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BRIEF COMMUNICATION

Influence of brassinosteroids on antioxidant enzymes activity in tomato under different temperatures

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Abstract

The effect of brassinosteroids (BRs) on catalase (EC 1.11.1.6), peroxidase (EC 1.11.1.7) and superoxide dismutase (SOD, EC 1.15.1.1) activity in tomato leaf discs was analyzed at 25 and 40 °C. Tomato leaf discs were preincubated for 24 h in Petri dishes with 24-epibrassinolide (EBR) or a polyhydroxylated spirostanic analogue of brassinosteroids (MH5). Both concentrations (10.60 and 2.12 nM) of EBR and MH5 stimulated the activity of SOD at 25 and 40 °C, the MH5-stimulated increase of this enzyme activity was greater. Peroxidase activity was unaffected at 25 °C, while at 40 °C this activity was enhanced by both compounds. The changes in catalase activity markedly depended on the structure BRs, doses and temperature. The results suggest a possible role of EBR and MH5 in the reduction of cell damage produced by heat stress due to induction of enzymatic antioxidants.

Additional key words: brassinosteroid analogue, catalase, heat stress, *Lycopersicon esculentum*, peroxidase, superoxide dismutase.

Brassinosteroids (BRs) are growth-promoting substances (Clouse and Sasse 1998) that have been associated with the protection of plants from salt (Hathout 1996, Vardhini and Rao 1997), heat (Zhu *et al.* 1996), drought (Li and Van Staden 1998) and chilling (Wilen *et al.* 1995). However, the mechanism by which brassinosteroids modulate plant heat stress responses is not understood. One mechanism that may be involved in the resistance to many types of stress is the increased activity of the antioxidant pathway. High contents of antioxidant enzymes have been found in response to heat, chilling, salinity, drought and wounding, as well as to oxidative stress, and they may have a general role in the acquisition of tolerance of plants to different environmental stresses (Sabehat *et al.* 1998, Hernández *et al.* 2000). Several studies have shown that BRs alter the antioxidant capacity of plants under stress conditions (Wilen *et al.* 1995, Chen *et al.* 1997, Li *et al.* 1998, Dhaubhadel *et al.* 1999).

The low abundance of BRs in natural sources has forced most workers to employ more readily available synthetic analogues (Ikekawa and Zhao 1991, Coll *et al.* 1995, Iglesias *et al.* 1996, 1997, Jomarrón 1995, Ramirez *et al.* 2000).

We examined the influence of EBR on some enzymatic antioxidants in tomato leaf discs with the aim to elucidate possible mechanisms that might be involved in the EBR-promoted antioxidant responses to room and high temperature. We further compare the influence of a spirostanic analogue (MH5) on these antioxidants to determine whether tested effects are related to different structure.

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Abbreviations: BRs - brassinosteroids; CAT - catalase, EBR - 24-epibrassinolide; MH5 - polyhydroxylated spirostanic analogue of brassinosteroids; POX - peroxidase, SOD - superoxide dismutase.
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Tomato (*Lycopersicon esculentum* Mill.) cv. Amalia seeds were germinated during three days in Petri dishes on filter paper containing distilled water at room temperature. Tomato seedlings were then grown on pots with peat and zeolite 1:1 (v/v) as substrate in a growth cabinet under 16-h photoperiod with the maximum/ minimum photosynthetically active radiation (PAR) 245/81 μmol m^{-2} s^{-1}. Day/night average temperatures and relative humidities were 25/18 °C and 60/85 %, respectively. Tomato leaf discs (1 cm diameter) were obtained from the 20-d-old tomato seedlings. 24-epi-brassinolide (EBR) was purchased from *Beak Consultants Ltd.* (Toronto, Canada) and the brassinosteroid analogue (MH5, a polyhydroxylated spirostanic analogue) was supplied by the Laboratory of Natural Products, University of Havana. Both growth regulators were dissolved in absolute ethanol, dimethyl-formamide and polyethylenglycol (16:1:1) to achieve a stock solutions which were stored at 4 °C.

Leaf discs (f.m. 0.25 g) were preincubated for 24 h in Petri dishes with either EBR or MH5 solution at two concentrations (10.60 and 2.12 nM). Leaf discs in Petri dishes containing distilled water were as controls. About 15 cm^3 were applied per dish, completely wetting the leaf discs. Half of leaf discs was incubated for 2 h at 40 °C (high temperature), and half at 25 °C (room temperature). Afterwards, samples were extracted for enzymatic determination.

Leaf tissue (0.25 g) was frozen and ground in liquid N_2, homogenized in 2 cm^3 of 50 mM potassium phosphate, pH 7.8, containing 0.1 mM Na_2EDTA, 1.5 % (m/v) polyvinylpolypyrrolidone (PVPP) and 0.1 % (v/v) Triton X100. The homogenate was centrifuged (10 000 *g*, 4 °C) for 10 min. The supernatant was collected in two samples of about 1 cm^3 and stored at -20 °C until enzymatic measurements were performed. Estimation of peroxidase activity was based on the Willstätter method quoted by Bergmeyer (1974). One unit of peroxidase activity was defined as the amount of enzyme that caused an increase of 0.1 units of absorbance per minute at 25 °C. SOD activity was measured according to Beyer and Fridovich (1987). One unit of SOD was defined as the amount of the extract that caused a 50 % decrease of the SOD-inhibitable nitrobluetetrazolium (NBT) reduction. Varying amounts of extract were used in the assay to attain a 50 % inhibition of NBT reduction. Catalase activity was measured via decomposition of H_2O_2 followed directly by decrease in absorbance at 240 nm. One unit of catalase was the amount of enzyme which decomposed 1 mmol H_2O_2 per minute at 25 °C (Aebi 1984).

The experiment was carried out twice. The data from the two experiments were averaged, processed by analysis of variance and expressed as means ± SE.

Both EBR and MH5 significantly increased SOD activity in leaves at both temperatures and no significant difference was observed between the two concentrations used (Fig. 1). MH5-treated discs showed substantially higher SOD activity than those treated by EBR (Fig. 1).

The peroxidase activity of both EBR and MH5 treated discs at 40 °C increased substantially (about 25 %) in comparison to leaf discs kept at 25 °C (Fig. 1). However, there was a marked decrease in the peroxidase activity of control discs when they were incubated at 40 °C for 2 h, indicating that this enzyme was affected by the heat-stress treatment. Both concentrations of EBR and MH5 (Fig. 1), provoked no variations in peroxidase activity at 25 °C, but both concentrations caused a significant increase at 40 °C.

EBR (10.60 nM) stimulated catalase activity in the discs held both at 25 and 40 °C. At 2.12 nM concentration stimulation of catalase activity was not observed (Fig. 1). Neither concentration of MH5 had any effect on catalase activity at 25 °C, but a significant increase was noted at 40 °C at both concentrations (Fig. 1).

Superoxide dismutase is a key enzyme in the detoxification of superoxide radicals. The increased SOD activity after EBR treatment at 25 °C suggests that EBR-promoted activation of SOD might decrease the possible toxic concentration of O_2^- radicals. As heat stress induces O_2^- and its product, H_2O_2, in plant tissues (Foyer *et al.* 1997, Dat *et al.* 1998), the EBR enhanced SOD activity at 40 °C would be a way of removing any excess O_2^- generated. However, the H_2O_2 produced might removed by catalase or by ascorbate peroxidase of the ascorbate-glutathione cycle (Foyer *et al.* 1997).

However, because catalase enzyme might be thermolabile, a significant reduction of catalase activity at 40 °C was expected. In contrast, in the presence of EBR catalase activity was enhanced. These increases in catalase activity might be important in eliminating H_2O_2 excess.

Kulaeva *et al.* (1991) reported that brassinosteroids can protect wheat leaf cells from heat stress. If it is assumed that the EBR-stimulated SOD and catalase activities led to enhance thermotolerance of tomato leaf discs in our experiment, then a mechanism of EBR-induced heat tolerance involving, directly or indirectly, activation of these enzymes would be operating. In this regard, it would be interesting to test whether EBR promotes changes in other enzymatic and/or non-enzymatic antioxidants.

In our experiment EBR also stimulated peroxidase activity at 40 °C but not at 25 °C, probably indicating an interaction between BRs and heat shock.

It was expected that the great structural differences between MH5 and EBR would provoke significantly different activity in the antioxidant enzymes tested. However, the MH5 caused a similar changes of SOD and peroxidase activity as EBR both at 25 and 40 °C, suggesting that the spirostanic structure without the common side chain patterns which occur in natural brassinosteroids elicit similar enzymatic antioxidant

responses. The implication of this observation may be that this polyhydroxylated spirostanic analogue could also trigger, directly or indirectly, the enzymatic detoxifying defense system as part of a possible MH5-induced termotolerance mechanism. An analysis of this hypothesis is in progress.

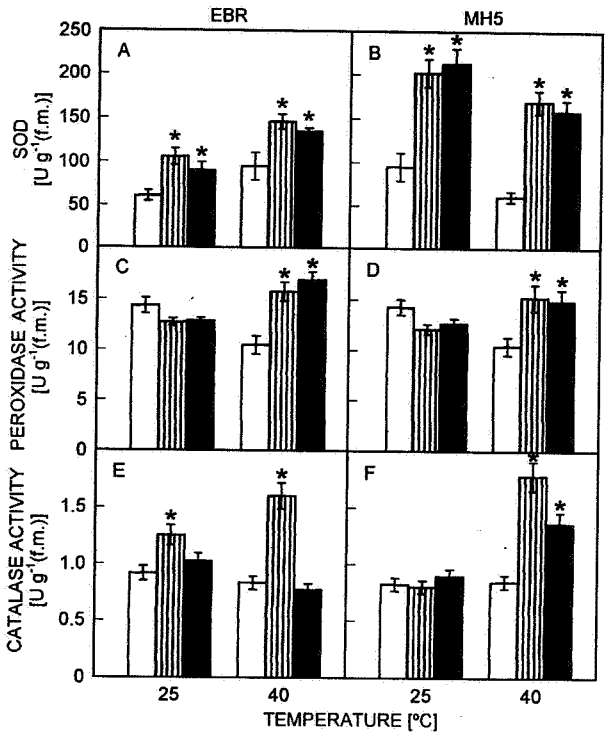

Fig. 1. Superoxide dismutase activity (SOD), peroxidase activity (POX) and catalase activity (CAT) of tomato leaf discs preincubated either with 24-epibrassinolide EBR (*A*) or a brassinosteroid analogue MH5 (*B*) at 25 °C and 40 °C. *Empty columns* - controls, *stripped columns* - 10.60 nM, *full columns* - 2.12 nM. *Bars* represent the SE (n = 6). *Asterisks* indicate statistically significant differences from control at $P < 0.05$.

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