A Major Jasmonate-Inducible Protein of Sweet Potato, Ipomoelin, is an ABA-Independent Wound-Inducible Protein

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Treatment of sweet potato plants cultured in vitro with a vapor of methyl jasmonate (MeJA) induced an accumulation in leaves of a large amount of protein with an apparent molecular mass of 18 kDa. This protein, designated ipomoelin, was purified, and the amino acid sequences of proteolytic fragments were determined. Screening a cDNA library of MeJA-treated leaves by oligonucleotide probes designed from the peptide sequences identified a clone that could code for a polypeptide with 154 amino acids. The deduced amino acid sequence of ipomoelin showed an overall amino acid identity of 25% with the salt-inducible SalT protein of rice. In addition, the C-terminal 70 amino acid sequence of ipomoelin showed about 50% identity with the C-terminal amino acid sequences of seed lectins from Moraceae. The gene for ipomoelin was present in a few copies in the genome of sweet potato. The mRNA for ipomoelin was detected in leaves and petioles, but not in stems and tuberous roots, of sweet potato plants grown in the field. Mechanical wounding of leaves induced ipomoelin mRNA both locally and systemically, while treatment of leaves with ABA, salt, or a high level of sucrose did not induce ipomoelin mRNA. By contrast, ABA-inducible mRNA for sporamin was not induced by MeJA. These results suggest that ipomoelin is involved in defensive reactions of leaves in response to wounding and that JA-mediated wound-induction of ipomoelin occurs independently of ABA.

Key words: Gene expression — Ipomoelin — Jasmonic acid — Sporamin — Sweet potato — Wounding.

Exogenous application of jasmonic acid (JA, 3-oxo-2-[(z)-2-pentenyl]-1-cyclopentaneacetic acid) or the methyl ester of jasmonic acid (MeJA) to plants causes induction of expression of a variety of plant genes and exerts diverse physiological effects such as inhibition of growth, promotion of leaf senescence, promotion of stomata closure and induction of potato tuber formation (for review see Koda 1992, Sembdner and Parthier 1993). Among the JA-inducible genes, those coding for wound-inducible proteinase inhibitors and other proteins involved in defensive reactions have been studied in detail (for review see Doares et al. 1995, Peña-Cortés et al. 1995). A rapid increase in the endogenous levels of JA occurs after wounding (Creelman et al. 1992, Doares et al. 1995), and inhibitors of the biosynthesis of JA inhibit wound-induced expression of the proteinase inhibitor gene (Ryan 1992, Peña-Cortés et al. 1993, 1995). Based upon these and other results, JA is regarded as one of the endogenous wound-induced signals involved in the expression of defensive genes such as those for proteinase inhibitors. Other wound-inducible signalling molecules such as systemin, oligogalacturonide, and ABA have been shown to cause increased levels of endogenous JA, and these molecules have been suggested to function upstream of JA in the wound-induced signalling cascade (Doares et al. 1995, Peña-Cortés et al. 1995).

In addition to nutritional storage compounds, storage organs of plants often store various forms of defensive compounds. Proteinase inhibitors in seeds, fruits, tubers, and other storage organs are regarded as examples of such defensive compounds. Up to 80% of the total soluble proteins of the tuberous roots of sweet potato is accounted for by sporamin which exhibits many of the properties expected of storage proteins. It is accumulated in vacuoles and undergoes rapid degradation in germinating tuberous roots (Maeshima et al. 1985). Furthermore, the expression of the multigene family for sporamin is inducible by high levels of sugars concomitant with the accumulation of β-amylase and of starch, other major storage materials in tuberous roots (Hattori et al. 1990, 1991, Nakamura et al. 1991, Takeda et al. 1995). The amino acid sequence of sporamin shares homology with legume Kunitz trypsin inhibitor and its related proteins from a variety of plant species (Hattori et al. 1989, Ishikawa et al. 1994a, b, Bradshaw et al. 1989), and some of these proteins are known to be wound-inducible. In addition, we have previously shown that polygalacturonic acid and ABA, both known to mediate wound-induction of proteinase inhibitors in tomato and potato, induce the accumulation of sporamin mRNAs in sweet potato (Ohto et al. 1992, Takeda et al. 1995). From these results, it seemed possible that sporamin is a
wound-inducible defensive protein. However, mechanical
wounding of leaves of sweet potato only occasionally in-
duced accumulations of sporamin (Hattori et al. 1989,
Ohto et al. 1992).

In this paper, we examined whether expression of
the gene for sporamin is inducible by MeJA. The data
presented in this paper indicate that sporamin is not a JA-
inducible protein. Instead, we found that MeJA induces
accumulation of a large amount of a novel protein, design-
nated herein as ipomoelin, in leaves of sweet potato. Me-
chanical wounding of leaves induced the accumulation of
ipomoelin mRNA both locally and systemically. Unlike
wound-inducible proteinase inhibitors of tomato and
potato, accumulation of ipomoelin mRNA could not be in-
duced by ABA. These results suggest that ipomoelin is a
wound-inducible defensive protein of leaves and that
wound-induction of ipomoelin is mediated by JA in an
ABA-independent manner.

Materials and Methods

Plant materials and various treatments—Sweet potato (Ipomoea bataas Lam. cv. Kokei No. 14) plants grown in the field of
Nagoya University Experimental Farm or cultured in vitro were
used. In vitro plants were cultured in a plastic container (Plant
Box; Verdy Co. Toyohashi, Japan) with Murashige-Skoog salts
and vitamins, 3% sucrose, and 50 mM Tris-HCl (pH 7.5) and 50 mM NaCl, and fractionated by gel
phoresis of RNA and Northern blot hybridization with 32 P-
labeled cDNA for ipomoelin and pMO23 cDNA for sporamin
(Hattori et al. 1985) were carried out as described previ-
ously (Hattori et al. 1992). For the binding of proteins to a column of immobilized D-galactose
(Endo), they were extracted from leaves of MeJA-treated plants
with phosphate-buffered saline (PBS; 0.01 M NaPi, pH 7.2,
and 0.15 M NaCl). The DE52 non-absorbed proteins were applied to a
column of immobilized D-galactose (Pierce) preequilibrated with
PBS. The column was washed with PBS, and then bound proteins
were eluted with PBS containing 0.25 M D-galactose. The amount
of protein eluted from the column was determined by the method
of Bradford (1976). MPA obtained from Sigma was used as a con-
trol.

Results

MeJA-induced accumulation of ipomoelin in leaves of
sweet potato—We treated sweet potato plants, that had
been grown axenically on agar medium, with a vapor of Me-
JA (1 µl per 70 cm3) for four days. Proteins extracted from
various tissues were analyzed by SDS-PAGE. As shown in
Figure 1, a new band of protein with an apparent molecu-
lar mass of 18 kDa accumulated in leaves of MeJA-treated
plants. This 18 kDa-protein was designated ipomoelin. A

Bio-Gel HTP (Biorad Laboratories). After washing the column
with the dialysis buffer and then with 0.01 M KPi (pH 7.2),
ipomoelin was eluted with 0.1 M KPi (pH 7.2).

Purified ipomoelin was precipitated with 10% trichloroacetic
acid (TCA) and washed three times with cold acetone. The precipi-
tated proteins were dissolved in 0.25 M Tris-HCl (pH 6.8) or
0.125 M Tris-HCl (pH 8.0), containing 1% SDS and 10% glycer-
ol and heated at 100°C for 3 min before digestion with protease
V8 (Endoproteinase Glu-C, sequencing grade, Boehringer) or
endoproteinase Lys-C (sequencing grade, Promega), respectively.
Polypeptides were fractionated by electrophoresis through 25%
SDS-polyacrylamide gel and transferred to a polyvinylidene difluo-
ride (PVDF) membrane. Their amino acid sequences were
quantitated by imaging analyzer (Fujix BAS2000; Fuji Photo
Film). Isolation of total DNA of sweet potato and genomic
Southern blot hybridization were carried out as described previ-
ously (Murakami et al. 1986).

DNA cloning.—The cDNA library of MeJA-treated leaves
was constructed from the poly(A)+-RNA fraction by using ZAP-
cDNA synthesis kit (Stratagene) and packaged in vitro (Giga-
pack II Gold; Stratagene). The cDNA clone for ipomoelin was screened
by hybridization with oligonucleotide probes designed from the
amino acid sequence of the proteolytic fragments of ipomoelin.
The following probes were used: probe 1, CCT(C/T)GTT(A/G)T-
A(A/G)TGT(N)GC(A/G)AT(AT/C); probe 2, AA(T/C)AT-
(T/C)/AGAT(C/T)/GG(N)NAT(T/C)AT(T/C/A)AT(T/C/A); probe
3, AA(A/G)/AA(A/G)GA(A/G)TA(T/C)/GG(N)CCC(N)AT(T/C)C-
GG, where N in parentheses indicates all four deoxyribonucleo-
tides. Plaque hybridization, in vivo excision of the cDNA insert, and
daN sequence were carried out by standard methods.

Results

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lar mass of 18 kDa accumulated in leaves of MeJA-treated
plants. This 18 kDa-protein was designated ipomoelin. A
Fig. 1 MeJA-induced accumulation of ipomoelin in sweet potato. Proteins extracted from leaf (L), petiole (P), stem (S), and root (R) of sweet potato plants that had been cultured in vitro (−MeJA) or plants after treatment with MeJA vapor for 4 days (+MeJA) were fractionated by SDS-PAGE and stained with Coomassie Brilliant blue. Lane T indicates proteins extracted from tuberous roots of field-grown plants. For each lane, the amount of protein applied to the gel was 30 μg. Lane MW indicates molecular weight standards. Filled-in arrowhead indicates the band of sporamin; the band of ipomoelin is indicated by an open circle.

band corresponding to ipomoelin could also be detected in petioles of MeJA-treated plants, although at significantly lower levels than in leaves. No MeJA-induced accumulation of ipomoelin was detected in stems or roots. Stems of sweet potato plants cultured in vitro in medium containing sucrose accumulate large amounts of sporamin (Hattori et al. 1990; Fig. 1). Treatment of plants with MeJA vapor did not induce the accumulation of sporamin in leaves and decreased the level of sporamin in stems. The level of a group of proteins with an apparent molecular mass of 55 kDa in leaves, which probably correspond to the large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase, decreased significantly after treatment with MeJA.

Purification of ipomoelin—We purified ipomoelin from leaves of sweet potato plants that had been treated with MeJA vapor for 4 days (Materials and Methods; Fig. 2). The purified ipomoelin fraction contained a trace amount of a 14.8 kDa-protein in addition to ipomoelin. About 1 mg of ipomoelin was obtained from 9 g of leaves of MeJA-treated plants.

Fig. 2 Purification of ipomoelin. Proteins in fractions of various stages of the purification of ipomoelin were separated by SDS-PAGE. N, crude extract from leaves of non-treated plants (30 μg); 1, crude extract from leaves of MeJA-treated plants (30 μg); 2, combined fractions from Sephadex G-100 (20 μg); 3, DE52 non-adsorbed fraction (20 μg); 4, combined fractions from hydroxylapatite column (10 μg).

Since the N-terminal amino acid sequence of the purified ipomoelin could not be determined by Edman degradation, the protein was denatured with TCA and SDS and then cleaved with various proteinases. Cleavage with V8 protease and endoprotease Lys-C yielded several distinct bands of cleaved fragments on SDS-PAGE (data not shown). These proteolytic fragments were transferred to a PVDF membrane, and the N-terminal amino acid sequences were determined for three of them. The V8-2 fragment gave a sequence of 26 amino acids, and the Lys-9 and Lys-10 fragments gave sequences of 25 and 16 amino acids, respectively.

Isolation of cDNA for ipomoelin—A cDNA library was constructed with poly(A)+-RNA from leaves of sweet potato plants that had been treated with MeJA vapor for 1 day. We also prepared mixed oligonucleotide probes 1 and 2 corresponding to MIANYKG and NIDGDII sequences, respectively, in the V8-2 fragment and probe 3 corresponding to KKEYGPYG sequence in the Lys-9 fragment (see Materials and Methods). By screening the library of 10⁶ independent clones with probes 1 and 3, we identified about 30 and 40 positive plaques, respectively. We could not identify any positive plaque by screening with probe 2, probably because of its low Tm. Among the positive plaques identified with probes 1 and 3, nine of them hybridized with both of the probes. They were further analyzed after in vivo excision of the cDNA insert. One of the clones, pIP-1, contained the longest cDNA for ipomoelin; other clones contained shorter 5'- and/or 3'-noncoding regions of the cDNA. However, the nucleotide sequence of these cDNAs did not differ from that of pIP-1 cDNA in the coding and noncoding regions (data not shown).

Figure 3 shows the nucleotide sequence and the deduced amino acid sequence of the cDNA for ipomoelin in pIP-1. The 720 bp-long cDNA consisted of 45 bp of 5'-noncoding region, 462 bp of a coding region that coded for a
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**Fig. 3** Nucleotide sequence of plP-1 cDNA and deduced amino acid sequence of ipomoelin. The first base of the ATG initiation codon of the cDNA insert of plP-1 is numbered as +1. The N-terminal amino acid sequences of peptide fragments Lys-9, Lys-10, and V8-2 are indicated by arrowheads and bold letters. The probes 1–3 were designed from the amino acid sequences that are underlined.

polypeptide with 154 amino acid residues, and 224 bp of a 3'-noncoding region followed by a string of A residues. The amino acid sequence deduced from cDNA contained all of the three amino acid sequences of the peptide fragments derived from ipomoelin (Fig. 3). The ATG initiation codon for the open reading frame was not preceded by an in-frame termination codon in the 5'-noncoding region. The mRNA for ipomoelin detected by Northern blot hybridization analysis migrated to almost identical position as the cDNA insert of plP-1 upon electrophoresis in agarose gel (data not shown). The calculated molecular weight and pI value of a polypeptide encoded by the open reading frame were 16,503 and 8.9, respectively.

Sweet potato DNA was digested with the restriction enzymes HindIII, BamHI and EcoRI and the Southern blot filters were hybridized with the 32P-labeled cDNA for ipomoelin as a probe (Fig. 4). For each restriction enzyme digests, the probe hybridized to a major band and several other minor bands. Since sweet potato contains a complex hexaploid genome constitution, the results may suggest that the gene for ipomoelin exists in a few copies per haploid.

**Structure of ipomoelin**—The overall structure of ipomoelin shared amino acid identities of 25 and 30% with...
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The amino acid sequences of SalT protein (Claes et al. 1990) and GOS9 protein (De Pater and Schilperoort 1992) of rice, respectively (Fig. 5A). The SalT and GOS9 proteins are proteins with 145 and 139 amino acid residues, respectively, and they share 59% amino acid identity. The SalT protein is a protein which is induced in sheaths and roots of rice by salt, air drying, ABA, polyethylene glycol, and NaCl (Claes et al. 1990), while GOS9 protein is deduced from one of the root-specific cDNA of rice (De Pater and Schilperoort 1992). Although the N-terminal part of these proteins contain Gly-rich repeat sequences similar to those present in several other osmotic stress-induced proteins (Claes et al. 1990), the corresponding sequences are only partially conserved in ipomoelin.

In addition to similarities to SalT and GOS9 proteins, the C-terminal 71 amino acid sequence of ipomoelin shared 51 and 48% amino acid identities with the C-terminal regions of the α-subunit of jacalin from Artocarpus integrifolia (Yang and Czapla 1993) and MPA from Macfura pomifera (Young et al. 1991), respectively (Fig. 5B). Jacalin and MPA are seed lectins with a high affinity for α-D-galactopyranosides, and they consist of the α- and β-subunits that are derived from one precursor protein (Fig. 5B; Yang and Czapla 1993). The sequence similarity of ipomoelin with these lectins was limited to the C-terminal regions of the proteins. The sequence similarity between SalT and the lectins was slightly less than that between ipomoelin and the lectins. The Trp residue of the α-peptide of MPA has been implicated in sugar-binding with a neighboring Tyr residue, either Tyr or Tyr (Young et al. 1991). In the sequence of ipomoelin, Trp and Tyr residues are present near the site corresponding to Trp of MPA (Fig. 5B).

During the purification of ipomoelin, we noticed that it does not migrate as a sharp peak in gel filtration chromatography with Sephadex G-100 and Superose-12HR (data not shown). Ipomoelin may have some affinity for the gel matrix. We examined the question of whether ipomoelin has any affinity for D-galactose. Proteins extracted from leaves of MeJA-treated plants were applied to a column of immobilized-D-galactose. After washing the column with PBS, the bound proteins were eluted with PBS containing 0.25 M D-galactose. As shown in Figure 6, the fraction eluted from the column by D-galactose showed only a single band of ipomoelin. However, most of the ipomoelin in the extract did not bind to the column. Under the same conditions, most of the MPA was bound to the column and eluted with D-galactose (Fig. 6). These results indicate that ipomoelin has only a weak affinity for D-galactose as compared to MPA.

Expression of the gene for ipomoelin—Total RNAs isolated from various organs of sweet potato were analyzed for the presence of ipomoelin mRNA by Northern blot hybridization analysis. In the field-grown sweet potato plants, ipomoelin mRNA was detected in leaves and, to a lesser level, in petioles, but not in stems or tuberous roots (Fig. 7). In sweet potato plants cultured in vitro, ipomoelin mRNA was detected in leaves. Upon treatment with MeJA vapor, a significant increase in the level of ipomoelin mRNA occurred in leaves. Ipomoelin mRNA was also detected in petioles of MeJA-treated plants. However, the MeJA-induced accumulation of ipomoelin mRNA did not take place in stems or roots.

When a solution of MeJA was applied to the cut-edges of leaf-petiole cuttings that had been excised from the field-grown sweet potato plants, accumulation of ipomoelin mRNA was induced. The maximum level of induction was observed when the solution contained about 60 μM MeJA (data not shown). Figure 8 shows the time course of the increase in the level of ipomoelin mRNA in leaf parts of the leaf-petiole cuttings after feeding with a solution of 60 μM MeJA. The increase in the level of ipomoelin mRNA appeared after about 1 h of lag-time and continued linearly for about 6 h.

We examined whether ipomoelin gene expression is inducible by mechanical wounding of leaves of the field-grown plants. As shown in Figure 9, accumulation of ipomoelin mRNA was detected in wounded leaves 6 h after wounding and the level of ipomoelin mRNA decreased at 12 h after wounding. A significant increase in the level of ipomoelin mRNA was also observed in the opposite non-wounded
Fig. 5 Comparison of the amino acid sequence of ipomoelin with other proteins. Parts of amino acid sequences compared among the proteins are shown schematically by the gray box within the whole structures of proteins. Amino acid residues that are identical with the sequence of ipomoelin are indicated by white letters in the gray box. (A) Comparison with SalT protein (Claes et al. 1990) and GOS9 protein (De Pater and Schilperoort 1992) of rice. (B) Comparison with prepro-jacalin (Yang and Czapla 1993) and α-chain of MPA (Young et al. 1991). The α-chains of jacalin and MPA locate at the C-terminus of their prepro-precursor (Yang and Czapla 1993).

half of the main vein of leaves (Fig. 9) and in neighboring non-wounded leaves (data not shown). These Northern blot membranes were washed to remove the 32P-labeled cDNA for ipomoelin and then rehybridized with 32P-labeled cDNA for sporamin. The increase in the level of sporamin mRNA was smaller than that of ipomoelin mRNA in these wounded leaf (Fig. 9). Although the wound-induction of ipomoelin mRNA was observed in
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Fig. 6 Binding of ipomoelin to an immobilized D-galactose column. (A) The DE52 non-absorbed fraction of the crude extract from leaves of MeJA-treated plants (---) was applied to a column of immobilized D-galactose that had been pre-equilibrated with PBS. After the column was washed with PBS, the bound proteins were eluted with PBS containing 0.25 M D-galactose. As a control, MPA (—) was applied to the same column. (B) Equal amounts of proteins (30 μg) in the DE52 non-absorbed fraction (lane CE), pass through the fraction (lane A), and proteins eluted by 0.25 M D-galactose (lane B) were fractionated by SDS-PAGE.

several other independent experiments, the wound-inducibility of sporamin mRNA varied, depending on the experiment (data not shown; Hattori et al. 1989, Ohto et al. 1992).

We did not detect the induction of ipomoelin mRNA after feeding of leaf-petiole cuttings with solutions of 20 μM ABA, 1% NaCl, or 6% sucrose (Fig. 10). In contrast, sporamin mRNA was induced by these treatments, consistent with our previous results (Nakamura et al. 1991, Ohto et al. 1992). Similar to the results obtained with in vitro plants (Fig. 1), MeJA did not induce sporamin mRNA in leaf-petiole cuttings of the field-grown plants (Fig. 10). Results similar to those shown in Figure 10 were obtained in several other independent experiments.

Discussion

JA induces accumulation of specific proteins in a variety of plant species. These JA-induced proteins are called JIPs and many of them are suggested to be involved in defensive function of plant cells against biotic and abiotic stress (for reviews see Reinbothe et al. 1994, Sembdner and Parthier 1993). Treatment of the whole plant of sweet potato with MeJA vapor induced accumulations of large amounts of ipomoelin in leaves (Fig. 1), and ipomoelin gene expression was induced when leaves were wounded (Fig. 9). Ipomoelin, a sweet potato JIP, does not have significant sequence similarities to JIPs reported previously. The overall amino acid sequence of ipomoelin showed low levels of sequence similarity with the SalT and GOS9 proteins of rice (Fig. 5A). Neither the function of the SalT protein, which is induced in roots of rice by a variety of stresses such as osmotic stress, air dryness, and salt (Claes et al. 1990), nor that of root-specific GOS9 protein (De Pater and Schilperoort 1992) is understood at present. Unlike SalT protein, ipomoelin was not induced by salt stress or by ABA (Fig. 10), and unlike SalT and GOS9 proteins, ipomoelin could be detected only in leaves and petioles (Fig. 1, 7). These results indicate that the mode of expression of the gene for ipomoelin is significantly different from those of the SalT and GOS9 genes in rice. The C-terminal amino acid sequence of ipomoelin showed higher levels of similarity to the C-terminal region of seed lectins from Moraceae (Fig. 5B). However, these similarities were limited to the C-terminal region of ipomoelin, and ipomoelin showed only a weak affinity for D-galactose (Fig. 6). It is suggested that ipomoelin is a novel defensive protein against wounding which functions specifically in leaves.

Wound-induction of ipomoelin mRNA was observed in wounded sites of leaves within 6 h after wounding and then the level of ipomoelin mRNA decreased 12 h after wounding (Fig. 9). Induction of ipomoelin mRNA was also observed at non-wounded sites of the wounded leaf (Fig. 9) and in the neighboring non-wounded leaves (data not shown). As reported for several other plant species such as soybean, potato, tomato and Arabidopsis (Creelman et al. 1992, Doares et al. 1995), wounding of leaves of sweet potato plants may result in increased levels of endogenous
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Fig. 7 Tissue-distribution of ipomoelin mRNA. Twenty micrograms of total RNA isolated from various organs of the field-grown sweet potato plants (A) and sweet potato plants cultured in vitro (B), without (−MeJA) or after treatment with MeJA vapor for 3 days (+MeJA), were analyzed for the presence of ipomoelin mRNA by Northern blot hybridization. L, leaf; P, petiole; S, stem; T, tuberous root; R, root.

JA, which in turn induces expression of the ipomoelin gene. In leaves of tomato, transient increases in the levels of proteinase inhibitor mRNA as well as endogenous JA have been observed upon wounding (Doares et al. 1995, Graham et al. 1986). The expression of proteinase inhibitor genes of potato and tomato is also induced by ABA, and wound-induction of these genes is blocked in mutant plants impaired in ABA synthesis (Peña-Cortés et al. 1989). Furthermore, wounding induces increases in the levels of ABA in both wounded and non-wounded leaves, and systemic increases in ABA can be induced by systemin or electrical current (Peña-Cortés et al. 1995). From these results, it is postulated that ABA is an integral component of the signalling pathway which functions upstream of JA in the wound-induction of proteinase inhibitor genes (Peña-Cortés et al. 1995).

In contrast to the proteinase inhibitor genes of potato and tomato, ipomoelin gene expression was not induced by ABA even though it was inducible by MeJA and wounding (Fig. 9, 10). These results suggest that wound-induction of ipomoelin gene expression occurs via an ABA-independent

Fig. 8 Induction of ipomoelin mRNA by MeJA in the leaf part of the leaf-petiole cuttings. Leaf-petiole cuttings from field-grown plants were treated with 6 × 10⁻⁷ M MeJA (+MeJA) or with water (−MeJA) for the indicated period of time in darkness. At each time point, leaf parts from more than 5 leaf-petiole cuttings were used for the isolation of RNA. Twenty micrograms of total RNA were analyzed for the level of ipomoelin mRNA by Northern blot hybridization. The relative radioactivities of the band corresponding to ipomoelin mRNA were quantitated by imaging analyzer (BAS2000). The values on the abscissa are in arbitrary units.

Fig. 9 Induction of ipomoelin mRNA in leaves of sweet potato by mechanical wounding. Half of leaves of the field-grown sweet potato plants were mechanically wounded by forceps. Six and twelve hours later, total RNA was isolated from the wounded halves of leaves (W) and the other halves of leaves adjacent to the wounded ones (NW) from five plants. Leaves harvested from non-wounded plants (C) served as controls. Twenty micrograms of total RNA was used to analyze the levels of ipomoelin mRNA and of sporamin mRNA by Northern blot hybridization. In the lower panel, total RNA on the gel was stained with ethidium bromide.
pathway. In Arabidopsis thaliana, AtLox1 and AtLox2 coding for lipoxygenase are inducible by MeJA. The MeJA-inducible expression of AtLox1 occurs in roots, but not in leaves, while the MeJA-inducible expression of AtLox2 occurs in leaves, but not in roots (Bell and Mullet 1993). Although substantial induction of AtLox1 expression by ABA occurs in roots, the wound-inducible AtLox2 gene does not respond to ABA (Bell and Mullet 1993). This pattern of expression of AtLox2 gene is similar to that of the regulation of ipomoelin gene expression. These results indicate that JA-mediated wound-induction of gene expression is not necessarily coupled with ABA. Neither the mechanism of action of ABA in mediating the wound-signal to JA-inducible gene expression nor the mechanism of activation of JA-signalling pathway in response to wounding is known at present.

Inspite of its induction by polygalacturonic acid and ABA (Ohto et al. 1992, Takeda et al. 1995), expression of the gene for sporamin was not induced by MeJA (Fig. 1, 10). Wound-induction of sporamin in leaves of sweet potato was observed only occasionally in our experiments, which may be due to different physiological conditions of the plants. When wound-induction of sporamin occurs, this wound-induced expression of sporamin gene may be mediated by a JA-independent pathway. In addition to its high-level expression in tuberous roots, expression of the gene for β-amylase occurs with that of the genes for sporamin in response to polygalacturonic acid, ABA, and sugars (Nakamura et al. 1991, Ohto et al. 1992, Takeda et al. 1995). The expression of the gene for β-amylase was also not induced by MeJA (data not shown). Dehydration and salt stresses, which probably involve ABA-signaling, also induced expression of the genes for sporamin and β-amylase (Fig. 10; manuscript in preparation). It seems that ABA affects expression of the genes for sporamin and β-amylase directly rather than through the activation of JA-signalling.

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