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Short Communication

Peroxide Levels and the Activities of Catalase, Peroxidase, and Indoleacetic Acid Oxidase during and after Chilling Cucumber Seedlings

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ABSTRACT

The activities of catalase, peroxidase, indoleacetic acid (IAA) oxidase and peroxide levels in cucumber plants during and after chilling were determined. During 96 hours at 5 C and 85% relative humidity, catalase activity declined, IAA oxidase activity increased, and peroxide concentrations increased. Peroxidase activity was not affected by chilling. When chilled plants were returned to 25 C to recover, enzyme activities and peroxide concentration were restored to their prechilling levels. The increase in peroxide and IAA oxidase activity may inactivate or destroy IAA and thus retard growth.

Chilling injury is characteristic of tropical and subtropical plants. Although symptoms of chilling injury have been described extensively (11, 15, 16), it seems desirable to investigate further the biochemical aspect of chilling injury and recovery. During chilling, soluble nitrogen increased in roots of cotton seedlings (10), and the concentration of some free amino acids increased in okra leaves (15). These studies measured changes only during the chilling period. Stewart and Guinn (22) reported that cotton plants returned to optimum conditions after chilling restored initial ATP concentrations when chilled 1 day, but not when chilled 2 days. When cotton seedlings were exposed to 5 or 10 C for 48 h, $^{14}CO_2$ fixation and protein synthesis increased immediately after chilling (14).

Recently, attention has been given to mechanisms of chilling injury. Lowered respiratory activity in mitochondria isolated from sensitive plants was correlated with a phase transition of lipid (12, 18). Rapid leakage of electrolytes was reported by Wright and Simon (24) and by Smith and Powell (21). The work reported in this paper was undertaken to study effects of chilling and recovery from chilling on peroxidase, catalase, IAA oxidase and H_2O_2 levels in cucumber cotyledons.

MATERIALS AND METHODS

Cucumber seeds (*Cucumis sativus* L. var. Marketmore) were washed in 5% Clorox solution for 1 min, washed, and then planted in Vermiculite. Seedlings were grown at 25 C in controlled growth chambers with a 14-h light period and light intensity of 5,292 μw cm^{-2} for 10 days. Seedlings were then placed either at 5 C or in identical growth chambers at 25 C and 85% RH.

At various intervals, cucumber cotyledons were excised and homogenized with (1:1, w/v) 0.2 M phosphate buffer (pH 7.0) in a cold mortar and pestle. The homogenate was strained through four layers of cheesecloth and centrifuged at 27,000*g* for 20 min in a refrigerated centrifuge. The supernatant was used for assays of peroxidase and catalase activities. For peroxidase assay, the technique of Putter (17) was modified in that 3.8 ml of the assay mixture contained 80 mM phosphate buffer (pH 7.0), 3.5 mM guaiacol, 0.156 mM H_2O_2, and 0.2 ml of enzyme. The rate change in *A* at 436 nm was measured using a Perkin-Elmer (Coleman 124) double-beam spectrophotometer. Catalase activity was determined as μmol of H_2O_2 degraded per min by the methods of Chance and Maehly (6) with the following modifications. The assay mixture contained 80 mM phosphate buffer (pH 7.0); 40 mM H_2O_2 was titrated with 0.005 N potassium permanganate.

The IAA oxidase activity was determined using a modified technique of Bohnsack and Albert (3). Fifty 5-mm-diameter leaf discs were punched from cotyledons and placed in incubation flasks containing 10 ml of 0.05 mM 2,4 dichlorophenol, 25 μg/ml IAA, and 150 mM citric acid phosphate buffer (pH 5.6). The flasks were placed in a water bath and the solution in the flask was tested for residual IAA. Aliquots of the solution were placed in test tubes with Salkowski reagent (40 ml of 35% perchloric acid, 1 ml of 0.5 M $FeCl_3$) and allowed to develop for 1 h. *A* was measured on a Perkin-Elmer (Coleman 124) spectrophotometer (540 nm). Residual IAA was determined against a standard curve. The leaf discs were removed from the flask, dried in an oven at 80 C, and weighed. The IAA oxidase activity was expressed as μg IAA degraded/h·g dry weight. Peroxide content was determined using the technique described by Brennan and Frenkel (5) except PVP was added to the homogenate.

RESULTS AND DISCUSSION

There were no significant differences in peroxidase activity in plants placed at 5 and 25 C for treatment or during recovery at 25 C. Changes in *A* were about 0.83 units/min regardless of treatment. These results indicate that the rate of H_2O_2 utilization by this enzyme is unchanged by chilling. Catalase activity declined during 96 h of chilling (Fig. 1A). A decrease in catalase activity during chilling with unchanged peroxidase activity could lead to the observed accumulation of H_2O_2 in tissue (Fig. 1B) since there was no increase in peroxidase activity to compensate for the slow H_2O_2 removal by catalase. When cucumber plants were transferred to the control temperature, catalase activity was restored to its normal levels within 48 h. It is not clear whether this is due to an increase in catalase activity, increased synthesis of the enzyme, or both. Peroxide concentration increased during 96 h of chilling probably in part because of the inhibition of catalase activity (Fig. 1B). In control plant tissue peroxides did not accumulate to a significant amount.

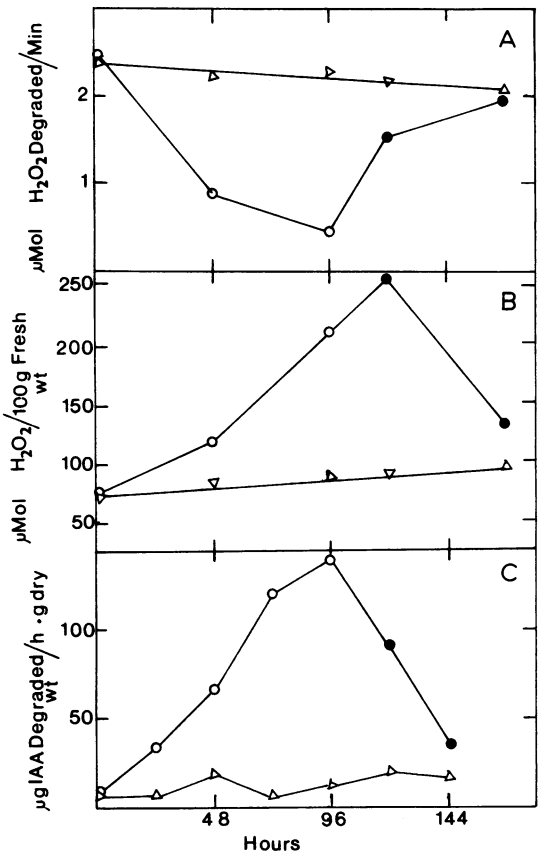

FIG. 1. Effects of chilling on: (A) catalase activity; (B) peroxide concentrations; (C) IAA oxidase activity. (O——O) chilled plants (5 C); (●——●) recovery (25 C); (Δ——Δ) control plants (25 C).

Peroxide accumulation may cause changes in plant metabolism in several ways. They may oxidize sulfhydryl groups (23), and in combination with superoxides they can form hydroxyl radicals which may be involved in the aging process (1). H_2O_2 may be involved also in the oxidative breakdown of IAA (20, 23). Omran (13) has shown that increased H_2O_2 levels inactivate IAA. This inactivation was reversed upon the introduction of catalase. In the present work, the growth of the chilled cucumber plants was very slow, peroxide level was high, and the catalase activity was low. However, when these plants were transferred to the control temperature, catalase activity and peroxide concentrations returned to normal within 48 h and plants resumed their normal growth rate after a week.

A marked increase in IAA oxidase activity was observed during 96 h of chilling (Fig. 1C). In view of the known regulation of cell growth by IAA and the inverse correlation of IAA oxidase activity with growth (8), it is likely that the enzymic destruction of IAA is important in regulating the amount of growth substance in plants. Hence, the increase in IAA oxidase may cause the destruction of IAA, therefore, affecting growth. Bolduc *et al.* (4) reported an increase in IAA oxidase activity during cold treatment of winter wheat seedlings. These results suggest that the increase in IAA oxidase activity by cold treatment should keep endogenous auxins at a low level. IAA oxidase may be an inducible enzyme (3, 8) and thus chilling is one of the stimuli that may cause its induction (2, 7, 9). Our results appear to be in agreement with the reported work. The increase in peroxide level during chilling probably stimulated IAA oxidase activity. When the cucumber plants were returned to the control temperature, IAA oxidase activity dropped to its normal level within 48 h. Since IAA oxidase may be part of an oxidizing complex of polyphenoloxidase and peroxidase (1), and since peroxidase has been implicated in lignification (19), the increased IAA oxidase activity during chilling could lead to an increase in lignification.

In conclusion, the accumulation of peroxides as a result of catalase inhibition may be a contributing factor in chilling injury. The increased activity of IAA oxidase may cause the retardation of growth generally observed during chilling. Chilled plants, when returned to 25 C, were able to restore their initial levels of metabolic activities after 96 h at 5 C.

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