ผลของสาร 7,8-dihydro-8α-20-hydroxyecdysone ต่อกระบวนการสังเคราะห์ด้วยแสง ปริมาณคลอโรฟิลล์ และโครงสร้างคลอโรพลาสต์ในมะเขือเทศพันธุ์ CH154 ภายใต้สภาวะแล้ง

Effect of 7,8-dihydro-8α-20-hydroxyecdysone on Photosynthesis, Chlorophyll Content and Chloroplast Structure in Tomato (*Solanum lycopersicum* cv. "CH154") under Drought Stress

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บทคัดย่อ

งานวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาผลของสาร 7,8-Dihydro-8α-20-Hydroxyecdysone (DHECD) ต่อความสามารถในการทนแล้งของต้นมะเขือเทศ การสังเคราะห์ด้วยแสง ปริมาณคลอโรฟิลล์และโครงสร้างคลอโรพลาสต์ โดยการจำลองสภาวะแล้งด้วยสาร Polyethylene Glycol 6000 (PEG) แบ่งเป็นการทดลองที่ 1 ศึกษาความสามารถในการทนต่อระดับความแล้งของพืชโดยศึกษาความยาวต้นและราก ปริมาณน้ำสัมพัทธ์ (Relative Water Content, RWC) ปริมาณคลอโรฟิลล์และแคโรทีนอยด์ ด้วยการจำลองสภาวะแล้ง จำนวน 4 ทรีตเมนต์ คือ 0 % 0.25 % 0.5 และ 1 % (w/v) PEG การทดลองที่ 2 ศึกษาผลของสาร DHECD ต่อสัดส่วนคลอโรฟิลล์ เอต่อบี Performance Index (PI) คลอโรฟิลล์ฟลูออเรสเซนซ์ (F_v/F_m) ค่าความเขียวของใบ (SPAD Value) และโครงสร้างคลอโรพลาสต์ภายใต้สภาวะแล้ง แบ่งออกเป็น 3 ทรีตเมนต์ คือ 1) พืชในสภาวะปกติที่พ่นด้วยสาร 0 μM DHECD 2) พืชในสภาวะแล้งที่ระดับ 0.5 % (w/v) PEG ที่พ่นด้วยสาร 0 μM DHECD และ 3) พืชในสภาวะแล้งที่ระดับ 0.5 % (w/v) PEG ที่พ่นด้วยสาร 50 μM DHECD ผลการทดลองพบว่า การทดลองที่ 1 พืชทนต่อระดับความแล้งสูงสุดที่ 0.5 % (w/v) PEG โดยมีความยาวต้นและ

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ราก RWC ปริมาณคลอโรฟิลล์เอและบี ในใบลดลงเท่ากับ 33.93 % 60.57 % 34.22 % 12.47 % และ 35.68 % เมื่อเทียบกับชุดควบคุม ตามลำดับ แต่ปริมาณแคโรทีนอยด์ เพิ่มขึ้น 47.20 % เมื่อเทียบกับชุดควบคุม การทดลองที่ 2 พบว่าหลังจากได้รับสภาวะแล้ง 12 วัน สาร DHECD ช่วยรักษาโครงสร้างคลอโรพลาสต์ในใบ เพิ่มสัดส่วนคลอโรฟิลล์ เอต่อบี ค่า PI F_v/F_m และ SPAD เท่ากับ 6.51 % 21.22 % 0.72 % และ 25.43 % ตามลำดับเมื่อเทียบกับพืชที่ได้รับสภาวะแล้งที่ไม่ได้พ่นด้วยสาร DHECD แสดงให้เห็นว่าสาร DHECD ส่งเสริมประสิทธิภาพของการสังเคราะห์ด้วยแสง ช่วยรักษาสภาพของโครงสร้างคลอโรพลาสต์และปริมาณคลอโรฟิลล์ของต้นมะเขือเทศภายใต้สภาวะแล้งได้

คำสำคัญ: มะเขือเทศ ความแล้ง บราสสิโนสเตียรอยด์ คลอโรฟิลล์ฟลูออเรสเซนซ์ คลอโรฟิลล์ คลอโรพลาสต์

Abstract

This research aimed to investigate the role of 7,8-Dihydro-8α-20-Hydroxyecdysone (DHECD) on drought tolerant ability, photosynthesis, chlorophyll content and chloroplast structure under drought by using Polyethylene Glycol 6000 (PEG) in tomato. The experiment 1 studied drought tolerant ability in tomato and measured shoot length, root length, Relative Water Content (RWC), chlorophyll and carotenoids contents. There were 4 treatments: 0 %, 0.25%, 0.5%, and 1 % (w/v) PEG whereas the experiment 2 determined the effect of DHECD on chlorophyll *a*/*b* ratio, performance index (PI), chlorophyll fluorescence (F_v/F_m), SPAD values and chloroplast ultrastructure in tomato under drought. There were 3 treatments: 1) control plants (0 %(w/v) PEG) and sprayed with 0 µM DHECD, 2) drought treated plants (0.5 % (w/v) PEG) and sprayed with 0 µM DHECD, and 3) drought treated plants (0.5 %(w/v) PEG) and sprayed with 50 µM DHECD. Experiment 1 results showed that shoot and root lengths, RWC, chlorophyll *a*, and chlorophyll *b* contents of drought treated plants (0.5 %(w/v) PEG) were decreased by 33.93 %, 60.57 %, 34.22 %, 12.47 % and 35.68 %, respectively when compared with control while carotenoids content increased by 47.20% when compared with control. Experiment 2 results showed that after 12 days of drought treatment, DHECD maintained chloroplast structure in leaves and increased chlorophyll *a*/*b* ratio, PI, F_v/F_m and SPAD values by 6.51 %, 21.22 %, 0.72 % and 25.43 %, respectively when compared with drought. These results indicated that DHECD application improves photosynthetic efficiency and maintains chlorophyll content and chloroplast structure in tomato under drought.

Keywords: Tomato, Drought, Brassinosteroids, Chlorophyll Fluorescence, Chlorophyll, Chloroplast

Introduction

Tomato (*Solanum lycopersicum*) is important economic crops of Thailand. Moreover, it contains many antioxidants such as carotenoids, ascorbic acid, vitamin E and phenolic compounds. Nowadays, drought is environmental stress that declines Relative Leaf Growth Rate (RGR) and increases lipid peroxidation and hydrogen peroxide (H_2O_2) level in plants. Plant growth regulators (PGR) application is one of the solutions for this problem. Brassinosteroids (BRs) are steroidal PGR that can promote seed germination,

induce cell elongation and increase yield of plants, such as 24-epibrassinolide (EBR). The previous study reported that EBR application reduced malondialdehyde (MDA) and H_2O_2 by enhancing antioxidant enzymes, such as superoxide dismutase (SOD), ascorbate peroxidase (APX), guaiacol peroxidase (GPOD) and catalase (CAT) in leaves of tomato under heat stress [1]. However, the commercial use of BRs is expensive. Therefore, in this study the researchers interested to study the effect of 7,8-dihydro-8α-20-hydroxyecdysone (DHECD), which similar structure to BRs on some physiological responses in tomato under drought. Previous study reported that DHECD alleviated heat stress by increasing net photosynthetic rate and decreasing lipid peroxidation and induced thermotolerance by improving antioxidant enzymes, such as SOD, APX, CAT and peroxidase (POD) for H_2O_2 scavenging in rice [2]. Accordingly, this research purposed to study DHECD on some physiological changes, such as chlorophyll *a*/*b* ratio, SPAD value and chlorophyll fluorescence parameters in order to improving the drought tolerance in tomato cv. "CH154".

Materials and Methodology

1. Plant material and growth conditions

Seeds of tomato (*Solanum lycopersicum* cv. "CH154") were sown in plastic pots containing Hoagland's nutrient solution. The plants were grown in greenhouse at Faculty of Science, Srinakharinwirot University, Bangkok. Sixty-five-day-old of tomatoes were used for the experiments. An experimental design was completely randomized design (CRD) using four replications per treatment.

Experiment I: To investigate of drought tolerance capacity in tomato under different levels of drought. Simulation of drought stress by polyethylene glycol (PEG) induces drought on the plants. PEG treatments were divided into four levels consisting of 0 %, 0.25 %, 0.5 % and 1 % (w/v). The leaf samples were harvested every 6 days for 24 days. Shoot and root lengths, relative water content (RWC), chlorophyll and carotenoids contents were measured.

Experiment II: To investigate the role of DHECD on chlorophyll content, chloroplast structure and efficiency of photosynthesis in tomato under drought. The plants were divided into three treatments and sprayed one time with DHECD at the beginning (day 0) as follows: 1) control plants and sprayed with 0 μM DHECD, 2) drought treated plants (0.5 % PEG) and sprayed with 0 μM DHECD and 3) drought treated plants (0.5 % PEG) and sprayed with 50 μM DHECD. The third and fourth tomato compound leaves (numbered basipetally) were harvested every 3 days for 12 days after drought treatment.

2. Determination of pigment contents

One hundred milligrams of leaf fresh weight in test tubes were added with 7 mL of dimethyl sulfoxide (DMSO) solution. After overnight incubation, extract liquid was boiled at 65 ˚C until leaf greenness and made up to 10 mL of total volume with DMSO. The extract was measured by using spectrophotometer (UNICO S1200, U.S.) at 480, 649 and 665 nm. Pigment contents contained chlorophyll *a*, chlorophyll *b* and carotenoids, which calculated with equations [3].

Chlorophyll *a* content (Chlo *a*) = 12.19 (A_{665}) - 3.45 (A_{649})

Chlorophyll *b* content (Chlo *b*) = 21.99 (A_{649}) - 5.32 (A_{665})

Chlorophyll *a*/*b* ratio = Chlo *a* / Chlo *b*

Carotenoids content = [1000(A_{480}) - 2.14(Chlo *a*) - 70.16(Chlo *b*)] / 220

3. Determination of relative water content (RWC)

Leaf discs of 6 mm diameters were excised from tomato leaves with cork borer. After recording fresh weight (FW), the discs were incubated in distilled water for 24 hours and then measured turgid weight (WT). Finally, the leaves were dried at 60 ˚C for 5 days and dry weight (DW) was recorded. Relative water content (RWC) was calculated by using standard equation as follows: RWC (%) = [(FW - DW) / (TW - DW)] × 100.

4. Measurement of SPAD value

The third and fourth tomato compound leaves (numbered basipetally) were selected. Each fully expanded mature leaf was recorded 3 times with a chlorophyll meter (SPAD-502, Konica Minolta, Japan). Each average SPAD value was calculated.

5. Measurement of chlorophyll fluorescence parameters

The third and fourth tomato compound leaves (numbered basipetally) were selected. Chlorophyll fluorescence parameters contained the maximum quantum efficiency of PSII (F_v/F_m) and performance index (PI) and were recorded by using a Pocket PEA chlorophyll fluorimeter (Hansatech, UK). Tomato leaves were pinched with leafclips for 30 min of dark adaptation. Minimal (F_0) and maximal (F_m) fluorescent emissions in dark-adapted state were determined for calculating F_v/F_m value [(F_m - F_0) / F_m].

6. Chloroplast ultrastructure observation

Terminal leaflets of third compound leaf (numbered basipetally) were selected. Tomato leaves were sliced into 1 mm^2 sections. The sections were immersed in mixture of 2.5 % glutaraldehyde and formaldehyde in phosphate buffer (pH 7.5) for 24 hours (primary fixation). Leaf sections were washed with 0.1 % buffer and fixed in 0.1 % osmic acid for 2 hours (secondary fixation). After dehydration in ethanol and embedding, ultra-thin sections were cut by using ultramicrotome (Leica EM UC7, Austria), stained with uranium acetate and lead citrate in series and examined under an electron microscope (Hitachi HT7700, Japan) at 80 kV.

7. Statistical analysis

The data were analyzed with One-way ANOVA of SPSS program (version 25). Mean differences of data were compared with method of Duncan's Multiple Range Test (DMRT) at $p < 0.05$ level of significance.

Results

In the experiment I, drought stress level at 0.5 % (w/v) PEG significantly decreased ($p < 0.05$) shoot and root lengths, chlorophyll *a*, chlorophyll *b* contents and RWC in tomato plants by 59.91, 76.72, 39.21, 70.79 and 12.31 %, respectively when compared with control while carotenoids content increased by 55.89 % after 24 days of drought when compared with control. The chlorophyll and carotenoids contents showed no significant difference between unstressed plants and 0.25 %(w/v) PEG-treated plants at day 18. The decline of length, chlorophyll content and RWC were higher in 0.5 % (w/v) PEG than 0.25 % (w/v) PEG. Nonetheless, shoot and root lengths were the lowest in 1 % (w/v) PEG. Moreover, pigment contents and RWC could not measure from day 18 to day 24 under 1 % (w/v) PEG because of leafless for sampling (Figure 1).

Chlorophyll *a*/*b* ratio, SPAD value, PI and F_v/F_m parameters of stressed plants with 0 µM DHECD decreased by 12.98, 22.48, 23.83 and 1.08 %, respectively at day 9 compared with control plants. The chlorophyll *a*/*b* ratio and SPAD value was higher in unstressed plants than stressed plants that sprayed with 0 µM DHECD whereas PI and F_v/F_m showed no significant difference at day 12. However, these parameters increased significantly ($p < 0.05$) in DHECD-treated plants from day 6 to day 12 by 6.51, 25.43, 21.22 and 0.72 %, respectively after 12 days of drought stress compared with stressed plants without sprayed with DHECD (Figure 2).

After 12 days of drought, the structure of chloroplast in stressed plant that sprayed with 0 µM DHECD showed small size, shrink shape, disappearing starch and thin grana lamellae (Figure 3b) while unstressed plant represented mature size, regular shape, generating starch and thick grana lamellae (Figure 3a). Nevertheless, chloroplast structure of stressed plant that sprayed with 50 µM DHECD was similar to unstressed plant (Figure 3c).

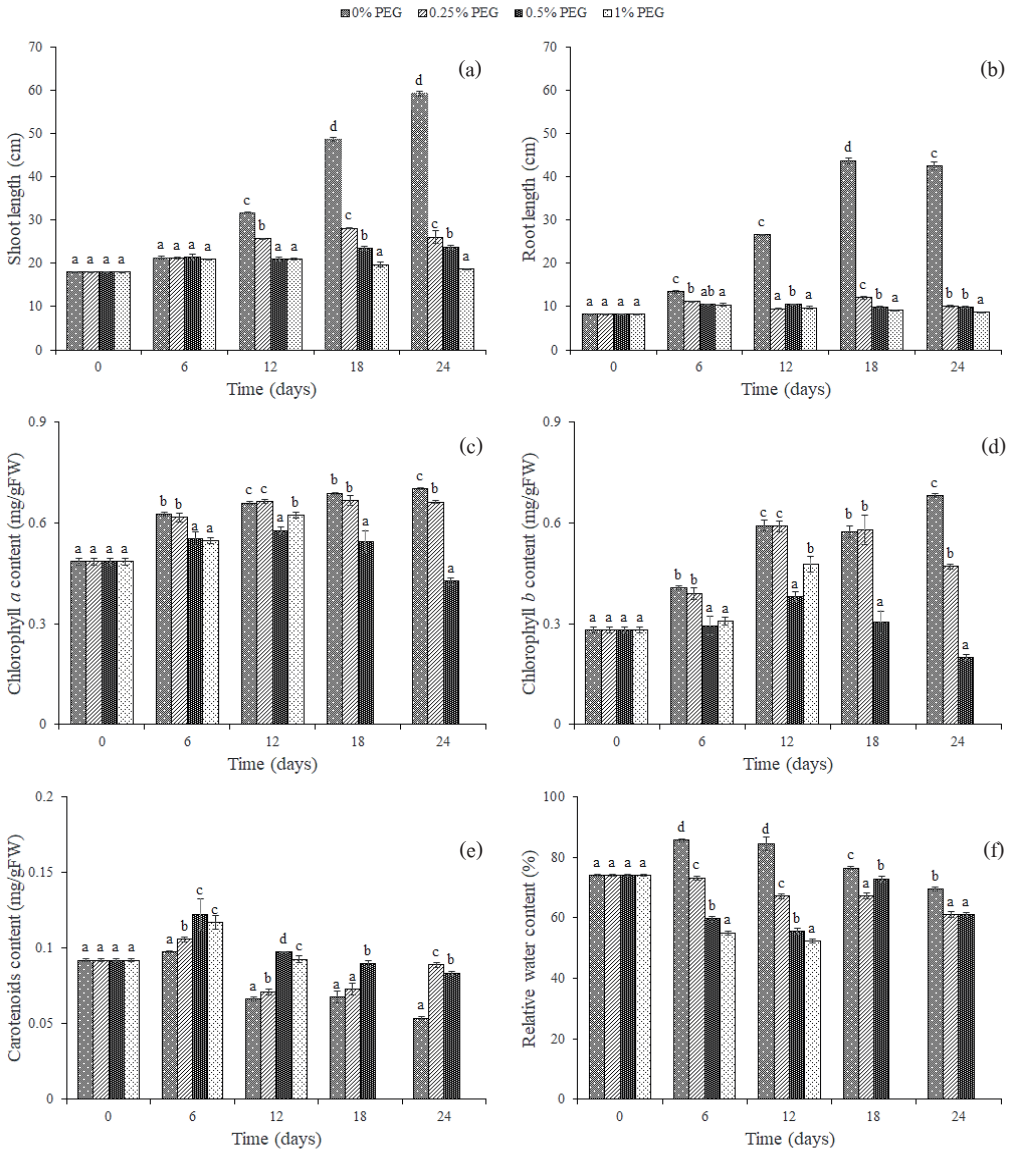

Figure 1 Effect of drought stress on shoot length (a), root length (b), chlorophyll *a* (c), chlorophyll *b* (d), carotenoids (e) and relative water contents (f) of tomato. Each histogram shows a mean ± standard error (SE) of four replications. The different letters within same time represent significant difference between treatments ($p < 0.05$).

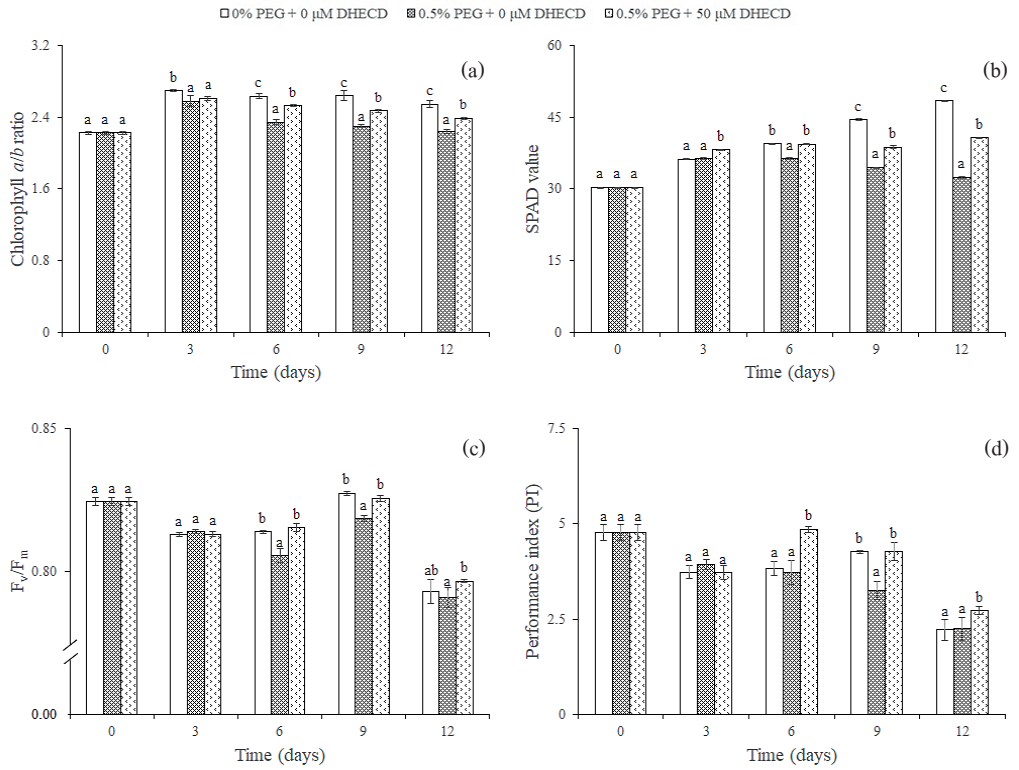

Figure 2 Effect of 7,8-dihydro-8α-20-hydroxyecdysone (DHECD) on chlorophyll *a*/*b* ratio (a), SPAD value (b), the maximum quantum efficiency of PSII; F_v/F_m (c) and performance index; PI (d). Each histogram shows a mean ± standard error (SE) of four replications. The different letters within same time represent significant difference between treatments ($p < 0.05$).

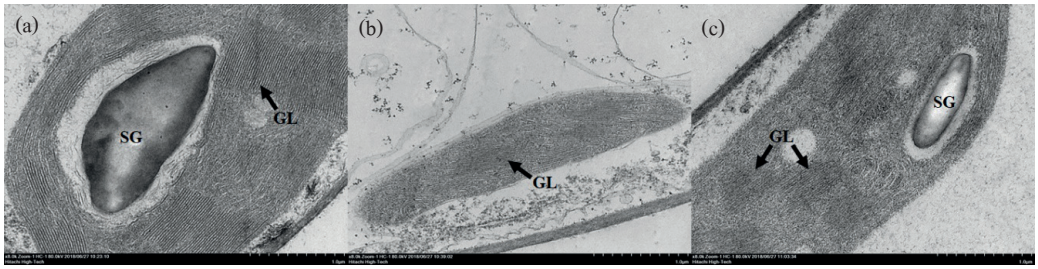

Figure 3 Effect of 7,8-dihydro-8α-20-hydroxyecdysone (DHECD) on the ultrastructure of chloroplast in tomato leaves. Control (0 % PEG) spray with 0 µM DHECD (a), drought (0.5 % PEG) spray with 0 µM DHECD (b) and drought (0.5 % PEG) spray with 50 µM DHECD (c). The third (numbered basipetally) fully expanded mature leaves were selected for transmission electron microscopy after 12 days of drought stress (grana lamellae; GL and starch grain; SG and scale bar for chloroplast ultrastructure are 1.0 µm.)

Discussion

Plants manifest multiple defense mechanisms involving various morphological and physiological alterations in response to drought stress. In this study, the shoot length, root length, relative water content (RWC), chlorophyll of tomato were declined under drought (Figure 1) because PEG interfered water absorption of root. It affected decreasing water within plant cells and incomplete mitosis, which led to inhibit cell elongation and cell division. Furthermore, drought stress decreased chlorophyll contents by enhancing transcription of chlorophyll degradation enzymes, such as chlorophyllase and pheophorbide *a* oxygenase. In contrast, carotenoids content increased because they protect photosynthetic apparatus against reactive oxygen species (ROSs), which produced from plants grown under abiotic stresses. The results showed that 0.5 % (w/v) PEG was the highest drought tolerant level of this tomato whereas 1 % (w/v) PEG was severe drought level that the plant could not tolerate to stress because this PEG concentration affected leafless tomato from day 18 to day 21. These results are consistent with those of other studies that have reported that high concentrations of PEG reduce the plant growth such as the germination percentages of lentil [4]. Therefore, the concentration of PEG at 0.5 % (w/v) was selected for experiment II.

Leaf greenness value (SPAD value), which positively correlated with chlorophyll contents is a parameter for estimating plant response to drought. The results showed that chlorophyll *a*/*b* ratio and SPAD value were decreased by drought stress. However, in this study found that exogenous DHECD maintained chlorophyll level and SPAD value in tomato plants under drought (Figure 2b).

Evaluation of the response of the photosynthetic apparatus to different abiotic stress condition has generally been performed by analyzing parameters describing the maximum quantum efficiency of photosystem II (PSII) photochemistry (F_v/F_m), electron transport rate (ETR), photochemical quenching (qP) and non-photochemical quenching (qN). F_v/F_m implied that plant exhibited different photosynthetic strategies to utilize the absorbed irradiance under drought stress [5]. Moreover, there are many studies suggest that performance index (PI) parameter, which quantifies the overall functionality of the electron flow through PSII, might be a sensitive parameter of plant homeostasis and it is also interesting to evaluate specific energy fluxes per reaction centre (RC) in plant. The result showed that unstressed plants can efficiently keep homeostasis of photosynthetic apparatus and the stressed plants that sprayed with DHECD had similar F_v/F_m after exposure to drought stress. While stressed plants were not sprayed with DHECD were lower F_v/F_m than the other (Figure 2c). This indicated a perturbation in PSII functional properties in drought. The result found that stressed plants were sprayed with 50 µM DHECD had higher PI than stressed plants that sprayed with 0 µM DHECD in drought stress (Figure 2d). Therefore, DHECD enhance the efficiency of photosynthesis in tomato plants under drought. These results were consistent with previous research, which reported that DHECD increased chlorophyll contents, SPAD value and F_v/F_m of rice (*Oryza sativa* L. cv. “Pathum Thani 1”) under heat stress [6].

Moreover, the changing in morphological traits such as chloroplast structure indicated plant displayed defects in osmotic stress. The result also showed that exogenous DHECD had maintained chloroplast structure of tomato plant under drought (Figure 3). Although, drought induced the generation of ROSs (e.g. hydrogen peroxide (H_2O_2) and hydroxyl radical) in chloroplast and ROSs ruined DNA, polyunsaturated fatty acid in lipids and amino acids in proteins. Therefore, DHECD alleviated drought stress by reduced lipid peroxidation, H_2O_2 content, and maintained structure of chloroplast in tomato leaves [7].

Conclusion

The study demonstrated that drought stress reduced shoot and root growth, chlorophyll content and water content in tomato leaves. The highest drought tolerant level of tomato (*Solanum lycopersicum* cv. "CH154") was 0.5 % (w/v) PEG. However, DHECD relieved drought stress in tomato by sustaining chloroplast structure and improving photosynthetic efficiency and chlorophyll accumulation in leaves. This study indicated that DHECD enhanced drought tolerance and it was a good candidate for the improvement of the photosynthetic efficiency in tomato plants under drought stress.

References

[1] Ogweno, J. O., Song, X. S., Shi, K., Hu, W. H., Mao, W. H., Zhou, Y. H., Yu, J. Q., & Nogués, S. (2008). Brassinosteroids alleviate heat-induced inhibition of photosynthesis by increasing carboxylation efficiency and enhancing antioxidant systems in *Lycopersicon esculentum*. *Journal of Plant Growth Regulation*, *27*(1), 49–57.

[2] Sonjaroon, W., Kaveeta, L., Chai-arree, W., Klinsakorn, S., Suksamrarn, A., & Jutamanee, K. (2016). Exogenous 7,8-dihydro-8α-hydroxyecdysone application improves antioxidative enzyme system, photosynthesis, and yield in rice under high-temperature condition. *Acta Physiologiae Plantarum*, *38*(8), 1–11. DOI: https://doi.org/110. 007/s11738-016-2205-8.

[3] Wellburn, A. R. (1994). The spectral determination of chlorophylls *a* and *b*, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. *Journal Plant Physiology, 144*(3), 307–313.

[4] Siahsar, B. A., Ganjali, S., & Allahdoo, M. (2010). Evaluation of drought tolerance indices and their relationship with grain yield of lentil lines in drought-stressed and irrigated environments. *Australian Journal of Basic and Applied Sciences, 4*(9), 4336–4346.

[5] Ceusters, N., Valcke, R., Frans, M., Claes, J. E., den Ende, W. V., & Ceusters, J. (2019).Performance index and PSII connectivity under drought and contrasting light regimes in the CAM orchid *Phalaenopsis. Frontiers in Plant Science, 10*, 1–15. www.frontiersin.org.

[6] Thussagunpanit, J., Jutamanee, K., Kaveeta, L., Chai-arree, W., Pankean, P., Homvisasevongsa,S., & Suksamrarn, A. (2015). Comparative effects of brassinosteroid and brassinosteroid mimic on improving photosynthesis, lipid peroxidation, and rice seed set under heat stress. *Journal of Plant Growth Regulation*, *34*(2), 320–331.

[7] Maneechote, P., Ke, X., Thongsomnuek, P., Keawlaong, S., Hngokchai, C., Suksamrarn, A.,Chaivisuthangkura, P., & Saeng-ngam, S. (2018). Effect of brassinosteroid mimic on chlorophyll content and lipid peroxidation of tomato (*Solanum lycopersicum*) grown under drought stress. *Khon Kaen Agriculture Journal, 46*(Supplement 1), 322–327.