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Adaptive Changes in ATPase Activity in the Cells of Winter Wheat Seedlings during Cold Hardening

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ABSTRACT

A cytochemical study of ATPase activity in the cells of cold hardened and nonhardened winter wheat (*Triticum aestivum* L. cv. *Nongke No. 1*) seedlings was carried out by electron microscopic observation of lead phosphate precipitation. ATPase activity associated with various cellular organelles was altered during cold hardening. (a) At 22°C, high plasmalemma ATPase activity was observed in both cold hardened and nonhardened tissues; at 5°C, high activity of plasmalemma ATPase was observed in hardened tissues, but not in unhardened tissues. (b) In nonhardened tissues, tonoplast and vacuoles did not exhibit high ATPase activity at either 22 or 5°C, while in hardened tissues high activity was observed at both temperatures. (c) At 5°C, ATPase activity of nucleoli and chromatin was decreased in hardened tissues, but not in nonhardened tissues. It is suggested that adaptive changes in ATPase activity associated with a particular cellular organelle or membrane may be associated with the development of frost resistance of winter wheat seedlings.

The relation of cell membrane systems to cold injury of plants has been demonstrated by many experiments (7, 15, 18). ATPase is an enzyme which is associated with membranes and plays an important role in physiological functions.

Recently, we reported that at 5°C high ATPase activity was observed in the tillering node cells of winter wheat seedlings hardened in late autumn and early winter (8). The fact indicates that ATPase activity at low temperature may be closely related to cold hardening. The present paper deals with further studies of ATPase activity at low temperature in winter wheat seedlings during cold hardening.

MATERIALS AND METHODS

Winter wheat, *Triticum aestivum* L. cv. *Nongke No. 1*, was used as the experimental material. Seedlings at two different growth stages were subjected to low temperature for cold hardening. One lot of seeds was directly sown in pots with sandy/loam soil and held at 15 to 20°C with 12 h daylength. Plants were subjected to cold hardening when they reached the 3-leaf stage after about 20 d. Another lot of seeds was soaked in water for 12 h, placed on moist filter paper in Petri dishes, and allowed to germinate at 25°C in the culture chamber in dark for 1 d before cold hardening. The young sprout about 1 cm in length and seedlings at the 3-leaf stage grown at 15 to 20°C were used as controls.

Cold Hardening and Hardiness Evaluation. Seedlings were subjected to cold hardening under the following conditions: (a) seedlings at the 3-leaf stage were hardened for 10 d at 2 to 7°C during the day with 9 h light and at 2°C in a refrigerator at night, and (b) young sprouts were hardened for 20 d at 2°C in a refrigerator in the dark.

Plants were transferred directly to −3°C and −8°C for 24 h and then were gradually thawed at 3°C. Cold hardiness was expressed as the percentage of survival as compared to unfrozen controls.

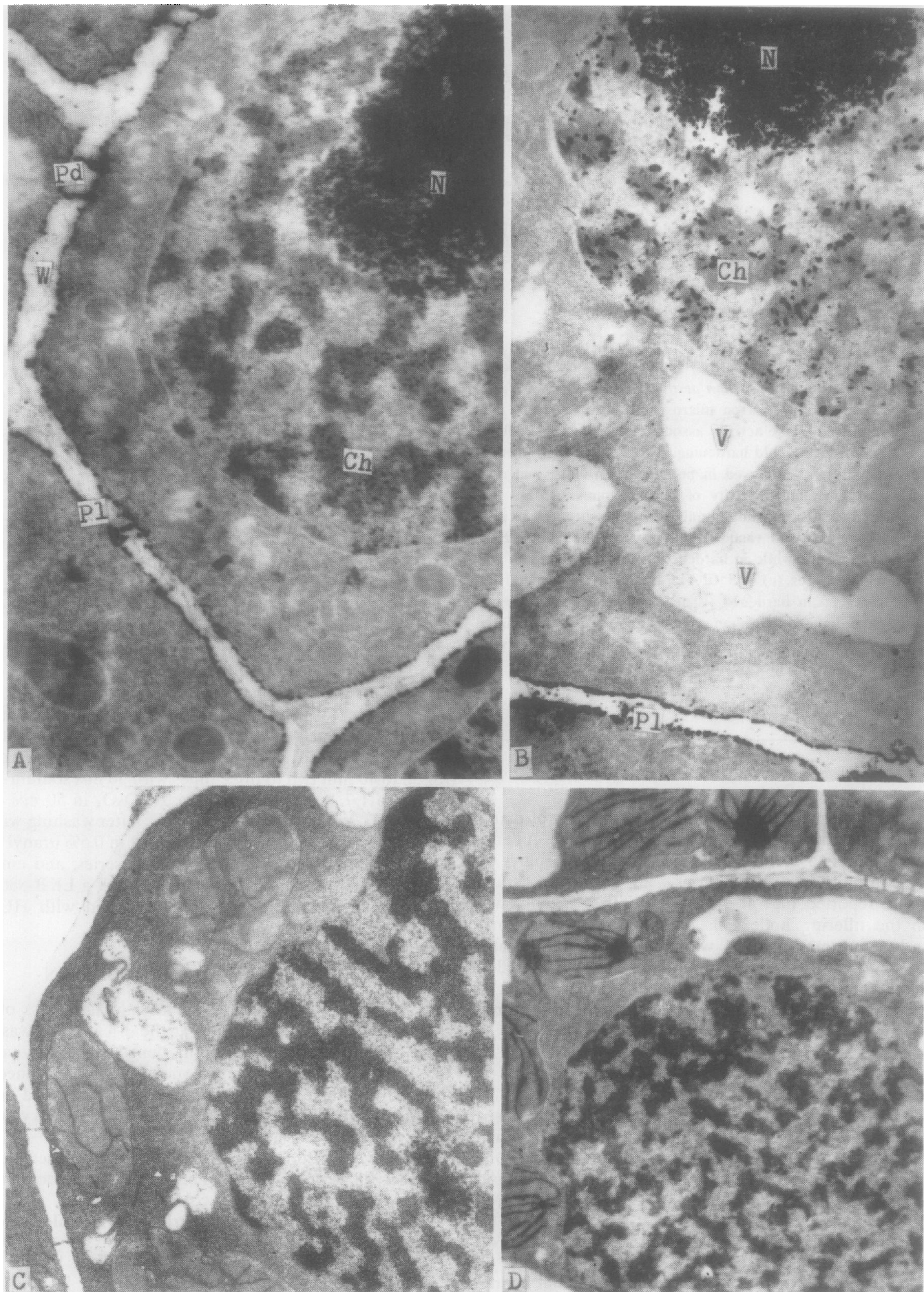
Cytochemical Reactions and Electron Microscopic Observations. The samples used for determining enzymic cytochemical reactions were prepared as follows. Young leaves about 1 cm in length were cut into 0.5 × 0.5 mm slices and fixed immediately with 4% formaldehyde-3% glutaraldehyde in 50 mM sodium-cacodylate buffer (pH 7.2) for 75 min at 22°C. After fixation, samples were washed twice, each time for 45 min, with 50 mM sodium-cacodylate buffer (pH 7.2), and further washed for 1 to 2 h with 50 mM Tris-maleate buffer (pH 7.2). Then the leaf slices were incubated in a modified Wachstein-Meisel medium (4, 8, 19) containing 3 mM $Pb(NO_3)_2$, 5 mM $MgSO_4$, and 2 mM ATP in 50 mM Tris-maleate buffer at pH 7.2. The incubation was carried out at 22°C for 2.5 h for one group of tissues and at 5°C for 5 h for a second set of tissues. Control slices were incubated in: (a) medium without ATP substrate, and b) medium with fluoride inhibitor (NaF 0.01 M). After incubation, leaf slices were washed for 30 to 60 min two to three times with 50 mM sodium-cacodylate buffer (pH 7.2), and postfixed in 2% OsO_4 in 50 mM cacodylate buffer (pH 7.2) overnight at 2 to 3°C. After washing with distilled H_2O for 3 h, the leaf slices were stained in 0.5% uranyl acetate for 45 min, dehydrated in graded ethanol series, and embedded in Epon 812. Ultrathin sections were cut with a LKB-8800 ultramicrotome, and examined and photographed with HU-11A and JEM-100CX electron microscopes.

RESULTS AND DISCUSSION

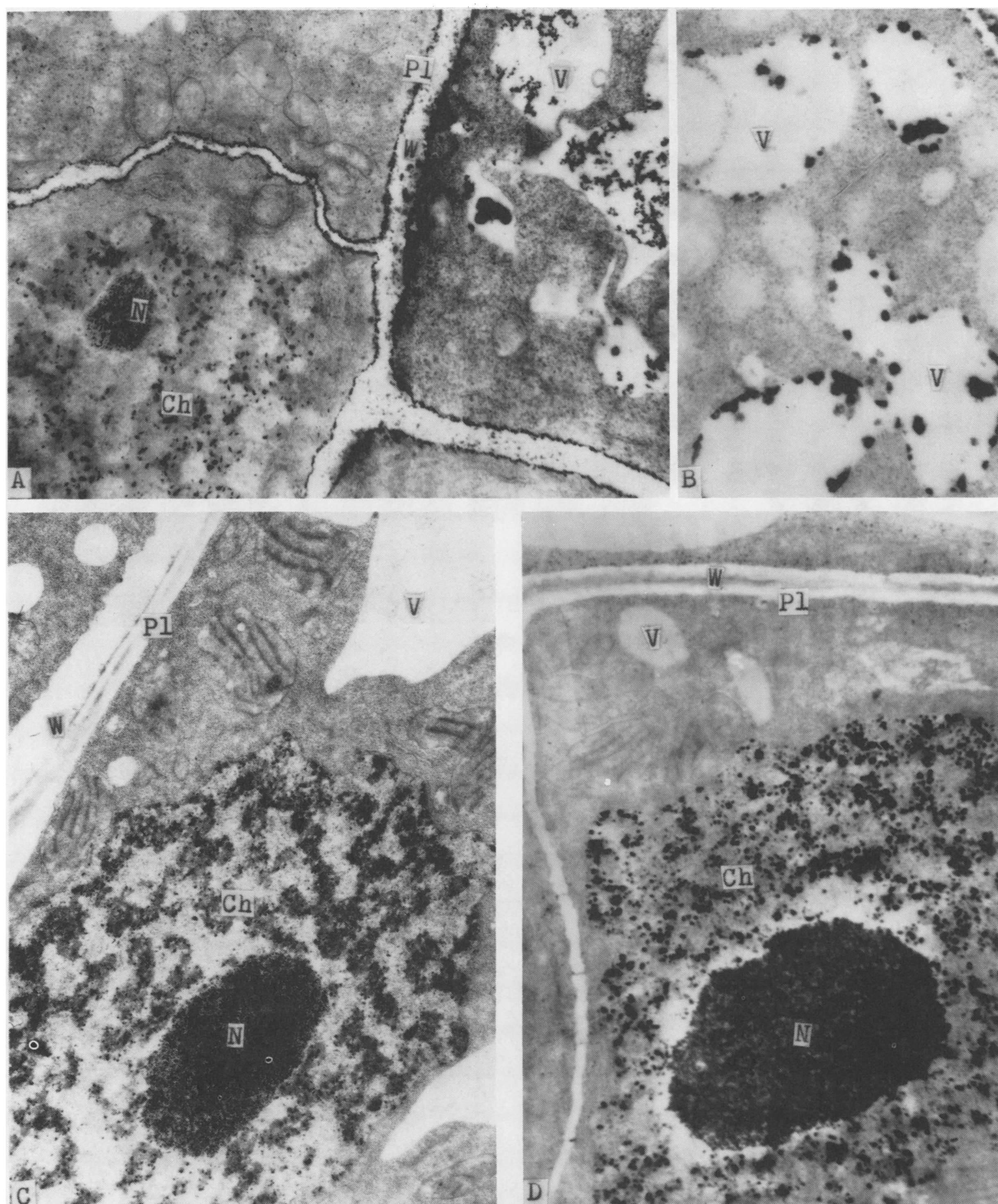
Increase in Cold Hardiness. The cold hardiness of seedlings transferred to low temperature at the 3-leaf stage was markedly increased after 10 d. All of the hardened plants survived freezing to −8°C for 24 h. In contrast, none of the nonhardened seedlings at the 3-leaf stage survived the same treatment, and only 13% of the plants survived freezing to −3°C for 24 h. When young sprouts were treated at 2°C for 20 d and then frozen at −8°C for 24 h, 91% survived. Again, all nonhardened seedlings died after freezing to either −8°C or −3°C for 24 h. An increase in cold hardiness of at least 5°C was achieved by cold acclimation.

ATPase Activity and Its Distribution in Cold-Hardened and Nonhardened Seedlings.

Incubated at 22°C. When the young leaf cells from the seedlings of control were incubated at 22°C for 2.5 h, lead phosphate deposits indicating ATPase activity were localized at the plasmalemma, plasmodesmata, nucleoli, and chromatin (Fig. 1, A and B), but were not found in the vacuoles, mitochondria, plastids, Golgi bodies, and ER (Fig. 1, A and B). When these samples were incubated in: (a) medium without ATP and (b) medium containing ATP and fluoride (NaF 0.01 M), there was little or no deposition of lead phosphate (Fig. 1, C and D). We inferred, therefore, that



FIGS. 1. A and B, the young leaf cells of nonhardened wheat seedlings at 3-leaf stage, 2.5 h incubation at 22°C. The lead phosphate deposits indicating ATPase activity reaction were localized at plasmalemma (Pl), plasmodesmata (Pd), nucleoli (N), and chromatin (Ch). W, cell wall; V, vacuoles. A, × 18,700; B, × 15,300. C and D, controls of ATPase activity reaction. C, slices were incubated in medium without ATP for 2.5 h at 22°C. D, slices were incubated in medium with 0.01 M NaF and ATP for 2.5 h at 22°C. Sections were stained with uranyl acetate and lead citrate. C, × 9,850; D, × 9,300.

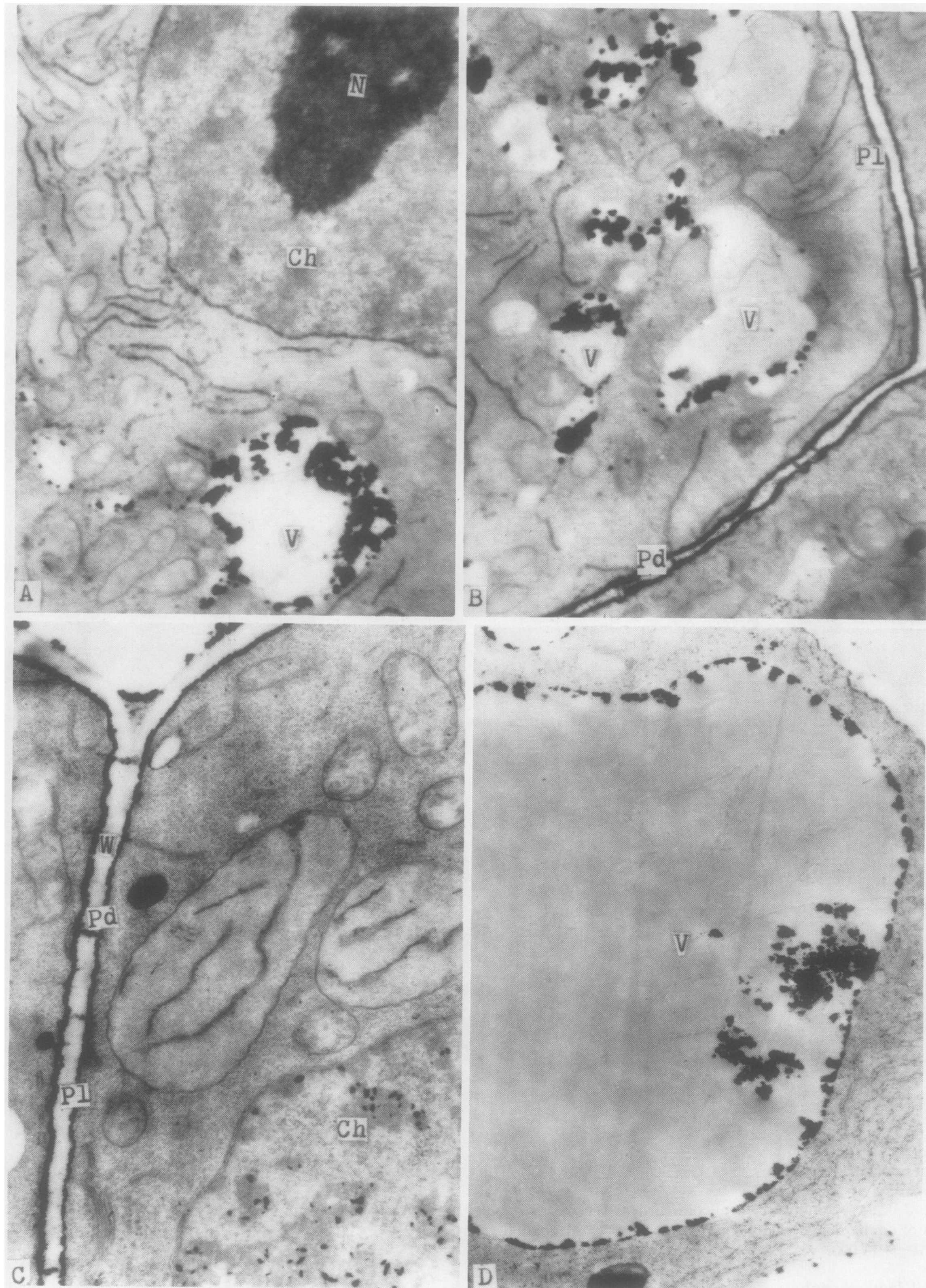


FIGS. 2. A and B, the young leaf cells of cold-hardened seedlings at 3-leaf stage were incubated for 2.5 h at 22°C. A, × 11,900; B, × 14,450. C, the young leaf cells of nonhardened seedlings at 3-leaf stage, 5 h incubation at 5°C; × 12,400. D, the young leaf cells of nonhardened young sprouts, 5 h incubation at 5°C; × 11,000.

the presence of lead phosphate deposits is the result of ATPase activity. In seedlings at the 3-leaf stage that had been cold-hardened for 10 d, reaction products of ATPase activity not only were localized at the plasmalemma, plasmodesmata, nucleoli, and chromatin, but also found on the tonoplast and in vacuoles (Fig. 2, A and B).

Incubated at 5°C. When the young leaf cells from nonhardened seedlings at the 3-leaf stage or young sprouts were incubated at 5°C for 5 h, significant ATPase activity was not observed in the plasmalemma, plasmodesmata, tonoplast, and vacuoles. Lead phosphate deposits, however, were found in nucleoli and chromatin of some cells (Fig. 2, C and D). The absence of ATPase activity on the tonoplast and in vacuoles was similar to the results observed in nonhardened seedling cells incubated at 22°C.

When the young leaf cells from cold-hardened seedlings at the 3-leaf stage were incubated at 5°C for 5 h, the sites of reaction products of ATPase activity in these cells were markedly different from those of nonhardened cells. The heavy lead phosphate



FIGS. 3. A and B, the young leaf cells of hardened seedlings at 3-leaf stage were incubated for 5 h at 5°C. A, × 11,900; B, × 10,700. C and D, the young leaf cells of hardened young sprouts, 5 h incubation at 5°C. C, × 17,000; D, × 16,000.

deposits were localized at the plasmalemma and plasmodesmata, and very high ATPase activity was also found on the tonoplasts and in the vacuoles, but ATPase activity was not found in the nucleoli and chromatin in most of the cells (Fig. 3, A and B). When the young leaf cells from cold-hardened young sprouts were incubated at 5°C for 5 h, the difference in ATPase activity between hardened and nonhardened cells was similar to that found in the seedlings at the 3-leaf stage. Very strong ATPase activity was shown at the plasmalemma, plasmodesmata, tonoplasts, and in vacuoles in hardened cells. In addition, reaction products of

ATPase activity were also found in the nucleoli and chromatin in most of the cells (Fig. 3, C and D).

From the above description, it is evident that the ATPase activity between cold-hardened and nonhardened winter wheat tissues incubated at 22 or 5°C is different. Following incubation at 22°C, ATPase activity was associated with the plasmalemma, plasmodesmata, nucleoli, and chromatin in both cold-hardened and nonhardened tissues, while ATPase activity associated with the tonoplasts and vacuoles was observed only in hardened tissues. Following incubation at 5°C, the differences in ATPase activity between hardened and nonhardened tissues were as follows: (a) ATPase activity at the plasmalemma and plasmodesmata was insignificant or absent in nonhardened tissues, while high ATPase activity was observed in cold-hardened tissues; (b) high ATPase activity in the nucleoli and chromatin was maintained in nonhardened tissues, but diminished in cold-hardened tissues; and (c) ATPase activity on the tonoplasts and in vacuoles in hardened tissues was as high as that at 22°C, while the tonoplasts and vacuoles in nonhardened tissues did not exhibit ATPase activity.

Studies on adaptive changes in some enzyme activities in plants during cold hardening have been reported (1, 5, 11–14, 17), but determinations of the activities of these enzymes were mostly carried out at the optimum temperatures. Only Kacperska-Palacz *et al.* (1974) and Kovacs *et al.* (1978) determined the activity of peroxidase at low temperatures. Their results indicated that the difference in the enzyme activity between cold-hardened and nonhardened samples was greater at low temperatures than at the optimum temperature. Our results are consistent with these observations.

Plasmalemma is a primary sensor of the cells responding to their environmental factors. Many investigators indicated that the plasmalemma may be the primary site of freezing injury (15, 18). Palta and Li (15) suggested that the primary site of sublethal freezing injury may be at the active ion-transport system of cell membranes. Recently, we studied the cytochemical changes in ATPase activity in tomato and cucumber seedlings during chilling stress. Even though the semipermeability of the cell membranes was maintained, the plasmalemma ATPase activity was greatly inhibited and inactivated during chilling (9). The present experimental results indicated that the cold-tolerant characteristic of plasmalemma ATPase activity in the cells of winter wheat seedlings could be developed during cold hardening. This seems to be of some significance. The increase of the cold-tolerance of plasmalemma ATPase activity not only enable it to avoid freezing injury, but also may be associated with the development of frost resistance of winter wheat seedlings. As well known, a close relationship exists between plasmalemma ATPase activity and active ion-transport function (2, 6, 16). Therefore, it is possible that if high plasmalemma ATPase activity can be maintained at low temperatures, ion transport will not be depressed, so that plants can function normally during the cold hardening process. During cold hardening, the ATPase at tonoplast and in vacuoles exhibited its high activity and this may be related to the accumulation of solutes in vacuoles. It has been demonstrated that the accumulation of sucrose in vacuoles of red beet tissue was associated with ATPase activity (3).

The absence of ATPase activity at the nucleoli and chromatin in hardened tissues of the winter wheat incubated at low temperature (5°C) may be related to the decrease of physiological activity of nucleoli and the rate of cell mitoses of the seedlings during low-temperature hardening in late autumn and early winter (10). It is possible that the ATPase activity plays a regulating role in the physiological activity of nucleoli and the rate of cell mitoses, since undoubtedly these processes require energy. As the ATPase activity is lowered at low temperature, the quantity of energy is decreased, the physiological activity of nucleoli and the rate of cell mitoses are also slowed down. It is well known that the decrease and cessation in both cell mitoses and growth is a preceded condition for the development of hardiness in plants (10).

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