한국환경과학회 봄 학술발표회 발표논문집 제11권(제1호), 468 - 471, 2002
Proceedings of the Korean Environmental Sciences Society Conference, May. 24~25, 2002

생물/생태-P2

Effects of low temperature and salicylic acid on chilling tolerance in cucumber seedlings

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1. Introduction

Plants native to warm regions are generally sensitive and injured to chilling temperatures. Chilling injury is a physiological disorder that occurs in sensitive plants subjected to non-freezing temperatures below 12℃ (Saltveit and Morris, 1990). A number of tolerance mechanism have been proposed on the physiological and biochemical changes associated with chilling injury (Markhart, 1986). Acclimation to chilling results in a lowering of the temperature at which the plant is damaged or killed by chilling temperature. The mechanisms of acclimation remains to be discovered about how'these metabolic changes coordinate to produce a level of low temperature tolerance during acclimation. Low temperature may cause various types of physiological damage and induces oxidative stress in the cell (Szalai et al., 1997). It was found that the level of some amino acids such as proline increased under stress conditions including cold stress (Naidu et al., 1991). There has been considerable interest on the role of salicylic acid (SA) in inducing tolerance to low temperature. SA has been implicated in protection against chilling (Janda et al., 1999). The present paper report on the effects of low temperature and SA on chilling tolerance, and describes chilling acclimation in seedlings of chilling sensitive cucumber. In the present work some of the role of the SA with special regard to changes in the proline contents in cucumber plants will be discussed as a possible factor responsible for increased chilling tolerance.

2. Materials and methods

Seeds of cucumber (*Cucurmis sativus* L.) were placed in vermiculite and grown at 25℃ for 3 days in the dark. The seedlings were then preexposed at either 14℃ for 3 d in the dark (acclimation period) followed by 7 days in the dark at 4℃ or directly transferred to 4℃ for 7 days in the dark (chilling period). Control seedlings did not receive a 14℃ treatment. The final growth analysis was done after the acclimated and nonacclimated seedlings were transferred to the initial growth conditions (25℃, 12 h photoperiod) and grown for 10 days. The number of surviving seedlings was counted and dry weights of the seedlings were measured both before and after 10-d grow-out period.

To determine whether exogenous application of SA affected chilling tolerance, seedlings were exposed to 0, 100, 500 or 1000 μM SA, 100 μM H_2O_2 and cycloheximide (CH). Seedlings were transplanted into pots filled with Hoagland solution and allowed to become established at 25℃ for 12 h as a recovery period from oxidative shock, after which they were transferred to 4℃ for 7 days. Long-term experiments were also carried out with two-week-old seedlings to examine necrotic injuries, survival rates and proline contents of the whole seedlings and of the third leaf. The seedlings were acclimated before chilling for 4 d, subsequent chilling at 5℃ mostly up to 5 days. The proline contents were determined according to Bates et al. (1973).

3. Results and discussion

Chilling damage in cucumber seedling was found to be dependent on both temperature and duration of exposure. Survival was unaffected by chilling stress less severe than 5℃ for 7 d, and shoot dry weights were reduced with increasing degree of chilling stress. Acclimation (Ac) significantly affected survival and shoot dry weights. The effect on survival was most evident in the 5℃ for 7 d treatment in which survival in the Ac treatment was 78% and that of control treatment was 20%.

Acclimation and SA treatment provided some degree of protection from chilling. Exposure of seedlings to the 14℃ acclimation temperature induced numerous changes that act in concert to increase chilling tolerance. The treatment with exogenous SA improved the growth and survival of chilled seedlings. The result indicated that SA is essential for survival in the face of chilling stress, and SA and acclimation have common effect. Even though SA improved the survival of chilled seedlings, it was not as effective as acclimation in inducing chilling tolerance. The inhibition of seedling growth and survival by CH, an inhibitor of protein synthesis, dramatically affected the ability of Ac seedlings to tolerate chilling stress. In unchilled seedlings, CH treatment had no effect on survival, although seedling weights were greatly reduced. AcCh seedlings showed a significant reduction in survival when treated with CH. Exogenous application of SA to CH-treated seedlings restored their ability to survive chilling stress. The addition of H_2O_2 to seedlings before the cold treatment showed relatively similar results with SA, indicating chilling tolerance probably due to the increased ability of certain antioxidant enzymes leading to increased chilling tolerance in young cucumber plants.

Chilling caused a large increase in free proline levels, regardless of a acclimation status. However, very little proline accumulation occurred during acclimation. There was a significant differences in the proline content in shoots and roots between SA-treated and control plants during the chilling period. Chilling on non-acclimated seedlings caused injuries that increased linearly with chilling duration. Injuries of acclimated seedlings were on the average smaller by half than those of the non-acclimated ones. During chilling of non-acclimated seedlings the proline content in the third leaf increased, and further chilling increased the proline accumulation. The addition of SA to plants during chilling treatment in the light and dark increased higher proline content in the light than that in the dark. The present results indicate that endogenous proline obviously plays a role in chilling tolerance of seedlings by stabilizing the water status.

4. Abstract

The present study was undertaken to investigate the effects of low temperature and salicylic acid (SA) on chilling tolerance in acclimated and nonacclimated cucumber seedlings. Acclimation significantly affected survival and shoot dry weights. Injuries of acclimated seedlings at the third leaf stage were on the average smaller by half than those of the nonacclimated ones. Chilling caused a large increase in free proline levels, regardless of acclimation status. Exogenous treatment with SA resulted in improvement in growth and survival of acclimated, chilled seedlings, indicating SA and acclimation have common effects. Cycloheximide treatment in the presence of SA restored acclimation-induced chilling tolerance. An elevated proline level was observed in cold-treated and SA- treated plants and the level was more pronounced in the light than in the dark at chilled temperature, indicating that endogenous proline may play a role in chilling tolerance.

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