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Effect of salicylic acid during heavy metal stress

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ABSTRACT The effect of salicylic acid (SA) on Cd-induced stress was investigated in young maize (hybrid Norma) plants. When SA and Cd were applied simultaneously, the damage was less pronounced than without SA. However, SA treatment itself also caused oxidative stress and damage to the root system, and inhibited the phytochelatin synthase enzyme, so when the SA treatment was used before the Cd stress, it accelerated the damaging effect.
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KEY WORDS

cadmium
phytochelatin
salicylic acid
Zea mays

Salicylic acid, which may act as a component of the signal transduction system important in defence mechanisms against pathogen attack (Raskin 1992), may also provide protection against certain abiotic stresses, as was shown for example in the case of heat stress in mustard seedlings (Dat et al. 1998) or chilling damage in maize (Janda et al. 1999). Cadmium is one of the most aggressive heavy metals and may induce oxidative stress in plants (Hegedűs et al. 2001). The aim of the present study was to investigate the effect of salicylic acid treatment on cadmium stress, under the same conditions where it was able to protect the plants against chilling injury.

Materials and Methods

Sterilized seeds of maize (*Zea mays* L., hybrid Norma) were allowed to germinate for 4 days at 26°C, and were then grown for 10 days in Hoagland solution at 22/20°C with a 16/8-h light/dark periodicity in a Conviron PGV-36 plant growth chamber with a relative humidity of 75 %. The photosynthetic photon flux density (PPFD) at leaf level was 200 µmol m^{-2} s^{-1}, provided by metal halide lamps. Some of the plants were treated with 0.5 mM $Cd(NO_3)_2$ for 1 day. Salicylic acid (0.5 mM) was added either 1d before or together with the Cd.

The chlorophyll fluorescence from the youngest fully expanded leaves was determined under growth conditions using a pulse amplitude modulated fluorometer (PAM-2000, Walz, Effeltrich, Germany).

The enzyme activities were measured as described by Janda et al. (2000).

Measurement of root viability: 0.1 g root was incubated in 1.5 ml 50 mM phosphate buffer pH 7.5 containing 0.8% triphenyltetrazolium chloride for 24 h. The roots were then transferred into 3 ml ethanol and kept at 60°C for 30 min. The absorption of ethanol was then measured at 485 nm.

The Cd content was measured according to Hegedűs et al. (2001).

The phytochelatin synthase activity was measured according to Chen et al. (1997).

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Results and Discussion

Chl-a fluorescence induction

The DF/F_m' chlorophyll-a fluorescence induction parameter, which indicates the quantum efficiency of photosystem 2 (PS 2) (Genty et al. 1989), was measured to detect the damaging effect of the 0.5 mM Cd treatment (Fig. 1). After 1 d this parameter decreased from 0.7 to 0.5 in Cd-treated plants. Treatment with 0.5 mM salicylic acid alone for 1d did not cause any significant change in DF/F_m'; however, after a second day this too decreased the quantum efficiency of PS 2. The most dramatic changes occurred when 1 d SA pre-treatment was followed by Cd treatment. When Cd and SA were applied simultaneously, they had hardly any effect on the DF/F_m' parameter.

Root viability

Root viability decreased drastically after 1 d of SA or Cd treatment. However, when both SA and Cd were applied (either together, or consecutively), the decrease in root viability was even more pronounced (data not shown).

Cd content

As expected, Cd accumulated mainly in the root. The Cd content was significantly lower in plants which were pre-treated with SA for 1 day before the Cd treatment or when SA and Cd were applied together (data not shown).

Antioxidant enzyme activities

The antioxidant enzyme activities in the root were hardly affected by 1 d of Cd treatment; only the decrease in guaiacol peroxidase (POD) activity was significant. Contrary to Cd, SA caused an increase in the POD activity of the roots. However, when Cd was added simultaneously with SA, it prevented any significant increase in POD activity. On the other hand, after pre-treatment with SA, the existing increase in POD activity was not affected by Cd. Ascorbate peroxidase (APX) activity was increased about two-fold by SA and this increase did not change when SA pretreatment was followed by 1 d of recovery or Cd treatment. However, when SA and Cd were added at the same time, their effect was

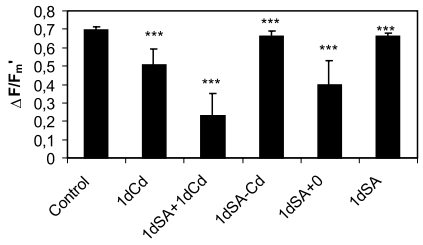

Figure 1. Changes in the quantum yield of PS 2 after 1 day 0.5 mM Cd treatment (1d Cd) or 1 day 0.5 mM salicylic acid treatment (1dSA). Some of the plants received 1 d SA pre-treatment before the Cd (1dSA+Cd), or SA and Cd were applied simultaneously (1dSA-Cd). In the 1dSA+O plants the 1 d SA pre-treatment was not followed by Cd treatment. (*** indicates a significant difference at the 0.001 level compared to control plants).

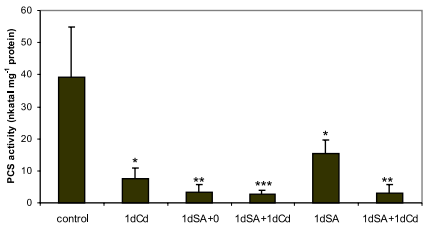

Figure 2. Changes in the phytochelatin synthase activity in the root of young maize plants treated with 0.5 mM salicylic acid and/or 0.5 mM Cd. For an explanation of the symbols see Fig. 1. (*, **, *** indicate significant differences compared to control plants at the 0.05, 0.01 and 0.001 levels, respectively.)

synergistic, resulting in a threefold increase in activity. Glutathione reductase (GR) activity was increased to the same extent by 1 d of SA or SA+Cd treatment, but dropped to almost the control level when SA treatment was followed by 1 d of recovery. However, when Cd was added after pre-treatment with SA, the GR activity remained at the same high level. The catalase activity in the root was very low and did not exhibit a significant change upon treatment with Cd and/or SA (data not shown).

The POD activity in the leaves was not affected by either of the treatments. In the case of catalase and APX only 1 d of SA treatment caused a significant decrease, and after 1 d of recovery the enzyme activities reached the control level. GR activity was increased by all the treatments to the same extent, and this high activity was maintained after 1 d of recovery following the SA pre-treatment as well (data not shown).

Phytochelatins

Phytochelatins (PCs) are enzymatically synthesized Cys-rich peptides, which are able to create chelates with heavy metals to decrease their damaging effects (Grill et al. 1989). PCs form a family of structures with increasing repetitions of the g-Glu-Cys dipeptide followed by terminal Gly [(g-Glu-Cys)$_n$-Gly], where n is generally in the range of 2 to 5. The substrate for PC biosynthesis is GSH. The transpeptidation of the g-Glu-Cys moiety of GSH onto initially a second GSH to form PC_2 is catalysed by PC synthase. The enzyme is active only in the presence of metal ions. The activity was detected in the roots and stems of tomato plants (Chen et al. 1997). The biosynthesis of PC is constitutive in nature (Grill et al. 1989).

The phytochelatin (PC) levels increased in the roots after 1 d of Cd treatment. The PC levels were hardly affected by other treatments. The PC synthase activity decreased in all cases compared to the control in the roots. Activities were lower in SA-pretreated plants than in treated ones both when it was applied alone and when it was followed by 1 d of Cd treatment. SA and Cd treatment together caused a greater decrease in the activity than the SA treatment or pre-treatment alone.

There were no significant changes in the PC levels in the leaves during the various treatments. PC synthase activity increased during the treatments, especially in the case of the SA+Cd treatment, compared to the control values. The lowest activity occurred after the SA treatment.

These results suggest that SA may inhibit Cd uptake, but may also cause damage to the roots, including a decrease in the phytochelatin synthase activity, so SA pre-treatment may accelerate the damaging effect of Cd.

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