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BRIEF COMMUNICATION

Effects of cold acclimation and salicylic acid on changes in ACC and MACC contents in maize during chilling

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Abstract

The effect of 0.5 mM salicylic acid (SA) pretreatment and of growing at hardening temperatures on chilling-induced changes in 1-aminocyclopropane-1-carboxylic acid (ACC) and malonyl 1-aminocyclopropane-1-carboxylic acid (MACC) was investigated in young maize (*Zea mays* L.) plants grown in hydroponic solution at 22/20 °C. Chilling at 5 °C caused an increase in ACC content; however, this increase was less pronounced in plants cold acclimated at 13/11 °C 4 d before the chilling treatment, and in those which were pretreated with SA for 1 d before the cold stress. Changes in MACC at low temperature showed no correlation with chilling tolerance in maize.

Additional key words: 1-aminocyclopropane-1-carboxylic acid (ACC), ACC oxidase, ACC synthase, cold stress, malonyl 1-aminocyclopropane-1-carboxylic acid (MACC), *Zea mays*.

Ethylene biosynthesis is affected by stress factors including low temperature (*e.g.* Wang *et al.* 1990). The increased activity of ACC synthase, catalysing the conversion of S-adenosyl-methionine to the immediate precursor, 1-aminocyclopropane-1-carboxylic acid (ACC), is responsible for the enhanced ethylene production after chilling (Wang and Adams 1982, Janowiak and Dörffling 1995). It was found, however, that low temperature affected the ACC content not only directly but also indirectly by inducing a water deficit in the chilling-sensitive inbred maize line. Higher ACC contents in the chilling-sensitive genotypes were accompanied by greater injuries.

Temperatures below 10 - 15 °C often cause damage to plants. In higher plants ethylene is synthetised from ACC by an iron-containing enzyme, ACC oxidase. The inactivation of ACC oxidase due to membrane damage resulting from low temperature stress may also contribute to the increased ACC content (Etani and Yoshida 1987). Furthermore, low temperature-enhanced ethylene production may continue after transfer to non-chilling conditions (Kimmerer and Kozlowski 1982).

Several studies have been made on the role of salicylic acid (SA) in defence mechanisms against pathogen attack (Raskin 1992). In addition, its accumulation during exposure to ozone or UV radiation has also been reported (Yalpani *et al.* 1994). It has also been shown that salicylic acid induced heat tolerance in mustard seedlings (Dat *et al.* 1998), and alleviated the toxic effects of heavy metals on membrane integrity in rice plants (Mishra and Choudhuri 1999). In previous studies it was shown that salicylic acid may also decrease the symptoms of low temperature damage in young maize plants (Janda *et al.* 1999).

SA may interfere with ethylene biosynthesis at several points. SA prevented the accumulation of ACC synthase transcripts induced by wounding (Li *et al.* 1992) and inhibited ethylene synthesis in pear suspension cultures

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Abbreviations: ACC - 1-aminocyclopropane-1-carboxylic acid; MACC - malonyl 1-aminocyclopropane-1-carboxylic acid; SA - salicylic acid.
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by blocking ACC oxidase (Leslie and Romani 1988). This latter effect may be due to the iron chelating properties of SA. The aim of the present work was to investigate the effects of salicylic acid pretreatment, which increased the chilling tolerance of the plants, on changes in ACC and MACC contents in maize during hardening and low temperature stress.

Sterilized seeds of maize (*Zea mays* L., hybrid Norma) were allowed to germinate for 4 d at 26 °C, then were grown for 10 d in Hoagland solution (250 cm^3) in a *Conviron PGV-36* plant growth chamber (Winnipeg, Canada) at a temperature of 22/20 °C (non-acclimated plants), relative humidity of 75 %, a 16-h photoperiod and a photosynthetic photon flux density (PPFD) at leaf level of 200 $\mu mol\ m^{-2}\ s^{-1}$, provided by metal halide lamps. Cold acclimation was carried out at 13/11 °C for 4 d before chilling. There was no significant difference between the temperature of the hydroponic solution and that of the air. The cold treatment was carried out in a chamber of the same type at 5 °C. One day before the cold treatment the 240 plants were divided into two identical groups, one of which was treated with 0.5 mM salicylic acid. All the plants were used for analyses.

The ACC and MACC contents were determined after chemical conversion to ethylene as described earlier (Tari and Nagy 1994). Samples of 2 g were taken from the shoots of 14-d-old maize seedlings and extracted with 10 cm^3 of 80 % ethanol. After centrifugation at 12 000 *g* for 20 min, the residue was reextracted. The supernatants were combined and evaporated to dryness in a vacuum evaporator. ACC was determined according to Lizada and Yang (1979). The efficiency of conversion of ACC to ethylene was 85 %. The reaction was not inhibited by 10^{-10} - 10^{-3} M SA. MACC was hydrolysed by refluxing the extract in 2 M HCl at 105 °C for 3 h. After neutralization with 10.5 M NaOH, the ACC content was determined. MACC concentrations were calculated from the difference in ACC concentration before and after hydrolysis.

Ethylene samples taken from the headspace of the incubation vessels were measured with a *Hewlett-Packard 5890 Series II* gas chromatograph (Palo Alto, USA) equipped with FID and a column packed with activated alumina. Flow rates were 35 $cm^3\ min^{-1}$ for He, 30 $cm^3\ min^{-1}$ for H_2 and 300 $cm^3\ min^{-1}$ for air. The oven, injector and detector temperatures were 100, 120 and 160 °C, respectively.

The results are the means of at least 3 but in most cases 5 measurements and were statistically evaluated using the *t*-test and *ANOVA* to assess significant differences ($P \leq 0.05$) between the means.

The ACC content in the leaves exhibited a progressive increase in untreated maize plants under chilling conditions (Fig. 1). This increase was less pronounced in plants pretreated with salicylic acid. These changes correlated well with the ethylene production of the seedlings on the first day of cold treatment (Janda *et al.* 1999). On average (plants chilled for 1 - 4 d) the ACC content in SA-treated plants was 53 % of the controls, although there was no statistically significant difference between the ACC contents of the non-chilled plants. In plants which were grown at 13/11 °C for 4 d, this cold stress (5 °C) did not cause a significant increase in the ACC content of untreated and SA-treated leaves after 4 d. These data support the concept that the endogenous ACC level may indicate the status of chilling stress in plant tissues.

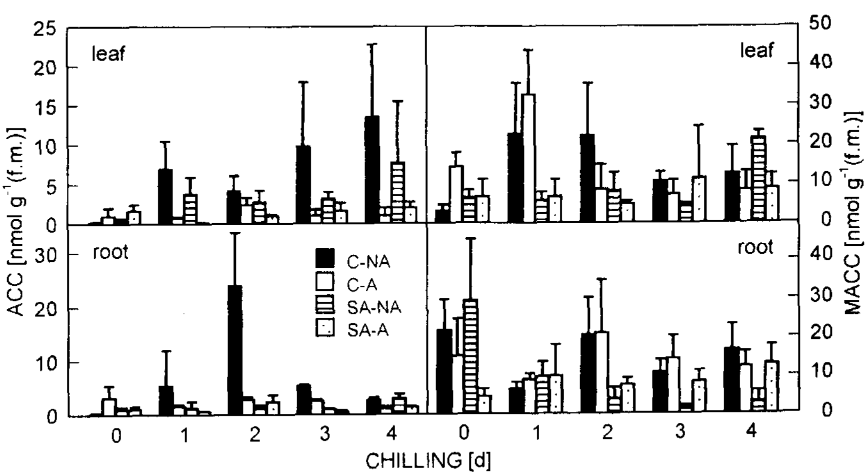

Fig. 1. Changes in the ACC and MACC contents in the youngest fully developed leaves and roots of young maize plants during 5 °C chilling stress. (C - control; SA - pretreated with 0.5 mM salicylic acid 1 d before the cold stress; NA - non-acclimated; A - acclimated)

Both hardening and the 0.5 mM salicylic acid treatment caused an increase in the ACC content of the roots (Fig. 1). The ACC content in the control plants showed a rapid increase for 2 d during chilling, after which it started to decrease, but it remained at a substantially higher level than before the cold treatment.

In plants treated with SA or grown at hardening temperature the ACC content did not change significantly even after 4 d of low temperature stress. In this case SA prevented the bulk accumulation of ACC in the roots at the outset of chilling in non-acclimated plants. The successful adaptation of the roots determines the regrowth capacity, ion and water uptake and/or transport of the seedlings after chilling. The elongation of the roots is very sensitive to ethylene, so a decrease in ACC content is very important in ensuring sufficient root growth for the successful acquisition of soil resources after chilling.

An increase in the ACC content due to low temperature stress was also observed by other authors in several inbred maize lines and hybrids (Janowiak and Dörffling 1995, 1996). This increase showed a high positive linear correlation with the chilling sensitivity of the genotype (Janowiak and Dörffling 1996). This correlation is supported by the observation that maize plants grown at hardening temperatures, which tolerate low temperatures (Janowiak and Dörffling 1995, Janda *et al.* 1998), showed a lower increase in the ACC content during low temperature stress. Similarly, the ACC accumulation was less pronounced in unhardened plants pretreated with salicylic acid. The decreasing effect of salicylic acid on ACC accumulation in the roots was especially remarkable during the first three days of chilling. The inhibition of ethylene biosynthesis by SA pretreatment may be involved in the adaptation of unhardened roots to chilling temperatures. In acclimated plants, however, SA treatment did not cause a further decrease in the ACC accumulation in the plant organs.

The MACC content showed an increase in the leaves due to the low temperature treatment (Fig. 1). In the unchilled plants the MACC content in the leaves was significantly higher in the acclimated plants than in the controls. The accumulation of MACC at low temperature was observed not only in maize but also in wheat plants (Macháčková *et al.* 1989). However, in contrast to ACC, changes in the MACC content were not highly correlated either to the chilling tolerance of maize (Janowiak and Dörffling 1996) or to the frost resistance of wheat genotypes (Macháčková *et al.* 1992).

The MACC content in the roots decreased during chilling in the non-acclimated plants (Fig. 1). In wheat plants MACC accumulates in the roots during hardening (Macháčková *et al.* 1992). In maize this accumulation could not be observed: the MACC concentration was never significantly higher in the cold acclimated plants than in the controls.

In conclusion, it was shown in the present work that not only low temperature acclimation, but also a 1-d pretreatment with 0.5 mM salicylic acid, which was earlier shown to decrease the symptoms of chilling injury, may decrease the accumulation of ACC under chilling conditions in young maize plants. These results support the hypothesis that ACC accumulation is a reliable marker of low temperature damage. The malonyl conjugate of ACC also changed during low temperature stress; however, these changes in the MACC content were not correlated with the chilling tolerance in maize.

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