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BRIEF COMMUNICATION

The effects of brassinosteroids on photosynthetic parameters in leaves of two field-grown maize inbred lines and their F1 hybrid

M. KOČOVÁ[1], O. ROTHOVÁ[1], D. HOLÁ[1]*, M. KVASNICA[2] and L. KOHOUT[2]

Charles University in Prague, Faculty of Science, Viničná 5, CZ-12843 Prague, Czech Republic[1]
Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo nám. 2, CZ-16610 Prague, Czech Republic[2]

Abstract

The effect of foliar spray with 10^{-12} M aqueous solutions of 24-epibrassinolide or a synthetic androstane analogue of castasterone on the activity of photosystem (PS) 1, the Hill reaction activity, the content of photosynthetic pigments and the specific leaf mass was examined for three different leaves developed after brassinosteroid (BR) treatment in two inbred lines of field-grown maize and their F1 hybrid. The brassinosteroids significantly affected neither the efficiency of photosynthetic electron transport, nor the content of chlorophylls or carotenoids.

Additional key words: carotenoids, chlorophylls, 24-epibrassinolide, heterosis, Hill reaction, photosystem 1.

More than 70 structurally and functionally related brassinosteroids (BRs) have been characterized from various natural sources since their discovery in 1979 (Grove *et al.* 1979). These organic compounds with structure based on polyhydroxylated sterols show multiple effects on plant physiology, development and growth and are now included among main groups of phytohormones. Several excellent reviews concerning BRs action in plants at the molecular, cellular or whole plant level have appeared in the last years (*e.g.* Wang and He 2004, Haubrick and Assman 2006, Bajguz and Hayat 2009). One of the diverse functions of BRs in higher plants is their possible involvement in the regulation of photosynthesis. The application of BRs enhanced the net photosynthetic rate in several plant species, *e.g., Brassica juncea* (Hayat *et al.* 2000, 2001, 2007, Ali *et al.* 2008b, Fariduddin *et al.* 2009a,b), *Cucumis sativus* (Yu *et al.* 2004, Xia *et al.* 2006), *Glycine max* (Zhang *et al.* 2008), *Lycopersicon esculentum* (Singh and Shono 2005), *Oryza sativa* (Farooq *et al.* 2009), *Vigna radiata* (Fariduddin *et al.* 2003, 2004, Ali *et al.* 2008a) or *Triticum aestivum* (Sairam 1994a,b, Ali *et al.* 2008c). The majority of these studies was made on very young plants cultivated in controlled conditions (often in combination with some unfavourable environmental factor), so a care should be taken when extrapolating these results to plants grown at natural conditions. Moreover, it is still not entirely clear, whether BRs primarily affect the activities or contents of the pigment-protein complexes of photosynthetic electron-transport chain, the chloroplast ATP synthase or the enzymes catalyzing the individual steps of CO_2 fixation, or whether they perhaps participate in the regulation of stomatal function, the synthesis or degradation of photosynthetic pigments or some other parts of photosynthetic processes (Yu *et al.* 2004, Hayat *et al.* 2007, Ali *et al.* 2008c, Ogweno *et al.* 2008, Fariduddin *et al.* 2009a,b). Thus, we have decided to examine the possible effects of applied BRs on the primary

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Abbreviations: AAC - 2α,3α,17β-trihydroxy-5α-androstan-6-one; BR - brassinosteroid; Car - carotenoids; Chl - chlorophyll; DAS - days after sowing; DCPIP - 2,6-dichlorophenol indophenol; DMF - *N,N*-dimethyl formamide; DMSO - dimethyl sulfoxide; 24-EPI - 24-epibrassinolide; HRA - Hill reaction activity; PAR - photosynthetically active radiation; PS - photosystem; SLM - specific leaf mass.

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* Corresponding author; e-mail: danahola@natur.cuni.cz

photochemistry (particularly the activity of PS 1, as this has not yet been previously studied) and the contents of photosynthetic pigments in leaves of field-grown maize.

Zea mays L. plants were cultivated at the experimental field of the Department of Genetics and Microbiology, Faculty of Science, Charles University in Prague, Czech Republic (54°04′ N, 14°25′ E, altitude 238 m) during four years (2004 - 2007). Kernels of two maize inbred lines (2023 and CE704) and their F1 hybrid (2023×CE704) were first sown into containers filled with garden soil and placed in glass-covered hot-beds at the end of April. After 4 weeks the seedlings were transplanted into the field in a regular pattern (70 × 50 cm). The leaves of plants were sprayed with tap water (control) or with 10^{-12} M aqueous solutions of either 24-epibrassinolide (24-EPI) or synthetic androstane analogue of castasterone (AAC) 41 d after sowing (DAS). BRs were synthesized as described by Kohout (1994). Experiments were arranged in a split-plot design with two replicates during each experimental season; each subplot consisted of 9 plants.

Photosynthetic parameters were measured in leaves 2, 4 and 6, counting the youngest fully developed leaf at the time of BRs treatment as leaf 0. The measurements were made at 72 DAS for leaf 2, 82 DAS for leaf 4 and 94 DAS for leaf 6; at this time points the respective leaves were fully developed and showed maximum photosynthetic efficiency. At each sampling date, the leaves from 9 plants per each treatment were cut off and the middle parts of leaf blades were used for all subsequent analyses. The isolation of chloroplasts and the polarographical measurements (Clark-type oxygen electrode, *Theta ′90*, Prague, Czech Republic) of the activities of photosystem (PS) 1 and Hill reaction (HRA) were carried out as described previously (Holá *et al.* 2007), *i.e* as the amount of oxygen consumed (or, in case of HRA, formed) by suspensions of isolated chloroplasts [Class II, type C according to Hall (1972) nomenclature] after the addition of artificial electron acceptors or donors: 0.25 mM DCPIP reduced by 10 mM sodium ascorbate, 0.1 mM methylviologen for the PS 1 activity measurements and 7 mM $K_3[Fe(CN)_6]$ for the HRA measurements (irradiance was 170 W m^{-2} of PAR). The content of photosynthetic pigments was determined spectrophotometrically (*Anthelie 2 Advanced*, *Secomam*, Ales, France) after their extraction from 6 leaf discs (diameter 8 mm) with *N,N*-dimethyl formamide (DMF; Wellburn 1994). The specific leaf mass (SLM) was determined as the dry mass of 6 leaf discs (oven-dried at 80 °C for 72 h) to their area ratio. Each genotype/BRs treatment was represented by 3 or 6 (SLM) technical replicates per each experimental replicate and the mean values were used for the statistical evaluation. The data from all four experimental years were pooled together and analyzed by two-way *ANOVA* with genotypes, BRs treatments and their interaction included as the possible sources of variation (*CoStat Version 6.204*, *CoHort* software, Monterey, CA, USA).

The results of statistical analysis revealed that the three maize genotypes significantly differ in all photosynthetic parameters examined (with the exception of Chl *a/b* ratio which did not differ at all, and the HRA measured in samples from leaf 4, where $P = 0.065$) (Table 1). The 2023×CE704 hybrid displayed the highest activity of PS 1 and HRA, as well as the highest content of photosynthetic pigments (Fig. 1), confirming again the previous findings that F1 hybrids of maize show positive heterosis not only for yield and morphology parameters, but for various photosynthetic parameters as well (*e.g.* Baer and Schrader 1985, Mehta *et al.* 1992, Holá *et al.* 1999, 2007, Ahmadzadeh *et al.* 2004, Kočová *et al.* 2009). The inbred line CE704 was characterized by the lowest content of Chls and Cars and usually also by the lowest PS 1 activity and HRA (Fig. 1). As regards SLM, this parameter did not significantly differ among genotypes when recorded for leaf 2, but for both younger leaves its values were lower in the 2023 inbred line compared to the other two genotypes (Fig. 1*C*).

Our treatment of plants with either 24-EPI or AAC had no effect on SLM or the content of Chls or Cars in maize leaves that developed after the application of BRs (Table 1, Fig. 1*D-F*), which agrees with findings of some authors who worked with non-stressed plants of various species (*e.g.* Yu *et al.* 2004, Janeczko *et al.* 2007, Ali *et al.* 2008c, Shahbaz *et al.* 2008, Zhang *et al.* 2008) but disagrees with others (Sairam 1994a,b, Hayat *et al.* 2001, 2007, Fariduddin *et al.* 2003, 2004, 2009a,b, Ali *et al.* 2008a,b). It is interesting to note that with the exception of Ali *et al.* (2008b,c) who worked with *Brassica juncea* or *Vigna radiata*, all authors who observed the positive influence of BRs on the content of Chls worked with 28-homobrassinolide, whereas those that found no effect treated their experimental plants with 24-EPI or with brassinolide. As no other common denominator among these studies (for example the mode of application, the concentration of BR, the age of plants during BRs treatment or during the measurements of Chls content) exists, it would seem that the type of the applied BR is the main determinant in the manifestation of BRs effect on the content of photosynthetic pigments, and plant species is probably the second deciding factor.

Similarly to the content of Chls or Cars, neither the activity of PS 1 nor the HRA were significantly affected by treatment of plants with 24-EPI or AAC, though 24-EPI slightly (but insignificantly) increased the HRA in the F1 hybrid (and, in case of lower leaf insertions, in the 2023 inbred line as well) (Table 1, Fig. 1*A-B*). The activity of PS 1 has not been previously examined in connection with the possible role of BRs in photosynthesis and our study presents the first evidence that the function of this pigment-protein complex is very probably not modified by these phytohormones. The HRA measured with $K_3[Fe(CN)_6]$ as an artificial electron acceptor can to a certain degree be also regarded as a measure of the activity of PS 1 (particularly when chloroplasts with mainly undamaged thylakoid membranes are used for its measurements, as in our case). However, $K_3[Fe(CN)_6]$ also accepts electrones from photosynthetic plastoquinones, *i.e.* our measurements of the HRA can partly represent the PS 2 activity

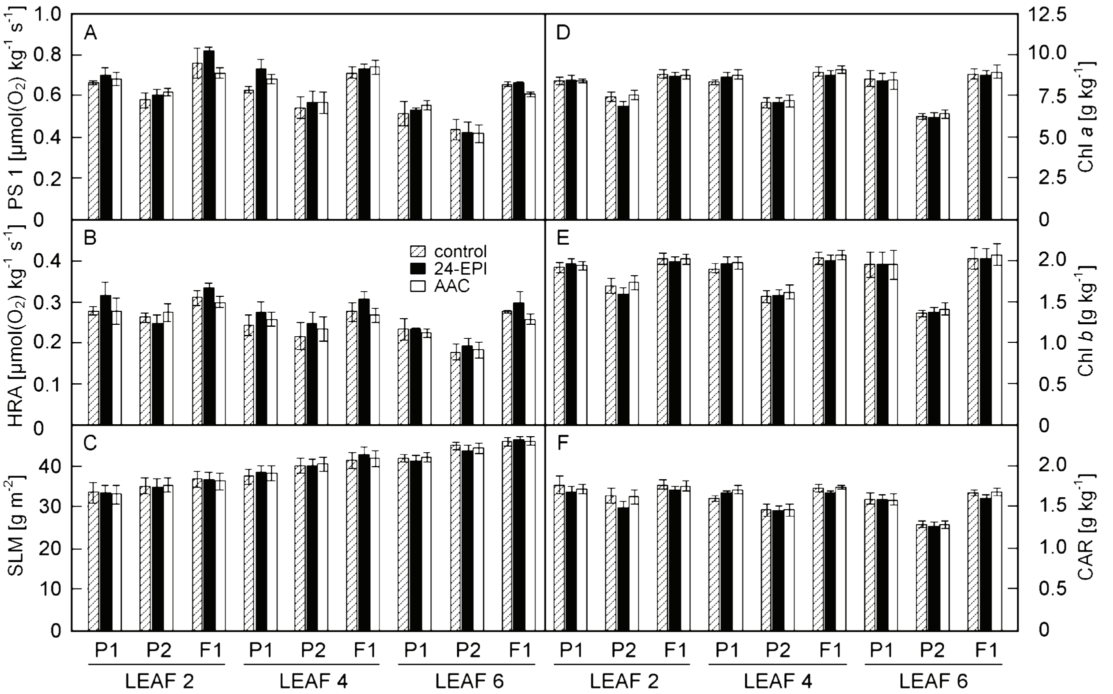

Fig. 1. The photosystem 1 activity (*A*), the Hill reaction activity (*B*), the specific leaf mass (*C*) and the content of chlorophyll *a* (*D*), chlorophyll *b* (*E*) and total carotenoids (*F*) in leaves of maize inbred lines 2023 (P1), CE704 (P2) and their hybrid 2023×CE704 (F1) grown at field conditions and treated with 10^{-12} M aqueous solutions of 24-epibrassinolide (24-EPI), synthetic androstane analogue of castasterone (AAC) or with tap water (control). The means ± SE were calculated from data pooled from four experimental seasons. Leaves are numbered beginning with the last fully developed leaf in the time of brassinosteroid treatment as leaf 0.

Table 1. The results of two-way analysis of variance (*ANOVA*) applied on the the specific leaf mass (SLM) and photosynthetic parameters in leaves of field-grown maize plants of three genotypes (inbred lines 2023 and CE704 and their hybrid 2023×CE704) treated with 10^{-12} M aqueous solutions of 24-epibrassinolide (24-EPI), synthetic androstane analogue of castasterone (AAC) or with tap water (control). Genotypes (G), brassinosteroid treatments (T) and their interaction (G×T) were included as the possible sources of variation for *ANOVA*. The levels of probability (*P*) are shown; the differences between individual genotypes or between 24-EPI, AAC and control treatments were statistically significant only in those cases where $P \leq 0.05$. Leaves are numbered beginning with the last fully developed leaf in the time of brassinosteroid treatment as leaf 0.

	Leaf 2			Leaf 4			Leaf 6		
	G	T	G×T	G	T	G×T	G	T	G×T
SLM	0.168	0.983	0.999	0.036	0.838	0.994	< 0.001	0.900	0.933
PS 1 activity	< 0.001	0.306	0.539	< 0.001	0.318	0.822	< 0.001	0.886	0.745
HRA	0.009	0.552	0.508	0.065	0.327	0.989	< 0.001	0.382	0.821
Chl *a*	< 0.001	0.456	0.655	< 0.001	0.545	0.915	< 0.001	0.885	0.998
Chl *b*	< 0.001	0.666	0.722	< 0.001	0.665	0.987	< 0.001	0.936	0.999
CAR	0.029	0.263	0.974	< 0.001	0.497	0.721	< 0.001	0.740	0.955
Chl *a/b*	0.972	0.829	0.991	0.405	0.954	0.958	0.175	0.941	1.000
Chls/CARs	< 0.001	0.277	0.884	< 0.001	0.607	0.697	< 0.001	0.694	0.908

as well. Several authors have examined the photosynthetic efficiency of PS 2 (mainly using Chl fluorescence measurements) in plants treated with BRs and usually either did not observe any effect of these compounds (Yu *et al.* 2004, Ali *et al.* 2008c, Ogweno *et al.* 2008) on the PS 2 activity or the effect was significant only in case these plants were at the same time exposed to some stressful conditions (Shahbaz *et al.* 2008, Zhang *et al.* 2008). Thus, it seems that the positive role of BRs in the regulation of photosynthesis is not associated with their effect on the primary photochemistry but that it concerns some other parts of photosynthetic processes.

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