Agriculture and Natural Resources 52 (2018) 234–240

Contents lists available at ScienceDirect

Agriculture and Natural Resources

journal homepage: http://www.journals.elsevier.com/agriculture-and-natural-resources/

Original Article

Impact of brassinosteroid mimic on photosynthesis, carbohydrate content and rice seed set at reproductive stage under heat stress

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A R T I C L E I N F O

Article history:
Received 28 September 2017
Accepted 29 December 2017
Available online 15 September 2018

Keywords:
7,8−Dihydro−5α,8α−20−hydroxyecdysone
High temperature
Starch
Stomatal factor
Sugar

A B S T R A C T

Brassinosteroid mimics have been shown to increase photosynthesis in plants and alleviate the effects of heat stress. 7,8-Dihydro-8α-20-hydroxyecdysone (αDHECD) is a brassinosteroid mimic, but there has been no reporting on the mechanism of αDHECD against heat stress. Therefore, the effect of αDHECD was investigated on photosynthesis and seed set in rice subjected to heat stress. In this experiment, rice plants were applied with water or 0.0001 μM αDHECD by foliar application before being subjected to heat stress during the reproductive growth stage. Then, plants were exposed to either a normal temperature regime of 30/26 °C day/night or to a high temperature regime of 40/30 °C day/night for 7 d. The results showed that αDHECD increased the net photosynthetic rate under the normal temperature regime. Under heat stress, αDHECD enhanced the net photosynthetic rate, stomatal conductance, transpiration rate and stomatal limitation, while decreasing the intercellular CO_2 concentration and efficiency of water utilization. αDHECD also increased the total soluble sugar content in rice leaves under the normal and high temperature treatments. The application of αDHECD also increased seed setting, seed weight and the sugar and starch contents in the straw and seed under heat stress. The results indicated that αDHECD is a brassinosteroid mimic that enhances photosynthetic performance, increases the carbohydrate contents in the straw and seed and improves rice seed set under high temperatures when it is applied in the reproductive growth stage.

Introduction

High temperature is one of the abiotic stresses which affect biochemical and physiological processes and decrease productivity in crop plants. Primarily, a high temperature reduces stomatal conductance, which subsequently decreases gas exchange and photosynthesis (Inzé and Montagu, 2002; Mohammed and Tarpley, 2009). Rice is a major food crop in Thailand. However, the reproductive stage of rice is most sensitive to high temperature (Krishnan et al., 2011). High temperature decreases pollen germination resulting in a poor seed set (Cao and Zhao, 2008). Moreover, heat stress has a significant impact on photosynthesis and can reduce the photosynthetic rate by about 40−60% in the reproductive stage (Oh-e et al., 2007). The reduction of photosynthetic performance can lead to a decrease in rice yield (Oh-e et al., 2007).

Plant growth regulators are one of the alternative methods that are widely used to increase agricultural production. Brassinosteroids (BRs) are a group of steroidal plant growth regulators that are involved in major physiological processes in plants, including stem elongation and pollen tube growth (Clouse and Sasse, 1998). BRs also induce synthesis of ethylene, xylem differentiation, synthesis of nucleic acid and proteins, activation of enzymes and photosynthesis (Clouse and Sasse, 1998; Hayat and Ahmad, 2003; Khripach et al., 2003; Sasse, 2003; Yu et al., 2004). At present, there are many derivatives of BRs which show activity similar to natural BRs, such as 24−epibrassinolide (EBR), and have been studied and used extensively to increase the yield and to mitigate the effects of biotic and abiotic stresses in various plants, including cucumber (Yu et al., 2004), melon (Zhang et al., 2013), eggplant (Wu et al., 2014), tomato (Ogweno et al., 2008) and rice (Thussagunpanit et al., 2015).

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https://doi.org/10.1016/j.anres.2018.09.001

Most natural BRs are produced in low quantities and are very expensive (Serna et al., 2012). Therefore, Suksamrarn et al. (2002) synthesized 7,8-dihydro-8α-20-hydroxyecdysone (DHECD) through catalytic hydrogenation of 20-hydroxyecdysone which was obtained from *Vitex glabrata* stem bark (Werawattanametin et al., 1986). The skeleton of the structure and functional groups of DHECD are similar to those of castasterone—a type of BR. A previous study indicated that DHECD exhibited some biological activities similar to the role of BRs in the plant (Homvisasevongsa, 2006). Moreover, DHECD could promote photosynthesis, antioxidative enzyme activity, the percentage of rice pollen germination and seed setting under heat stress conditions (Sonjaroon et al., 2016; Thussagunpanit et al., 2013). Moreover, BR mimic substances have also been discovered and in recent studies, many derivatives of BRs have been synthesized. Suksamrarn et al. (2002) reported that the DHECD structure was changed to 7,8-dihydro-8α-20-hydroxyecdysone by adding 5% Na_2CO_3 and MeOH which changed the H bond at the C−5 position of DHECD. The αDHECD structure has a *cis* nature at C−5 in the A/B ring (Fig. 1) whereas DHECD possesses a trans-A/B ring. αDHECD has been shown to reduce dwarfism in the BR-biosynthesis-deficient mutant *det2* and also downregulated the expression of the BR biosynthesis genes *DWF4* and *CPD* in *Arabidopsis*. Moreover, αDHECD induced dephosphorylated BIL1/BZR1 accumulation which enhanced BR signaling as a master transcription factor (Thussagunpanit et al., 2017). Therefore, αDHECD is similar to BRs in their effects on plants at the molecular level. However, there are only a few studies on how αDHECD affects the actual physiological processes in plants (Thussagunpanit et al., 2017). In particular, the effects of αDHECD on photosynthesis and yield in plants under heat stress have not been reported. Our current study focused on αDHECD application in rice (*Oryza sativa* L. cv. PathumThani) under high temperature stress. The objective of this study was to investigate the effect of αDHECD on photosynthesis, the contents of sugar and starch and the yield of rice under high temperature conditions.

Materials and methods

Plant material and brassinosteroid mimic treatments

Rice seedlings (*Oryza sativa* L. cv. PathumThani 1) were grown in plastic pots with a 1:1 mixture of clay and organic substance with 3 kg dry substrate per pot and one plant per pot. The experiment was conducted in a greenhouse at the Department of Botany, Kasetsart University, Bangkok, Thailand (13°50′N, 100°34′E). Prior to the treatments, the seedlings were grown at 600 μmol/m^2/s photosynthetic photon flux density (PPFD) at 30/25 °C (12 h day/12 h night) and 50–60% relative humidity. The water in the pot was controlled throughout the experiment. Rice seedlings received chemical fertilizer in three periods: 0.48 g nitrogen (N) at 15 d after sowing, 6.57 g N at 55 d after sowing, and 2.97 g N at 75 d after sowing, according to the recommendations by Department of Agriculture (2005).

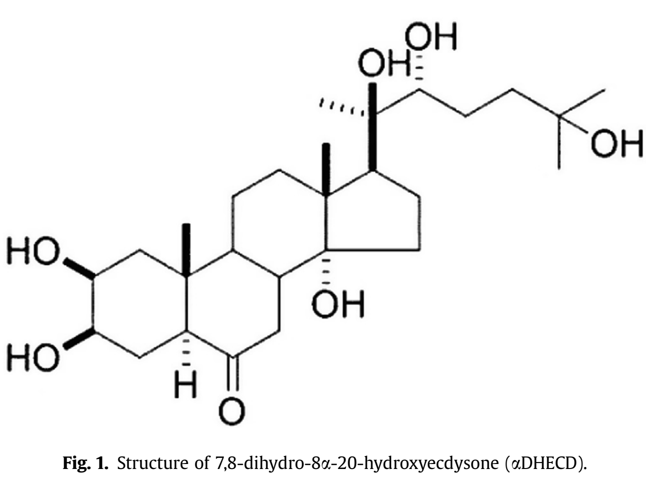

Fig. 1. Structure of 7,8-dihydro-8α-20-hydroxyecdysone (αDHECD).

The experiment was conducted using a completely randomized design with four replications. The seedlings were assigned to two temperature treatments: normal temperature (30/25 °C for 12 h day/12 h night) and high temperature (40/30 °C for 12 h day/12 h night). Other abiotic factors were controlled at 600 μmol/m^2/sPPFD and 50–60% relative humidity for 12 h day/12 h night. With a factorial design of two factors (temperatures and application of αDHECD), this experiments consisted of four treatments: application of water at normal temperature (C), 0.001 μM of αDHECD at normal temperature (αD), distilled water at high temperature (SC) and 0.001 μM αDHECD at high temperature (S+αD). Prior to spraying, distilled water and 0.001 μM αDHECD solutions were mixed with 0.25% Tween-20 surfactant. The spray was applied at the rate of 20 mL per plant.

Rice plants were sprayed with either distilled water or αDHECD at the normal temperature at 80 d after planting. Subsequently, all treatments were transferred in their respective treatments for 7 d. The photosynthetic gas exchange and total soluble sugar were measured at 84, 86, 88 and 90 d after sowing and data on seed setting were collected at harvest (120 d after sowing). Lastly, the total soluble sugar and starch contents were analyzed in the straw and rice seed after harvest.

Measurement of photosynthetic leaf gas exchange in rice

Gas exchange parameters were measured on rice leaves, using a portable LI-6400 system (Li-cor Inc.; Lincoln, NE, USA). The gas exchange measurement was performed during 0900–1100 h with the artificial saturating photo photon flux density at 1400 μmol/m^2/s, the CO_2 concentration at 400 ppm, the relative humidity at 65%, the air flow rate into the assimilation chamber at 500 μmol/s and the leaf area used in the measurements was 1.5 cm^2. The net photosynthetic rate (P_N), stomatal conductance (g_s), transpiration rate (E) and intercellular CO_2 concentration (C_i) were obtained from the portable photosynthesis systems (LI-6400). The water use efficiency (WUE) was calculated from P_N and E. The stomatal limitation (L_S) was calculated using the 1-(C_a/C_i) equation.

Determination of total soluble sugar and starch content in leaf, straw and rice seed

The total soluble sugar and starch contents were measured, using the anthrone method (Turakainen et al., 2004; Yemm and Willis, 1954). For the total soluble sugar, 0.1 g of plant tissues (fresh leaves, straw and rice seed) was homogenized in 2.5 mL of 80% ethanol for 10 min. Then, the supernatant was collected from the extraction of the sample. The homogenization was repeated two more times. After that, 4.5 mL of anthrone reagent was added to the supernatant and incubated in hot water for 20 min. Finally, the sample was subjected to spectrophotometry at 620 nm to determine the total soluble contents from a glucose standard plot and calculated on a fresh weight basis.

For starch content determination, the precipitate from the extraction of the sugars was hydrolyzed by incubation with 50% (v/v) perchloric acid overnight at room temperature. After that, 4.5 mL of anthrone reagent was added to the supernatant and incubated in hot water for 20 min. Finally, the sample was subjected to spectrophotometry at 620 nm to determine the sugars content from a glucose standard plot and the starch content was calculated on a fresh weight basis.

Measurement of dry matter and grain yield

The rice plants were harvested at age 120 d. The rice yield was determined as the number of spikelets per panicle, the grain weight of filled seed and the percentage of filled seed. Then, the leaf and culm in each treatment were dried at 80 °C for 7 d using the hot air oven, and the dry matter was weighed.

Statistical analysis

The analysis of variance between treatments was performed using the SAS statistical package (SAS Institute, 2003). All results were presented as the mean ± standard error. Means differing at the 0.05 level were considered significant. Tukey's test was applied as a *post hoc* comparison for pairwise differences.

Results

Photosynthetic leaf gas exchange

Application of αDHECD significantly increased P_N compared to the control on day 0 before the exposure to heat stress (Fig. 2A). Under heat stress, P_N decreased by 52.1% on day 5 and 56.9% on day 7 compared to the control treatment (Fig. 2A). From day 3 to

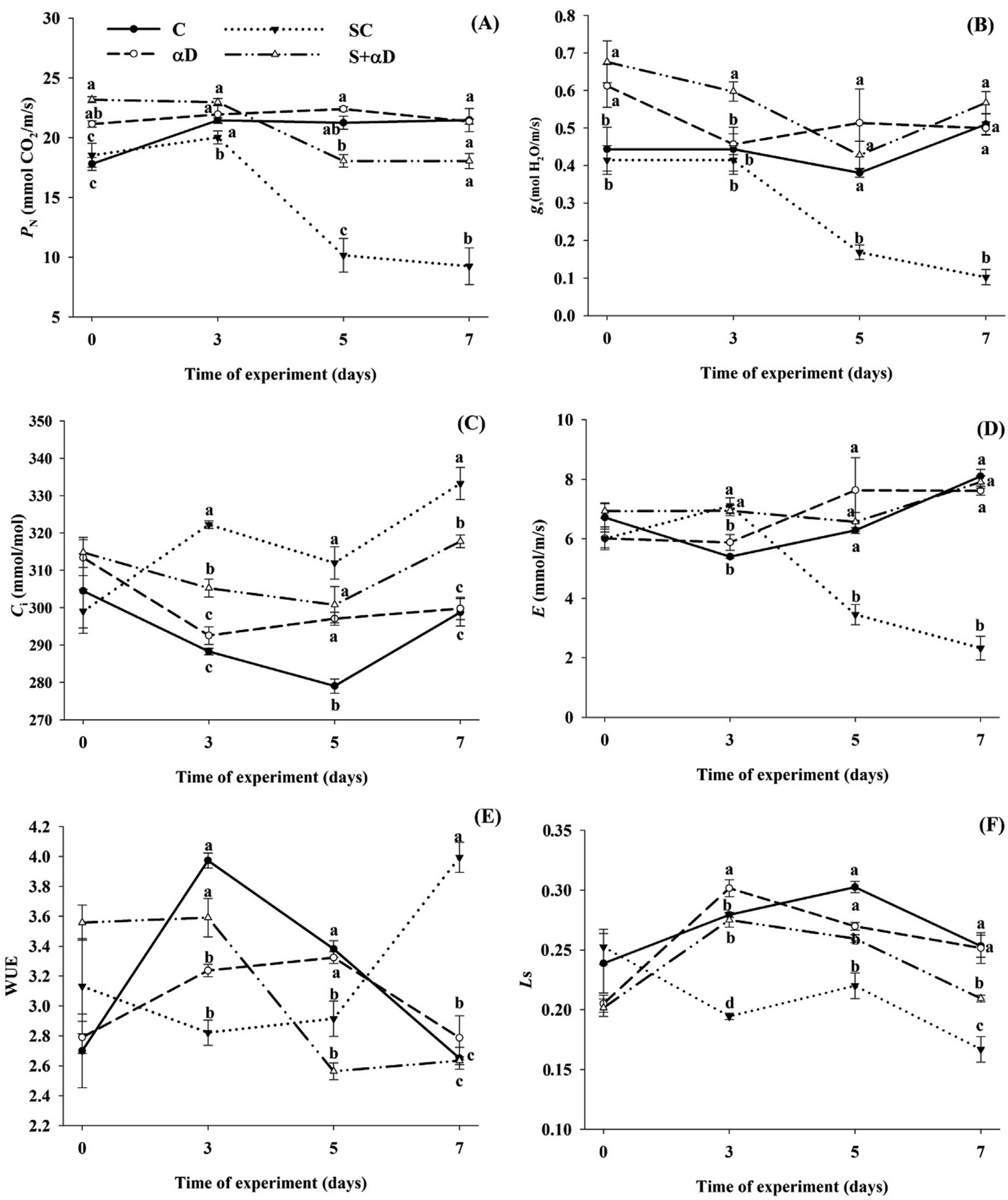

Fig. 2. Effects of 7,8-dihydro-8α-20-hydroxyecdysone (αDHECD) on rice leaves under high temperature regime: (A) net photosynthetic rate (P_N); (B) stomatal conductance (g_s); (C) intercellular CO_2 concentration (C_i); (D) transpiration rate (*E*); (E) water use efficiency (WUE); (F) stomatal limitation (*Ls*). Data are mean ± SE, with n = 5. Times with the same lowercase letter are not significantly different at 0.05 levels according to Tukey's test. (C: unstressed control plant, αD: plant with αDHECD, SC: stressed control plant, S+αD: stressed plant with αDHECD).

day 7, αDHECD significantly improved the P_N compared with the stressed control by 14.7%, 43.7% and 48.8%, respectively (Fig. 2A). The g_s reduced under heat stress from day 5 to day 7 by 66.3% and 80.3%, respectively (Fig. 2B). Application of αDHECD significantly improved g_s, compared to the stress control treatment throughout the experiment (Fig. 2B). Heat stress increased C_i compared with the control plant treatment from day 3 to day 7, whereas αDHECD application lowered C_i compared to the stressed control plant on day 3 and day 7 (Fig. 2C). The *E* was reduced under heat stress (Fig. 2D), while αDHECD significantly improved *E* compared to the stressed control plant treatment from day 5 to day 7 by 47.7% and 71.4%, respectively (Fig. 2D). Rice exposed to high temperature had improved WUE on day 7 (Fig. 2E), whereas αDHECD reduced the WUE from day 5 to day 7 under heat stress (Fig. 2E). Moreover, the stomatal limitation (*Ls*) suddenly reduced after exposure of the rice to heat stress from day 3 to day 7. Application of αDHECD resulted in a higher L_S than the stressed control plant from day 3 to day 7 (Fig. 2F).

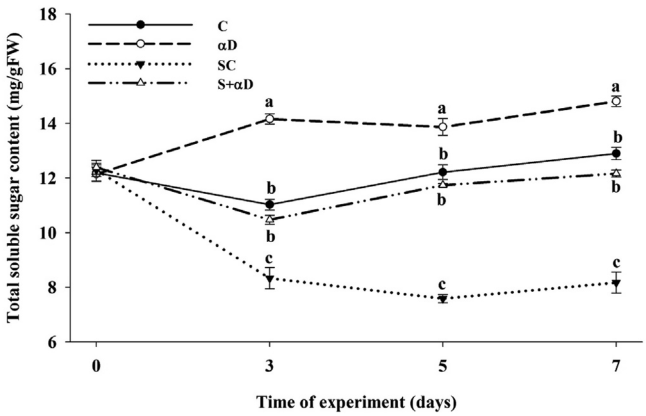

Fig. 3. Effects of 7,8-dihydro-8α-20-hydroxyecdysone (αDHECD) on the total soluble sugar content of rice leaves under normal and high temperature regimes. Data are mean ± SE, with n = 5. Times with the same lowercase letter are not significantly different at 0.05 level according to Tukey's test. (C: unstressed control plant, αD: plant with αDHECD, SC: stressed control plant, S+αD: stressed plant with αDHECD).

Total soluble sugar in rice leaf

Under normal temperature, the application of αDHECD significantly increased the total soluble sugar content compared to the control plants from day 3 to day 7, by 22.1%, 11.9% and 12.88%, respectively (Fig. 3). Under the high temperature, the sugar content in rice leaves reduced from day 3 to day 7 when compared to the control plants in the normal temperature, by 24.38%, 37.8% and 36.6%, respectively (Fig. 3). Plants in the αDHECD application had a higher total soluble sugar content than the SC treatment from day 3 to day by 7, 20.4%, 35.3% and 32.7%, respectively (Fig. 3).

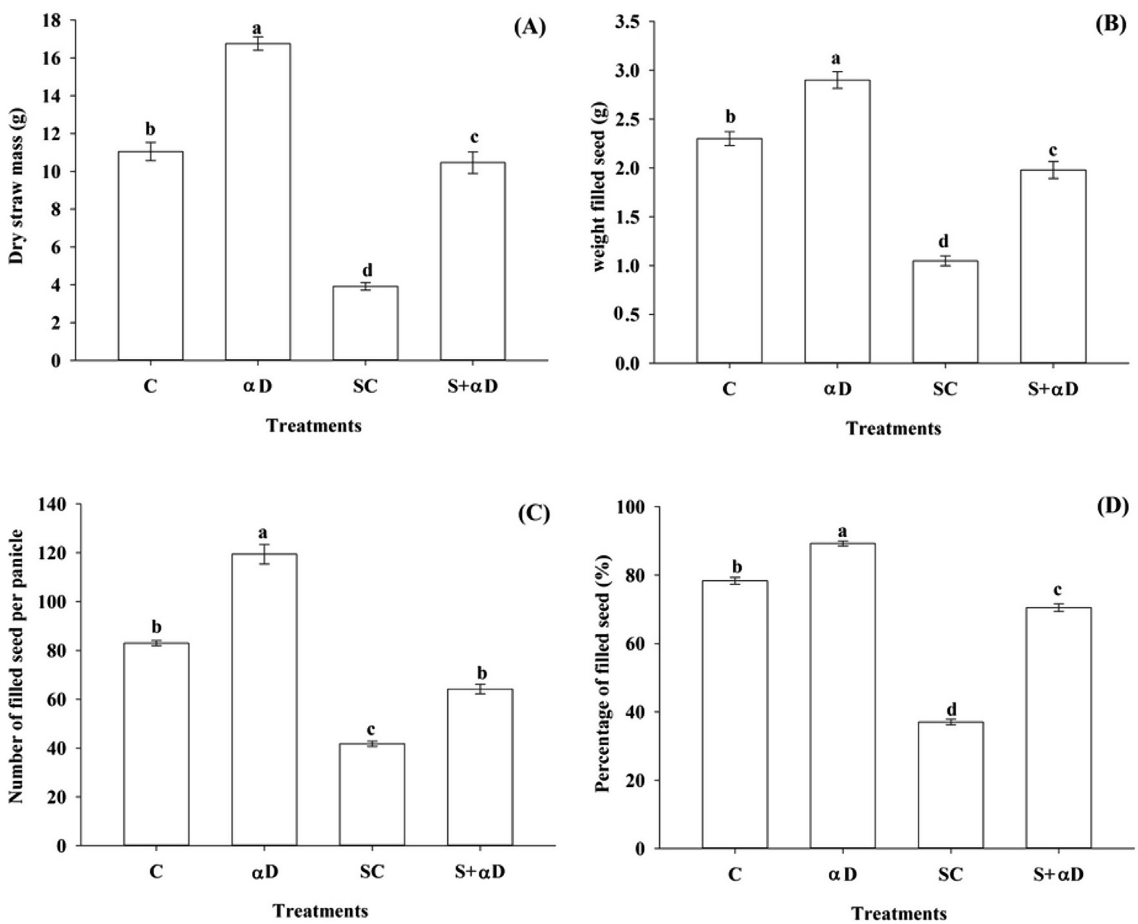

Fig. 4. Effects of 7,8-dihydro-8α-20-hydroxyecdysone (αDHECD) on rice under high temperature regime: (A) dry straw mass; (B) weight filled seed; (C) number of filled seed per panicle; (D) percentage of filled seed. Data are mean ± SE, with n = 5. Columns with the same lowercase letter are not significantly different ($p > 0.05$) according to Tukey's test. (C: unstressed control plant, αD: plant with αDHECD, SC: stressed control plant, S+αD: stressed plant with αDHECD).

Dry matter and grain yield

Under normal temperature, application of αDHECD significantly increased the dry straw mass, weight of filled seed, number of filled seeds per panicle and the percentage of filled seeds compared with the control group by 1.5, 1.2, 1.4 and 1.1 times, respectively (Fig. 4A–D). The rice yield was reduced under high temperature (Fig. 4A–D). αDHECD significantly improved all rice yield parameters, compared to the SC treatment, by 2.6 times for the dry straw mass, 1.8 times for filled seed weight, 1.5 times for the number of filled seeds per panicle and 1.9 times for the percentage of filled seeds.

Total soluble sugar and starch content in straw and seed

Application of αDHECD significantly increased the straw sugar content by 1.4 times the control under normal temperature (Fig. 5A). The straw and seed had starch contents 1.5 and 1.1 times that of the control treatment when αDHECD was applied (Fig. 5C,D). Under the high temperature conditions, the sugar contents in the straw and seed and seed starch were lower than the control treatment at normal temperature by 1.5, 1.3 and 1.2 times, respectively (Fig. 5A,B,D). Application of αDHECD significantly improved the sugar and starch contents in the straw and seed compared with the SC treatment, with increases of 1.8 times for the sugar content in straw, 1.1 times for the sugar content in seed, 1.4 times for the starch content in straw and 1.1 times for the starch in seed under heat stress (Fig. 5A–D).

Discussion

These results showed that the photosynthetic gas exchange parameters were reduced when the rice was exposed to high temperature stress. These suggested that when rice is exposed to heat stress, stomatal closure decreased the uptake of CO_2 into the leaves. Reduction of the solubility of CO_2 relative to O_2 within the leaf tissue results in decreased availability of CO_2 as a substrate for CO_2-concentrating mechanisms (Li et al., 2007; Salvucci and Crafts-Brandner, 2004; Singh and Shono, 2005). Application of αDHECD increased P_N, g_s, E and L_s values, while decreasing C_i and WUE values under high temperature (Fig. 2). αDHECD could improve g_s which is a measure of the stomatal opening. The increase in g_s involved increasing P_N (Zhang et al, 2013). On the other hand, C_i is one of the factors affecting photosynthetic processes (Whiteman and Koller, 1967). In the current study, αDHECD application reduced C_i under high temperature when compared with stress-controlled plants (Fig. 2C). Thus, the reduction in the intercellular space CO_2 found in this study indicated that rice leaves could use more of the CO_2 for carbon dioxide fixation in the Calvin cycle (Hola, 2011). Moreover, the stomatal conductance was also related to the transpiration rate (E). The results showed that the reduction of g_s coincided with a decrease in E under heat stress (Fig. 2D). Thus, the value of E reversed with g_s (Fig. 2B,D). The increase in WUE indicated that the plants used less water due to a reduced transpiration rate. It has been reported that increasing the CO_2 concentration within the leaf enables effective stomatal control which decreases the transpiration rate and

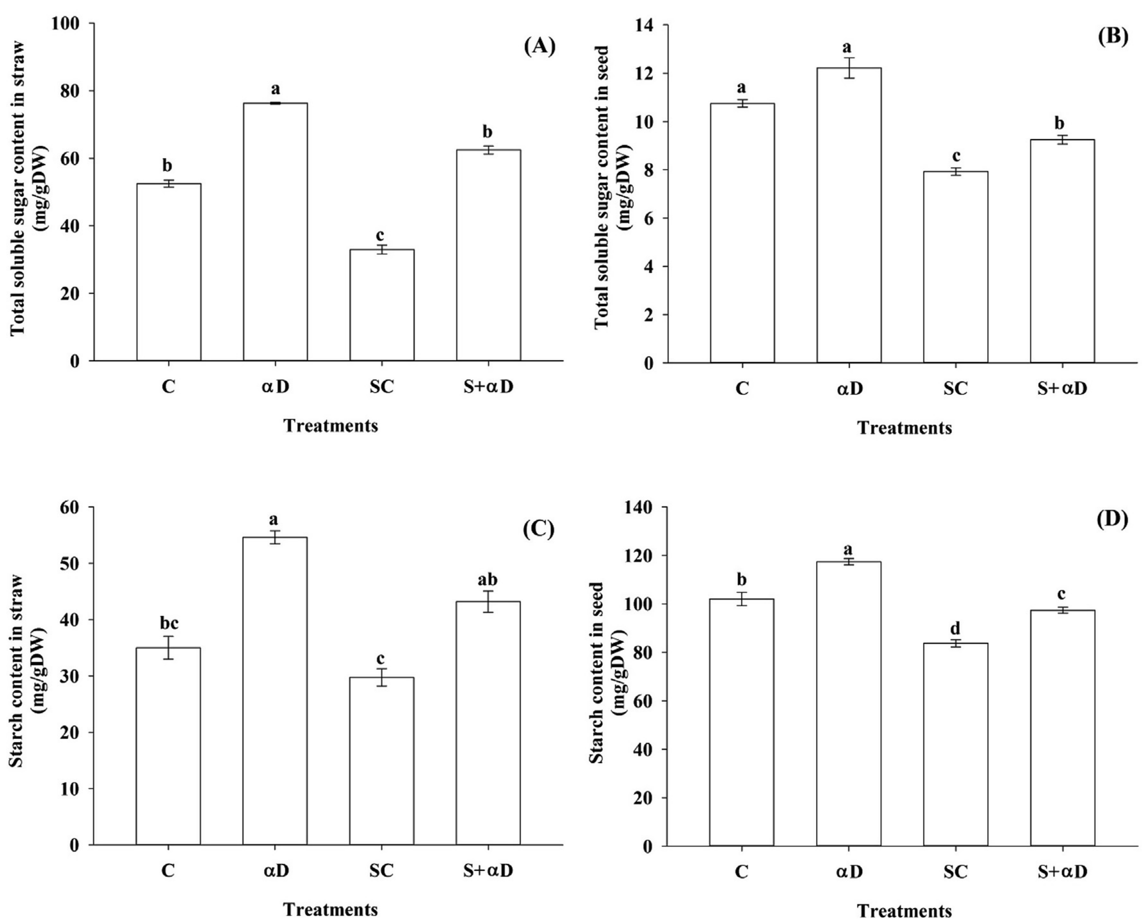

Fig. 5. Effects of 7,8-dihydro-8α-20-hydroxyecdysone (αDHECD) on rice under high temperature regime: (A) total soluble sugar content in straw; (B) total soluble sugar content in seed; (C) starch content in straw; (D) starch in seed. Data are mean ± SE, with n = 5. Columns with the same lowercase letter are not significantly different ($p > 0.05$) according to Tukey's test. (C: unstressed control plant, αD: plant with αDHECD, SC: stressed control plant, S+αD: stressed plant with αDHECD).

consequently increases WUE (Xu and Zhou, 2008). Moreover, the results showed that rice exposed to heat stress had a lower L_S value (Fig. 2F). The decrease in L_S leads to an increase in the CO_2 concentration which can lead to a reduction in Rubisco activity because the amount of Rubisco required to maintain the same assimilation rate decreases (Drake et al., 1997; Woodrow, 1994). αDHECD could increase L_S which showed adaptation in the rice leaves under high temperature. All of the photosynthetic parameters suggested that the exposure to αDHECD increased the photosynthetic performance in rice under heat stress.

αDHECD increased the total soluble sugar content in rice leaves under heat stress (Fig. 3). This result was consistent with Vardhini and Rao (1998) who demonstrated that the application of BR on peanut plants could elevate the total soluble sugar content as a result of an increased photosynthetic rate. The application of αDHECD increased the photosynthetic rate which might have resulted in increased starch accumulation. In previous studies on other BRs, the BRs have been shown to improve the yield of wheat (Holâ et al., 2010), soybean (Zullo and Adam, 2002) and rice (Ramraj et al., 1997). Moreover, DHECD, another BR-mimic, also increased rice yield under heat stress (Thussagunpanit et al., 2015). In the current study, αDHECD increased the dry straw mass, the number of fertile seeds, the number of filled seeds per panicle and the percentage of filled seeds under heat stress. The increased yield in plants was a consequence of the increase in photosynthesis which led to transfer of the carbon partition from the plant biomass to the harvested organ (Richards, 2000). The results indicated that αDHECD has similar effects as BRs and DHECD, which can also increase the yield of rice. In addition, the study of the total soluble sugar and starch contents in the rice straw and seed showed that the application of αDHECD significantly improved the sugar and starch contents in the straw and seed under heat stress. The high sugar and starch contents resulted from the high rates of P_N in the αDHECD treatment. A previous study showed enhanced sink strength and phloem uploading which led to flux sugar from the source to the sink organs (Sasse, 2003). Moreover, BRs increased sucrose transport to the rice endosperm which subsequently increased the yield (Wu et al., 2008). Therefore, improvement in the rice seed set, grain weight, and the sugar and starch contents in seed resulted from the increased sugar and starch contents in the straw dry matter which was caused by improving photosynthesis through using αDHECD.

In conclusion, αDHECD is a BR mimic which has similar effects on plants to natural BRs. The current study demonstrated that the foliar application of αDHECD significantly improved the photosynthetic rate, stomatal conductance, transpiration rate and stomatal limitation, while decreasing the intercellular CO_2 concentration and efficiency of water utilization under heat stress. αDHECD also increased the total soluble sugar content in rice leaves under heat stress, as a result of photosynthetic improvement. αDHECD increased the seed setting and seed weight of rice under high temperature conditions. Moreover, the application of αDHECD significantly improved the sugar and starch contents in the straw and seed under heat stress. The application of αDHECD increased the photosynthetic rate which affected the sugar content in the leaves and led to starch accumulation in the rice under heat stress.

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgments

This research was supported by a graduate scholarship provided by the National Research Council of Thailand (NRCT) for the 2017 fiscal year. This work was also supported by the Graduate School, Center for Advanced Studies in Tropical Natural Resource, NRU-KU, Kasetsart University, Bangkok, Thailand. Partial support from the Center of Excellence for Innovation in Chemistry, Office of the Higher Education Commission is gratefully acknowledged. Moreover, this work was also supported by the Thailand Research Fund (RDG6050067). Sincere gratitude is also expressed to Dr. Ekaphan Kraichak for suggestions that improved the manuscript.

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