THE EFFECT OF ABSCISIC ACID ON THE FREEZING TOLERANCE IN LOLIUM TEMULENTUM L.

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ABSTRACT

This study was conducted to clarify the effect of abscisic acid (ABA) on the development of freezing tolerance in *Lolium temulentum* L. We compared the changes of freezing tolerance and endogenous ABA content by the ABA treatment with those by the hardening treatment. Exogenous ABA applications (7.5 x 10-5 M) increased freezing tolerance, but did not completely substitute for the hardening process. This treatment resulted in great increase in endogenous ABA content, as compared with the control or the hardening treatment. Since the relationship between endogenous ABA content and freezing tolerance was not parallel, it is considered that freezing tolerance is not dependent on endogenous ABA level alone in seedlings

KEYWORDS

Abscisic acid, Freezing tolerance, Hardening, *Lolium temulentum* L.

INTRODUCTION

Many plants become more resistant to freezing temperatures when exposed to non-freezing temperatures, a process known as hardening. The mechanisms involved in hardening are not well understood. Several studies have suggested that ABA may be involved in the hardening process. Two lines of evidence indicate that ABA is involved in the induction of freezing tolerance. First, an increase in the endogenous levels of ABA has been observed when plants are exposed to low temperature (Chen and Li, 1982). Secondly, exogenous applications of ABA have been shown to increase the cold hardiness in certain plants (Chen and Gusta, 1983). We recently reported that *L.temulentum* could increase freezing tolerance by hardening, and that the N-terminal amino acid sequence of one hardening-induced protein was similar to that of ABA-inducible protein of alfalfa (Tase et al., 1996). However, it is not clear in *Lolium* species whether freezing tolerance can be induced by ABA at normal temperature.

To clarify the effect of ABA on the freezing tolerance in *L.temulentum*, we investigated the time courses for the changes of freezing tolerance and endogenous ABA content during exogenous application of ABA as compared with those of hardening.

MATERIALS AND METHODS

L.temulentum strain PI176624 introduced from the United States Department of Agriculture was used for experiments. Seeds were sown in seedling boxes (15 x 6 x 10 cm), at the rate of 16 seedlings per box. The boxes were maintained in a growth cabinet which was kept at 20½C / 13½C, day / night with 16 h photoperiod. When the seedlings were raised to the 3-4 leaf stage, they received exogenous ABA applications or a hardening treatment. Each experiment was repeated at least three times with 3 replications. An ABA solution was sprayed on leaves every 12 h for the entire test period. ABA (cis-trans mixed isomer, Sigma Chemical Co.) was dissolved in ethanol and diluted with distilled water to 7.5x10-5 M. The control seedlings were sprayed with distilled water only. The hardening treatment was carried out as described previously (Tase and Kobayashi, 1996). Non-hardened control seedlings were maintained under the standard temperature and photoperiod conditions described above. Seedlings from the ABA or hardening treatment together with untreated control were collected from the cabinet periodically for the freezing tolerance test. Freezing tolerance was determined by the estimation of LT50 value (the temperature required to kill 50 % of the seedlings) as described previously (Tase and Kobayashi, 1996). During treatments, ABA was extracted periodically from leaves of five seedlings in each species. ABA was assayed by the phytodetek assay kit (Phytodetek-ABA, Idetek Inc.) using a monoclonal antibody specific for 2-cis-(+)-ABA. All assays were replicated three times.

RESULTS AND DISCUSSION

Changes in ABA- and hardening-induced freezing tolerance (Fig.1) The control seedlings had LT50 values ranging from -4.0°C to -5.0°C over the experimental period. Either treatments induced a significant increase in freezing tolerance compared to the control. After 1 day of ABA treatment, LT50 values changed rapidly from -4.5°C to -5.1°C. Then, after 14 days of ABA treatment, LT50 value decreased to -7.0°C. Further application of ABA showed no additional decrease in LT50 values. On the other hand, LT50 values of seedlings hardened at 2°C were also lowered from -4.0°C to -5.0°C after 1 day. Subsequent LT50 values decreased to -8.2°C. Thereafter, LT50 values remained nearly constant during hardening treatment. From these results, it was indicated that the exogenous ABA treatment could clearly induce a higher level of freezing tolerance than the control treatment, but could not induce the same degree of freezing tolerance as the hardening treatment.

Accumulation of endogenous ABA during ABA or hardening treatment (Fig.2). The control treatment seedlings maintained stable ABA content ranging from 5.0 to 16.9 ng per g fresh weight over the experimental period. The ABA content of ABA-treated seedlings increased rapidly to 40 ng per g fresh weight after 3 days as compared with the control. ABA content then continued to accumulate, and reached its highest measured value of 275 ng per g fresh weight at the end of the 28 days of treatment. The hardening treatment also caused a gradual increase in the endogenous ABA content during the 14 days of the hardening treatment, although ABA content of hardened seedlings was lower than that of ABA-treated ones. ABA content of ABA-treated seedlings at the end of the treatment was 16- and 6-fold higher than that of control and hardening treatments, respectively. Several workers reported that, when plants were exposed to low temperature, an increase in freezing tolerance was observed and ABA contents were increased 1.5 to 10-fold as compared with controls, depending on the species (Daie and Campbell, 1979). From this point of view, the endogenous ABA level induced by ABA treatment in this study seems to be extremely high. However, in spite of the accumulation of such plentiful endogenous ABA contents, the freezing tolerance was higher in the hardening treatment than that of the ABA treatment. Since the relation between ABA content and freezing tolerance was not parallel, it is considered that freezing tolerance is not dependent on endogenous ABA level alone in seedlings. Perhaps when endogenous ABA accumulates to a certain minimum level in seedlings, the mechanism of hardening is activated, and freezing tolerance is increased. However, further increase of freezing tolerance seems to be accomplished by another metabolic pathway induced by low temperature.

In this study, we showed that exogenous application of ABA in *L.temulentum* could not completely substitute for hardening, but did show an improvement freezing tolerance. It is considered that endogenous ABA, when accumulated to more than a certain level, has an important role in hardening of *Lolium* seedlings. ABA appears to be very useful as a clue to understand the mechanism of hardening.

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Figure 1
Changes in ABA- and hardening-induced freezing tolerance of *L. Temulentum* seedllings.

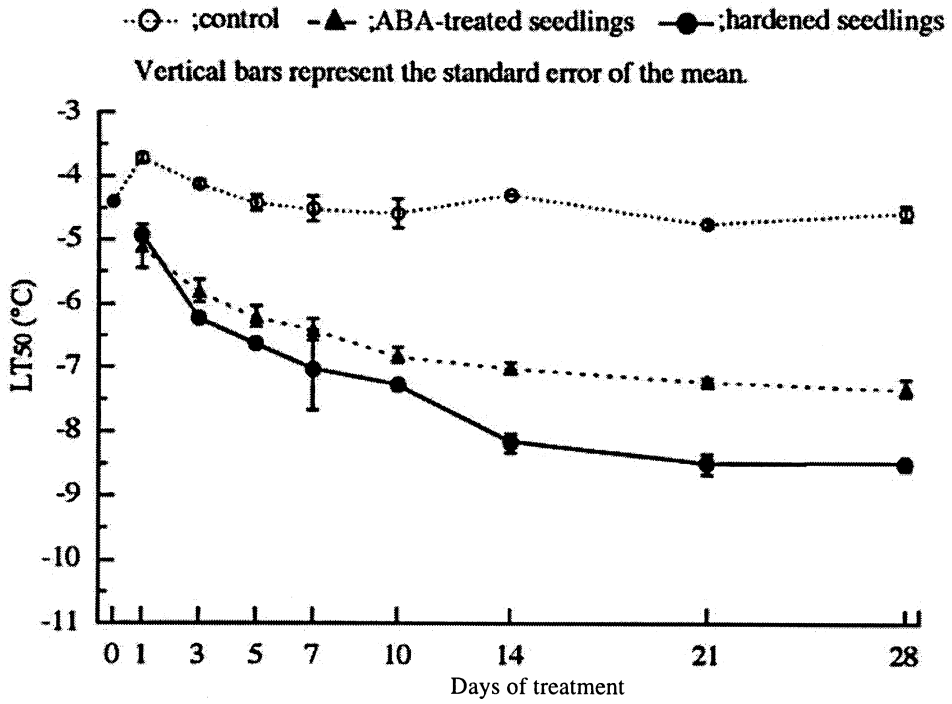

Figure 2
Accumulation of endogenous ABA during exogenous ABA or hardening treatment in *L. temulentum* seedlings. Symbols and vertical bars see Figure 1