots (Hubbard, 2019).

It may be more effective to water plants a little more often than to water them all at once (Tang & Chai, 2020). As shown in Fig. 4, it is better to avoid over 50% moisture soil. To prevent microbial development, plants should be sometimes allowed to dry out slightly and watered only when the soil surface feels dry. It is not recommended to water at a fixed time, but more attention should be paid to the state of succulent plant and specific weather conditions.

The parameter of Fv/Fm is the intrinsic quantum yield of PSII and commonly provides a good indicator (Butler, 1978; Genty *et al*., 1992). If the leaves of a plant are not stressed, the value of Fv/Fm is highly consistent and has values of ~0.83, and correlates to the maximum quantum yield of photosynthesis (Demmig & Björkman, 1987). The value of Fv/Fm is ranged from 0.793 (80% moisture) to 0.813 (30%) on water level (Fig. 6). The value of Fv/Fm is ranged from 0.798 (pH 4.5) to 0.808 (pH 6.5) on pH level (Table III).

In this study, plant leaves with moisture or PH outside the normal range showed low Fv/Fm values. It was assumed that low Fv/Fm values reflected under the stress conditions (Jägerbrand & Kudo, 2016).

E. aureum contains the broad spectrum of phytochemicals and secondary metabolite including alkaloids and cardiac glycosides which has been investigated by preliminary analysis of the ethanol explant extract (leaves and root) (Lalitha *et al*., 2010). These secondary contents are popular because they exhibit functionality. For example, *E. aureum* has detoxification potential for formaldehyde and nicotine and can be used for self-regenerating bio-filter system for indoor air purification (Weidner & Silva, 2006). However, Calcium oxalate crystals are being involved in *E. aureum*. So it is used as effective natural pesticides, it also involved in calcium regulation. Someone needs to be careful that *E. aureum* is not consumed by house pets (Meshram & Srivastava, 2014). Other toxicity symptoms such as oral irritation, allergic reaction can be present (Palmer & Betz, 2006). The plant is listed as "toxic to cats, toxic to dogs" by the American Society for the Prevention of Cruelty to Animals (ASPCA), because of the presence of insoluble raphides.

Many studies confirmed that leaf chlorophyll content decreased with increasing water stress degree (Ashkavand *et al*., 2015). This study showed lower chlorophyll content in E. aureum (0.517 mg/g =51.7 mg/100 g) on 30% moisture, Table I). Bohn and Walczyk (2004) reported that spinach had

69.1 mg/ 100 g chlorophyll content, and iceberg lettuce had 19 mg/100 g of chlorophyll. Huh (2022) reported found 0.548 mg/g of chlorophyll in leafy vegetables (*Allium tuberosum*). *A. tuberosum* was grown in natural soils with pH values of 6.5 and it is same as *E. aureum* (Table III).

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