

The Exogenous Application of Brassinosteroids to *Zea mays* (L.) Stressed by Long-Term Chilling Does Not Affect the Activities of Photosystem 1 or 2

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Received: 24 August 2009 / Accepted: 7 April 2010 / Published online: 11 May 2010
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Abstract The effect of various concentrations of exogenously applied 24-epibrassinolide (E) and $2\alpha,3\alpha,17\beta$ -trihydroxy- 5α -androstan-6-one (A) on the activities of Photosystem 1 and the Hill reaction, the contents of photosynthetic pigments, and the growth of plants was examined in young maize (*Zea mays* L.) plants subjected to long-term chilling stress or grown in normal-temperature conditions. Neither the activity of Photosystem 1 nor the Hill reaction activity of plants was in any way affected by the treatment with brassinosteroids (BRs), which suggests that the photosynthetic complexes of thylakoid membranes are not the primary site of the influence of BRs on photosynthesis. An extremely low (10^{-14} M) concentration of A applied to the nonstressed plants significantly increased the length of their 4th to the 7th leaves and their height, as well as the contents of chlorophylls *a* and *b* and total carotenoids. However, under chilling conditions, this positive effect was significant for the chlorophyll content only and higher concentrations of BRs (10^{-12} , 10^{-10} , 10^{-8} M) usually had no effect at all.

Keywords Brassinosteroids · Carotenoids · Chlorophylls · Growth · Hill reaction · Low-temperature stress · Photosynthesis · Photosystem 1 · *Zea mays*

Introduction

Brassinosteroids (BRs) are polyhydroxylated steroids that are able to act at extremely low concentrations and are pleiotropic with respect to their effect on plant development, morphology, and physiology (Rao and others 2002). In addition to their role in promoting plant growth and development, BRs possess the ability to protect plants against various biotic and abiotic stressors and together with other plant hormones have been shown to participate in regulation of stress responses (recently reviewed by Bajguz and Hayat 2009).

The positive effect of BRs on stressed plants can result from various changes in plant cells, with one of these changes being the modification of the efficiency of photosynthetic processes. The exogenous application of BRs has been shown to ameliorate a stress-induced decrease in the net photosynthetic rate (P_N) in several plant species, for example, *Triticum aestivum* (Sairam 1994; Ali and others 2008c; Shahbaz and others 2008), *Brassica juncea* (Hayat and others 2007; Ali and others 2008b; Fariduddin and others 2009a, b), *Vigna radiata* (Ali and others 2008a), *Lycopersicon esculentum* (Ogweno and others 2008), or *Glycine max* (Zhang and others 2008). However, the exact causes of this phenomenon are far from clear. BRs could prevent loss of photosynthetic pigments, for example, by activating enzymes that participate in chlorophyll biosynthesis (or an induction of their synthesis), as suggested by Hayat and others (2007), Ali and others (2008a, b), or Fariduddin and others (2009a, b). Another possibility is that BRs improve the efficiency of photosynthetic carbon fixation, for example, by overcoming stomatal limitations and, thus, increasing the internal concentration of CO_2 available for photosynthetic enzymes (Ali and others 2008b), although Shahbaz and others (2008) reported that

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the BR-induced increase or decrease in stomatal conductance in salt-stressed wheat plants was not related to any significant changes in substomatal CO₂ concentration. BRs could also induce synthesis and/or activation of carbonic anhydrase that catalyzes the interconversion of CO₂ and HCO₃⁻ (Hayat and others 2007), increases the activation state of ribulose-1,5-carboxylase/oxygenase (Rubisco) (Ali and others 2008c; Ogwen and others 2008), or protects enzymes involved in the regeneration of ribulose-1,5-bisphosphate (Ogwen and others 2008).

The possible role of BRs in improved efficiency of primary photosynthetic processes in plants subjected to unfavorable conditions is yet more obscure, as some authors have described a positive response of the primary photochemistry of stressed plants to exogenously applied BRs (Janeczko and others 2005; Ogwen and others 2008; Zhang and others 2008), whereas others did not observe any such phenomenon (Ali and others 2008c; Shahbaz and others 2008). Moreover, all these studies have addressed only the question of the effects of BRs on the Photosystem (PS) 2 complex; as far as we know, no one has yet attempted to study the possible effect of BRs on PS 1. We have thus decided to examine the response of this photosynthetic complex to BRs and to compare it with the effect of these hormones on Hill reaction activity (HRA), the content of photosynthetic pigments, and selected morphological and growth parameters in maize plants subjected to optimum or suboptimum (long-term chilling) conditions.

Materials and Methods

Kernels of maize (*Zea mays* L., inbred line 2023) were sown into containers (35 cm × 15 cm × 6 cm) filled with garden soil (10 plants per container, the total number of plants = 2000) and allowed to germinate in the greenhouse at 21–25/17–22°C day/night for 10 days. At day 11, seedlings with fully developed 1st leaves were divided into two groups. One group (approximately half of the seedlings) was transferred into the cold greenhouse (chill stress), the other half of the seedlings were transferred into the warm greenhouse (control), and the plants were allowed to develop for another 50 days. The temperature in the cold greenhouse was maintained at 8°C during night and early morning, gradually increased to 23°C from 9:00 to 15:00 (central European time), and then decreased again to 8°C from 15:00 to 22:00. Similarly, in the warm greenhouse, the night temperature was 17°C, increased to 29°C at 15:00, and again decreased in the afternoon and early evening until 22:00. Plants were grown at natural irradiance conditions and watered daily as necessary, and the relative humidity was maintained at 60/80% day/night in both greenhouses.

The stock solutions of 24-epibrassinolide (E) and 2 α ,3 α ,17 β -trihydroxy-5 α -androstan-6-one (A) were prepared by dissolving these BRs in 1 cm³ ethanol, adding 1 cm³ dimethyl sulfoxide, and diluting with distilled water to 10⁻⁴ M concentration. This stock solution was then diluted with distilled water to 10⁻⁸, 10⁻¹⁰, 10⁻¹², and 10⁻¹⁴ M concentrations that were used for the treatment of plants. Foliar spray with the appropriate aqueous solution of BRs or with distilled water was applied to plants at the time of their transfer into the cold or warm greenhouses (that is, day 11) and again at day 25. The experiments were in four replicates with a completely randomized design, and each temperature/BR treatment combination was represented by at least 25 plants per replicate. At day 39 (counting from the date of sowing, DAS), the majority of the photosynthetic measurements were made, whereas the evaluation of morphological and growth parameters was done both before and after this day until the plants were 60 days old.

At 39 DAS, the 3rd leaves were cut off from 12 plants in each temperature/BR treatment replicate and mesophyll chloroplasts were isolated from the middle part of the leaf blade as described by Holá and others (2003). Chloroplast suspensions were stored at 0°C in the dark and used for polarographic measurements of the activities of PS 1 and HRA (Clark-type oxygen electrode, Theta'90, Czech Republic) as described by Holá and others (2003), that is, as the amount of oxygen consumed (PS1) or produced (HRA) by irradiated chloroplast suspensions after the addition of artificial electron acceptors and/or donors. The contents of chlorophylls (Chl) *a* and *b* and the content of total carotenoids (Car) were determined spectrophotometrically (Wellburn 1994) and the ratios of Chl *a/b* and total Chl/Car were calculated.

The heights of whole plants (that is, the distance from the ground to the ligule of the youngest fully developed leaf) and the numbers and lengths of fully developed leaves were recorded in regular intervals (7 days) starting 1 week after the initial BR treatment (that is, 18 DAS). The lengths of the 4th to 7th leaves were measured in the control plants only as these leaves were not yet fully developed in the chill-stressed plants by the end of the experiments. These measurements were made on six randomly selected plants in each temperature/BR treatment replicate.

The data from all measurements were first subjected to two-way ANOVA with interactions, and then data from the control and the chill-stressed plants were analyzed individually by one-way ANOVA. The statistical significance of the differences among individual BR treatments was ascertained using the least significant difference (LSD) test. All statistical evaluations were made with the CoStat v6.204 statistical software (CoHort Software, Monterey, CA, USA).

Results and Discussion

Both PS1 activity and HRA were negatively affected by the exposure of plants to long-term chilling (Tables 1, 2), which is in good agreement with previously published results (for example, Kudoh and Sonoike 2002; Zhang and Scheller 2004; Holá and others 2003). The contents of Chls *a* and *b*, as well as the total Car content, also significantly decreased in leaves of the chill-stressed plants, with carotenoids less affected than Chls (Tables 1, 2). The degradation of photosynthetic pigments, particularly Chls, is another symptom frequently associated with chilling-induced stress (Haldimann 1998). With respect to plant morphology, the long-term chilling significantly decreased both the length of the 2nd and 3rd leaves (Tables 1, 3) and the plant height (Fig. 1; Table 1), which agrees with our previously published results (for example, Holá and others 2003).

The treatment of plants with BRs did not significantly change the values of the PS1 or the HRA activity in either the stressed plants or the controls (Tables 1, 2). This finding is, in our opinion, the most important result of our study. The effect of BRs on the activity of PS1 has not been previously analyzed, although this photosystem is just as important to the proper functioning of photosynthetic

processes as PS2 (in some plants subjected to the combination of chilling and light, the activity of PS1 can be the limiting factor for the efficiency of primary photochemistry; Zhang and Scheller 2004). The HRA parameter is, to a certain degree, a measure of the activity of PS2. The possible influence of BRs on the activity of this photosynthetic complex has been studied more, but the results are often conflicting. The majority of authors who examined the possible effect of BRs on primary photochemistry (usually by analysis of chlorophyll fluorescence) did not find any significant effect when plants were grown under optimum conditions (for example, Ali and others 2008c; Ogwen and others 2008; Shahbaz and others 2008), but in some cases the efficiency of PS2 was improved by treatment with BRs in plants stressed, for example, by drought (Zhang and others 2008), salinity (Shahbaz and others 2008), or the presence of cadmium (Janeczko and others 2005). As far as we know, no data exist as yet on the effects of BRs on the PS2 photochemistry in plants stressed by low temperature, and our results do not seem to point to the possibility that the efficiency of this photosystem can be improved by BR treatment in such environmental conditions (at least not in maize). We thus strongly support the view of Yu and others (2004) that the photosynthetic complexes of thylakoid membranes indeed are not the primary site of BR influence

Table 1 Results of the analysis of variance (ANOVA) of the data for selected photosynthetic and morphological parameters of chill-stressed (stress) or nonstressed (control) maize plants

Parameter	Two-way ANOVA			One-way ANOVA	
	T	BRs	T × BRs	Control BRs	Stress BRs
Photosystem I activity	0	0.173	0.979	0.711	0.178
Hill reaction activity	0	0.853	0.962	0.903	0.924
Chlorophyll <i>a</i> content	0	0	0.009	0	0
Chlorophyll <i>b</i> content	0	0	0.002	0	0.001
Carotenoids content	0	0.002	0.495	0.043	0.083
Chlorophyll <i>a/b</i> ratio	0.074	0.570	0.463	0.524	0.517
Chlorophyll/carotenoids ratio	0	0	0.035	0	0.001
Plant height (18-day-old plants)	0	0	0	0.001	0
Plant height (25-day-old plants)	0	0	0	0.001	0
Plant height (32-day-old plants)	0	0.001	0	0.001	0.004
Plant height (39-day-old plants)	0	0.001	0	0.001	0.330
Plant height (46-day-old plants)	0	0.002	0.003	0.011	0.245
Plant height (53-day-old plants)	0	0.024	0.021	0.045	0.740
Plant height (60-day-old plants)	0	0.030	0.010	0.024	0.500
Final length of the 1st leaf	0.607	0.270	0.235	0.512	0.043
Final length of the 2nd leaf	0	0.594	0.013	0.219	0.012
Final length of the 3rd leaf	0	0.721	0.001	0.083	0.015

Temperature conditions (T) or brassinosteroid treatments (BRs) and their interaction were included as the possible sources of variation for two-way ANOVA. One-way ANOVA was then individually applied to the data from either stressed or control plants. The levels of statistical significance (*P*) are shown

on photosynthesis and that any eventual changes in the parameters associated with the efficiency of photosynthetic electron transport are probably more of a secondary nature and caused indirectly by, for example, the increased efficiency of the Calvin cycle that results in an increased demand for ATP and NADPH production.

The effect of BRs on the content of photosynthetic carotenoids has also not been examined much. Our results seem to indicate that these pigments are affected by BRs to a lesser extent than Chls (Table 2), which is in good agreement with the results published by other authors (Janeczko and others 2005; Cevahir and others 2008).

The application of 10^{-14} M A significantly increased the contents of Chls *a* and *b* in plants grown in both normal and suboptimal temperature conditions, and the same was true for 10^{-12} or 10^{-10} M solution of A or 10^{-14} M E solution sprayed on the control plants (Table 2). The plants stressed by long-term chilling and treated with other concentrations of BRs were not significantly affected by this treatment compared to the plants treated with water only, and in no case (even with the use of 10^{-14} M A) did the treatment of the chill-stressed plants with BRs increase the content of Chls to the level observed in the control plants. Similar dependence of the BR effect on the concentration of BR used was found for plant morphology, where the control plants sprayed with 10^{-14} M solution of either E or A showed significantly greater lengths of the 5th, 6th, and 7th leaves and greater height by the end of the monitoring period compared to the untreated plants (Fig. 1d; Table 3). On the other hand, the application of 10^{-8} M A or E decreased the lengths of the 4th to the 7th leaves and the height of the control plants (Fig. 1; Table 3). The lengths of the individual leaves on the plants subjected to suboptimal temperatures were not significantly affected by BR treatment (with the exception of the application of 10^{-12} M E, which decreased the length of the 3rd leaf), and the effect of BRs on the height of these plants was significant only where the 10^{-14} M solution of E was used (or, during the first week after BR application, the same concentration of A) (Fig. 1; Table 3). It was shown previously that the effective concentration range for any BR can be very narrow, differ quite substantially among plant species, and depend on both the type of BR applied and the mode of its application (for example, Cevahir and others 2008; Fariduddin and others 2009b).

We can thus conclude that although the exogenously applied BRs can perhaps somehow alleviate the negative effect of long-term chilling stress on photosynthesis in maize plants by diminishing the degradation of chlorophylls (and even in this case, the positive effect of BRs on the contents of these photosynthetic pigments manifests itself only when extremely low concentrations of BRs are used and strongly depends on the type of BR), they do not

Table 2 Effect of 24-epibrassinolide (E) and 2 α ,3 α ,17 β -trihydroxy-5 α -androstan-6-one (A) on the activity of Photosystem 1, Hill reaction activity, contents of chlorophylls *a* and *b*, and total carotenoids in leaves of chill-stressed (stress) or nonstressed (control) maize plants

Treatment	Photosystem I activity ($\mu\text{mol O}_2 \text{ kg}^{-1} \text{ DM s}^{-1}$)		Hill reaction activity ($\mu\text{mol O}_2 \text{ kg}^{-1} \text{ DM s}^{-1}$)		Chlorophyll <i>a</i> content ($\text{mg kg}^{-1} \text{ DM}$)		Chlorophyll <i>b</i> content ($\text{mg kg}^{-1} \text{ DM}$)		Carotenoids content ($\text{mg kg}^{-1} \text{ DM}$)	
	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress
Water	0.91 ± 0.03 ^b	0.62 ± 0.01 ^c	0.31 ± 0.01 ^a	0.18 ± 0.01 ^d	13.98 ± 0.40 ^c	8.14 ± 0.12 ^e	4.06 ± 0.10 ^{cd}	2.26 ± 0.05 ^f	2.50 ± 0.05 ^b	1.97 ± 0.02 ^e
10 ⁻⁸ M E	0.94 ± 0.05 ^{ab}	0.65 ± 0.04 ^c	0.28 ± 0.02 ^{abc}	0.20 ± 0.01 ^{cd}	14.10 ± 0.42 ^{bc}	8.95 ± 0.35 ^{de}	4.03 ± 0.15 ^{cd}	2.45 ± 0.08 ^{ef}	2.49 ± 0.11 ^b	2.13 ± 0.11 ^c
10 ⁻¹⁰ M E	0.94 ± 0.07 ^{ab}	0.65 ± 0.01 ^c	0.34 ± 0.06 ^a	0.22 ± 0.05 ^{bed}	14.10 ± 0.78 ^{bc}	8.82 ± 0.18 ^e	4.02 ± 0.14 ^{cd}	2.44 ± 0.11 ^{ef}	2.67 ± 0.13 ^b	2.13 ± 0.06 ^e
10 ⁻¹² M E	0.91 ± 0.02 ^{ab}	0.59 ± 0.05 ^c	0.31 ± 0.01 ^a	0.18 ± 0.01 ^d	14.11 ± 0.35 ^{bc}	7.39 ± 0.23 ^e	4.02 ± 0.08 ^{cd}	2.23 ± 0.07 ^f	2.51 ± 0.06 ^b	1.94 ± 0.04 ^e
10 ⁻¹⁴ M E	0.94 ± 0.05 ^{ab}	0.65 ± 0.04 ^c	0.28 ± 0.02 ^{abc}	0.20 ± 0.01 ^{cd}	15.52 ± 0.62 ^b	8.35 ± 0.43 ^e	4.59 ± 0.10 ^b	2.30 ± 0.10 ^f	2.57 ± 0.09 ^b	1.98 ± 0.12 ^e
10 ⁻⁸ M A	0.86 ± 0.04 ^b	0.56 ± 0.02 ^c	0.30 ± 0.01 ^{ab}	0.18 ± 0.01 ^d	13.71 ± 0.23 ^c	8.10 ± 0.40 ^e	3.95 ± 0.06 ^d	2.24 ± 0.08 ^f	2.52 ± 0.03 ^b	1.94 ± 0.06 ^e
10 ⁻¹⁰ M A	1.02 ± 0.08 ^a	0.64 ± 0.03 ^c	0.33 ± 0.07 ^a	0.20 ± 0.06 ^{cd}	14.95 ± 0.91 ^{bc}	8.25 ± 0.42 ^e	4.67 ± 0.17 ^b	2.32 ± 0.21 ^f	2.60 ± 0.12 ^b	2.02 ± 0.02 ^e
10 ⁻¹² M A	0.91 ± 0.09 ^{ab}	0.58 ± 0.04 ^c	0.32 ± 0.02 ^a	0.18 ± 0.02 ^d	15.44 ± 1.14 ^b	8.00 ± 0.36 ^e	4.44 ± 0.40 ^{bc}	2.20 ± 0.11 ^f	2.75 ± 0.20 ^{ab}	2.07 ± 0.09 ^e
10 ⁻¹⁴ M A	0.86 ± 0.04 ^b	0.56 ± 0.02 ^c	0.30 ± 0.01 ^{ab}	0.18 ± 0.01 ^d	19.77 ± 0.92 ^a	10.45 ± 0.19 ^d	5.80 ± 0.42 ^a	2.92 ± 0.05 ^e	2.98 ± 0.13 ^a	2.16 ± 0.05 ^e
LSD ($P \leq 0.05$)	0.13		0.09		1.63		0.49		0.26	

DM dry matter

Plants were sprayed with aqueous solutions of brassinosteroids (concentrations 10^{-8} to 10^{-14} M) or with distilled water. The mean values ± SEM ($n = 6$) are given with the LSD values. Values followed by different letters significantly differ at $P \leq 0.05$

Table 3 Effect of 24-epibrassinolide (E) and 2 α ,3 α ,17 β -trihydroxy-5 α -androstan-6-one (A) on the final lengths (mm) of the 2nd to the 7th leaves of chill-stressed (stress) or nonstressed (control) maize plants

Treatment	Leaf 2		Leaf 3		Leaf 4	Leaf 5	Leaf 6	Leaf 7
	Control	Stress	Control	Stress	Control	Control	Control	Control
Water	153 ± 5 ^{abcd}	134 ± 3 ^{defgh}	269 ± 8 ^{abc}	191 ± 5 ^{fg}	383 ± 7 ^a	450 ± 7 ^{bcd}	439 ± 8 ^{bc}	391 ± 5 ^b
10 ⁻⁸ M E	154 ± 9 ^{abcd}	118 ± 4 ^h	285 ± 14 ^{ab}	161 ± 4 ^{gh}	398 ± 8 ^a	441 ± 5 ^{cde}	407 ± 6 ^{cd}	339 ± 7 ^c
10 ⁻¹⁰ M E	145 ± 9 ^{cdefg}	144 ± 7 ^{cdefg}	253 ± 12 ^{bcd}	212 ± 9 ^{ef}	360 ± 8 ^{ab}	427 ± 6 ^{de}	430 ± 6 ^{bc}	376 ± 9 ^b
10 ⁻¹² M E	173 ± 7 ^a	132 ± 6 ^{defgh}	296 ± 7 ^a	147 ± 4 ^h	399 ± 14 ^a	476 ± 14 ^{ab}	ND	ND
10 ⁻¹⁴ M E	157 ± 5 ^{abc}	132 ± 10 ^{efgh}	269 ± 6 ^{abc}	210 ± 13 ^{ef}	387 ± 6 ^a	485 ± 7 ^a	475 ± 9 ^{ab}	446 ± 10 ^a
10 ⁻⁸ M A	142 ± 4 ^{cdefg}	129 ± 6 ^{fgh}	241 ± 10 ^{cde}	192 ± 9 ^{fg}	328 ± 30 ^b	419 ± 2 ^e	366 ± 38 ^d	321 ± 19 ^c
10 ⁻¹⁰ M A	141 ± 6 ^{cdefg}	150 ± 3 ^{bcdef}	248 ± 9 ^{bcd}	219 ± 5 ^{def}	357 ± 9 ^{ab}	436 ± 12 ^{cde}	446 ± 8 ^{abc}	402 ± 9 ^b
10 ⁻¹² M A	167 ± 17 ^{ab}	124 ± 6 ^{gh}	295 ± 31 ^a	187 ± 10 ^{fgh}	402 ± 26 ^a	469 ± 13 ^{abc}	ND	ND
10 ⁻¹⁴ M A	151 ± 4 ^{abcde}	133 ± 4 ^{defgh}	269 ± 7 ^{abc}	195 ± 8 ^{fg}	402 ± 14 ^a	486 ± 18 ^a	494 ± 29 ^a	454 ± 25 ^a
LSD ($P \leq 0.05$)	27		48		45	38	67	71

ND leaves were not yet fully developed at the end of the measurements

Plants were sprayed with aqueous solutions of brassinosteroids (concentrations 10⁻⁸ to 10⁻¹⁴ M) or with distilled water. The mean values ± SEM ($n = 6$) are given with the LSD values. Values followed by different letters significantly differ at $P \leq 0.05$

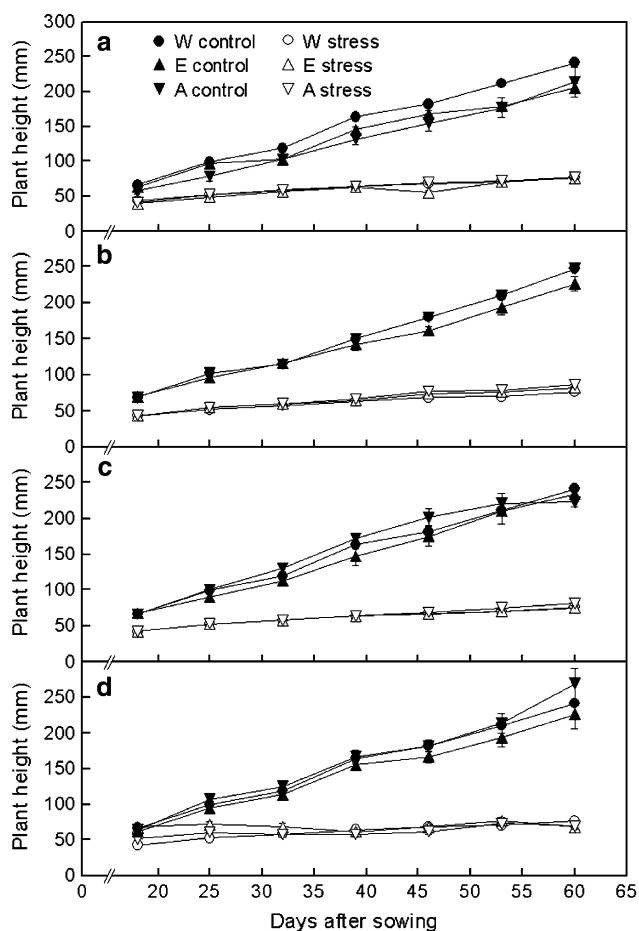


Fig. 1 The effect of brassinosteroids on the height of chill-stressed (stress) or nonstressed (control) maize plants. Plants were sprayed with 10⁻⁸ M (a), 10⁻¹⁰ M (b), 10⁻¹² M (c), or 10⁻¹⁴ M (d) aqueous solutions of 24-epibrassinolide (E), 2 α ,3 α ,17 β -trihydroxy-5 α -androstan-6-one (A), or distilled water (W). The mean values ± SEM ($n = 6$) are shown

improve the efficiency of primary photosynthetic processes and the activities of Photosystem 1 or 2.

Acknowledgments The authors are grateful to Monika Benešová for her help with the measurements of photosynthetic and morphological parameters, and to Dr. Jaroslav Poruba from the CEZEA Breeding Station in Čejč, Czech Republic, for the supply of maize kernels. This study was supported by grant KJB601110611 from the Grant Agency of the Academy of Sciences of the Czech Republic and grant MSM0021620858 from the Ministry of Education, Youth and Sports of the Czech Republic.

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