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Induction of a Pea Cell-Wall Invertase Gene by Wounding and Its Localized Expression in Phloem[1]

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A full-length cell-wall invertase cDNA obtained from pea (*Pisum sativum* L.) seedlings was cloned previously. The gene exhibits tissue-specific expression, and both its transcript and enzyme activities are abundant only in root tissues. Mechanical wounding dramatically induced the accumulation of the cell-wall invertase mRNA in detached or intact leaves, stems, and roots. In both detached and intact tissues mRNA started to accumulate 3 h after wounding and in detached tissues (except root tissues) was much stronger and lasted longer compared with that in intact pea plants. The induction of cell-wall invertase by wounding was not systemic, since no significant increase of transcript was found in the unwounded tissues remote from the site of wounding. Accumulation of this cell-wall invertase was induced by abscisic or jasmonic acid, and in situ hybridization studies show that this invertase mRNA is differentially localized in wounded plant tissue, being most abundant in the phloem. mRNA accumulation was limited mainly to the wounded area; no significant increase was detected in the unwounded portions of the wounded stem segments. The results suggest that, as part of the wounding response, this cell-wall invertase may provide energy through hexose availability to companion cells in the phloem.

Plants react to mechanical injury and pathogen infection by mounting a defense response that is characterized by the expression of a set of genes involved mainly in wound healing and the prevention of pathogen invasion. These responses include reinforcement of the cell wall by deposition of callose, lignin, and Hyp-rich glycoproteins; synthesis of the antimicrobial phytoalexins; production of proteinase inhibitors and lytic enzymes such as chitinases and glucanases (Hildmann et al., 1992); and increased respiration (Collinge and Slusarenko, 1987). Increased metabolic activity requires an enhanced flow of metabolizable substrates into the cells. Suc plays an important role in the transport of carbohydrates in plants; the turgor pressure that results from the Suc concentration gradient between source and sink organs drives Suc movement in phloem. Before it can be utilized, Suc must be cleaved into hexoses either by invertase (β-fructofuranosidase, EC 3.2.1.26) or by Suc synthase (EC 2.4.1.13). Thus, Suc-hydrolyzing enzymes play an important role in controlling net Suc movement (Patrick, 1990).

High activity of both soluble and insoluble invertases is induced by the wounding of storage roots in carrot, sweet potato, and red beet (Bacon et al., 1965; Edelman and Hall, 1965; Ricardo and ap Rees, 1970; Matsushita and Uritani, 1974; Leigh et al., 1979). Invertase activities are also stimulated by pathogen infection in corn (Billett et al., 1977) and wheat (Krishnan and Pueppke, 1988). Sturm and Chrispeels (1990) found that both cell-wall invertase activity and the level of invertase mRNA can be induced by wounding or bacterial infection in carrot storage roots and leaves. The homology of the carrot cell-wall invertase enzyme to the levan-hydrolyzing enzyme levanase suggests that it may hydrolyze the slime coat of bacterial pathogens, thereby inhibiting bacterial growth directly or making the pathogen susceptible to further defense reactions (Lorenz et al., 1995).

The isolation of a full-length cDNA for a pea (*Pisum sativum* L.) cell-wall invertase was recently reported (Zhang et al., 1996). Utilization of a part of this sequence has made it possible to investigate not only the expression of the gene but also the changes in its expression under a variety of experimental conditions in several tissues of the pea plant. Both enzyme activity and the level of mRNA transcript are higher in root tissues than in leaf or stem tissues, suggesting that this cell-wall invertase may play an important role in regulating root sink strength. Changes in expression were detected in the transcript following in vitro and in vivo mechanical wounding, as well as after application of ABA or JA. The localization of mRNA transcripts in wounded and unwounded pea tissues was examined by in situ hybridization. The data reported here, along with subsequent physiological analyses, should make it possible to examine the function of this cell-wall invertase in the context of carbohydrate partitioning under normal and stress conditions.

MATERIALS AND METHODS

Plant Materials and Reagents

Two varieties of pea (*Pisum sativum* L.) were used: the tall phenotype Alaska, homozygous or heterozygous for the internode length gene *Le*, and the dwarf Little Marvel, homozygous for the internode length genotype *le* (Cohn et al., 1994). The pea plants were grown in a greenhouse

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Abbreviation: JA, jasmonic acid.

under natural light conditions. ABA, GA_3, and JA were purchased from Sigma.

Wounding of Pea Plants

For wounding of detached tissues (in vitro), the fourth and fifth internodes of the stems and whole roots of 2-week-old pea seedlings were harvested and cut into 2- to 4-mm segments and kept on wet, sterile Whatman 3MM paper in the dark. Tissue (1 g) harvested at designated times after wounding was frozen in liquid nitrogen, and control tissue (time 0) was frozen directly after cutting. For wounding of tissues in intact plants (in vivo) the fourth and fifth internodes and the first and second true leaves were gently abraded with a fingernail file. For the study of the potential systemic transmission of the wounding signal from one part of the plant to another, the upper sixth internode and the third and fourth true leaves above the cotyledons were selected for RNA extraction and northern blotting analysis.

ABA and JA Application

ABA solutions (100 μM ABA in sterile water with 0.01% [v/v] ethanol and 2 drops of Tween 20 per L) were applied to plants by aerial spraying until the entire plant surface was uniformly moistened. Plants were sprayed in this way every 6 h for the entire 24-h test period. JA was dissolved in *N,N*-dimethylformamide (100 mM stock solution) and subsequently diluted in water to obtain a 100 μM solution. GA was dissolved directly in water to obtain a 100 μM solution. Both GA and JA solutions were applied to plants as described previously for the ABA solutions. "Petiole feeding" experiments were performed by submerging whole leaves, including the petioles, in water or 100 μM solutions of ABA, GA, or JA for 24 h in the dark.

RNA Extraction and Northern Blot Analysis

Total RNA was isolated from wounded or unwounded tissues according to the method described by Promega. Total RNA (10 μg/lane) was separated on 1.2% agarose gels containing formaldehyde (Sambrook et al., 1989). RNA gel-blot analyses were performed on nylon membranes (Zeta-Probe GT, Bio-Rad) with the probe ^{32}P-labeled by random priming (Promega). A partial-length invertase cDNA (PcI-5) produced by PCR from dwarf pea cDNA was used as a probe (Zhang et al., 1996). Prehybridization was done at 65°C in 0.25 M phosphate buffer (pH 7.2) and 7% SDS. Hybridization was carried out in the same buffer overnight at 65°C. The blots were washed twice with 50 mM phosphate buffer and 1% SDS at 65°C for 30 min. Under these hybridization conditions, only one cell-wall invertase gene hybridizes with this probe (L. Zhang, N.S. Cohn, and J.P. Mitchell, unpublished data). The same northern blot was stripped of invertase probe and rehybridized to ^{32}P-labeled H1 cDNA (Lawton and Lamb, 1987) as an internal standard. Autoradiographs were scanned with a computing densitometer (model 300A, Molecular Dynamics, Sunnyvale, CA) and were quantitatively analyzed by densitometric measurement of the PcI-5-hybridized bands normalized with the H1 measurements (Image Quant, version 3.2, Molecular Dynamics).

In Situ Hybridization

Control and 12-h-wounded (cut segments) root, leaf, and stem tissues from 2-week-old dwarf pea seedlings were fixed in 4% paraformaldehyde solution at 4°C for 24 h. After paraffin embedding, the tissues were sectioned at 10 μm thickness. Probes for the in situ hybridization were prepared using the partial-length cell-wall invertase cDNA produced by PCR (PcI-5), which was cloned into a pCR vector (Invitrogen, San Diego, CA). Both ^{35}S-labeled antisense and sense RNA probes were synthesized by in vitro transcription using SP6 and T7 polymerases as described by the manufacturer (Novagen, Madison, WI). The sense strand probes were used to detect nonspecific binding. In situ hybridization was performed according to the instructions of the manufacturer (Novagen). Labeled RNA probe (5×10^5 cpm) in 100 μL of hybridization solution was used for each slide, which was dipped in NTB-2 (Kodak) autoradiography emulsion and exposed at 4°C for 3 weeks before developing.

RESULTS

Accumulation of Cell-Wall Invertase mRNA in Detached Pea Stem and Root Segments

To determine whether wounding increases the levels of mRNA for cell-wall invertase in pea plants, total RNA was isolated from the in-vitro-wounded dwarf pea tissues at designated times. The level of invertase mRNA was determined by probing the RNA gel blot with the cell-wall invertase cDNA PcI-5. As shown in Figure 1A, unwounded dwarf pea stems had no detectable cell-wall invertase mRNA. There was no signal at 1 h, but at 3 h invertase mRNA started to accumulate and was maximally induced 24 h after wounding. Based on densitometric measurements, the invertase expression level at 24 h was 17 times higher than that at 3 h. Unwounded dwarf plant roots (Fig. 1B) had a weak RNA signal after longer exposure of the film (data not shown), and invertase mRNA was maximally induced 3 h after wounding, followed by a rapid decline during the next few hours. At 6 h the densitometry reading was one-half that at 3 h, and at 12 h the reading was at base level. Results similar to those of the dwarf plants were found in the stems of the tall plants (Fig. 1C). There was no detectable mRNA in wounded stem tissues at 1 h, and the induction of mRNA appeared 3 h after wounding, reaching its maximum level at 24 h and gradually decreasing thereafter. The same result was obtained with roots from tall plants (data not shown).

Induction of Cell-Wall Invertase mRNA in Wounded Leaf and Stem Tissues of Intact Pea Plants

The effect of mechanical wounding (with a fingernail file) was examined in the stems and leaves of intact, 2-week-old, tall pea plants. Total RNA was isolated from these tissues at intervals and analyzed by northern blotting

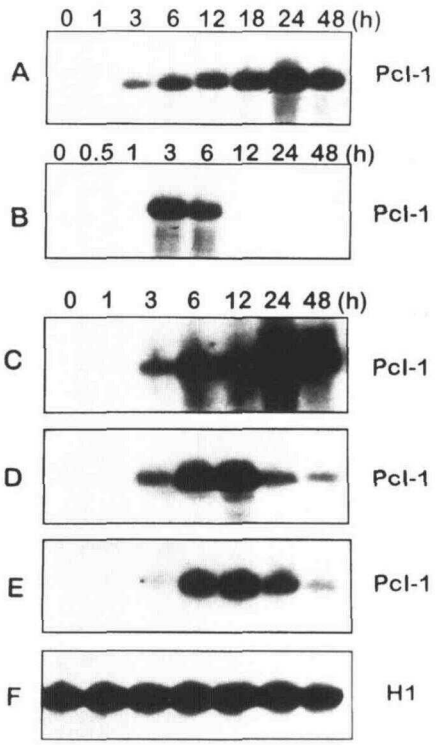

Figure 1. Time-course accumulation of cell-wall invertase mRNA in detached and intact dwarf or tall pea tissues in response to wounding. Tissues were obtained from 2-week-old seedlings. The times 0 to 48 h refer to the times after treatment when RNA was extracted. Total RNA (10 μg) was separated by electrophoresis, blotted onto a nylon membrane, and hybridized with ^{32}P-labeled PcI-5. PcI-1 identifies the mRNA transcript labeled with the PcI-5 probe. A, Dwarf pea stem wounded in vitro; B, dwarf pea root wounded in vitro; C, tall pea stem wounded in vitro; D, tall pea leaf wounded in *vivo*; E, tall pea stem wounded in vivo; F, H1 cDNA probe hybridized with RNA gel blot E as an internal control. Each set of samples (A–E) was stripped and rehybridized with H1.

with PcI-5 as a probe (Fig. 1, D and E). As expected, no signal was detectable in RNA that was isolated from leaves and stems prior to wounding (lane 0). Clear hybridization bands were obtained in the RNA samples from both stem and leaf tissues 3 h after wounding, although minimally in stems (Fig. 1E). The levels of cell-wall invertase mRNA increased gradually and reached a maximum 12 h after wounding in both stem and leaf tissues. Based on densitometric analysis of the hybridization bands, the leaf samples were 3 to 5 times stronger than the stem samples at 3, 6, and 12 h. Similar results were also found in dwarf pea plants (data not shown). The consistency of equal loading of the mRNA samples was established by probing the stripped RNA gel blot with H1 cDNA, which was unaffected by wounding (Fig. 1F). The H1 hybridizations are shown for Figure 1E only but were done for all samples.

Systemic Transmission of the Wounding Signals in Leaves and Stems

It was interesting to determine the extent of systemic induction of expression of the cell-wall invertase gene in the unwounded tissue of Alaska pea plants in which the leaves or stems had been wounded. The fourth and fifth internodes and the first and second true leaves of 2-week-old pea seedlings were wounded with a fingernail file. After 12 h RNA was isolated from the wounded leaves and stems, as well as from the unwounded sixth internode and the third and fourth true leaves. After electrophoretic separation and transfer to nylon membranes these RNAs were analyzed by northern blotting with PcI-5 as a probe. The results are shown in Figure 2. No signal was detectable in RNA that was isolated from leaves or stems prior to wounding, whereas strong signals were obtained in RNA that was isolated from 12-h-wounded leaf and stem tissue. Densitometry indicated that the signal in the wounded leaf tissue was 2.6 times stronger than that in the wounded stem. However, only a low level of invertase mRNA was detected in the samples of RNA that were isolated from the unwounded sixth internode located just above the wounded tissues, and there was no detectable signal in upper unwounded leaves. Similar results were also found in dwarf pea plants (data not shown).

Induction of Cell-Wall Invertase Gene by ABA and JA

Several studies have indicated that ABA and JA are involved in wound-induced gene expression (Hildmann et al., 1992; Peña-Cortés et al., 1995). To determine whether the cell-wall invertase gene can be induced by ABA or JA, detached leaves, including their petioles, from 2-week-old Alaska pea seedlings were incubated with 100 μM solutions of ABA or JA for 24 h in the dark. As a control, detached leaves of the same age were incubated with water or 100 μM solution of GA. After 24 h RNA was isolated from different hormone-treated and control leaves and analyzed for the expression of the cell-wall invertase gene by RNA blot analysis. As shown in Figure 3, cell-wall invertase mRNA accumulated in ABA-incubated and JA-incubated detached leaves. Control leaves incubated with water or 100 μM solution of GA did not show any accumulation of cell-wall invertase mRNA. Spraying intact plants with 100 μM solution of ABA or JA, however, produced signals much weaker than those from petiole feeding experiments (data not shown). Identical responses were obtained by using dwarf pea plants (data not shown). Cell-wall invertase enzyme activity in leaf extracts can also be induced by wounding, ABA, or JA treatment, although there is a lag of about 20 h between mRNA appearance and increased en-

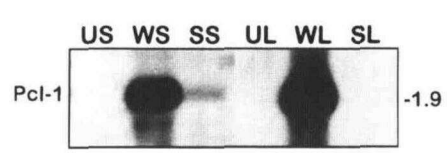

Figure 2. Effect of wounding on the expression of the cell-wall invertase gene in tall pea plants. Total RNA (10 μg) isolated from a nonwounded stem (US), a 12-h-wounded stem (WS), a systemic stem (SS), an unwounded leaf (UL), a 12-h-wounded leaf (WL), and a systemic leaf (SL) was separated by electrophoresis, blotted onto nylon membrane, and hybridized with ^{32}P-labeled PcI-5. PcI-1 (1.9 kb) identifies the mRNA transcript labeled with the PcI-5 probe.

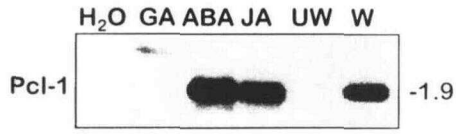

Figure 3. Cell-wall invertase mRNA expression in pea leaves upon wounding, GA feeding, ABA feeding, and JA feeding through the petiole. Total RNA (10 μg) isolated from H_2O-incubated (H_2O); GA-incubated (GA); ABA-incubated (ABA); JA-incubated (JA); non-wounded (UW), and 24-h-wounded (W) leaves was separated by electrophoresis, blotted onto a nylon membrane, and hybridized with ^{32}P-labeled Pcl-5. Pcl-1 (1.9 kb) identifies the mRNA transcript labeled with the Pcl-5 probe.

zyme activity (D. Kim, J.P. Mitchell, and N.S. Cohn, unpublished data).

Cell-Wall Invertase mRNA Localization in Tissues of Wounded and Unwounded Pea Plants

Localization of cell-wall invertase mRNA in both wounded and unwounded tissues from 2-week-old dwarf pea plants was studied by in situ hybridization (Figs. 4 and 5). A ^{35}S-labeled RNA sense probe was used to detect nonspecific binding. The light regions in Figure 4, 2A, are due to the light-scattering effects of the thick cell walls of xylem vessels and phloem fibers. In both wounded leaf and stem tissues there was a differential distribution of cell-wall invertase mRNA, with the heaviest label in the phloem tissue (Figs. 4 and 5, 2C and 2D). Some cell-wall invertase mRNA was also detected in epidermal cells and xylem tissue. In unwounded stem tissues very weak labeling was detected in phloem tissue (Fig. 4, 2B), and no significant signals were detected in unwounded leaf tissues (Fig. 5, 2B). In 12-h-wounded stems there was a great increase of cell-wall invertase mRNA in phloem tissue (Fig. 4, 2C and 2D). The accumulation of cell-wall invertase mRNA was mainly limited to the wounded area, with no significant increase detected in the unwounded parts of the wounded stem segments (data not shown). In 12-h-wounded leaves there was a dramatic increase of cell-wall invertase mRNA in the phloem tissue along the midveins, with the strongest signals at the tips of the midveins (Fig. 5, 2C and 2D). Weak signals were observed in the phloem tissues of the unwounded and 12-h-wounded roots (data not shown).

DISCUSSION

Pea Cell-Wall Invertase Gene Is a Wound-Inducible Gene

Expression of the cell-wall invertase gene, as determined by northern blot hybridizations, was not found in the flowers, flower buds, young seeds, fruits, stems, or leaves in unwounded plants (L. Zhang, N.S. Cohen, and J.P. Mitchell, unpublished data). However, expression is found constitutively in the root and can be dramatically induced by wounding detached or intact stem, leaf, and root tissues. There is a gradual accumulation of cell-wall invertase mRNA in wounded detached stem tissues, with the maximum expression 24 h after wounding. In wounded detached root tissues, however, cell-wall invertase mRNA increased to a maximum level only 3 h after wounding and decreased afterward. In unwounded roots and in roots examined at 0.5, 1, 12, 24, and 48 h after wounding, there is a constitutive low level of expression producing measurable amounts of invertase (data not shown).

The root is a strong sink organ that depends on Suc from source organs. In detached root tissues, however, with increased incubation time Suc concentration will gradually decrease. In turn, this may induce the repression of the cell-wall invertase gene. The induction of cell-wall invertase gene expression by Suc as well as Glc was reported by Roitsch et al. (1995) in a *Chenopodium rubrum* cell suspension system. We also found that the cell-wall invertase gene can be induced in detached leaves by Suc treatment (L. Zhang, N.S. Cohen, and J.P. Mitchell, unpublished data). In stress situations such as wounding or pathogen infection,

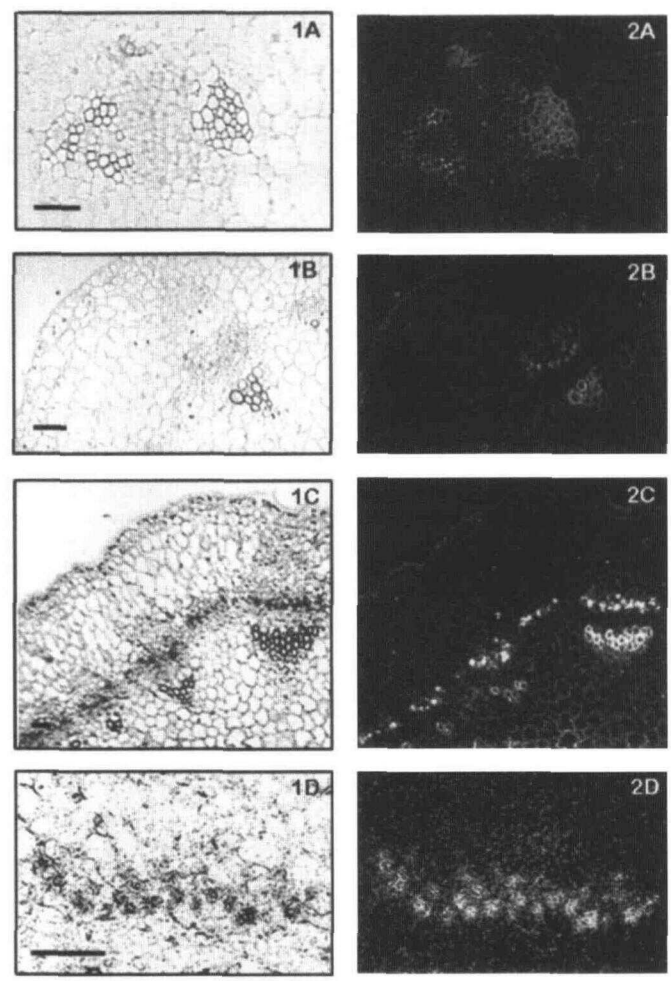

Figure 4. Cell-wall invertase mRNA localization in unwounded and 12-h-wounded stem tissues from dwarf pea plants. The cross-sections of stem were hybridized with ^{35}S-labeled sense (row A) or antisense (rows B–D) RNA probes prepared from the partial-length cell-wall invertase cDNA (Pcl-5). 1A, 12-h-wounded stem tissues; 1B, unwounded stem tissues; 1C, 12-h-wounded stem tissues; 1D, 12-h-wounded stem tissues. Photographs in column 1 were taken using bright-field optics; photographs in column 2 were taken using dark-field optics with the same magnification. The bars = 80 μm.

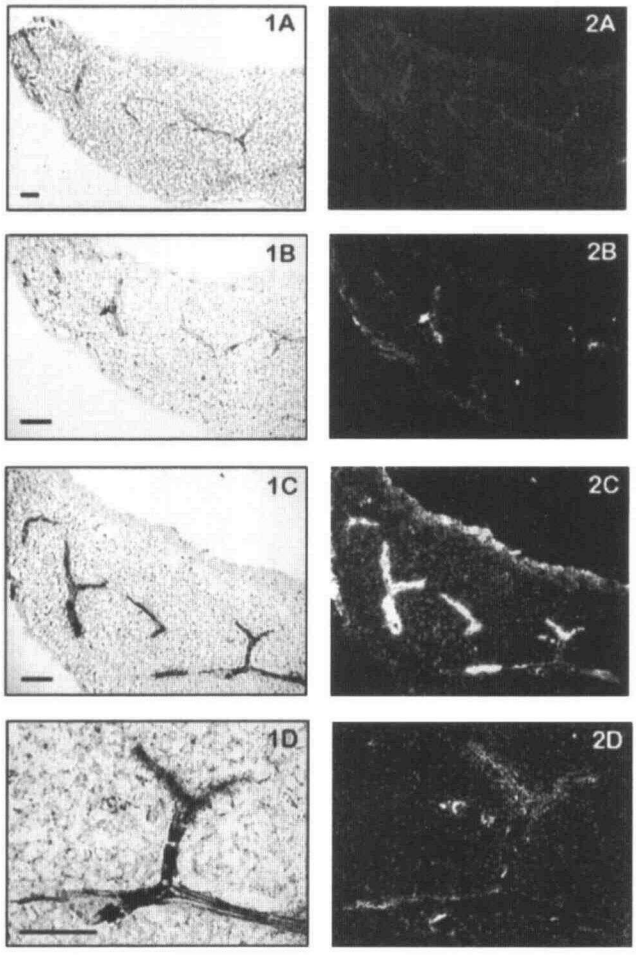

Figure 5. Cell-wall invertase mRNA localization in unwounded and 12-h-wounded leaf tissues from dwarf pea plants. The longitudinal sections of leaf were hybridized with ^{35}S-labeled sense (row A) or antisense (rows B–D) RNA probes prepared from the partial-length cell-wall invertase cDNA (PcI-5). 1A, 12-h-wounded leaf tissues; 1B, unwounded leaf tissues; 1C, 12-h-wounded leaf tissues; 1D, 12-h-wounded leaf tissues. Photographs in column 1 were taken using bright-field optics; photographs in column 2 were taken using dark-field optics with the same magnification. The bars = 80 μm.

cells have a high demand for hexoses to fulfill the energy and carbon requirements needed for an adequate response (Herbers et al., 1996).

In intact tall and dwarf plants mechanical wounding can induce the expression of the cell-wall invertase gene in both leaf and stem tissues. However, cell-wall invertase gene expression is much lower in wounded stem tissues of intact plants than in detached stem tissues (6- to 7-fold lower, based on densitometry). Furthermore, cell-wall invertase gene expression in wounded leaves of intact plants is much higher than in wounded stems. The difference in the expression level of the gene may be related to the area and/or intensity of wounding. Compared with the stem, the leaf has a larger wounded area, perhaps accounting for the higher gene expression in wounded leaf tissue.

Induction of Cell-Wall Invertase by Wounding Is Not Systemic

Some wound-inducible genes such as proteinase inhibitor 2 can be activated not only in the area close to the wound site but also in undamaged tissues remote from the site of wounding (Hildmann et al., 1992). This systemic induction in unwounded tissue can move both acropetally and basipetally in potato plants. Northern blot results show that the induction of cell-wall invertase gene expression by wounding in peas is not systemic. This result was obtained consistently in several independent experiments with both tall and dwarf plants, demonstrating that the activation of the cell-wall invertase gene is limited to the wounded area; in situ hybridization results also support this conclusion. Cell-wall invertase mRNA is abundantly localized in the wounded section of stem tissues. Upon wounding, plants enter a state of high metabolic activity that may lead to healing of the wounded area and prevention of subsequent pathogen invasion (Herbers et al., 1996). Cells in the wounded area have a high demand for hexoses, and cell-wall invertase is found in the apoplast, serving to hydrolyze Suc into hexoses (Sturm and Chrispeels, 1990).

The wounding signals that induce the production of cell-wall invertase mRNA are localized and not transmissible; they can reach only a limited area around the damaged tissues. Some wounding signals, such as oligogalacturonides isolated from plant cell walls, can induce the expression of proteinase inhibitor genes when supplied through the cut petioles of excised leaves (Walker-Simmons et al., 1984), but these signals may not be systemic, because their mobility through the phloem is limited (Baydoun and Fry, 1985).

Accumulation of Cell-Wall Invertase Can Be Induced by ABA and JA

Results of several studies point to the involvement of ABA and JA in wound-induced gene expression (Hildmann et al., 1992; Peña-Cortés et al., 1995). Exogenous application of ABA has been shown to induce a systemic pattern of proteinase inhibitor 2 mRNA accumulation identical with that induced by mechanical wounding. The application of methyl jasmonate can also induce a significant accumulation of proteinase inhibitor 2 transcripts, both in wild-type and in ABA-deficient plants (Hildmann et al., 1992), thus supporting a role for JA as an intermediate in the signaling pathway that leads from ABA accumulation in response to wounding to transcriptional activation. In our study cell-wall invertase gene expression in leaf was induced by the exogenous application of ABA or JA. Different methods of ABA and JA application (spraying or petiole feeding) produced similar effects on cell-wall invertase gene expression, but petiole feeding can produce stronger signals than spraying. The difference may be due to the absorption of a greater amount of ABA or JA with petiole feeding than with spraying intact plant leaves.

The application of GA_3 has no effect on the cell-wall invertase gene in pea leaves or internodes. According to the wounding signal transport pathway proposed by

Peña-Cortés et al. (1995), mechanical injury may generate changes in plasma membrane potential, creating electrical signals that may propagate throughout the plant and acting as the systemic inducing signal. Wound-induced action potential may cause variation of ion concentration, leading to the activation of a mechanism that elicits the release of the active, systemically mobile, small oligopeptide systemin. The systemin and/or electrical signal may induce the production of ABA, which may turn on the biosynthesis of JA, and JA, in turn, stimulates the production of the wound-inducible mRNA. Why the induction of the pea cell-wall invertase mRNA by wounding is not systemic, but can by regulated by ABA or JA, is not clear. Similar results were found in potatoes by Hildmann et al. (1992); two of four ABA/wound-responsive genes were not systemically inducible. All four genes were strongly induced by JA in both wild-type and ABA-deficient potato plants. Their explanation is that ABA is not directly involved in the signal pathway as a long-range signal but has an indirect function in the inductive pathway.

Cell-Wall Invertase mRNA Is Abundant in Phloem Tissue

Suc is transported to the sink organs through the phloem and is taken up by plant cells either after hydrolysis into Glc and Fru or as Suc. Some investigators have suggested that cell-wall-bound invertase may play a role in phloem loading and/or unloading by maintaining a steep Suc concentration gradient between the source and sink regions of a plant (Eschrich, 1980; Morris and Arthur, 1985). Tomlinson et al. (1991) found that the soluble invertase activity in vascular bundles isolated from grapefruit was higher than that in surrounding tissues, indicating a role for this enzyme in Suc translocation, but no invertase activity has been detected in sieve tube sap (Lehmann, 1973; Geigenberger et al., 1993). Using an immunocytochemical localization study, Wu et al. (1993) found that pea cell-wall invertase enzyme accumulated primarily in the cell walls, with only a few immunogold particles observed in the cytoplasm of the cell. The density of immunogold particles was abundant in phloem cells.

Until our study no information was available, to our knowledge, about the location of invertase mRNA. The results reported here, however, show that cell-wall invertase mRNA is localized principally in the phloem tissue. Cell-wall invertase mRNA was also detected in epidermal cells and xylem, the latter possibly representing only a nonspecific binding in some of the vessel walls. The distribution of cell-wall invertase mRNA is consistent with the localization of the invertase enzyme (Wu et al., 1993). The physiological significance of cell-wall invertase in the phloem tissue may be (a) the maintenance of a steep Suc concentration gradient between the source and sink regions of a plant or (b) the provision of hexose, and thus energy, for the metabolism of companion cells. Similar hypotheses for the physiological significance of Suc synthase in companion cells have been proposed (Nolte and Koch, 1993).

Overexpression of yeast invertase in the apoplast of transgenic tomato plants resulted in the inhibition of Suc export in leaves and, in turn, inhibition of photosynthesis (von Schaewen et al., 1990; Dickinson et al., 1991; Sonnewald et al., 1991; Heineke et al., 1992). The dramatic increase of pea cell-wall invertase mRNA in the phloem tissue along the midveins, with the strongest signals at the tips of midveins, suggests the possibility that the export of Suc may be greatly inhibited in wounded leaves. The increase of invertase mRNA in epidermal cells may be because they are the actual wounded cells, but the presence of small quantities of invertase mRNA in xylem tissues is not understood. There is no significant increase in cell-wall invertase mRNA in the unwounded parts of wounded plants. Consistent with the low expression level of mRNA in normal pea tissues detected by northern blotting analysis, no significant amount of mRNA was detected in any of the unwounded tissues of stems, leaves, or roots. Even in 12-h-wounded root tissues, in situ hybridization studies did not show significant increases of the cell-wall invertase mRNA in phloem tissues. This is consistent with the northern hybridization results of the in vitro wounded pea root tissues; northern blotting analysis revealed that the mRNA transcripts of the cell-wall invertase gene already decreased to a normal level in root tissues 12 h after wounding.

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