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Antagonistic Effect of Salicylic Acid and Jasmonic Acid on the Expression of Pathogenesis-Related (PR) Protein Genes in Wounded Mature Tobacco Leaves

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Salicylic acid (SA) and jasmonic acid (JA) are essential compounds in the pathogen- and wound-signaling pathways accompanying induced expression of acidic and basic pathogenesis-related (PR) protein genes, respectively. However, on the effect of exogenously supplied SA and JA in induction of *PR* gene expression, conflicting results have been obtained using various plant materials at different developmental stages. There is no clear evidence on these effects in the presence of both signals at the same time. We analyzed the effect of SA on wound- and JA-induced basic *PR* gene expression and that of JA on SA-induced acidic *PR* gene expression in mature tobacco leaves. Wound-induced accumulation of transcripts for all 4 basic *PR* genes tested was enhanced in the presence of MeJA, and inhibited in the presence of SA. On the other hand, expression of all 3 acidic *PR* genes tested was induced by SA and was inhibited by MeJA. Using antibodies raised against acidic PR-1 and PR-2 proteins, these effects were confirmed at the protein level. These results indicated that JA works as an inducer of basic *PR* genes, and also as an inhibitor for acidic *PR* genes, while SA does the opposite.

Key words: Gene expression — Jasmonic acid — Pathogenesis related (PR) proteins — Salicylic acid — Tobacco plant — Wound signal transduction.

In the cultivars resitsant to pathogen, the infected host plant cells kill themselves to enclose invading pathogen in tissue. This phenomenon has been observed in the interaction of many pathogens and host plants and called as hypersensitive reaction (HR, Goodman and Novacky 1994) or response. HR depends on the interaction between a resistance (*R*) gene of host plant and the pathogen (Baker et al. 1997). As a result of HR, necrotic local lesions are developed in the infected tissue accompanying induction of salicylic acid (SA) (Malamy et al. 1990, Métraux et al. 1990) and subsequent activation of pathogenesis-related (PR) protein genes (Bol et al. 1990, Ohshima et al. 1990). Synthesis of jasmonic acid (JA) is induced by wounding in tobacco (Seo et al. 1995) and JA has been proposed as a key molecule in wound signal transduction pathways and to be produced via the octadecanoid pathway (Farmer and Ryan 1992). Ethylene as well as JA is induced in HR and reported to be an inducer of PR protein (Eyal et al. 1992, Seo et al. 1997).

PR proteins that are induced in pathological or related situations (Van Loon 1985) are classified into families on the basis of amino acid sequences, serological relationship, cellular localization, and/or enzymatic and biological activities. In addition to 5 well-studied families (PR-1 to PR-5) from tobacco, a further 6 families (PR-6 to PR-11) were proposed for classifying PR proteins (Van Loon et al. 1994). The 5 well-known families have both acidic and basic types without exception (Memelink et al. 1990, Ohashi and Ohshima 1992). Both types are induced after HR, however, they respond quite differently to wounding or treatment with SA, auxin and cytokinins (Ohashi and Ohshima 1992).

Some basic types are effectively induced by wound stress such as rubbing the leaf surface and cutting the leaf blade and localize in vacuole, however some acidic types are not induced by wounding (Memelink et al. 1990). Some basic types are found constitutively in root, but some acidic types are not (Memelink et al. 1990). Expression of acidic *PR-1* and *-2* genes is reported to be induced by SA (Ohshima et al. 1990, Hennig et al. 1993) and these gene products are localized in the intercellular spaces (Parent and Asselin 1984, Hosokawa and Ohashi 1988). Thus, the response of acidic *PR* genes to SA, JA or wounding is quite different from that to basic *PR* genes as well as cellular localization of these gene products. However, there was also a report that SA induces expression of a basic PR protein gene in young tobacco plants (Ward et al. 1991) and that SA and JA synergically induce expression of an acidic *PR* gene in tobacco seedlings (Xu et al. 1994). Thus, there is some controversy surrounding the relationship between the inducers and the responding PR protein genes. To date, SA- or JA-induced *PR* gene expression has been only partially studied using different analysis systems. It was reported that SA blocks JA biosynthesis in tomato leaves

Abbreviations: HR, hypersensitive reaction or response; IgG, immunoglobulin G; JA, jasmonic acid; MeJA, methyl jasmonic acid; PI-II, proteinase inhibitor-II; PR, pathogenesis-related; SA, salicylic acid; SAG, salicylic acid β-glucoside; TMV, tobacco mosaic virus; WIPK, wound-induced protein kinase.

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(Peña-Cortés et al. 1993), on the other hand, SA production is inhibited by JA in wounded transgenic tobacco plants containing a rice small G protein gene (Sano et al. 1996). Thus, production of SA and JA are antagonistically inhibited by JA and SA, respectively. Could there be such an antagonistic relationship of SA and JA in the induced expression of both types of *PR* genes? To fully understand the mechanisms of SA and JA signaling pathways, experiments using at least several kinds of acidic and basic *PR* genes in the same experimental system are required. Thus, we report here on the relationship of SA and JA in induced expression of acidic and basic *PR* genes probed by 7 tobacco *PR* protein genes using discs from mature tobacco leaves.

Materials and Methods

Plant material—Tobacco plants (*Nicotiana tabacum* cv. Samsun NN) were grown in a temperature-controlled greenhouse at 20°C to 30°C. Leaf discs, 17 mm in diameter, were punched out from fully expanded upper leaves of 2 month-old plants, and floated on water or solutions containing sodium salicylate (pH 7.0) and/or methyl jasmonic acid (MeJA) at 20°C under continuous light (90 μE m^{-2} s^{-1}).

Northern blot analysis—Total RNA was isolated by the ATA-method (Gonzalez et al. 1980). Fifteen μg RNA per lane was subjected to electrophoresis and blotted onto a nylon membrane (Hybond-N, Amersham), and hybridized with [^{32}P]-labeled DNA probes. Specific probes for each *PR* gene were newly synthesized by PCR. The synthetic primers were designed to synthesize the DNA fragments containing the 3'-untranslated region for acidic *PR-1* (*PR-1a*; Matsuoka et al. 1987), acidic *PR-2* (*PR-N*; Linthorst et al. 1990), acidic *PR-3* (*PR-P*, Payne et al. 1989), basic *PR-1* (*PRB-1b*; Eyal et al. 1992), basic *PR-2* (Linthorst et al. 1990), basic *PR-5* (*osmotin*; Nelson et al. 1992), and *PR-6* (*proteinase inhibitor II* (*PI-II*); Balandin et al. 1995) cDNA, respectively. Hybridization was performed overnight at 42°C in a solution containing 50% formamide and the membrane was washed twice for 15 min in 6 × SSC containing 0.5% SDS and then in 2 × SSC containing 0.2% SDS at 42°C. The membranes were then exposed to Fuji X-ray film with an intensifying screen at −80°C. The levels of transcripts were quantitatively analyzed by an image analyzer (BAS 2000, Fujix).

Western blot analysis—Leaf discs were homogenized with an equal volume of extraction buffer (50 mM phosphate buffer, pH 7.0, 1 mM EDTA, 10 μM A-PMSF, 0.5 μg ml^{-1} leupeptin, 2 mM DTT), then centrifuged for 10 min at 10,000 × *g*. The supernatant was used as crude extract for immunoblotting. For analysis of PR-1 proteins, crude extracts denatured by SDS (Laemmli 1970) were subjected to tricine-SDS-PAGE as described by Schägger and Jagow (1987). For analysis of PR-2 proteins, crude extracts were subjected to native PAGE (15% acrylamide, pH 8.9) as described by Gabriel (1971). After electrophoresis, proteins were blotted onto a polyvinylidene difluoride membrane (Millipore) by semi-dry electroblotting (Kyhse-Anderson 1984). Bands for each PR protein were immunologically detected using polyclonal antibody as described by Ohashi and Matsuoka (1985). Antibodies against purified tobacco acidic PR-1a, and PR-N, one of the acidic PR-2 proteins, were newly raised in rabbits and used as the first antibody. Alkaline phosphatase conjugated anti-rabbit IgG was used as the second antibody. These antibodies can recognize not only acidic PR-1a or PR-N protein but other acidic and basic PR-1 proteins or PR-2 proteins, respectively.

Quantitative analysis of SA, SAG, JA, MeJA and ethylene—Free SA, SAG, JA and MeJA were extracted and quantitated as described by Seo et al. (1995). For quantitative analysis of ethylene, a set of leaf discs from 8 healthy leaves which had been incubated in a large moisture box were transferred to a sealed 100 ml Erlenmeyer flask and incubated at 25°C for 1 h, and 1-ml gas samples were withdrawn with a gas-tight syringe and subjected to gas-chromatography (Shimaze GC-9A equipped with an alumina column and a flame inonization detector).

Results

SA inhibits wound-induced accumulation of the transcript for basic PR-1 gene—Freshly prepared tobacco leaf discs were incubated with or without 200 μM SA, and levels of the transcripts for acidic and basic *PR-1* genes were determined using each specific probe (Fig. 1). Transcripts of acidic *PR-1* genes were detected neither in healthy nor wounded leaves, but in the presence of SA, they were found at 12 h and increased thereafter. Transcript of the basic *PR-1* gene was detected at 12 h and increased with time after discing, but the level was clearly repressed in the presence of SA. For example, it was decreased to 20% of control by 200 μM SA at 48 h. These results indicate that SA is not only an inducer for acidic *PR-1* genes but also an inhibitor for the expression of the basic *PR-1* gene.

Induction of endogenous signal compounds, SA, JA and ethylene, after wounding—Accumulation of the transcript for the basic *PR-1* gene is wound-inducible and is inhibited by SA as shown in Fig. 1B. Wounding induces increase in endogenous JA in tobacco leaves (Seo et al. 1995). To study wound-induced levels of predicted signal molecules for *PR* gene expression, SA, JA and ethylene were quantitatively measured at the same time in the discs which were cut out from fully expanded leaves of adult tobacco plants. The levels of SA, which is the sum of free SA and salicylic acid β-glucoside (SAG), were kept at almost a constant level (0.5 μM) after wounding (Fig. 2A). Ethylene production was increased by wounding to around 1–2 nl h^{-1} (g FW)$^{-1}$ (Fig. 2B), which was about 2 orders lower than the effective concentration generally used for induction of basic *PR* genes (Eyal et al. 1992) or the induced amount in the tobacco leaves after HR by pathogen attack (De Laat and Van Loon 1982). Under our conditions, exogenously supplied ethylene at 5 ppm did not induce basic *PR* gene expression (data not shown). On the other hand, endogenous levels of JA plus MeJA were increased to 1.7 nmol (g FW)$^{-1}$ at 3 h after wounding and peaked at 4 at 12 h (Fig. 2A), at which concentration basic *PR* gene expression was induced when MeJA was exogenously supplied to freshly prepared leaf discs (Fig. 3).

Effect of SA and/or MeJA on the expression of acidic and basic PR-1 genes—To study dose responses of SA and

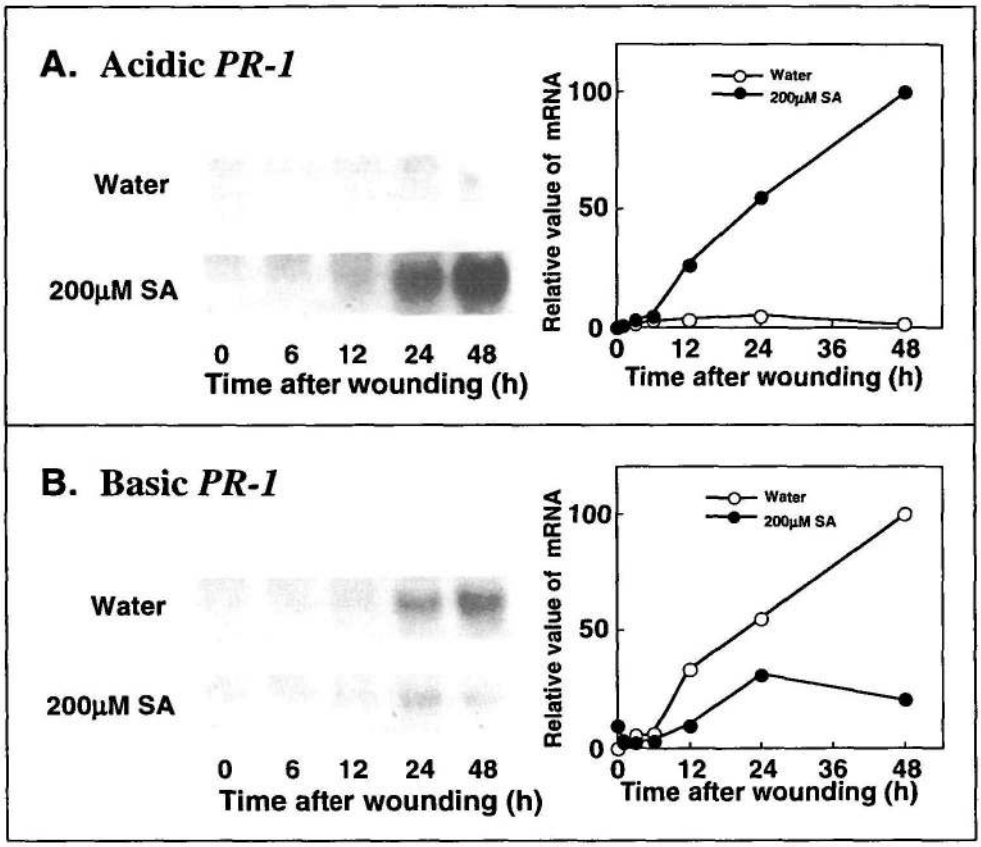

Fig. 1 Effect of SA on the expression of acidic and basic *PR-1* genes after wounding. Tobacco leaf discs were floated on water or 200 μM SA solution. After incubation at 20°C under continuous light (90 μE m^{-2} s^{-1}) for 2 d, total RNA was isolated and 15 μg RNA for each lane was subjected to RNA gel blot analysis, using probes specific for acidic (A) and basic (B) *PR-1* genes. Radioautograms are shown in the left. The level of each transcript was quantified with an image analyzer (BAS 2000, Fujix) and shown in the right as a value relative to transcripts isolated from leaf discs floated on 200 μM SA for 2 d for acidic *PR-1* gene and floated on water for 2 d for the basic *PR-1* gene.

JA for the expression of two types of *PR-1* genes, leaf discs were floated on water or solution containing various concentrations of SA and/or MeJA. Transcript levels of both types of *PR-1* genes were analyzed 2 d after the treatment (Fig. 3). In an SA concentration dependent manner, the signal for acidic *PR-1* genes was increased almost proportionally. However, the increase was clearly inhibited in the presence of MeJA, even at only 1 μM (Fig. 3A). For example the level induced by 50 μM SA was repressed to 80 and 50% in the presence of 5 and 20 μM of MeJA, respectively. On the other hand, a considerable level of the transcript for the basic *PR-1* gene was induced by wounding, and the level was effectively increased in the presence of MeJA in a concentration dependent manner (Fig. 3B). As shown in Fig. 2, the induced concentration of endogenous JA in tobacco leaves was 4 nmol (g FW)$^{-1}$ 12 h after wounding. This is enough to induce the basic *PR-1* gene expression as exogenously applied MeJA. Actually, addition of MeJA at 1 μM caused a 70% increase in the level of transcript (Fig. 3B). The induction of the basic *PR-1* gene expression by wounding can therefore be explained mainly by the increased levels of jasmonate with no direct contribution of ethylene. This wound- and MeJA-induced expression of the basic *PR-1* gene was inhibited by SA in a concentration dependent manner (Fig. 3B). Interestingly, Fig. 3A and 3B which shows the accumulated levels of mRNA for acidic and basic types of *PR-1* genes in the presence of various amount of SA and JA are a reverse mirror image. These results show that JA has a positive enhanced effect on the expression of the basic *PR-1* gene and an antagonistic effect on SA-induced expression of the acidic *PR-1* gene. And SA has quite the opposite effect. Thus, it was clearly shown that SA and JA induce the expression of two different types of *PR-1* genes respectively, and also inhibit another type of *PR-1* gene expression.

Inhibition of SA-inducible expression of acidic PR genes by MeJA and of MeJA-inducible expression of basic PR genes by SA—Results in Fig. 3 show that the expression

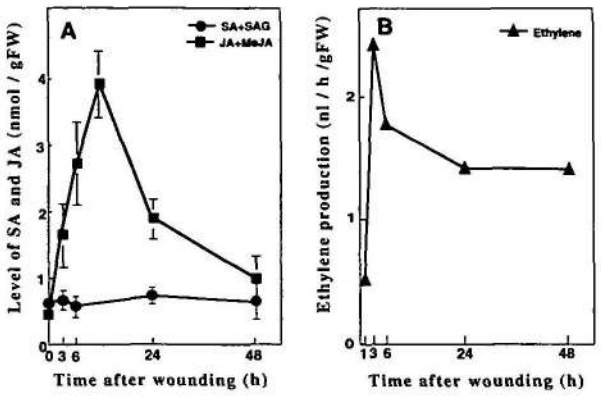

Fig. 2 Increase in endogenous SA, JA and ethylene after wounding in tobacco leaves. Leaf discs were cut out from mature healthy leaves and contents of salicylates (SA and SAG; ●) and jasmonates (JA and MeJA; ■) were determined after various incubation periods (A). Each value is the mean ± SD of three independent experiments. For ethylene determination, a set of leaf discs were prepared simultaneously from 8 healthy leaves and incubated for various periods in a moisture box. Then they were transferred to a flask and the amount of ethylene produced in 1 h was determined (▲) (B). Mean values with parallel experiments are shown.

of acidic or basic *PR-1* genes was induced by SA or MeJA respectively, and inhibited by MeJA or SA in a dose-dependent manner, respectively. To analyze whether expression of acidic and basic *PR* genes is controlled by a common regulation mechanism as in *PR-1* genes, levels of transcripts for acidic and basic types of several *PR* genes were determined using DNA probes specific for each gene. Results in Fig. 4A–C show that all acidic *PR* genes (*PR-1, 2* and *3*) tested were induced by SA, and the induction was inhibited in the presence of MeJA in a dose response manner without exception, although acidic *PR-2* gene responded more sensitively to lower concentrations of SA and was less inhibited by lower concentrations of MeJA than the other two acidic PR genes.

Expressions of 3 additive basic *PR* genes (*PR-2, 5* and *6*) as well as the basic *PR-1* gene were induced by discing which is a simple wound treatment, and the levels of the transcripts gradually elevated with increase in MeJA (Fig. 4D–F). Those increases were inhibited by SA in a concentration dependent manner without exception. The results clearly show that expressions of all acidic *PR* genes used in these experiments are regulated positively by SA and negatively by JA, and in contrast all basic *PR* genes tested are regulated positively by JA and negatively by SA. Tobacco *PI-II* gene, which was recently grouped to *PR-6* (Van Loon et al. 1994) was shown to be under a similar regulatory mechanism as basic *PR-1, -2* and *-5* genes.

Effect of SA and JA on accumulation of PR proteins —We described that SA and JA regulate the two types of *PR* genes at the transcriptional level. Next, we confirmed whether the levels of transcripts correctly reflected the levels of individual PR proteins by Western blot analysis (Fig. 5). Because SamsunNN tobacco has three genes for acidic PR-1 proteins (*PR-1a, 1b* and *1c*) of similar molecular weights that hardly separate on conventional SDS-PAGE (Matsuoka and Ohashi 1984), we used the tricine-SDS-PAGE system (Schägger and Jagow 1987). Despite

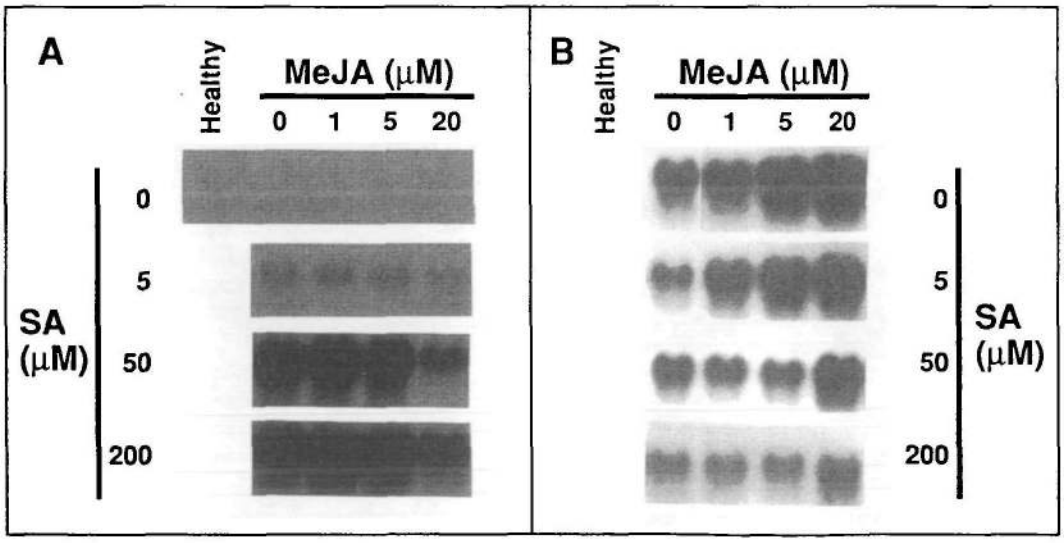

Fig. 3 Effect of SA and/or JA treatment on expression of *PR-1* genes. Total RNA from leaf discs incubated with various levels of SA and/or MeJA was isolated at 2 d after the treatment. Results of RNA gel blot analyses of acidic (A) and basic (B) *PR-1* gene are shown. Experimental conditions were as described for Fig. 1.

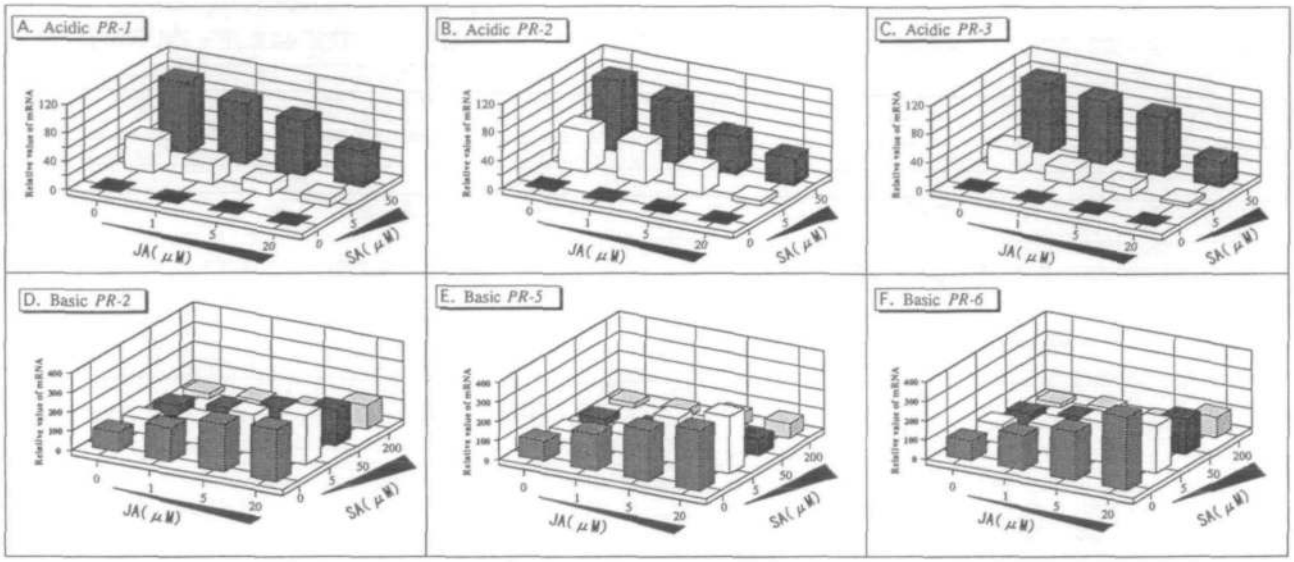

Fig. 4 Antagonistic effect of SA and JA on induction of acidic and basic *PR* genes in wounded tobacco leaves. PCR fragments corresponding to the 3′-untranslated region of acidic *PR-1* (A), *PR-2* (B) and *PR-3* (C) genes, and for basic *PR-2* (D), *PR-5* (E) and *PR-6* (F) genes were used as probes. Experimental conditions were as described for Fig. 3. The level of each transcript is shown as a value relative to transcripts isolated from leaf discs floated on 50 μM SA for acidic *PR* genes or floated on water for basic *PR* genes.

their similar molecular weights, the three proteins were clearly separated by this system (Fig. 5A). Whereas the acidic type of PR-1 proteins were not detected in healthy, wounded and MeJA-treated leaves, significant amounts were detected in SA-treated leaf discs (Fig. 5A). In both the SA and MeJA treated leaf discs, the levels of acidic PR-1 proteins were lower than that in the SA-treated leaf discs. Similar results were obtained for the PR-2 proteins (PR-2, N and O) in native gel electrophoresis (Fig. 5B), suggesting that the inhibitory effect of JA on SA-induced expression of acidic *PR* genes is basically the same at the translational as well as the transcriptional level.

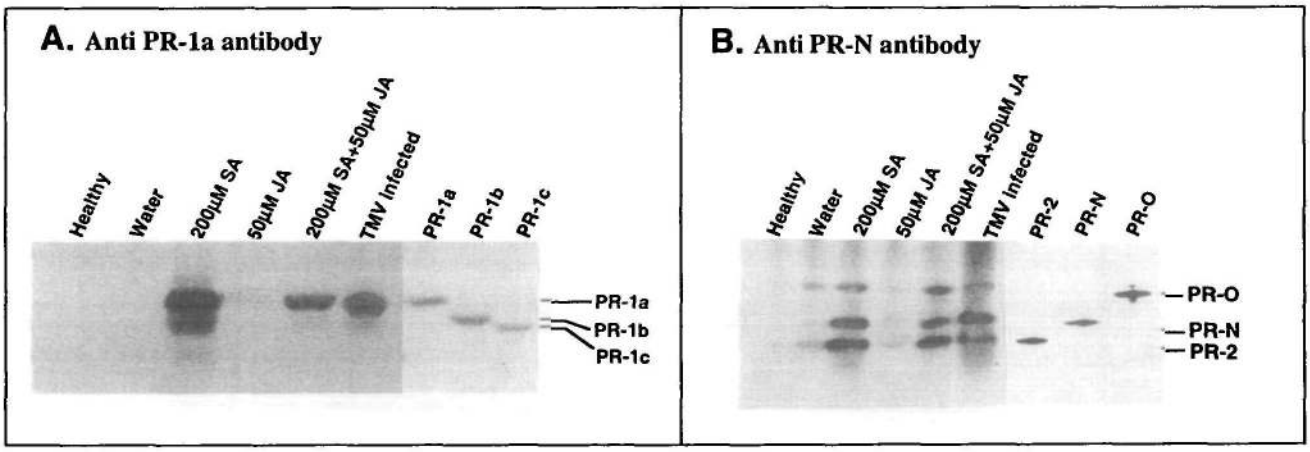

Fig. 5 Antagonistic effect of SA and JA on induction of acidic and basic PR proteins. Proteins were extracted from leaf discs which were floated on water or on 200 μM SA, 50 μM MeJA, 200 μM SA and 50 μM MeJA solution for 2 d at 20°C under continuous light (90 μE m^{-2} s^{-1}). As negative and positive controls, extracts from healthy leaves and leaves at which local lesions formed after TMV infection were used. Extract equivalent to 1.5 mg FW was loaded on each lane for all experiments except for TMV infected leaves, for which extract equivalent to 0.75 mg FW was used. After tricine-SDS-PAGE, PR-1 proteins were immunologically detected with anti tobacco PR-1a antibody (A). PR-2 proteins were detected with anti-PR-N antibody after native PAGE (B). Purified PR-1a, 1b and 1c proteins (100 ng each) were used as standard PR-1 proteins, and visualized by CBB staining (A). Purified PR-2, N and O proteins (100 ng each) were used as standard PR-2 proteins, and visualized by silver staining (B). The position of standard PR proteins are indicated in right margin.

Discussion

The results described here showed very clearly positive and negative effects of SA and JA on the expression of acidic and basic *PR* genes in mature tobacco leaf disc system. Sensitivity to the two signals differed a little between the genes, however, the basal mode of response to SA and JA on gene expression was almost the same in all 3 acidic *PR* genes or the opposite in all 4 basic *PR* genes used; SA works as an antagonist of JA-induced expression of basic *PR* genes, and JA as an antagonist of SA-induced expression of acidic *PR* genes.

Here, we proposed a model for the wound-induced signal transduction pathway and signal cross-talk for the expression of two types of PR protein genes in mature tobacco leaf disc system (Fig. 6). Production of JA by wounding (route 1; Farmer and Ryan 1992, Seo et al. 1995) and inhibition of JA production by SA or acetylsalicylic acid (route 2; Peña-Cortés et al. 1993, Doares et al. 1995) are well-known phenomena. In wild type tobacco plants, wounding does not induce SA production. However, recent studies showed that, in the presence of high levels of cytokinins, wounding abnormally induced production of SA in tobacco leaves (route 3; Sano et al. 1994, 1996, Sano and Ohashi 1995) and the SA production was inhibited in the presence of MeJA (route 4; Sano et al. 1996). In the present study, we showed new or confirmed proposed pathways leading to *PR* gene expression (routes 5, 6, 7 and 8). SA-induced acidic *PR* gene expression has been documented in a number of reports on tobacco (route 7; White 1979, Ohshima et al. 1990, Ward et al. 1991) and other plant species. On JA-induced basic *PR* gene expression, route 5, there have been the reports on basic *PR-5* (Xu et al. 1994) and *PR-6* (Doares et al. 1995). In the present study, we found that route 5 applies to basic tobacco *PR-2* genes as well as *PR-1, 5* and *6* genes. Route 6, inhibition of JA-induced basic *PR* gene expression by SA, was newly proposed by us for basic tobacco *PR-1, 2* and *5* genes. Because inhibition of wound- or JA-induced expression of proteinase inhibitor gene (*PR-6* gene) by acetylsalicylic acid has been reported in tomato plants (Doares et al. 1995, O'Donnell et al. 1996), this route could be considered a general pathway in plants. Route 8, SA-induced acidic *PR* gene expression inhibited by JA, was newly demonstrated using 3 acidic *PR* genes at various concentrations of SA and JA. These results clearly indicate that SA and JA are the inducers for acidic and basic *PR* genes, respectively, and also work as antagonists against the expression of the other type of *PR* genes, respectively. The relationship between SA and JA shown in Fig. 6 is quite symmetrical. It is symbolic that in Fig. 3A and B the levels of transcripts of acidic and basic *PR-1* genes at various concentrations of SA and JA are a reverse mirror image, and the relationship is also clearly shown in other families of PR genes (Fig. 4).

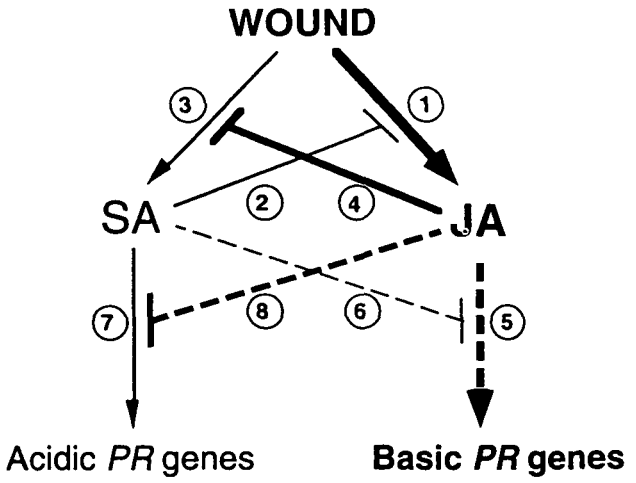

Fig. 6 Proposed pathways of wound-signal transduction on the expression of two types of *PR* genes in mature tobacco leaves. Solid lines indicate the proved pathways, and broken lines the proposed pathways which were elucidated by this work. Bold lines indicate the main normal wound-signaling pathway and the inhibitory effect by JA (See Discussion).

In the wound-signaling pathway, the role of ethylene has not been evaluated completely in contrast to JA. The endogenous level of JA detected 12 h after wounding, 1–4 nmol $(g\ FW)^{-1}$ in tobacco leaves (Fig. 2), was enough to induce basic *PR* gene expression as an exogenously supplied concentration of MeJA (Fig. 3, 4). Thus JA is considered a real signal molecule in wounding for the expression of basic *PR* genes. The levels of gaseous ethylene recovered after wounding were as low as 1 nl h^{-1} g^{-1} leaf discs in our experimental system and the levels of ethylene produced would not be enough for induction of basic *PR* genes. Some reports have described gaseous ethylene as a positive inducer of basic *PR* genes at 100 ppm (Eyal et al. 1992). Ethylene could work co-operatively with JA to induce basic *PR* gene expression (Xu et al. 1994). Recently we found a positive role for ethylene in induced expression of basic *PR* genes in tobacco plants (in preparation) with similar results for *PI* gene reported in tomato plants (O'Donnell et al. 1996). That SA inhibits ethylene synthesis (O'Donnell et al. 1996) suggests a role for ethylene similar to that of JA. This evidence indicates a co-operative role for JA and ethylene in basic *PR* gene expression even if the detected levels of ethylene in wounded leaf tissue are small.

That the pathways for SA-induced acidic *PR* gene expression and JA-induced basic *PR* gene expression are separate suggests different mechanisms of the two types of *PR* gene expression. First, wounding did not induce production of JA and induced an abnormal production of SA in transgenic tobacco plants in which the endogenous gene for a tobacco MAP kinase, WIPK (wound-induced protein kinase), was silenced (Seo et al. 1995), indicating the gene

necessary for JA signaling and SA signaling is switched on in the absence of the function of WIPK. Second, reports on the effect of inhibitors for protein kinase or phosphatase on *PR*-gene expression in tobacco leaves indicate that protein phosphorylation events are positively involved in wound and ethylene-induced basic *PR* gene expression (Raz and Fluhr 1993) and dephosphorylation events in SA-induced acidic *PR* gene expression (Conrath et al. 1997). The contrasting relationship of SA and JA shown in the present study will shed light on not only the wound-signaling but also the pathogen-signaling pathway and two types of *PR* gene expression.

It was believed that *PR* gene expression is regulated developmentally showing a constitutive expression in flower organs (Lotan et al. 1989). In the presence of high levels of cytokinin, wounding switched on the SA signaling pathway instead of the JA signaling pathway in mature tobacco leaves (Sano et al. 1996). Cytokinins and auxins inhibit the expression of basic *PR* genes (Shinshi et al. 1987) and activate acidic *PR* genes (Ohashi and Matsuoka 1987). These findings explain the contradiction between the results of Xu et al. (1994) and the present data because they used seedlings. Using intact tobacco seedlings, their data showed that basic *PR-5* gene expression was induced cooperatively by SA and JA, by SA, and not by MeJA. Intact whole seedling contains some organs in which the levels of plant hormones are variable. Because the response of acidic and basic types of *PR* genes in each organ would be variable, total levels of each *PR* gene expression in the seedlings are expected to be different from that in mature leaves as a matter of course. Studies on the effect of SA and JA on *PR* gene expression in different organs at different developmental stages are needed.

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