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ARTICLE

Exploration of cold signalling related to ascorbate and salicylic acid in *Arabidopsis thaliana*

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ABSTRACT TLow temperature is a significant limiting factor on plant development in the temperate zones. We tried to explore certain steps of the cold-signalling focused on salicylic acid (SA) and ascorbic acid (AA) using Arabidopsis thaliana as a model. The results of the freezing survival tests showed that both the wild type and mutant plants had better freezing tolerance after cold hardening treatment. SA deficient plants showed a higher survival rate. Two of the polyamines, putrescine and spermine showed only significant changes under the cold hardening. Significant differences were found in the bound SA level between the temperature treatments, but it did not correlate with the AA level and the survival rates.
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KEY WORDS

Arabidopsis thaliana
ascorbic acid
cold stress
freezing test
salicylic acid

Various mechanisms were evolved in plants to respond the changes in their environment, such as low temperature stress, which is one of the most important limiting factors in the spread of plants species or cultivated plant varieties. Low temperature has a strong impact on plant growth and development (Ruelland et al. 2009).

The development of cold hardiness to survive the freezing temperatures during winter requires prolonged periods of cold. During these periods wide range of adapting processes are stimulated includes changes in the membrane composition (Szalai et al. 2001), accumulation of osmoprotectants (Konstantinova et al. 2002), or changes in the redox status of plants (Szalai et al. 2009), which may also lead to improved antioxidant capacity (Janda et al. 2003). Cold stress causes an elevated level of the reactive oxygen species (ROS). This oxidative burst is one of the earliest responses of plant cells (Colville and Smirnoff 2008) and the degradation of the membrane lipids result the major source of the ROS (Bhattacharjee 2005). The role of the SA in stress adaptation has already described (Klessig et al. 2000). SA can protect maize plants against chilling injury (Janda et al. 1999) but less work has been published on the in vivo level of SA during stress.

Arabidopsis thaliana is an ideal model plant studying the cold-signalling but the results can be generalised to a limited extent (Soitamo et al. 2008).

In this work, the investigation of interaction the salicylic acid (SA) and ascorbic acid (AA) was planned during cold hardening and their role in the survival using various AA and SA mutant and transgenic Arabidopsis plants. The other aim of the work was to study the possible reasons the differentiation in the survival rates.

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Materials and Methods

Arabidopsis thaliana cv. Columbia (Col-0; wild-type) and five other mutant and transgenic lines were used for the experiments (vtc 2-3: mutant with low AA; Glo: transgenic plant with AA overproduction; sid2: chloroplastic SA synthesis deficient; eds5: inhibited SA transport from the chloroplast; NahG: transgenic plant with elevated SA degradation). Plants were grown at 21°C, PPFD: 250 µmol m^{-2} s^{-1}. The cold hardening was at 4°C for 4 d. The freezing test was carried out in the dark for 1 d at – 10 °C with a 0.5°C/h cooling gradient. Plants were re-acclimated at 4°C for 1d, and were allowed to recover for 2 weeks at room temperature. The survival rate is (survivors / total plants) x 100.

The fluorescence measurement was performed according to Janda et al. (1994). Extraction and the determination of the activity of various antioxidant enzymes (APX; CAT; GR) took place as reported by Janda et al. (1999). Total-AA content was determined according to Leipner et al. (1997), the SA according to Meuwly and Métraux (1993), and the polyamine (PA) level according to Smith and Davies (1985).

Results

There were no significant changes in the chlorophyll-a flurorescence parameters and the degree of the membrane destruction.

The lowest survival rate were observed at the vtc 2-3 and Glo mutants. NahG plants had a similar ones to the Col-0 plants, while the SA deficient mutants (eds5, sid2) had the highest one.

Under control temperature conditions, both the free and the bound SA was significantly higher in vtc 2-3 and Glo plants. Significantly lower level of the bound SA was found in the cases of the eds5, sid2 and NahG.

Cold caused a significant decrease of the free SA in the vtc 2-3 plants. Significant elevations of the bound SA were found in the Col-0, vtc 2-3, NahG, eds5 and sid2.

The activity of the antioxidant enzymes were also investigated. The GR showed an elevated activity in all cases during cold hardening. The APX activity increased significantly only in the Col-0. An activity drop-down were observed in NahG. The CAT activity increased in the vtc 2-3 and NahG plants.

The SA deficient plants showed a higher level of total-AA at control temperature, while it decreased in eds5, NahG and Glo during cold stress.

Only two of the PAs, putrescine and spermine changed significantly during cold-hardening. The putrescine increased in all genotypes, while the spermine was decreased.

Discussion

As it was expected, Arabidopsis plants with various genetical background used altered ways to compensate the negative effect of cold.

Our results show that cold can alter the endogenous SA level both in SA and AA deficient Arabidopsis plants. The AA deficient mutants had a lower survival, which were correlated to an elevated free and bound SA level. The survival of the NahG and Col-0 plants were similar. The chloroplastic SA metabolism deficient mutants (sid2 and eds5) showed the highest survival rate. It may caused by either the elevated putrescine level or the relatively high total-AA content during cold hardening. There were observed a significant elevation in the GR activity and a slight increase in the APX activity, respectively. The AA level and the GR activity may refer to the role of the ascorbate-glutathione cycle against cold stress, similar results were found by Pang and Wang (2010).

Our measurements show that temperature can effect on the PA content in Arabidopsis. Generally, the putrescine elevated which may caused by its early synthesis, as it was reported Gardiner et al. (2010). The spermine was lower during cold hardening.

In conclusion, cold-hardening was proven as an important factor enhancing the frost tolerance in Arabidopsis. Correlation between the higher survival rate and the decreased SA level is not completely understood. It might be compensated by either the AA or the action of the antioxidant enzymes. Furthermore, the results suggest that PAs have a pivotal role against cold stress in all of the investigated plants.

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