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Involvement of calcium and calmodulin in the acquisition of SA-induced thermotolerance in cucumber seedlings

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1. Introduction

High temperature is one of the major abiotic stress limiting plant yield and distribution in many regions of the world. The acquisition of thermotolerance is usually associated with prehardening plants at an elevated, but nonlethal temperature. Considerable evidences have correlated this acquired thermotolerance with the increased synthesis of heat shock proteins(HSPs). High temperature can alter the integrated system of enzymic and nonenzymic antioxidants involved in the detoxification of active oxygen species(Paoiacci et al., 1997). Enhanced thermotolerance has recently been described in plants treated with salicylic acid(SA). Tissues from potato microplants grown on acetylsalicylic acid were shown to have enhanced thermotolerance(Dat et al., 1998). There is also evidence for interactions between SA and the antioxidant system. Changes in antioxidant enzymes and metabolites have been reported SA treatment. SA can directly inhibit the activity of the H_2O_2-scavenging enzymes, catalase and ascorbate peroxidase. Further investigation of these newly discovered effects of SA may prove rewarding, as plants are believed to use the antioxidant system to defend against oxidative stress and enhance their abiotic stress tolerance(Foyer et al., 1997). In recent years, it has become clear that Ca^{2+} is not only a macronutrient, but also a major intracellular messenger involved in the mediation of many diverse physiological processes in plants, and calmodulin plays a pivotal role in the calcium messenger system(Bush, 1995). Ca^{2+} has also been found to take part in the perception and regulation of responses of plants to environmental stress. The cytosolic free Ca^{2+} level often shows a significant increase in plant cells under various stress, and increase of stress in cytosolic free Ca^{2+} level, has been considered as an adaptive signal(Monroy and Dhindsa, 1995). Under heat stress, Ca^{2+} mediated second messenger systems may be involved in heat shock responses. Pretreatment of hypocotyl segments and etiolated seedlings with Ca^{2+} or Ca^{2+} chelator EGTA resulted in changes in the synthesis of HSPs(Wu et al., 1992). In addition, some of the HSPs have been found to be calmodulin-binding proteins(Lu et al., 1995).

In the present study, we investigated the effects of exogenous SA treatment on thermotolerance of cucumber seedlings, possible involvement of Ca^{2+} and calmodulin in the SA-induced thermotolerance and their relation to the antioxidant system.

2. Materials and methods

Plant material: Cucumber seeds were pre-soaked for imbibition in distilled water(control) or salicylic acid, aqueous solution of $CaCl_2$, EGTA, verapamil and chlorpromazine(CPZ) at 25℃ for 12 h. Then seeds were germinated with distilled water in the dark at 25/20℃(day/night) for 2.5 days(60 h).

Growth conditions and measurement: Germinating seedlings were transferred onto filter papers wetted with SA solution(0, 100, 500 and 1000 μM) and grown for an additional day(24 h) in the dark. The seedlings were transferred at 50℃ in the 10 h, and returned to 25/20℃ for 8 days for recovery, then their survival percentage was counted. Electrolyte leakage in roots of cucumber seedlings was measured with a conductometer. Ascorbate peroxidase activity in cotyledons of seedlings was measured spectrophotometrically as described by Nakano and Asada(1987). The level of lipid peroxidation (in terms of MDA) in cotyledons of seedlings was measured with the meathod of Dhindsa et al.(1981).

3. Results and discussion

When 2.5-day-old cucumber seedlings were subjected to heat stress(50℃), the survival percentage decreased significantly. However, SA treatment led to an obvious increase in the survival percentage of the seedling. Heat treatment led to the leakage of electrolytes from roots and the loss of vitality in cucumber cotyledons. Pretreatment of cucumber seedling with SA alleviated significantly the leakage of electrolytes from root tips and the loss of vitality in cotyledons. The results indicated that SA treatment enhanced the thermotolerance of seedlings. EGTA-and SA-pretreated seedlings showed lower survival percentage than those with SA treatment alone. In addition, pretreatment with Ca^{2+} channel blockers verapamil also lowered the SA-enhanced survival percentages at 50℃ as compared with SA treatment alone. On the other hand, petreatment of seeds with $CaCl_2$, EGTA and verapamil has little effect on the germination of seeds and early growth of seedlings. Measurement of electrolyte leakage from root tips and vitality change in cotyledons under heat stress also showed similar results. Pretreatment of seeds with CPZ showed little effect on the SA-induced thermotolerance. Calcium pretreatment of seeds, which enhances SA-induced thermotolerance, further increased SA-induced APX activity in the seedlings in comparison with SA treatment alone. In contrast,

pretreatment of seeds with Ca^{2+} chelator EGTA, which weakened SA-induced thermotolerance, also lowered the SA-induced APX activity. After the seedlings were heat-treated at 46℃ for 2 days, APX activity showed a general decline. However, the seedlings with SA treatment alone maintained higher levels of APX activity than those without SA treatment after the heat stress. Furthermore, calcium plus SA treatment enabled the seedlings to maintain relatively higher APX activity than those with SA treatment alone. On the contrary, EGTA plus SA pretreatment aggravated the loss of APX activity under heat stress as compared with SA treatment alone. MDA content in the seedlings showed little difference among various treatments. However, after the seedlings were heat-treated for 2 days, their MDA content increased remarkably. The seedlings with SA treatment alone maintained a significantly lower MDA level than those without SA treatment, and calcium plus SA treatment further lowered the heat stress-induced increase of MDA level in the cucumber seedlings as compared with SA treatment alone. In contrast, EGTA plus SA pretreatment weakened the effect of SA treatment and enhanced the heat stress-induced increase of MDA level in the cucumber seedlings as compared with SA treatment alone. These results suggest that the SA-induced thermotolerance is mediated by Ca^{2+} and requires the entry of extracellular Ca^{2+} into cells across the plasma membrane. Antioxidant enzyme systems could take part in the final generation of the SA-induced thermolerance.

4. Summary

SA treatment significantly increased thermotolerance in cucumber seedlings. Pretreatment of seeds with $CaCl_2$ solution enhanced the SA- induced thermotolerance. On the contrary, pretreatment with the Ca^{2+} chelator EGTA lowered this SA-induced thermotolerance. In addition, pretreatment with Ca^{2+} channel blocker verapamil also weakened the SA-induced thermotolerance. However, the calmodulin antagonist chlorpromazine(CPZ) had little effect on the SA-induced thermotolerance. Measurement of activity of the antioxidant enzyme APX and the level of lipid peroxidation (in term of MDA) indicated that heat stress induced an oxidative stress in cucumber seedlings. SA treatment induced higher activities of APX and a lower level of lipid peroxidation. Ca^{2+} pretreatment further enhanced the SA-induced increase in APX activity and lowered the heat stress-induced lipid peroxidation, but EGTA pretreatment had a contrary effect. These results suggest that Ca^{2+} and calmodulin may be involved in the acquisition of the SA-induced thermotolerance; antioxidant enzyme system take part in the final generation of the SA-induced thermotolerance.

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