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Effects of brassinosteroid and brassinosteroid mimic on photosynthetic efficiency and rice yield under heat stress

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Abstract

Brassinosteroids (BRs) have been reported to counteract various stresses. We investigated effects of exogenously applied brassinosteroid, 24-epibrassinolide (EBR), and brassinosteroid-mimic compound, 7,8-dihydro-8α-20-hydroxyecdysone (DHECD), on the photosynthetic efficiency and yield of rice (*Oryza sativa* L. cv. Pathum Thani 1) under heat stress. Solutions (1 nM) of EBR and DHECD were separately sprayed onto foliage of individual rice plants during their reproductive stage. Five days after the application, the plants were transferred to the day/night temperature regime of 40/30ºC for 7 days and then allowed to recover at normal temperature for 7 days. We demonstrated that both DHECD and EBR helped maintain the net photosynthetic rate. The DHECD and EBR application enhanced stomatal conductance, stomatal limitation, and water-use efficiency under the high-temperature regime. DHECD- and EBR-treated plants showed an increase in the nonphotochemical quenching that was lower than that in the control plants. Moreover, DHECD and EBR treatments maintained the maximal quantum efficiency of PSII photochemistry and the efficiency of excitation capture of the open PSII center. Furthermore, the treatments with DHECD or EBR resulted in higher chlorophyll content during the heat treatment compared with the control plants. The paddy field application of 1 nM EBR and/or 1 nM DHECD at the reproductive stage during the hot season could increase the rice yield, especially, the number of filled seeds. DHECD and EBR enhanced total soluble sugar and reducing sugar in straw and more starch was accumulated in rice seeds. Consequently, our results confirmed that DHECD showed biological activities mimicking EBR in the improvement of photosynthetic efficiency and in rising the rice yield under heat stress.

Additional key words: 7,8-dihydro-8α-20-hydroxyecdysone; 24-epibrassinolide; chlorophyll fluorescence; gas exchange; high temperature.

Introduction

Brassinosteroids (BRs) belong to a group of steroidal plant growth regulators (Clouse and Sasse 1998, Fujioka and Yokota 2003). They were first discovered as brassinolide isolated from the pollen of bees foraging on *Brassica napus* (Grove *et al.* 1979). BRs are effective at low concentrations in all parts of plants and they are required for normal plant growth and development (Sasse 1997). BRs induce a broad spectrum of cellular responses, such

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Abbreviations: BR(s) – brassinosteroid(s); C_i – intercellular CO_2 concentration; C_i/C_a – concentration ratio of intercellular CO_2 and ambient CO_2; DHECD – 7,8-dihydro-8α-20-hydroxyecdysone; DHT – days after heat stress; DM – dry mass; E – transpiration rate; EBR – 24-epibrassinolide; ETR – electron transport rate; F_0 – minimal fluorescence yield of the dark-adapted state; F_0' – minimal fluorescence yield of the light-adapted state; F_m – maximal fluorescence yield of the dark-adapted state; F_m' – maximal fluorescence yield of the light-adapted state; F_s – steady-state fluorescence yield; F_v/F_m – maximal quantum yield of PSII photochemistry; F_v'/F_m' – the efficiency of excitation capture of open PSII center; FM – fresh mass; g_s – stomatal conductance; L_s – stomatal limitation; NPQ – nonphotochemical quenching; P_N – net photosynthetic rate; q_P – photochemical quenching coefficient; RE7 – 7 days of recovery; WUE – water-use efficiency; Φ_{PSII} – effective quantum yield of PSII photochemistry.
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elongation, vascular differentiation, pollen tube growth, induction of ethylene biosynthesis, proton pump activation, as cell expansion, cell division, stem regulation of gene expression, increase of photosynthesis, and adaptation responses to biotic and abiotic stresses (Clouse and Sasse 1998, Dhaubhadel *et al.* 1999, Khripach *et al.* 2000, Krishna 2003, Yu *et al.* 2004).

Unfavorable environmental conditions, especially high temperature and high solar energy, can lead to heat stress that strongly affects plant productivity. Heat stress impacts directly on the photosynthetic gas exchange (Singh and Shono 2005), PSII photochemistry (Sharkey 2005), and decreases the electron transport rate of PSII (Wise *et al.* 2004). The application of BRs can increase the leaf photosynthetic efficiency under heat stress conditions (Singh and Shono 2005). There are some hypotheses suggesting BRs can increase plant photosynthesis; for example, BRs enhanced the initial Rubisco activity, increased the quantum efficiency of PSII, and decreased chlorophyll (Chl) fluorescence (Fariduddin *et al.* 2009, Hayat *et al.* 2010, Yu *et al.* 2004).

The natural BR, 24-epibrassinolide (EBR), is a C-24 epimer of brassinolide (Ikekawa *et al.* 1988), which has been used generally in field trials to increase the crop yield (Khripach *et al.* 2000, Zullo and Adam 2002). The extraction of natural BRs from plants provides low quantities when compared with the fresh mass of plant samples and the synthesis of BRs is expensive (Grove *et al.* 1979, Yokota *et al.* 1982, Serna *et al.* 2012). BR mimics are compounds that show activity similar to natural BRs and are synthesized in order to reduce the cost of BRs. Suksamrarn *et al.* (2002) synthesized 7,8-dihydro-8α-20-hydroxyecdysone (DHECD), the BR-mimic compound, by catalytic hydrogenation of 20-hydroxyecdysone, which was obtained from *Vitex glabrata* stem bark (Werawattanametin *et al.* 1986). Our preliminary experiments demonstrated that DHECD exhibited biological activities similar to those of BRs in plants. DHECD was active in the rice laminar inclination test but it was less active than brassinolide (Homvisasevongsa 2006). Moreover, DHECD increased the percentage of rice pollen germination and the percentage of rice seed setting similarly to EBR (Thussagunpanit *et al.* 2013).

Thus, DHECD may improve photosynthetic efficiency and the yield of rice. The objective of this study was to explore the role of DHECD on the heat stress alleviation in rice, based on the changes in photosynthesis and the yield component by comparison with EBR.

Materials and methods

Preparation of chemicals: The brassinosteroid (EBR) and the BR-mimic compound (DHECD) were used as chemical substances. EBR was purchased from *Ruina International Co. Ltd*., China, and its formula is $C_{28}H_{48}O_6$. DHECD was chemically modified from 20-hydroxyecdysone, which was obtained from *Vitex glabrata* (Werawattanametin *et al.* 1986, Suksamrarn *et al.* 2002). The stock solutions of EBR and DHECD, each of 1 mM, were prepared by dissolving each compound in 0.01% ethanol; the solutions were stored in a refrigerator at 4ºC. The solutions of 1 nM EBR or DHECD were prepared from the stocks by the dilution with 0.01% ethanol.

Preparation of plants: Seeds of rice (*Oryza sativa* L. cv. Pathum Thani 1) were sown in 500 cm^2 plastic pots containing soil that was mixed with fertilizer ($N:P_2O_5:K_2O$ = 16:16:16) at the rate of 10 mg kg^{-1}(soil). Plants were grown in a greenhouse at the Department of Botany, Kasetsart University, Bangkok, Thailand (13°50'41.6"N, 100°34'14.7"E). Healthy seedlings were selected and grown in the greenhouse (30/25ºC day/night regime with natural irradiance). The 1 nM solutions of DHECD and EBR were prepared and 15 ml of each solution was separately sprayed onto individual plants at the age of 78 d (reproductive stage; R_2). The BR solutions and 0.01% ethanol as the control were mixed with 0.025% Tween-20 prior to use. Five days after the BR treatment, the plants treated with the BRs and the control plants were exposed to 40/30ºC day/night temperature for 7 d in a growth chamber under irradiance of 300 μmol(photon) m^{-2} s^{-1} and 75% relative humidity as the heat stress treatment; then, the plants were transferred to normal greenhouse conditions at 30/25ºC day/night for further growth. The pots were arranged in a completely randomized design with five replicates for each experiment.

Photosynthetic efficiency measurement: Photosynthetic gas exchange and Chl fluorescence were measured after 0, 1, 3, 5, and 7 d of the heat stress (DHT) and after 14 DHT, which included last 7 d of recovery (RE7). The measurements were applied on the flag leaves.

Leaf gas exchange was measured using an infrared gas exchange analyzer (*LI-6400*, *Licor Inc.*, Lincoln, NE, USA) from 09.00 to 11.00 h. The net photosynthetic rate (P_N), stomatal conductance (g_s), transpiration rate (E), and intercellular CO_2 concentration (C_i) were measured on 0.7 × 3.0 cm^2 of leaf area using an external CO_2 concentration of 400 ppm, PPFD of 1,000 μmol m^{-2} s^{-1}, relative air humidity of approximately 65–70 %, flow rate at 500 μmol s^{-1}, and the leaf temperature was maintained at 30ºC. The stomatal limitation (L_s) and the water-use efficiency (WUE) were calculated as $1 - C_i/C_a$ and P_N/E, respectively (Zhang *et al.* 2013).

Chl fluorescence was measured using a pulse amplitude modulation fluorometer (*PAM-2100*, *Walz*, Effeltrich, Germany) from 11.00 to 12.30 h. The minimal (F_0) and maximal fluorescence yield of the dark-adapted state (F_m) in the leaves were assessed after 30 min of dark adaptation.

F_0 was determined by a weak modulated light that did not induce any significant variable fluorescence and F_m was measured by a 0.8-s pulse of the saturated light of 8,000 μmol(photon) m^{-2} s^{-1}. After that, the leaf was illuminated with actinic light of 500 μmol(photon) m^{-2} s^{-1} for 3 min. The steady-state fluorescence (F_s) was recorded when the leaf reached a steady state of photosynthesis. A second 0.8-s saturating light was applied to determine the maximal fluorescence of the light-adapted state (F_m'). Then, the actinic light was turned off and the minimal fluorescence of the light-adapted state (F_0') was determined by illumination with 3-s of far-red light. The maximal quantum yield of PSII photochemistry (F_v/F_m), the efficiency of excitation capture of the open PSII center (F_v'/F_m'), the photochemical quenching coefficient (q_P), the effective quantum yield of PSII photochemistry (Φ_{PSII}), and nonphotochemical quenching (NPQ) were calculated according to Lichtenthaler *et al.* (2005) as $(F_m - F_0)/F_m$, $(F_m' - F_0')/F_m'$, $(F_m' - F_s)/(F_m' - F_0')$, $(F_m' - F_s)/F_m'$, and $(F_m - F_m')/F_m'$, respectively. The electron transport rate (ETR) was calculated as $\Phi_{PSII} \times PPFD \times 0.5 \times 0.84$ (Krall and Edwards 1992).

Chl quantification: The index of relative Chl content was estimated as a SPAD unit on the flag leaf of rice by using a Chl meter (*SPAD-502*, *Konica, Minolta Sensing Inc.*, Japan). The SPAD value was measured at ten positions of each flag leaf. The Chl content in the leaves was calculated by the calibration of the SPAD unit from the equation [$y = 0.0561\ x - 0.4926$], where y is Chl content [mg g^{-1}(FM)] and x is the SPAD unit (Islam *et al.* 2009).

Paddy field cultivation and rice yield measurement: Rice plants were grown in a paddy field at Samkhok, Pathum Thani, Thailand (14°4'4.4"N, 100°31'24.6"E) during the hot season from February to May 2012. Mutual rice seeds were sown homogenously in the paddy field at approximately 200 seeds per m^{-2}. Ammonium phosphate fertilizer ($N:P_2O_5:K_2O$ = 16:20:0) was applied at the rate of 12.50 g m^{-2} 20 d after sowing and urea fertilizer ($N:P_2O_5:K_2O$ = 46:0:0) was applied at the rate of 6.25 g m^{-2} at the panicle initiation stage (R_0; 59 d after sowing). The water height level in the paddy field was controlled to approximately 10 cm and water was drained 10 d before rice harvesting. The light irradiance, temperature, and relative humidity in the paddy field were recorded every 15 min by microstation data logger sensors (*Watchdog 1450*, *Spectrum Technologies Inc.*, USA). The experiment was conducted in a randomized complete block design with four experimental plots and each plot had an area of $5 \times 6\ m^2$. The treatments consisted of control plants (0.01% ethanol), 1 nM EBR-treated plants, and 1 nM DHECD-treated plants. Rice plants were sprayed with the respective chemicals mixed with 0.025% Tween-20 at 70 d after sowing (reproductive stage; R_2) using 5 L of each solution per plot. Panicles were harvested at 113 d after sowing. The number of seeds per panicle, seed mass, and dry straw mass were evaluated.

Sugar and starch contents analysis: Rice straw, husk, and seeds were collected after harvesting. Plant samples were crushed and extracted with 80% ethanol at 65ºC for 1 h. The extraction was repeated three times in the same manner. The supernatants were used to estimate the total soluble sugar and reducing sugar contents by anthrone reagent (Fales 1951) and dinitrosalicylic acid reagent (Miller 1959), respectively. The plant pellets were used to analyze the starch content according to Sheoran and Saini (1996).

Statistical analysis: The statistical analysis of variance and mean differences among brassinosteroid treatments and the control were estimated at $p<0.05$ according to *Duncan*'s new multiple range test.

Results

Gas exchange: The changes in photosynthesis showed that heat stress decreased the P_N, while both DHECD- and EBR-treated plants maintained the higher P_N when compared with the control plants (Fig. 1*A*). DHECD- and EBR-treated plants exhibited higher values of g_s and E than control plants. However, the application of DHECD resulted in values for g_s and E that were slightly lower than those for EBR (Fig. 1*B*,*C*). Moreover, both DHECD- and EBR-treated plants increased P_N, g_s, and E values during the RE7 period under normal temperatures to levels that were similar to 0 DHT (Fig. 1*B*,*C*). The C_i of all treatments increased sharply at high temperature. However, both the DHECD- and EBR-treated plants showed significantly lower heat stress than the control plants after 5 DHT (Fig. 1*D*). On the other hand, heat stress decreased the L_s. The DHECD and EBR treatments significantly enhanced L_s after 5 DHT (Fig. 1*E*). The WUE of all treatments decreased after being subjected to high temperature. However, DHECD and EBR could maintain a high level of WUE from 5 DHT (Fig. 1*F*).

Chl fluorescence: This study found that high temperature significantly increased the F_0 values of control plants at 7 DHT, but the DHECD- and EBR-treated plants maintained F_0 values similar to 0 DHT (Fig. 2*A*). The changes in F_m were in contrast to F_0 as F_m in all treatments decreased after being subjected to the high temperature regime. Nonetheless, the DHECD and EBR applications maintained higher F_m values than in the control plants after 5 DHT (Fig. 2*B*). The F_v/F_m and F_v'/F_m' of the control plants were reduced after the plants were exposed to the heat stress. In the DHECD and EBR treatments, the rice

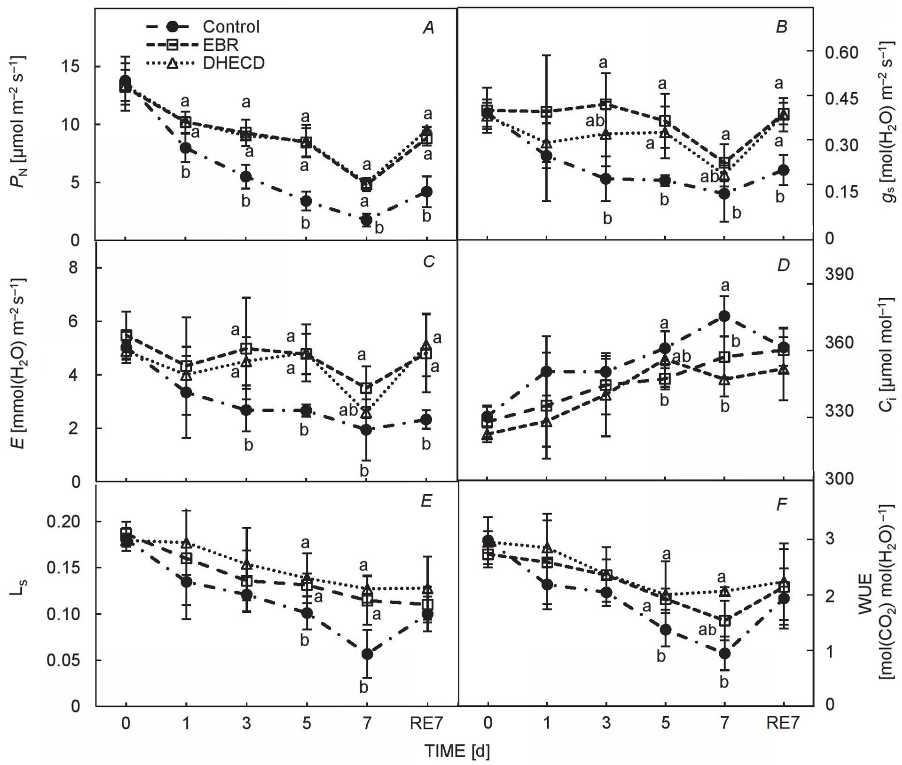

Fig. 1. Changes in (*A*) net photosynthetic rate (P_N), (*B*) stomatal conductance (g_s), (*C*) transpiration rate (*E*), (*D*) intercellular CO_2 concentration (C_i), (*E*) stomatal limitation (L_s), and (*F*) water-use efficiency (WUE) in control plants, EBR-treated plants, and DHECD-treated plants during the heat stress. Data are the means of five replicates with SD shown by vertical error bars. *The same letters* indicate no significant difference at $p<0.05$ according to *Duncan*'s new multiple range test.

showed higher F_v/F_m and F_v'/F_m' than in the control plants and the values of F_v/F_m and F_v'/F_m' in all treatments increased after recovery (Fig. 2*C,D*). The changes in q_P and the Φ_{PSII} were not significantly different among all treatments but the DHECD- and EBR-treated plants tended to increase q_P and Φ_{PSII} (Fig. 2*E,F*). Furthermore, NPQ in the control plants significantly increased after the heat stress treatment and the maximum NPQ value was 0.28 on 7 DHT. The DHECD- and EBR-treated plants showed an increase in NPQ that was lower than that in the control plants, but DHECD showed lesser effect on the maintenance of low NPQ than the EBR application at 1 and 3 DHT (Fig. 2*G*). Moreover, the results showed that the DHECD- and EBR-treated plants slightly improved the ETR under high temperature (Fig. 2*H*).

Chl content: In this study, we observed the changes of the Chl content in the same flag leaves. The results demonstrated that heat stress strongly decreased leaf Chl content after exposing the plants to high temperature. The DHECD and EBR applications alleviated Chl loss when compared with the control plants. Nevertheless, the Chl content of all treatments showed no significant difference after RE7 under normal temperature (Table 1).

Rice yield: DHECD- and EBR-treated rice was grown during the hot season in Thailand to investigate the yield. The mean daytime temperature recorded from planting to harvesting was 33ºC; the nighttime temperature was 27.5ºC, while the mean daytime and nighttime temperatures during the 7-day period after BR spraying were 33.6ºC and 28.9ºC, respectively (Table 2). The application of EBR or DHECD in the paddy field increased the rice yield when compared with the control plants. Foliar spraying with DHECD or EBR significantly increased the dry straw mass (Table 3). Furthermore, we found that the amount of filled seeds of DHECD- and EBR-treated plants was higher than that in the control plants. On the other hand, plants treated with DHECD or EBR produced less unfilled seeds. However, DHECD and EBR had no effect on the total seed numbers (Table 3). The application of DHECD and EBR could increase the rice seed mass, with the EBR-treated plants having a greater seed mass than the DHECD-treated plants (Table 3).

Sugar and starch content: The application of DHECD and EBR enhanced the increase in the total soluble sugar and reducing sugar contents in rice straw, but the DHECD-treated plants showed sugar contents slightly lower than in the EBR-treated plants (Table 4). Furthermore, the DHECD and EBR treatments significantly enhanced the starch content in the straw, husk, and seeds (Table 4).

Discussion

BRs are plant hormones that regulate plant growth and development and alleviate abiotic stress including heat stress (Fujioka and Yokota 2003, Krishna 2003). In the current study, the application of DHECD improved the photosynthetic efficiency under heat stress by maintenance of the high P_N as well as the EBR application (Fig. 1*A*). The improvement in photosynthesis under heat stress by DHECD and EBR was accompanied by the high g_s and high E (Fig. 1*B,C*). The g_s was correlated with the adjustment of net photosynthesis (Zhang *et al.* 2013), which could be caused by the amount of carbon dioxide available for fixation by photosynthetic enzymes (Holá 2011). The greater P_N in the DHECD- and EBR-treated plants was likely due to more open stomata because the high P_N under heat stress with BRs was associated with a decrease in the C_i as well as with the increase in L_s and WUE (Fig. 2*D–F*). The decline of C_i in the DHECD- and EBR-treated plants indicated that the foliar application of DHECD or EBR resulted in plants being highly adaptable to high temperatures (Singh and Shono 2005). Several reports have investigated the changes in stomatal factors associated with the exposure of plants to exogenous BRs, but the results of these studies have been rather ambiguous (Singh and Shono 2005, Qayyum *et al.* 2007, Shahbaz *et al.* 2008). It can be summarized that changes in photosynthesis may be related to g_s (Shahbaz *et al.* 2008) or C_i (Qayyum *et al.* 2007) or both (Singh and Shono 2005). The factors depend on the type of BR and the plant

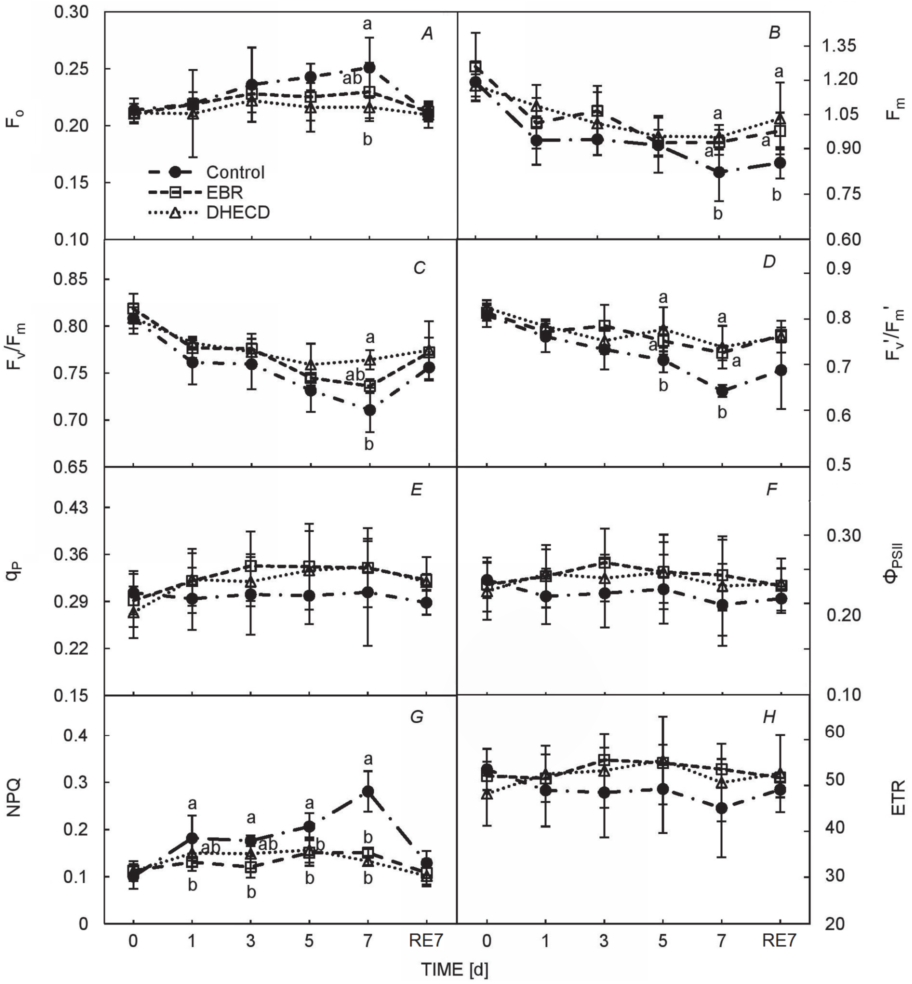

Fig. 2. Changes in (*A*) minimal fluorescence yield of the dark-adapted state (F_0), (*B*) maximal fluorescence yield of the dark-adapted state (F_m), (*C*) maximal quantum yield of PSII photochemistry (F_v/F_m), (*D*) the efficiency of excitation capture of open PSII center (F_v'/F_m'), (*E*) photochemical quenching coefficient (q_P), (*F*) effective quantum yield of PSII photochemistry (Φ_{PSII}), (*G*) nonphotochemical quenching (NPQ), and (*H*) electron transport rate (ETR) in control plants, EBR-treated plants and DHECD-treated plants during the heat stress. Data are the means of five replicates with SD shown by vertical error bars. *The same letters* indicate no significant difference at $p<0.05$ according to *Duncan*'s new multiple range test.

Table 1. Effect of brassinosteroids on chlorophyll contents calculated from SPAD values. Data are the means of five replicates with SD. Means of each time after heat stress with *the same letter* in the same column are not significantly different at $p<0.05$ according to *Duncan*'s new multiple range test. FM –fresh mass; EBR – 24-epibrassinolide; DHECD – 7,8-dihydro-8α-20-hydroxyecdysone.

Treatment	Chlorophyll content [mg g^{-1}(FM)] Time after heat stress [day]					
	0	1	3	5	7	RE7
Control	1.15 ± 0.12	1.03 ± 0.13^{b}	0.92 ± 0.09^{b}	0.85 ± 0.01^{b}	0.76 ± 0.09^{b}	0.58 ± 0.12
EBR	1.20 ± 0.09	1.20 ± 0.09^{a}	1.09 ± 0.08^{a}	1.03 ± 0.05^{a}	0.88 ± 0.07^{a}	0.67 ± 0.09
DHECD	1.14 ± 0.04	1.10 ± 0.05^{ab}	1.07 ± 0.05^{a}	0.93 ± 0.13^{ab}	0.85 ± 0.13^{a}	0.64 ± 0.13

Table 2. Photon flux density (PPFD), air temperature (T_{air}), and relative humidity (RH) in a paddy field of 'Pathum Thani 1' rice during the hot season (February to May 2012) at Samkhok, Pathum Thani, Thailand. ^{A} Data are the means of 113 days with SD. ^{B} Data are the means of 7 days with SD. nd – not determined. EBR – 24-epibrassinolide; DHECD – 7,8-dihydro-8α-20-hydroxyecdysone.

Parameter	Throughout the whole experiment [113 d] Daytime mean ^{A}	max	min	Nighttime mean ^{A}	max	min
PPFD [μmol m^{-2} s^{-1}]	1,208.53 ± 367.80	1,879.00	433.48	nd	nd	nd
T_{air} [ºC]	32.98 ± 2.55	37.38	27.92	27.53 ± 1.81	31.00	23.80
RH [%]	66.19 ± 7.55	80.80	54.18	84.60 ± 5.74	96.23	74.42
	7 days after spraying with EBR or DHECD Daytime mean ^{B}	max	min	Nighttime mean ^{B}	max	min
PPFD [μmol m^{-2} s^{-1}]	1,057.21 ± 310.90	1,387.88	433.48	nd	nd	nd
T_{air} [ºC]	33.61 ± 2.43	37.38	29.29	28.93 ± 0.82	30.10	27.57
RH [%]	68.31 ± 7.51	79.84	59.25	83.18 ± 3.70	90.23	79.03

Table 3. Effects of EBR and DHECD application on "Pathum Thani 1" rice yield in a paddy field during the hot season (February to May 2012). Means with the same letter in the same column are not significantly different at $p<0.05$ according to *Duncan*'s new multiple range test. Each value is the mean ± SD. ^{A} Dry straw mass, seed mass, and number of panicles per m^{2} were measured by sampling rice plants four times in a 1 × 1 m^{2} subset of the 5 × 6 m^{2} plot area (n = 16). ^{B} Number of seeds per panicle were calculated by sampling five panicles per 1 × 1 m^{2} sample from each plot (n = 20).

Treatment	Dry straw mass ^{A} [g m^{-2}]	Number of seeds per panicle ^{B} Total seed	Filled seed	Unfilled seed	Seed mass ^{A} [g m^{-2}]
Control	645.18 ± 9.48^{b}	99.78 ± 6.04	78.36 ± 3.98^{b}	7.13 ± 2.06^{a}	373.00 ± 18.02^{c}
EBR	734.25 ± 43.02^{a}	104.77 ± 5.00	90.81 ± 8.42^{a}	4.65 ± 0.10^{b}	504.40 ± 25.76^{a}
DHECD	754.75 ± 57.24^{a}	112.18 ± 5.64	98.73 ± 4.20^{a}	4.48 ± 1.44^{b}	429.60 ± 50.90^{b}

Table 4. Effects of EBR and DHECD application on the total soluble sugar, reducing sugar, and starch contents in straw, husk, and seed of "Pathum Thani 1" rice in the paddy field during hot season (February to May 2012). Means with the same letter in the same column are not significantly different at $p<0.05$ according to *Duncan*'s new multiple range test. Each value is the mean ± SD. EBR – 24-epibrassinolide; DHECD – 7,8-dihydro-8α-20-hydroxyecdysone.

Carbohydrate contents [mg g^{-1}(DM)]	Treatment	Rice organs Straw	Husk	Seed
Total soluble sugar content	Control	34.20 ± 4.54^{b}	5.37 ± 1.12	9.20 ± 0.77
	EBR	70.82 ± 9.34^{a}	5.66 ± 0.28	9.94 ± 0.46
	DHECD	58.02 ± 2.88^{a}	5.33 ± 0.74	9.26 ± 0.96
Reducing sugar content	Control	30.48 ± 3.76^{b}	1.20 ± 0.47	0.84 ± 0.22
	EBR	52.30 ± 10.71^{a}	1.15 ± 0.31	0.89 ± 0.35
	DHECD	42.51 ± 9.36^{a}	1.21 ± 0.42	0.81 ± 0.10
Starch content	Control	39.89 ± 7.00^{b}	44.70 ± 7.04^{b}	80.56 ± 9.67^{b}
	EBR	50.83 ± 1.83^{a}	55.16 ± 6.40^{a}	108.48 ± 3.02^{a}
	DHECD	51.88 ± 1.95^{a}	52.77 ± 2.87^{a}	102.72 ± 13.86^{a}

species (Holá 2011). The present study suggested that the changes in the P_N after the DHECD or EBR application were associated with both g_s and C_i (Fig. 1*B*,*D*).

The high temperature inhibited the photosynthetic rate due to photosynthetic apparatus disruption (Berry and Björkman 1980). In this study, the F_v/F_m of the control plants decreased to its lowest value during all days after the heat-stress treatment (Fig. 2*C*), which was associated with the increase in F_0 (Fig. 2*A*) and the decrease in F_m (Fig. 2*B*). Plants that showed the decrease in F_v/F_m and F_v'/F_m' suffered photoinhibitory damage as the result of the high-temperature stress (Gamon and Pearcy 1989). The decrease in F_v/F_m indicated that plants were injured by heat stress which was closely related to the integrity of the chloroplasts and the proportion of the photosynthetic capacity (Krause and Weis 1991). However, the results also demonstrated that the DHECD- and EBR-treated plants had higher F_v/F_m and F_v'/F_m' values than the control plants (Fig. 2*C*,*D*), which suggested that the DHECD and EBR applications reduced the overexcitation of PSII by heat stress. NPQ is often used to estimate the ability of plants to safely dissipate excessive excitation energy and indicates the heat dissipation that cannot be used in the photosynthetic electron transport (Guo *et al.* 2006). In this study, the NPQ of the control plants was the highest during the heat stress (Fig. 2*G*), showing that the plants did not utilize the energy for photochemistry (Guo *et al.* 2006, Dai *et al.* 2009). The unutilized energy becomes a potential source of damage to the plant as it can lead to the formation of reactive oxygen species, such as singlet oxygen radicals (Vasil'ev *et al.* 1998). The F_v'/F_m' and NPQ values of the DHECD- or EBR-treated plants were lower than those in the control plants (Fig. 2*D*,*G*), which indicated that the application of DHECD or EBR reduced the heat dissipation resulting in the utilization of energy absorbed by antenna pigment in PSII for photosynthesis (Guo *et al.* 2006, Zhang *et al.* 2006). The decrease in the NPQ can maintain the excitation energy transfer from the PSII antennae to the PSII reaction centers and can retain the values of q_p under stress conditions (Calatayud and Barreno 2004). The results showed that there was no significant difference among the changes in q_p and Φ_{PSII} in all treatments; however, the BR-treated plants tended to increase q_p and Φ_{PSII} (Fig. 2*E*,*F*). The increase in the q_p values indicated a decrease in the reduced quinone A (Q_A) fraction of PSII, which meant a lower susceptibility to photoinhibition (Hichem *et al.* 2009). Calatayud and Barreno (2004) reported that the decline of photochemical quenching was not necessarily associated with the increase in nonphotochemical quenching. Our study demonstrated that the application of DHECD or EBR significantly reduced only the NPQ and could not significantly increase q_p in rice plants under heat stress. The ETR represents the relative quantity of electrons passing through PSII during the steady-state photosynthesis (Dai *et al.* 2009). The control plants under heat stress showed a tendency toward lower ETR compared with the DHECD- and EBR-treated plants (Fig. 2*H*). Moreover, we showed that high temperature continually decreased the leaf Chl content as observed from the SPAD value (Table 1). Therefore, the decrease in ETR might be the result of Chl loss and the reduction in excitation capture efficiency (Dai *et al.* 2009). Moreover, the recent report described that EBR could induce the structural reorganizations of thylakoid membrane by the decrease of grana stacking extention which cause the unstacked thylakoid membrane and EBR also enhanced the energy redistribution between PSII and PSI. This process was shown as the adaptation to abiotic stress in plants (Dobrikova *et al.* 2014). The application of DHECD might enhance the structural flexibility of thylakoid membranes causing the alleviation to heat stress similar to EBR treatment.

In the paddy field experiment, the nighttime temperature was quite similar to the high nighttime temperature of 30ºC, which was set in the growth chamber experiment. Mohammed and Tarpley (2009, 2010) reported that seasonally high nighttime temperatures during the reproductive stage of rice ranged above 25ºC and this can reduce the rice yield and quality. Moreover, a mean air temperature in the paddy field exceeding 28ºC could decrease the rice yield and the photosynthetic rate because the high temperature induced the acceleration of leaf senescence (Oh-e *et al.* 2007).

Several researches reported that the activity of BRs could increase crop yield (Singh and Shono 2005, Wu *et al.* 2008, Divi and Krishna 2009, Serna *et al.* 2012). In the current study, the application of DHECD and EBR increased the number of filled seeds and the seed mass of rice in the paddy field (Table 3). Richards (2000) reported that the increase in the yield in plants occurred due to the increase in the photosynthetic rate per unit area and carbon partitioning from the plant biomass to the harvested organ. Our results showed that the treatments with DHECD and EBR not only increased the number of filled seeds and the seed mass, but also increased the dry straw mass of the rice biomass (Table 3). Singh *et al.* (2010) reported that a temperature higher than 2.5ºC above the optimum temperature in rice during the reproductive stage caused a strong decrease in the rice biomass and yield including the number of seeds per panicle and the 1,000-seed mass. The DHECD and EBR treatments enhanced the contents of total soluble sugar and reducing sugars in the rice straw (Table 4). The high sugar contents were associated with the maintenance of high rates of P_N in the DHECD- and EBR-treated plants (Fig. 1*A*). Moreover, the DHECD and EBR applications increased the starch content in the rice seeds compared with the control plants (Table 4). BR applications have been reported to enhance sink strength and phloem uploading which suggests stimulation of assimilate flux from source to sink organs (Sasse 2003). This might imply that DHECD and EBR increased the content of photosynthates that led to the increased crop yield. Wu *et al.* (2008) reported that BRs affect the grain mass because they mediate sucrose transport to the rice endosperm. Therefore, BRs improved the assimilate allocation from source to sink and resulted in the higher yield compared with the control (Table 3).

In conclusion, the study demonstrated that DHECD had similar effects in the improvement of the photosynthetic rate as the EBR treatment. The DHECD and EBR applications maintained high stomatal conductance, stomatal limitation, and water-use efficiency as well as the maximal quantum yield of PSII photochemistry, and the alleviation of nonphotochemical quenching increased in rice plants exposed to heat stress. DHECD and EBR counteracted high temperatures during the hot season by increasing the number of filled seeds and seed mass. Moreover, DHECD and EBR enhanced the contents of total soluble sugar and reducing sugar in the straw as well as the starch content in seeds. DHECD, the brassinosteroid mimic, which showed biological activities that mimicked EBR, was a good candidate for the improvement of the photosynthetic efficiency and the rice yield under heat stress.

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