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ARTICLE

Abscisic acid may alter the salicylic acid-related abiotic stress response in maize

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ABSTRACT The effect of abscisic acid (ABA) treatment on chilling tolerance and salicylic acid (SA)-related responses was investigated in young maize seedlings. Although the pre-treatment of plants with ABA slightly decreased the chlorophyll content, it also reduced the level of chilling injury caused by 6 days of cold treatment at 5°C. Under normal growth conditions increased levels of bound SA and of bound ortho-hydroxycinnamic acid (oHCA) were observed in the leaves during ABA treatment. In the roots ABA did not affect the free and bound SA levels, but increased the amount of free and bound oHCA. The activity of glutathione-S-transferase (GST) increased on the 3rd day of ABA treatment, while it did not change when followed by cold stress, compared to the control leaves. In the roots the activities of glutathione reductase, GST and ascorbate peroxidase (APX) increased during the ABA treatment, and those of GST and APX were also stimulated when ABA pre-treatment was followed by cold stress, compared to the control roots. Our results suggest that an overlap may exist between the ABA-induced cold acclimation and the SA-related stress response. **Acta Biol Szeged 55(1):155-157 (2011)**

KEY WORDS

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Zea mays L.

Maize seedlings are injured by temperatures below 10°C, which often occur in spring shortly after sowing and germination. Much of the injury to plants caused by abiotic stresses is associated with oxidative damage at the cellular level and protection against oxidation is an important component in the survival.

Abscisic acid (ABA) is considered to be one of the most important hormones involved in the plant response to cold stress. The protective mechanism of ABA against chilling stress is linked to its capacity for stabilising the water status by increasing root hydraulic conductivity and by closing the stomata, although ABA also induces antioxidant enzymes and modulates polyamine levels (Kumar et al. 2008).

Salicylic acid (SA) was also shown to enchance the chilling tolerance of various species. This enchanced tolerance was accompanied by increased activites of certain antioxidant enzymes, including gluthatione reductase and guaiacol peroxidase (Horváth et al. 2007). Cold acclimation also caused an increase in the level of ortho-hydroxycinnamic acid (oHCA).

Crosstalk between ABA and SA has recently discussed, but the relationship has not yet been completely elucidated (Okamoto et al. 2009). One major question is how exogenous ABA pre-treatment affects the levels of endogenous SA and its putative precursor, oHCA, under normal and stress conditions in maize seedlings.

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Materials and Methods

Ten-day-old maize plants growing in hydroponic solution in a plant growth chamber were treated with 0, 0.05 or 0.1 mM ABA for 1, 2 or 3 days. After the 2-day pre-treatment with or without ABA, some of the plants were cold-treated at 5°C for 1 day without changing the medium (2+1d cold).

The electrolyte leakage, as a measure of membrane damage, was determined according to Szalai et al. (1996).

SA was measured according to Meuwly and Métraux (1993) and enzyme activities according to Ádám et al (1995).

The total chlorophyll content was measured on the 3rd leaves using a SPAD-502 chlorophyll meter (Minolta Camera Co., Ltd, Japan).

The data were statistically evaluated using the standard deviation and t-test methods.

Results

Chlorophyll content and electrolyte leakage

The decrease in control plants subjected to 1 day of cold was similar to that found in the non-treated 3-day-old control. The chlorophyll content was slightly lower in the leaves of ABA-treated plants than in the control plants, and this decrease depended on the duration and concentration of the ABA treatment. However, when 2-day ABA treatment was followed by 1 day at 5°C, the chlorophyll levels were similar to that of control plants exposed to 1 day of cold.

Since cold stress under these conditions did not cause any change in the electrolyte leakage after 1 day, the treatment was continued for another 5 days. On the 6th day of cold treatment the electrolyte leakage from the leaves treated with 0.1 mM ABA was reduced.

Free and bound SA and oHCA contents

ABA treatment at normal temperature (22°C) increased the free SA contents only after 1 day at 0.1 mM concentration, while increases in the bound SA contents were significant still after 2 and 3 days. ABA increased the total SA level at 22°C but suppressed the low temperature-induced SA production in the leaves. In the roots the amount of free and bound salicylic acid did not change during the treatments.

In the leaves, ABA treatment and/or cold stress did not affect the free oHCA levels. In the leaves of control plants the bound oHCA content increased during the experiment. While the bound oHCA content increased in the case of 0.1 mM ABA treatment or cold treatment alone, it decreased when the ABA pre-treatment was followed by cold stress. In the roots of 2-day-old controls, the 1-day cold treatment alone caused an increase in the amount of bound oHCA. ABA treatment increased the level of free and bound oHCA at normal temperature at both concentrations, but when ABA treatment was followed by cold treatment the amount of free oHCA did not change, while the amount of bound oHCA showed a slight decrease.

Antioxidant enzymes

Exposure to ABA treatment for 3 days resulted in an increase in the GST activity in the leaves, compared to the control. Cold treatment alone slightly increased the activity of GST, but pre-treatment with ABA did not cause a further significant increase.

The activity of glutathione reductase (GR) increased from the first day of ABA treatment in the roots, compared to the control and it still remained above that of the control. The activity of GR increased at low temperature, and ABA pre-treatment did not affect this level.

The GST activity increased after treatment with ABA, either alone or combined with cold, but was not affected by cold stress alone. Similar changes were observed in the case of APX.

Discussion

ABA treatment increased the level of bound SA in the leaves under normal growth conditions. This is in accordance with another study, where the treatment of pea plants with an ABA biosynthesis inhibitor resulted in the disappearance of the SA peak during heat acclimation (Liu et al. 2006).

When plants treated with ABA for 2 days were exposed to chilling, the SA levels decreased, in contrast to their unchilled counterparts. A possible reason for this is that ABA-treated plants do not require a further enhancement of the SA-related pathway. Although the exact mechanism of the cross-talk between the ABA and SA signalling pathways is unclear, it is also possible that ABA inhibits the activity of SA-glucosyltransferase thus increasing the level of free SA (Liu et al. 2006). However, treatment with a high concentration of ABA without stress may induce general stress responses including SA synthesis, and the rate of generation of bound SA from the newly synthesised SA is probably higher than the inhibitory effect of ABA on SA-glucosyltransferase. It was also found that ABA negatively regulates SA synthesis by the transcriptional regulation of at least isochorismate synthase (de Torres Zabala et al. 2009).

ABA accumulation triggers the increased generation of reactive oxygen sepcies, which, in turn, leads to the up-regulation of the antioxidant defence system (Jiang and Zhang 2002). In the present study the activities of the antioxidant enzymes changed mainly in the roots during both ABA treatments, either alone or with cold treatment. It was also found that cold treatment significantly increased the amount of free and bound oHCA in the roots upon ABA treatment, while ABA had very little effect on SA levels in the roots. Since oHCA has been demonstrated to have antioxidant properties (Foley et al. 1999), these results suggest that the increase in the oHCA content may play a role in the antioxidative response to abiotic stresses, as found in the case of NaCl treatment (Szalai and Janda 2009).

In conclusion, this study demonstrates that the application of exogenous ABA increases the chilling tolerance of maize seedlings, through an increase in antioxidant enzyme activities. ABA treatment was also able to substitute for cold acclimation in part. ABA treatment induced changes in the endogenous SA and oHCA contents, and suggest that the SA-related stress response may overlap with the ABA-induced cold acclimation.

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