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Changes in Isoperoxidases during Cold Treatment of Dormant Pear Embryo[1,2]

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ABSTRACT

The number of isoperoxidases and the intensity of certain isozymes increased with increasing periods of stratification of pear (*Pyrus communis* cv. Bartlett) embryos. The presence of GA_3 or kinetin during stratification enhanced the activity of certain isoperoxidases, and these enhancements were blocked in the presence of ABA which by itself had an inhibitory effect. Enhancement in isoperoxidases of pear embryos during stratification was inhibited by 6-methylpurine and cycloheximide; and in the presence of either of these two inhibitors, stratification failed to release the dormancy. Pear embryos germinated for 3 days showed changes in the pattern of isoperoxidases depending on the length of stratification.

Peroxidase may be important in the regulation of plant growth and development (2, 3, 12). Good correlation between seed viability and peroxidase activity was reported by MacLeod (9). Peroxidase activity increased during stratification in embryos of European mountain ash (1); however, no attempt has been made to study the isoperoxidase during stratification of seeds with a cold requirement. Rychter and Lewak (12) reported recently that the appearance of faster moving isoperoxidases in apple embryo was stimulated by stratification, GA_3, and BA and inhibited by ABA or coumarin. Unfortunately, the peroxidases were isolated after 3 days of germination and the hormones were added at the beginning of germination. It is not certain whether the changes in isoperoxidases being measured were a result of growth alone, growth plus stratification, or growth plus hormonal treatment. The present study investigates the changes of isoperoxidases in pear embryos during stratification and germination. The data show changes in the isoperoxidase pattern during germination and stratification with or without the presence of hormones.

MATERIALS AND METHODS

Pear seeds, *Pyrus communis* cv. Bartlett, were soaked at 5 C or 25 C on moist blotters for various lengths of time. Before and after the above treatments, seeds were soaked for 10 min in 2% sodium hypochlorite solution and then washed thoroughly with distilled H_2O. Embryos were then dissected out. In some experiments pear seeds were stratified (5 C) in the presence of 20 μM hormone solution for each hormone, or of an inhibitor solution such as 100 μg/ml 6MP[3] or 20 μM CH. Five 3-day germinated or 10 ungerminated embryos were ground in a mortar with 0.5 ml of phosphate buffer (50 mM, pH 7.8). The homogenate was centrifuged at 20,000g for 10 min. Saturated sucrose solution (0.1 ml) was added to each supernatant. All operations were carried out at 4 C.

The electrophoresis was performed by a procedure adapted from McCown *et al.* (10). The 5.5% running gels with 4.4% spacer gels were prepared in glass tubes (0.6 × 7.8 cm) which were coated with diluted Photoflo solution. The gels were preelectrophoresed for 1 hr before applying 0.1 ml of supernatant (about 0.8 mg protein, except in germination experiment) on the top of each gel. The gel electrophoresis was conducted at 4 mamps/tube for approximately 2 hr in the 0.16 M tris-glycine buffer, pH 8.3, at 4 C. After electrophoresis, the gels were soaked in 1 M acetate buffer, pH 5, at 25 C for 30 min and then stained for isoperoxidases using benzidine as an electron donor. The relative intensities of the bands were visually estimated or recorded using a Gilford scanning attachment at 420 nm. The analyzed solutions represented the same number of embryos. There were no significant changes in soluble protein content of pear embryos during 31 days of stratification. An increase in sample volume or the number of embryos used did not result in additional isozymes.

In some experiments, 10 pear embryos stratified for various lengths of time were germinated at 25 C on two layers of filter paper in 5 ml of water for 3 days before the enzyme extraction. In other experiments embryos stratified with or without the presence of an inhibitor (CH or 6MP) were washed with 50 ml of distilled H_2O by shaking for 1.5 hr with one change of H_2O at the first 0.5 hr. The embryos were then germinated in distilled H_2O or 20 μM GA_3 solution for 3 days.

RESULTS

Seven anodic isoperoxidases were detected in the extract from the nondormant pear embryos which were stratified for 30 days (Fig. 1B). However, only four anodic isoperoxidases were found in the extract from the dry pear embryos (Fig. 1A). No cathodic isoperoxidase was found in the dry pear embryo (Fig. 1C). Two cathodic isoperoxidases were observed, however, after 30 days of stratification (Fig. 1D). These two isozymes were not observed after 21 days of stratification (data not shown). Later studies were conducted only with the anodic isozymes.

The number of isoperoxidases and the intensity of certain isozymes increased with increasing periods of stratification (Fig. 2). After 1 day of stratification the isozyme pattern was essentially the same as that found in the dry embryos (Fig. 1A). The first peak without a number on the top is the interphase between spacer gel and running gel. During stratification four different types of changes in isozymes were observed: (*a*) the activity of isoperoxidase 1 remained more or less unchanged; (*b*) an increase in activity of isoperoxidase 3 occurred during the

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[2] Approved by the Director of the New York State Agricultural Experiment Station as Journal Series Paper No. 2226.
[3] Abbreviation: 6MP: 6-methylpurine; CH: cycloheximide.

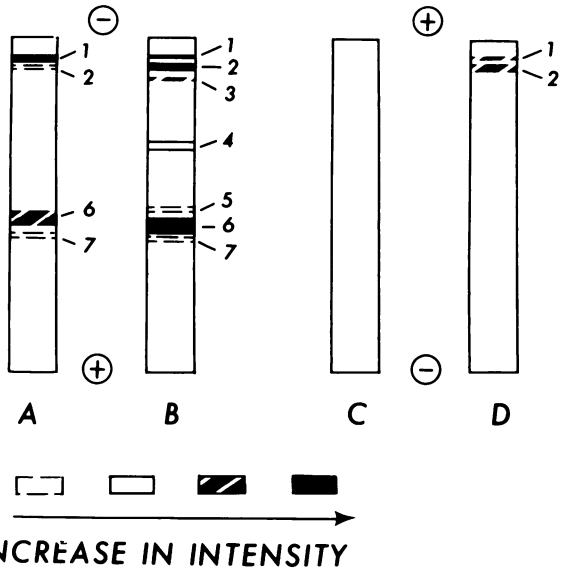

FIG. 1. Isoperoxidases of dormant and nondormant pear embryos. Isozymes were extracted from dry (A and C) or 30 day-stratified embryos (B and D). A and B: anodic isoperoxidases; C and D: cathodic isoperoxidases.

first 8 days, then remained about the same; (*c*) changes were not observed during the first 8 days, but an increase in activities of isoperoxidases 4, 5, 6, and 7 occurred at a later stage; and (*d*) the activity of isoperoxidase 2 increased progressively. When the seeds were kept at 25 C for 8 days on moistened blotters, the isozyme pattern remained unchanged as compared to that of dry embryos (data not shown). After 30 days, the isozyme pattern was similar to that of embryos stratified for 8 days; however, the levels of isoperoxidases were generally lower.

The activities of isoperoxidases were affected by the presence of plant hormones during stratification. The presence of 20 μM GA_3 enhanced the activities of all isozymes except isozyme 4 which varied in intensity from experiment to experiment (Fig. 3). Activities of certain isozymes were also enhanced by kinetin treatment (20 μM). The presence of 20 μM ABA inhibited the activities and/or synthesis of many isozymes and caused complete disappearance of isozyme 7. Inhibition of ABA was not prevented by the presence of 20 μM GA_3 or 20 μM kinetin, alone or in combination.

The appearance of several isozymes was inhibited in the presence of 100 μg/ml 6MP or 20 μM CH during stratification (Fig. 4). In the water control seven isozymes were observed; only three isozymes were observed in the presence of 6MP or CH. After 30 days of stratification in the presence of 100 μg/ml 6MP, and after the removal of 6MP by washing, these embryos behaved in a manner similar to that of unstratified embryos. They failed to germinate in distilled H_2O and their dormancy was partially broken by GA_3 (data not shown). The presence of CH during stratification gave similar results as 6MP (data not shown).

Pear embryos germinated for 3 days showed changes in the pattern of isoperoxidases depending on the length of stratification (Fig. 5). The intensity of fast moving isoperoxidases decreased as the stratification time increased. On the other hand, new isozymes appeared and activities of slow moving isozymes were promoted by an increase in stratification time.

DISCUSSION

Pear embryos require about 1 month of stratification to break the dormancy completely. Growth potential of these embryos is highly dependent on the length of stratification (8). The number of isoperoxidases and the intensity of certain isozymes increased with increasing periods of stratification (Figs. 1 and 2). Not only did changes occur during stratification, but the isoperoxidase pattern also changed after 3 days of germination (Figs. 1 and 5) and these changes were dependent on the length of stratification. The different isoperoxidases may be assumed to play different roles during seed after-ripening and seed germination.

Both kinetin and GA_3 can partially release the dormancy in pear embryos (7). ABA has been shown to induce dormancy in pear embryos (7). The presence of 20 μM GA_3 and 20 μM kinetin during stratification enhanced the activities of certain isoperoxidases and the presence of 20 μM ABA, showed an inhibitory effect (Fig. 3). These observations were similar to those found in peroxidases of other systems, such as lentil embryonic axis (3) and apple seedlings (12). The presence of ABA during stratification is known to inhibit subsequent germination of a cold-requiring seed (4). The inhibitory effect of ABA on isoperoxidases was not prevented by the presence of 20 μM GA_3 or 20 μM kinetin, alone or in combination. However, ABA inhibition of ^{32}Pi and ^{3}H-UTP incorporation into RNA of intact pear embryo and into RNA synthesized by the cell-free preparation of pear embryos (5, 7), was reversed by these hormones, alone or in combination.

The enhancement of isoperoxidases during stratification was sensitive to the presence of 6MP (Fig. 4) or CH. Thus, certain isoperoxidases could be synthesized during stratification. Isoperoxidases have been shown to be synthesized *de novo* in the embryonic axis of lentil (6). After 6MP or CH was removed by washing, the embryos remained dormant and were similar to the unstratified embryos in that GA_3 only partially released their dormancy. Peroxidases may be involved in the control of some phase of pear seed germination. However, it is quite possible that 6MP and CH may also affect other processes.

In the study of hazel seeds, the gibberellin content remained low during stratification but increased markedly after the transfer to 20 C (11, 14). Thus the cold treatment "prepares" hazel seeds for germination and hormonal changes occur mainly at germinating temperature. This seems not to be the case in pear embryos, since the ability of pear embryos to synthesize RNA was affected by length of stratification and hormone treatment in a parallel fashion, suggesting that cold treatment leads to an

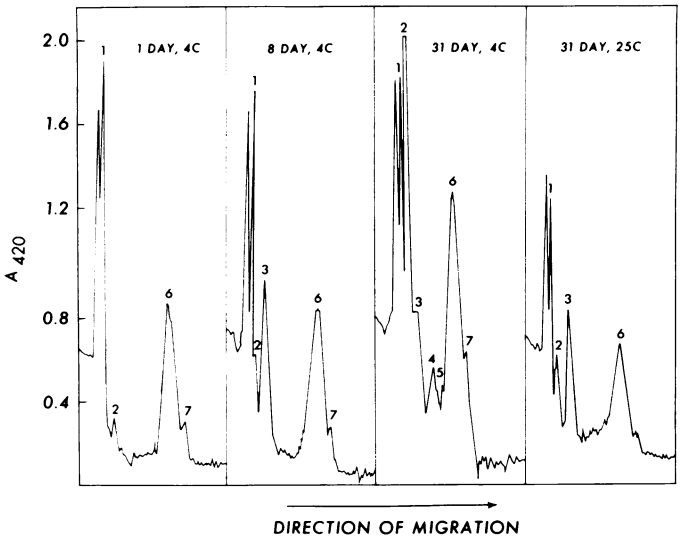

FIG. 2. Changes in isoperoxidases during stratification. Peroxidases were extracted from pear embryos kept at 4 C or 25 C on moistened blotters for various times as stated.

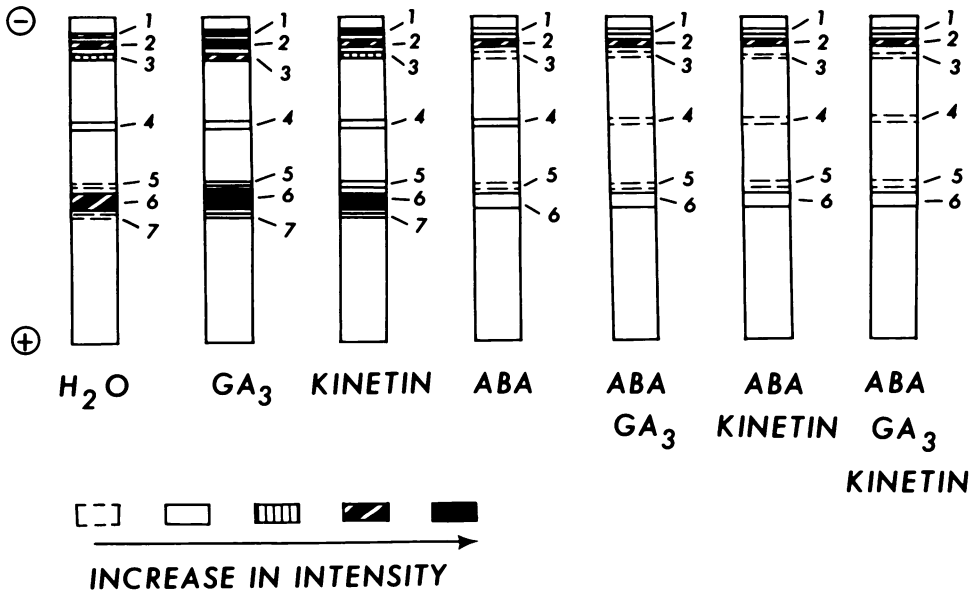

FIG. 3. Hormonal effects on the isoperoxidase patterns. Peroxidases were extracted from pear embryos stratified with or without the presence of hormones (20 μM for each) for 30 days.

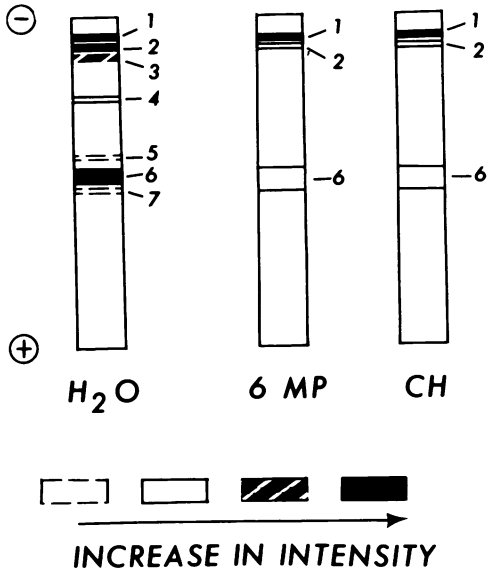

FIG. 4. Effects of 6MP and CH on the isoperoxidase patterns. Peroxidases extracted from embryos stratified in presence of 6MP (100 μg/ml) or CH (20 μM) for 31 days.

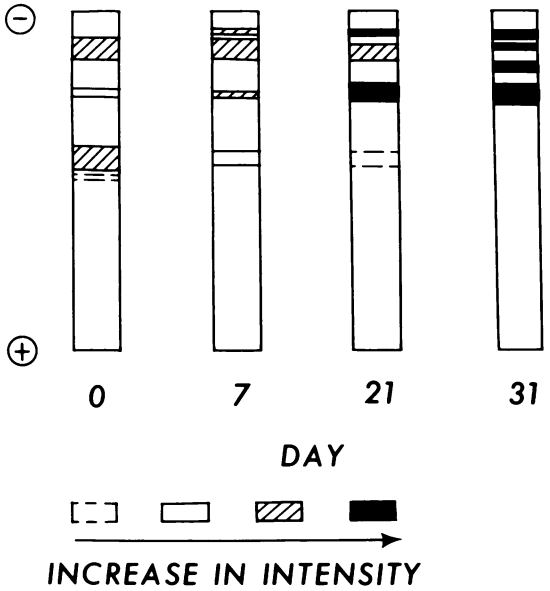

FIG. 5. Change in isoperoxidase patterns during germination of stratified embryos. Pear embryos were stratified for various lengths of time and germinated for 3 days. The soluble protein contents of 0.1-ml samples are 1, 1.1, 1.3, 1.5 mg respectively.

increase in the level of hormones (5, 7, 8). Increases in activities of peroxidases, as reported here, chromatin-bound RNA polymerase (5) and aminoacyl-tRNA synthetases (13) have been observed in pear embryo during stratification.

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