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Rapid Communication

Localized Wounding by Heat Initiates the Accumulation of Proteinase Inhibitor II in Abscisic Acid-Deficient Plants by Triggering Jasmonic Acid Biosynthesis[1]

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To test whether the response to electrical current and heat treatment is due to the same signaling pathway that mediates mechanical wounding, we analyzed the effect of electric-current application and localized burning on proteinase inhibitor II (Pin2) gene expression in both wild-type and abscisic acid (ABA)-deficient tomato (*Lycopersicon esculentum* Mill.) and potato (*Solanum phureja*) plants. Electric-current application and localized burning led to the accumulation of Pin2 mRNA in potato and tomato wild-type plants. Among the treatments tested, only localized burning of the leaves led to an accumulation of Pin2 mRNA in the ABA-deficient plants. Electric-current application, like mechanical injury, was able to initiate ABA and jasmonic acid (JA) accumulation in wild-type but not in ABA-deficient plants. In contrast, heat treatment led to an accumulation of JA in both wild-type and ABA-deficient plants. Inhibition of JA biosynthesis by aspirin blocked the heat-induced Pin2 gene expression in tomato wild-type leaves. These results suggest that electric current, similar to mechanical wounding, requires the presence of ABA to induce Pin2 gene expression. Conversely, burning of the leaves activates Pin2 gene expression by directly triggering the biosynthesis of JA by an alternative pathway that is independent of endogenous ABA levels.

Pin2 accumulates in tomato (*Lycopersicon esculentum* Mill.) and potato (*Solanum phureja*) leaves when subjected to mechanical wounding or pathogen attack, and these proteins are implicated in the plant defense mechanism (Bowles, 1990; Ryan, 1990). Gene expression of proteinase inhibitors, however, is controlled by environmental and developmental conditions. Pin2 genes are constitutively expressed in organs such as potato tuber and floral buds of both potato and tomato plants (Peña-Cortés et al., 1991) and are induced in leaves following mechanical wounding. The wound-induced Pin2 gene activation occurs not only in the damaged tissue (local) but also in the more distal nontreated leaves (systemic) (Peña-Cortés et al., 1988). This suggests the existence of a signal that moves from the injured tissue to the upper or lower leaves and leads to the systemic induction of Pin2 gene expression. Several mediators, both chemical and physical, have been suggested as the putative "systemic signal." Phytohormones such as ABA (Peña-Cortés et al., 1989; Hildmann et al., 1992) and JA (Farmer and Ryan, 1990, 1992; Peña-Cortés et al., 1993), the peptide systemin (Pearce et al., 1991), and oligosaccharides (Ryan, 1987) all have been demonstrated to be chemical signals. Recently, evidence was provided that supports the proposed role of systemin as a mobile wound signal (McGurl et al., 1994). Additionally, reduction of auxin concentrations seems to play a role in the signal transduction pathway that mediates wound-induced Pin2 gene expression (Kernan and Thornburg, 1989; Thornburg and Li, 1991). Moreover, hydraulic and electrical signals, classified as physical signals, have been implicated in wound-induced gene expression. The appearance of variation potentials throughout most of the shoot has been reported following localized wounding by heating or burning (Pickard, 1973) in several plants (Wildon et al., 1989; Malone and Stankovic, 1991). Furthermore, Wildon et al. (1992) reported that mechanical wounding and localized burning generate electrical signals that are propagated through the plant, thereby inducing Pin2 gene expression systemically. However, to our knowledge no definitive evidence about the nature and properties of this systemic signal has been provided. It is also unknown whether the same signal mediates both local and systemic Pin2 gene activation. Furthermore, the involvement of several simultaneous signals or the coordinated action of physical and chemical signals cannot be ruled out.

Recently, we demonstrated that applying electric current to tomato leaves activated Pin2 gene expression in a local and systemic manner (Herde et al., 1995). Additionally, heat and electric-current application led to an increase in endogenous levels of ABA in systemic, nontreated leaves (Peña-Cortés et al., 1995), with the levels being similar to those observed upon wounding. Nevertheless, gas-exchange measurements demonstrated a different behavior

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Abbreviations: JA, jasmonic acid; LOX, lipoxygenase; Pin2, proteinase inhibitor II.

of photosynthetic activity, such as assimilation and transpiration rates following heat treatment. These results suggest that the heat-induced Pin2 gene expression may be regulated by a pathway that is different from the pathway mediating wound- or electric-current-induced gene expression (Herde et al., 1995).

Several reports suggest the involvement of ABA and JA in wound-induced gene expression (Peña-Cortés and Willmitzer 1995). Indeed, exogenous application of either ABA (Peña-Cortés et al., 1991; Hildmann et al., 1992) or JA (Farmer and Ryan 1990; Peña-Cortés et al., 1993) initiates Pin2 mRNA accumulation in the absence of wounding. In addition, mechanical damage leads to an increase of endogenous levels of ABA and JA in both tomato and potato plants (Peña-Cortés and Willmitzer, 1995). Furthermore, ABA-deficient plants that become impaired in ABA biosynthesis do not respond to wounding or the peptide systemin. In this case there was no accumulation of Pin2 transcript, ABA, or JA (Peña-Cortés et al., 1996).

To get a broader insight into the involvement of ABA and JA in the signal transduction pathway mediating electric-current- and heat-induced Pin2 gene expression, we decided to investigate the effect of these stimuli on Pin2 gene expression and on the endogenous levels of ABA and JA in tomato and potato wild-type plants, as well as in ABA-deficient plants. We addressed the following questions: Do electrical current and heat treatment induce Pin2 gene activation in ABA-deficient plants? and Do these stimuli alter endogenous ABA and/or JA levels in wild-type or ABA-deficient plants?

The results show that heat treatment, like wounding or electric-current application, leads to a local and systemic activation of Pin2 gene expression and to a local and systemic accumulation of ABA and JA in wild-type plants. Surprisingly, this form of injury also causes a local and systemic accumulation of Pin2 transcript and JA in ABA-deficient plants. Pretreatment of whole plants with aspirin, an inhibitor of JA biosynthesis, abolished the accumulation of Pin2 mRNA following heat treatment. The results strongly suggest the presence of an alternative signal transduction pathway mediating heat-induced Pin2 gene expression, which initiates the accumulation of JA independently of endogenous levels of ABA.

MATERIALS AND METHODS

Tomato (*Lycopersicon esculentum* Mill. cv Moneymaker) and ABA-deficient *sitiens* plants were grown in the greenhouse (26°C day/20°C night, 70–80% RH, and 14 h of light). Potato (*Solanum phureja*) and ABA-deficient droopy plants were grown under greenhouse conditions (22°C day/18°C night, 60–80% RH, and 12 h of light). All measurements were performed on 21- to 28-d-old plants.

Current Application

A direct current power supply was used and 10 V was provided for 30 s during electric stimulation as described by Herde et al. (1995).

Mechanical Wounding

Dialysis clamps were applied as described by Sanchez-Serrano et al. (1986).

Localized Wounding by Heat

Heat stimulation was performed on the tip of a leaf. Approximately 1 cm^2 was burned, according to Malone and Stankovic (1991).

Gel Blot Analysis of RNA

Plant total RNA was isolated and subjected to electrophoresis (10 μg of RNA per slot) in agarose-formaldehyde gels as described by Logemann et al. (1987). Blotting and hybridization conditions were as described by Amasino (1986). Probes used for radioactive labeling consisted of potato Pin2 (cDNA 1; Sanchez-Serrano et al., 1986), TAS 14 cDNA fragments (Godoy et al., 1990), PRP1-1 (pathogen defense gene from potato; Taylor et al., 1990), and small subunit of Rubisco cDNA (rbcS) (Eckes et al., 1986). Each experiment was independently repeated at least five times. The northern blots and ABA/JA quantitation shown in the various figures are representative of the average situation. ABA/JA quantitation is given with sample SDs (n = 5).

ABA and JA Quantitation

Endogenous ABA and JA concentrations were determined as described by Peña-Cortés et al. (1989) and by Knöfel et al. (1990), respectively.

Aspirin Treatment

Whole tomato plants were cut at the base of the stem and supplied with water alone or aspirin (1 mM) for 3 h as described by Peña-Cortés et al. (1993).

RESULTS

Localized Wounding by Heat Initiates Pin2 Accumulation in Both Wild-Type and ABA-Deficient Plants

Mechanical wounding and heat treatment promote Pin2 accumulation in both treated and nontreated systemic tissues (Wildon et al., 1992; Peña-Cortés et al., 1995). Both stimuli may generate electrical signals that are propagated through the vascular tissue of the plants, triggering the Pin2 gene expression in the nontreated systemic tissues (Wildon et al., 1992). Recently, we reported the induction of Pin2 gene expression in tomato leaves following electric-current application. As with damage from mechanical wounding and heat treatment, current application was able to activate Pin2 gene expression in both local and systemic leaves of tomato plants (Herde et al., 1995). How this electrical signal is propagated and transduced, leading to the gene activation, remains unknown. Phytohormones such as JA and ABA have been implicated in the regulation of the events mediating wound response (Farmer and Ryan, 1992; Peña-Cortés and Willmitzer, 1995). By using ABA-deficient plants impaired in ABA biosynthesis (Tay-

lor et al., 1988; Duckham et al., 1989), the involvement of ABA in the signal transduction chain mediating wound-induced Pin2 gene activation was demonstrated (Peña-Cortés et al., 1991; Hildmann et al., 1992). These plants did not respond to mechanical injury through the accumulation of Pin2, which suggests a pathway that depends on normal ABA concentrations. It is more interesting that JA and its methyl ester were able to induce Pin2 gene expression in ABA-deficient plants, suggesting that the site of action of JA is located downstream of the action site of ABA in the mechanism mediating wound-inducible gene expression (Peña-Cortés and Willmitzer, 1995). To examine whether electric-current- or heat-induced Pin2 gene expression are mediated by a chain of events similar to that which mediates the wound response, we investigated the effect of these stimuli on Pin2 gene expression in both wild-type and ABA-deficient tomato and potato plants.

Whole potato and tomato plants were mechanically wounded, treated with electrical current, or burned at the tip of a leaf. After 6 h, the time when wound-induced JA accumulation reaches its highest levels (Peña-Cortés et al., 1993), treated (Fig. 1, lane L) and nontreated (Fig. 1, lane S) systemic leaves were harvested and analyzed for Pin2 gene expression by northern blot. Since the effect of electric-current and heat treatment on tomato plants was already reported (Herde et al., 1995), the results presented in this study were obtained with wild-type potato plants.

As expected, mechanical damage of the leaves led to a local and systemic accumulation of Pin2 mRNA in wild-type (Fig. 1A, Wounding) but not in ABA-deficient plants (Fig. 1, B and C, Wounding). Extended exposure of the autoradiograms did not reveal an accumulation of the Pin2 mRNA in ABA-deficient plants following either wounding or electric-current application (data not shown). Similarly, electric-current application activated Pin2 gene expression locally and systemically only in wild-type (Fig. 1A, Current) but not in ABA-deficient plants (Fig. 1, B and C, Current). Heat treatment of the leaves initiated local and systemic accumulation of Pin2 mRNA in both wild-type (Fig. 1A, Heat) and ABA-deficient (Fig. 1, B and C, Heat) plants. These results suggest that electric-current-induced Pin2 gene activation in both tomato and potato plants requires normal levels of ABA, whereas heat-induced Pin2 gene activation is regulated by an alternative pathway that is not dependent on normal levels of ABA. To prove the specificity of the Pin2 gene expression following electric-current or heat treatment and to exclude the possible influence of water stress, the same blot was reprobed with the tomato cDNA clone of TAS-14, which displays a well-characterized ABA and water-stress response (Godoy et al., 1990), and the rbcS cDNA (Eckes et al., 1986), which is down-regulated following mechanical wounding (Peña-Cortés et al., 1988). Figure 1 shows that none of the stimuli used led to the accumulation of TAS-14 or to a relevant decrease of rbcS mRNA. Thus, 6 h after the treatment none of these stimuli had strongly affected rbcS gene expression. However, 10 h after treatment reduction of rbcS mRNA accumulation was comparable with early reported data (Peña-Cortés et al., 1988). Analysis of total RNA from leaves harvested at times shorter than 6 h does not show a difference in the rate of accumulation of Pin2 mRNA following the various stimuli (data not shown).

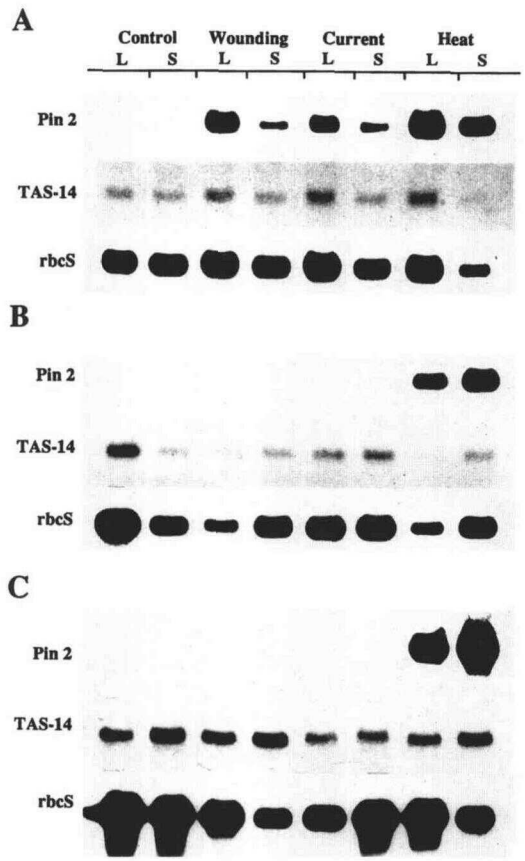

Figure 1. Mechanical wounding and electric-current and heat treatment initiate the local and systemic accumulation of Pin2 mRNA. Wild-type potato (A), ABA-deficient potato (B), and tomato (C) plants were wounded (Wounding), treated with electric current (Current), or treated with heat (Heat). Six hours after the treatment total RNA was isolated from the treated leaves (L) and from the leaves located distal (S) to the treated ones. The autoradiogram shows the result of a RNA gel blot hybridization of total RNA (10 μg per slot) against radioactive Pin2 (Sanchez-Serrano et al., 1986), TAS-14 (Godoy et al., 1990), and rbcS cDNA (Eckes et al., 1986).

Heat Increases ABA Levels Only in Wild-Type Plants

Mechanical damage and exogenous application of the peptide systemin lead to an activation of Pin2 gene expression by increasing endogenous levels of both ABA and JA in tomato and potato wild-type plants (Peña-Cortés et al., 1995). To get a broader insight about the effect of heat and electric-current treatment on the internal levels of these hormones, leaves of treated wild-type tomato and ABA-deficient plants were analyzed for their ABA and JA contents. Hormone concentration was measured 6 h after the treatment in both the directly treated leaves (local) and in the leaves located distal (systemic) to the treated ones. As a control, intact tomato plants were wounded mechanically. As expected, both ABA (Fig. 2, wild type) and JA (Fig. 3, wild type) accumulated in tomato wild-type plants

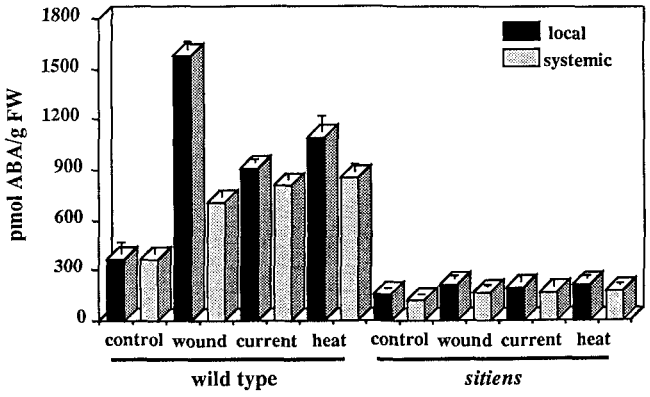

Figure 2. ABA contents in tomato plants. Wild-type and ABA-deficient *sitiens* plants were mechanically wounded (wound), treated with electric current (current), or treated with heat (heat). Both directly treated (local) and nontreated distal leaves (systemic) were harvested after 6 h. Endogenous levels of ABA were determined as described in "Materials and Methods" and indicated as means (n = 5) with SDs. FW, Fresh weight.

following wounding, whereas ABA-deficient plants did not show substantial changes in ABA (Fig. 2, *sitiens*) or JA (Fig. 3, *sitiens*) levels. As with wounding, electric-current treatment led to an increase of ABA and JA in both local and systemic leaves of wild-type plants but not in the ABA-deficient plants (Figs. 2 and 3, current). Similarly, heat treatment promoted an increase of ABA and JA in both tomato (Figs. 2 and 3, wild type) and potato (data not shown) wild-type plants to levels comparable with those observed following mechanical damage or electric-current application. Heat treatment also initiated the accumulation of JA in both the treated and systemic leaves of ABA-deficient tomato (Fig. 3, *sitiens*) and potato droopy plants (Fig. 4), which is slightly lower than the levels observed in the wild-type plants.

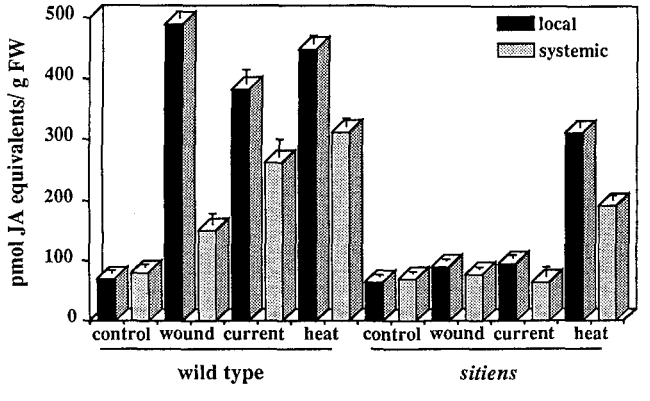

Figure 3. Effect of wounding and electric-current and heat treatment on endogenous levels of JA. Wild-type and ABA-deficient *sitiens* plants were mechanically wounded (wound), treated with electric current (current), or treated with heat (heat). Both directly treated (local) and nontreated distal leaves (systemic) were harvested after 6 h. Endogenous levels of JA were determined as described in "Materials and Methods" and indicated as means (n = 5) with SDs. FW, Fresh weight.

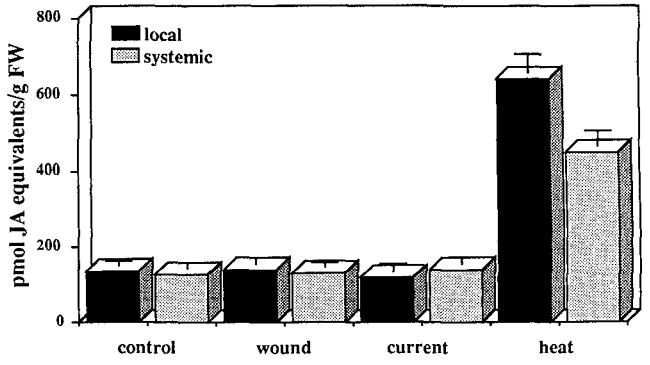

Figure 4. Effect of wounding and electric-current and heat treatment on endogenous levels of JA. ABA-deficient droopy plants were mechanically wounded (wound), treated with electric current (current), or treated with heat (heat). Both directly treated (local) and nontreated distal leaves (systemic) were harvested after 6 h. Endogenous levels of JA were determined as described in "Materials and Methods." FW, Fresh weight.

Aspirin Inhibits Heat-Induced Pin2 Gene Expression

Different LOX inhibitors repress the activation of certain genes that require the biosynthesis of JA (Staswick et al., 1991; Staswick, 1992). Doherty et al. (1988) demonstrated that the wound response in plants can be inhibited by aspirin and related hydroxy-benzoic acids. In addition, we and others have recently confirmed that aspirin (or salicylic acid) inhibits wound-induced Pin2 gene expression (Doares et al., 1995) by blocking the wound-induced accumulation of JA (Peña-Cortés et al., 1993). To confirm the involvement of endogenous JA in Pin2 gene activation following heat treatment, whole tomato wild-type plants were cut at the base of the stem and supplied with either water (Fig. 5, lane 1) or a solution containing 1 mM aspirin for 3 h (Fig. 5, lanes 2–9). The plants were subsequently burned and kept in water for 6 h (Fig. 5, lanes 4–9), after which the directly treated leaves and the distal leaves were examined for Pin2 gene expression. Northern blot analysis of total RNA showed that aspirin prevented the accumulation of Pin2 mRNA following heat treatment (Fig. 5, lanes 4–9, representing three different plants). The same blot was hybridized with a potato cDNA encoding for PRP-1 (Taylor et al., 1990), and it revealed the activation of these genes as a result of the treatment with aspirin (Fig. 5, lanes 2 and 3). Localized burning did not affect the aspirin-induced PRP-1 gene expression (Fig. 5, lanes 4–9). Quantitation of the endogenous levels of JA in the same samples used for the gene expression analysis shows that the aspirin treatment leads to an inhibition of the heat-induced accumulation of JA and that the values are similar to those observed before treatment (data not shown). These results strongly suggest that localized burning of the leaf leads to an activation of Pin2 gene expression by triggering the biosynthesis of JA.

DISCUSSION

In this work we describe the effect of electric-current and heat treatment on Pin2 gene expression and on the endogenous contents of ABA and JA in both wild-type and

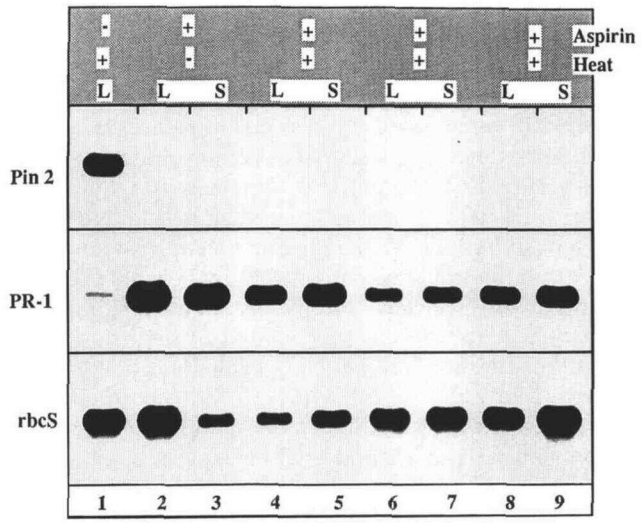

Figure 5. Aspirin blocks the heat-induced Pin2 gene expression. Whole, wild-type tomato plants were cut at the base of the stem and supplied with water alone (lane 1), 1 mM aspirin (lanes 2 and 3), or 1 mM aspirin for 3 h and subsequently treated with heat (lanes 4–9) representing three different plants. Both directly treated (L) and non-treated systemically induced leaves (S) were harvested after 6 h. The RNA gel blot was hybridized against Pin2, PRP-1 (Martini et al., 1993), and rbcS cDNA probes.

ABA-deficient potato and tomato plants. Examination of Pin2 gene expression by northern blot analysis revealed that application of electric current initiates the local and systemic accumulation of Pin2 mRNA, ABA, and JA in wild-type plants but not in ABA-deficient plants. These results indicate that wound-induced and electric-current-induced Pin2 gene expression share some common steps, which may require the presence of normal endogenous levels of ABA to trigger the later steps involved in gene activation. Furthermore, application of electric current or wounding led to an accumulation of JA only in the wild-type plants but not in ABA-deficient plants. These data strongly suggest that wound-induced or electrically induced JA accumulation may require certain levels of ABA that exist in the wild-type plants but not in the ABA-deficient plants, which support the hypothesis that the site of JA action is located after the site of ABA action in the signal chain mediating the activation of Pin2 gene expression (Peña-Cortés and Willmitzer, 1995).

Local burning of potato and tomato leaves results in a local and systemic accumulation of Pin2 mRNA in wild-type plants. In contrast to mechanical damage and electric-current application, heat treatment leads to a local and systemic activation of Pin2 gene expression in ABA-deficient potato and tomato plants. Analysis of the expression of a water-stress- and ABA-responsive gene such as TAS-14 reveals that none of the three stimuli affect the expression of this gene, which suggests that the increased levels of ABA, as a result of wounding, electric-current, or heat treatment, are either not enough to activate water-stress-induced gene expression or ABA accumulates in a location different from that induced following water-stress conditions.

Measurements of endogenous levels of ABA and JA following heat treatment reveal an increase in the level of both hormones in wild-type plants, suggesting an activation of both ABA and JA biosynthesis. ABA-deficient plants do not respond to this stimulus by accumulating ABA, which supports their inability to synthesize this hormone under any stress condition (Taylor et al., 1988; Duckham et al., 1989). ABA-deficient plants accumulate JA upon heat treatment, suggesting an activation of JA biosynthesis that does not require elevated levels of ABA. Since exogenous application of JA does not lead to an accumulation of ABA (Peña-Cortés et al., 1996), the accumulation of this hormone in wild-type plants upon heat treatment suggests that this stimuli has the capacity to activate two biosynthetic pathways independently (i.e. ABA and JA biosynthesis). Furthermore, the accumulation of JA in ABA-deficient plants suggests that heat treatment is able to activate JA biosynthesis independently of the internal levels of ABA and also supports the hypothesis that wound-induced (or electric-current-induced) JA accumulation requires a previous increase (or at least a certain level) of endogenous ABA, which does not occur in ABA-deficient plants. As in wild-type plants, aspirin treatment blocks the heat-induced Pin2 gene expression in the ABA-deficient plants (data not shown). This suggests that the levels of JA accumulated following heat treatment, although lower than the levels accumulated in wild-type plants, seems to be sufficient for triggering Pin2 mRNA accumulation. Recently, we demonstrated that exogenous application of ABA leads to an increase in JA levels in tomato and potato plants (Peña-Cortés et al., 1996). Furthermore, the effect of ABA on linolenic acid, the precursor of JA, metabolism has already been demonstrated (Abian et al., 1991) as well as the activation of LOX gene expression (Melan et al., 1993), which is involved in the wound-induced accumulation of JA (Bell et al., 1995). These results indicate that ABA may influence the early steps involved in JA biosynthesis by the release of linolenic acid or through the conversion of linolenic acid to 13S-hydroperoxy linolenic acid by the action of LOX.

Involvement of endogenous JA in regulating gene expression has been well documented (Sembdner and Parthier, 1993). For instance, LOX inhibitors block the activation of vegetative storage protein genes following wounding and petiole girdling (Staswick et al., 1991). Additionally, aspirin and salicylic acid prevent wound-induced Pin2 mRNA accumulation by inhibiting JA biosynthesis (Peña-Cortés et al., 1993). In this work we confirm the inhibitory effect of aspirin on JA biosynthesis and describe the capability of this substance to repress the heat-induced accumulation of Pin2 mRNA. Several studies have demonstrated the role of salicylic acid and aspirin in the plant defense response against pathogens (Malamy and Klessig, 1992; Raskin, 1992; Vernooij et al., 1994). Application of exogenous salicylic acid to tobacco plants leads to an induction of systemic acquired resistance and accumulation of pathogen-related proteins, thus increasing the capability of the plants to confront virus infection (Enyedi et al., 1992; Gaffney et al., 1993).

As illustrated in Figure 5, our results confirm the activation of pathogenesis-releated gene expression in tomato plants following aspirin treatment. The fact that aspirin concurrently inhibits wound-induced JA biosynthesis and activates pathogen-related protein gene expression reveals the presence of two, partially different signal transduction pathways mediating wound and pathogen responses. Additionally, these results suggest a crucial role for aspirin in the regulation of these signaling pathways. Thus, similar to salicylic acid (Doares et al., 1995), aspirin may simultaneously turn off the wound response through inhibiting JA biosynthesis, and turn on the pathogen response by activating pathogen-related protein gene expression. This accords with recent results that indicate that the pathogen response and the acquired resistance occur independent of JA in barley plants (Kogel et al., 1995).

JA is synthesized in plants from linolenic acid by an oxidative pathway that is similar to the one that leads to the synthesis of eicosanoids in animals. Indeed, the chemical structure of JA is very similar to that of prostaglandins (Vick and Zimmermann, 1987). It is interesting that eicosanoid synthesis is also triggered by certain traumatic conditions, such as mechanical, chemical, or other types of injury (Ferreira, 1977; Euler, 1988). The structural similarity between JA and eicosanoids and the fact that both JA and eicosanoids accumulate following stress conditions strongly suggest the existence of a closely related pathway in animal and plant cells and support the assumption that some common features may be involved in the modulation of both biosynthetic pathways.

Mechanical damage, electric-current application, and heat treatment all activate Pin2 gene expression locally and systemically. Although heat treatment and mechanical damage have been demonstrated to generate electrical signals that may be propagated through the vascular tissue, thereby leading to the systemic activation of Pin2 gene expression (Wildon et al., 1989, 1992), no definitive evidence has been provided that shows that both stimuli are mediated by the same chain of events. Recently, gas-exchange measurements of photosynthetic parameters such as assimilation and transpiration rates before, during, and after heat treatment, electric-current application, or mechanical damage demonstrated different classes of responses (Peña-Cortés et al., 1995). Indeed, mechanical wounding or electric-current stimulation activated two characteristic time constants in the gas-exchange relaxation kinetics. Conversely, heat stimulation led to only one major time constant, suggesting a possible alternative pathway regulating heat-induced Pin2 gene expression (Herde et al., 1995). Rapid and systemic hydraulic events, called "hydraulic signals," are triggered by localized wounds in different plants, including tomato (Boari and Malone, 1993). These signals are transmitted from the wound site but they cannot by themselves induce proteinase inhibitors (Malone et al., 1994a). A rapid hydraulic signal does not provide a specific signal for wounding, which suggests that these signals per se are not the proteinase inhibitor inducing factor (Malone et al., 1994b). Nevertheless, the hydraulic signal seems to be an essential requirement for the systemic induction of proteinase inhibitors by localized treatments (Malone et al., 1994a).

More recently, it was shown that long-distance wound signals in tomato can pass freely through heat-killed regions, indicating that neither electrical transmission nor phloem transport is involved (Malone and Alarcón, 1995). Since xylem remains fully functional after heat treatment, the authors concluded that xylem is the primary route for wound signals in tomato, postulating the hydraulic-dispersal model of systemic wound signaling. Nevertheless, no definitive evidence has been provided that shows that the transmission of electrical events is limited to the phloem, so we cannot exclude the possibility that electrical events are also propagated through the xylem or another tissue. Thus, burning of the leaf may cause a massive hydraulic event, which, in an unknown form, may influence the stability of the membrane triggering the biosynthesis of JA and the subsequent activation of Pin2 gene expression. Accepting this hypothesis and considering that mechanical damage or electric-current application do not activate JA accumulation or Pin2 genes in ABA-deficient plants, we postulate that either (a) the response to both stimuli is mediated by a different pathway in which a component in the signal pathway is missing (i.e. ABA) or (b) that the treatment conditions are not strong enough to lead to the hydraulic events generated by heat treatment. Rapid, systemic, hydraulic signals are also transmitted from leaves subjected to mechanical damage, but they are much smaller in magnitude than those observed following scorching of a single leaflet. Additionally, multiple mechanical wounding induces larger hydraulic signals than those following single wounds (Malone et al., 1994a). Multiple damage of ABA-deficient plants did not initiate Pin2 mRNA accumulation or JA accumulation (data not shown), which indicates that hydraulic signals alone are not sufficient to activate Pin2 gene expression systemically. This result confirms early observations described by Malone et al. (1994a). Furthermore, these data suggest that localized wounding by burning leads to the release of an additional signal (e.g. chemical signal) involved in the activation of Pin2 gene expression by a signaling pathway independently of ABA levels.

Different conditions used to damage the ABA-deficient plants in a massive form did not lead to an accumulation of Pin2 mRNA in the tissues of these plants (data not shown). If the heat treatment represents a more vigorous effect than mechanical wounding or electric-current application, we would expect to see differences in wild-type plants. For instance, higher levels of JA and ABA after heat treatment would be expected. However, the levels of both ABA and JA are even lower after heat treatment than after mechanical wounding in these plants (Figs. 2 and 3). Furthermore, comparison of the levels of JA in ABA-deficient plants after heat treatment and ABA treatment (Peña-Cortés et al., 1996) shows that heat-induced JA accumulation is lower than the levels observed following ABA treatment. These results suggest that heat treatment does not lead to an activation of the pathways involved in wound response in a manner stronger than mechanical wounding.

The results presented in this report support the existence of an alternative chain of events mediating heat-induced Pin2 gene expression that does not require the involvement of ABA. This pathway involves the activation of the JA biosynthetic pathway by localized burning of the leaves. Studies using aspirin suggest that heat treatment either by itself, by generating hydraulic signals, or by releasing a factor triggers the JA biosynthetic pathway in a step located before hydroperoxide dehydrase, presumably affecting lipase or LOX activity (Vick and Zimmermann, 1983). Additionally, we reported the inhibition of heat-induced Pin2 gene expression by aspirin treatment and the simultaneous activation of pathogenesis-related gene expression in tomato plants, suggesting a role for aspirin (salicylic acid) in the regulation of two different pathways.

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