Research Note

Transient Accumulation of Jasmonic Acid During the Synchronized Hypersensitive Cell Death in *Tobacco mosaic virus*-Infected Tobacco Leaves

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Jasmonic acid (JA) transiently accumulated during temperature-dependent synchronous necrotic lesion formation in *Tobacco mosaic virus*-infected tobacco leaves. The accumulation of JA was preceded by activation of a tobacco mitogen-activated protein kinase, WIPK, which functions upstream of JA in wound signal transduction pathways.

The hypersensitive reaction (HR) is a plant defense system against pathogen attack. HR is characterized by the rapid death of infected cells accompanying the formation of necrotic lesions in which pathogens are thought to be enclosed. Events slightly preceding the appearance of lesions are an increase in the endogenous levels of salicylic acid (SA) (Malamy et al. 1992; Yalpani et al. 1991) and ethylene (De Laat and Van Loon 1983) and the subsequent accumulation of transcripts for pathogenesis-related (PR) proteins (Guo et al. 2000; Malamy et al. 1992). Because exogenously supplied SA and ethylene induce accumulation of transcripts for PR proteins (Brederode et al. 1991; Eyal et al. 1992; Linthorst et al. 1990; Memelink et al. 1990), SA and ethylene are thought to be endogenous signals in the PR gene expression during HR (Dong 1998; Yang et al. 1997).

Jasmonic acid is a signal molecule in wound responses of plants (Creelman and Mullet 1995; Wasternack and Parthier 1997). Exogenously supplied JA induces the expression of genes encoding basic PR proteins at the transcript and protein levels (Niki et al. 1998; Schweizer et al. 1997; Xu et al. 1994), suggesting that JA is a candidate for inducers of this type of PR protein. Little has been published, however, on changes in endogenous JA levels during HR. In *Alternaria brassicicola*-inoculated leaves of *Arabidopsis thaliana* plants, endogenous levels of JA began to increase coincident with an accumulation of the transcript of JA-inducible plant defensin gene (Penninckx et al. 1996). Kenton et al. (1999) used *Pseudomonas syringae* pv. *phaseolicola*-infected tobacco leaves to

determine that JA accumulated 3-h postinoculation, paralleling the occurrence of enhanced electrolyte leakage, which is an HR-related event.

In the HR-inducing systems described above, fungal and bacterial pathogens gradually move from infected cells to uninfected cells, resulting in the asynchronous induction of HR. To study the profiles of JA induction during HR, we used a synchronous HR-inducing system. The HR of tobacco to *Tobacco mosaic virus* (TMV) occurs in cultivars carrying the *N* gene (*NN* tobacco) but not in cultivars lacking this gene (*nn* tobacco) (Holmes 1938). The *N* gene-mediated HR is induced at temperatures below 25°C but not above 28°C (Kassanis 1952; Weststeijn 1981). When TMV-infected *NN* tobacco leaves are incubated at temperatures above 28°C and transferred to temperatures below 25°C, HR is induced synchronously in the infected tissue (Weststeijn 1981).

To synchronously induce HR, TMV-inoculated leaves of Samsun NN, an *NN* tobacco cultivar, were incubated at 30°C for 40 h, and then at 20°C. In the infected leaves, the endogenous level of JA began to increase 5 h after the temperature shift, reaching a maximum at 9 h, and gradually declined to the basal level by 36 h (Fig. 1). We found that with the use of this synchronous system, enhanced electrolyte leakage is detectable 8 h after the temperature shift and simultaneously with the appearance of necrotic lesions (Ohashi and Shimomura 1976; Ohtsubo et al. 1999) (Fig. 1). Thus, the accumulation of JA preceded the appearance of necrotic lesions. No significant increase in endogenous JA levels was seen in TMV-inoculated leaves of Samsun nn, an *nn* tobacco cultivar, and in mock-inoculated leaves of both cultivars, suggesting that neither TMV-infection nor the temperature shift causes JA accumulation.

A possible role of JA accumulation during HR is to induce expression of genes encoding basic PR proteins. We observed that transcripts of the JA-inducible basic PR-1 gene started to accumulate 6 h after TMV-inoculated Samsun NN leaves were shifted from 30 to 20°C (Fig. 2A). This accumulation was 1 h later than the accumulation of JA (Fig. 1), suggesting that the accumulation of JA precedes the accumulation of transcripts of the basic PR-1 gene.

Mitogen-activated protein (MAP) kinases are signal molecules that play a central role in the transduction of diverse extracellular stimuli. Plant MAP kinases also have been implicated in defense responses against pathogen attack (Hirt 1997; Zhang and Klessig 1999). Our recent study showed that activation of a MAP kinase, WIPK, is required for JA accumulation in wounded tobacco plants (Seo et al. 1999). To examine activation of WIPK in our synchronous system, a time-course analysis was made of the level of WIPK activity after the temperature shift. Crude leaf extracts were immunoprecipitated with an anti-WIPK antibody (Seo et al. 1999), and the immune complexes were subjected to the kinase assay with myelin basic protein (MBP) as an artificial substrate. In TMV-inoculated, but not mock-inoculated Samsun NN leaves, a change in the kinase activity of WIPK was detectable 4 h after the temperature shift, reaching a peak at 8 h and declining gradually thereafter (Fig. 2B). The timing of the initial increase in kinase activity was 1 h earlier than the initial increase in the endogenous

JA levels. There was no MBP kinase activity of WIPK in TMV- or mock-inoculated Samsun nn leaves (data not shown). Consistent with our observation, WIPK is activated 4 h after the temperature shift from 32 to 22°C for TMV-infected leaves of Xanthi nc, an *NN* tobacco cultivar (Zhang and Klessig 1998). These time-course analyses indicate that during the *N* gene-mediated HR, the activation of WIPK precedes the accumulation of JA. This is supported by the observation that JA treatment did not activate WIPK (Kumar and Klessig 2000).

The idea of cross talk between defense responses to pathogen attack and wounding has been discussed previously (Doares et al. 1995; Felton et al. 1998; Sano et al. 1994). On the basis of this concept, it was suggested that the JA-signaling pathway in HR occurs very early in the plant resistance response to pathogens and may have features in common with wound responses. It is therefore possible that the accumulation of JA during HR in TMV-infected tobacco is caused by activation of WIPK.

Fig. 1. Levels of jasmonic acid (JA) in *Tobacco mosaic virus* (TMV)- and mock-inoculated tobacco leaves after temperature shift. Upper, fully expanded healthy leaves of 2-month-old Samsun NN (NN) and Samsun nn (nn) tobacco plants were detached, inoculated with 10 µg of TMV per ml or buffer only (Mock), incubated at 30°C for 40 h, and shifted to 20°C at time 0. All of the inoculated leaves were set on wet filter paper in transparent boxes and incubated under continuous illumination at an intensity of 120 µE·m⁻²·s⁻¹. Leaves were harvested at the indicated time after the temperature shift and used for quantitation of JA, which was extracted as described (Lehmann et al. 1995) and analyzed by gas chromatography-mass spectrometry selected ion monitoring as described (Seo et al. 1999). [³H₂](±)-JA was used as an internal standard (Nojiri et al. 1992). Values are the mean ± standard deviation from three independent measurements. All data are corrected for losses. Arrow indicates the earliest time point where electrolyte leakage was observed.

Fig. 2. Levels of basic pathogenesis-related (PR)-1 transcripts and myelin basic protein (MBP) kinase activity of WIPK in *Tobacco mosaic virus* (TMV)- and mock-inoculated tobacco leaves after the temperature shift. Upper, fully expanded healthy leaves of 2-month-old Samsun NN tobacco plants were detached, inoculated with 10 µg of TMV per ml or buffer only (Mock), incubated at 30°C for 40 h, and shifted to 20°C at time 0. Leaves were harvested at the indicated time after the temperature shift and used for RNA gel blot analysis or myelin basic protein (MBP) kinase assay of WIPK. **A**, RNA gel blot analysis. Aliquots of 20 µg of total RNA per lane were fractionated by gel electrophoresis, transferred to nylon membranes, and subjected to hybridization with the basic PR-1 cDNA probe (Niki et al. 1998). To standardize RNA loading, the blot was stained with methylene blue (rRNA). **B**, MBP kinase activity. Soluble proteins containing 50 µg of total protein were immunoprecipitated with 5 µg of the anti-WIPK antibody, and the level of MBP kinase activity was measured in the immunoprecipitate (Seo et al. 1999). Arrow indicates the position of the phosphorylated MBP. **A and B**, The experiments were repeated three times with similar results.

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