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Short communication

Systemic accumulation of 12-oxo-phytodienoic acid in SAR-induced potato plants

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Abstract

In potato plants induced for systemic resistance by infiltration with *Pseudomonas syringae* pv. *maculicola*, 12-oxo-phytodienoic acid (OPDA) accumulated in infiltrated leaves as well as in non-treated leaves of infected plants. In contrast, jasmonic acid (JA) levels increased only in infiltrated leaves, suggesting that the biosynthetic precursor of JA, OPDA, might play a role in systemic acquired resistance.

Abbreviations: JA – jasmonic acid; OPDA – 12-oxo-phytodienoic acid; SAR – systemic acquired resistance.

Systemic resistance in potato can be induced by elicitors such as arachidonic acid (AA; Cohen et al., 1991; Coquoz et al., 1995), jasmonic acid (JA; Cohen et al., 1993) or by infection with pathogens (Strömberg, 1995; Kombrink et al., 1996). Development of necroses on potato leaves infiltrated with avirulent *Pseudomonas syringae* pv. *maculicola* correlates with systemic activation of defense genes as well as with a reduction in disease symptoms upon subsequent infection with *Phytophthora infestans* (Kombrink et al., 1996; Kombrink and Schmelzer, 2001).

In contrast to other plants, putative signaling compounds involved in systemic acquired resistance (SAR) in potato are unknown. Salicylic acid (SA) and JA have been identified in tobacco, cucumber and Arabidopsis as signal molecules in induced resistance responses, such as SA-dependent pathogen-induced systemic resistance (Dong, 1998), JA/ethylene-mediated rhizobacteria-induced systemic resistance (Pieterse et al., 1998) and wound-induced systemic resistance (Schweizer et al., 1998). Recent models suggest considerable crosstalk between the SA- and JA/ethylene-dependent signaling pathways, and inhibitory (Gupta et al., 2000), neutral (Imanishi et al., 2000) and additive (van Wees et al., 2000) effects have been described.

In comparison to other plants, potato contains rather high levels of SA (Coquoz et al., 1995). The apparent lack of systemic SA accumulation in AA-induced SAR (Coquoz et al., 1995) and, on the other hand, the inability to obtain SAR in SA-deficient *nahG* potato plants (Yu et al., 1997) suggests a unique function of SA for SAR in potato.

With respect to activation of pathogen-defense responses in potato plants, little is known about JA and its derivatives, the jasmonates, and its precursor 12-oxo-phytodienoic acid (OPDA) and related compounds, collectively called octadecanoids. JA originates from α-linolenic acid (LeA) which is converted in three enzymatic steps to OPDA. Removal of the double bond within the cyclopentanone ring and three steps of β-oxidation yield (+)-iso-JA which is converted into the more stable (−)-JA (Wasternack and Parthier, 1997). Despite detailed knowledge about JA- and wound-induced signal transduction and gene expression in solanaceous plants

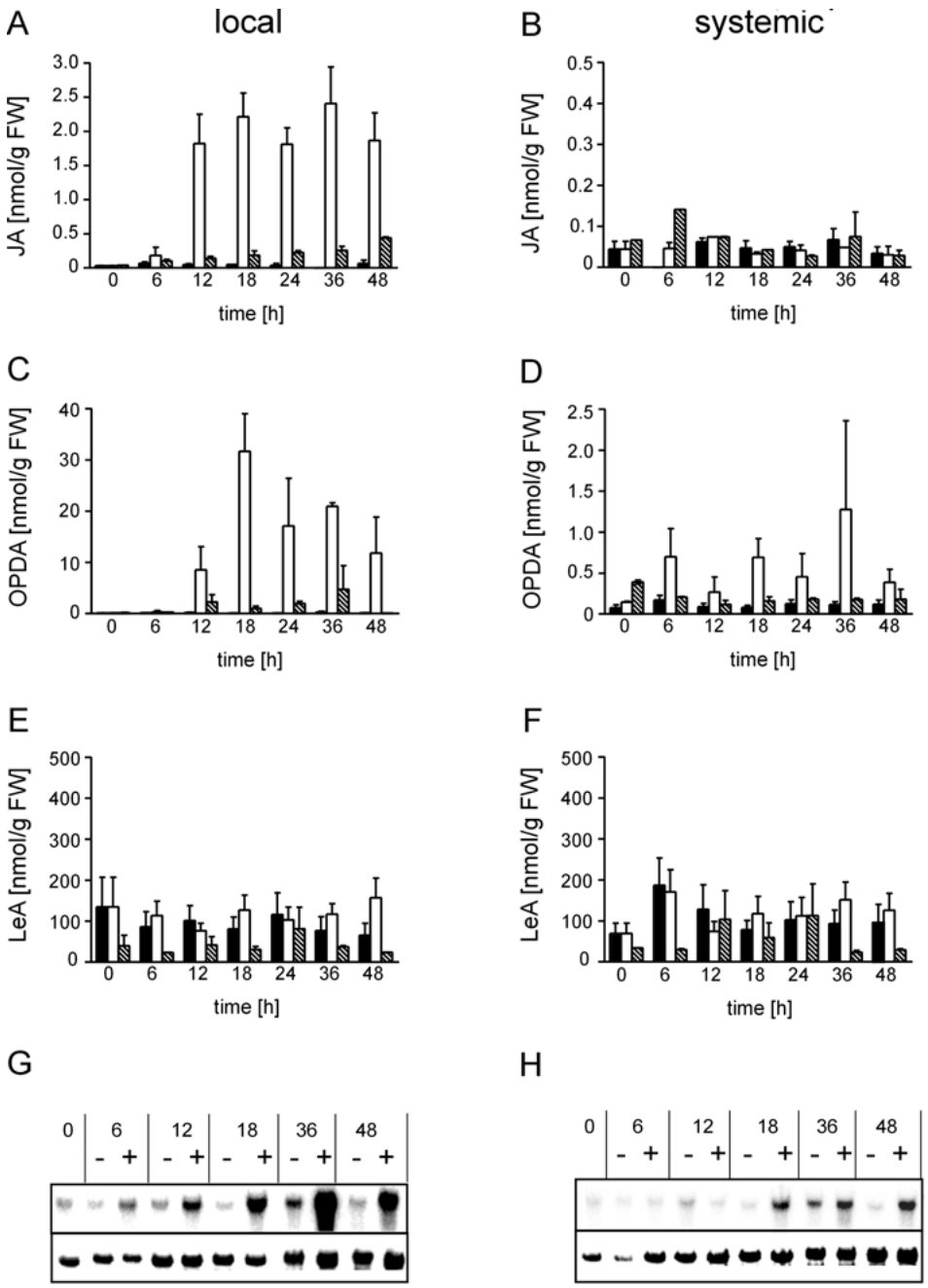

Figure 1. Local (A, C, E) and systemic (B, D, F) accumulation of JA (A, B), OPDA (C, D) and LeA (E, F) in leaves of potato plants infiltrated with $MgCl_2$ (black bars), *P. syringae* pv. *maculicola* (white bars) or Eosin (hatched bars). The mean values of five independent experiments are shown. Error bars indicate standard deviations. G and H show local and systemic accumulation of chitinase A transcripts (upper panel) in $MgCl_2$-(−) or bacteria-infiltrated (+) potato leaves at the time points indicated. As a loading control, the gel was stained with ethidiumbromide (lower panel).

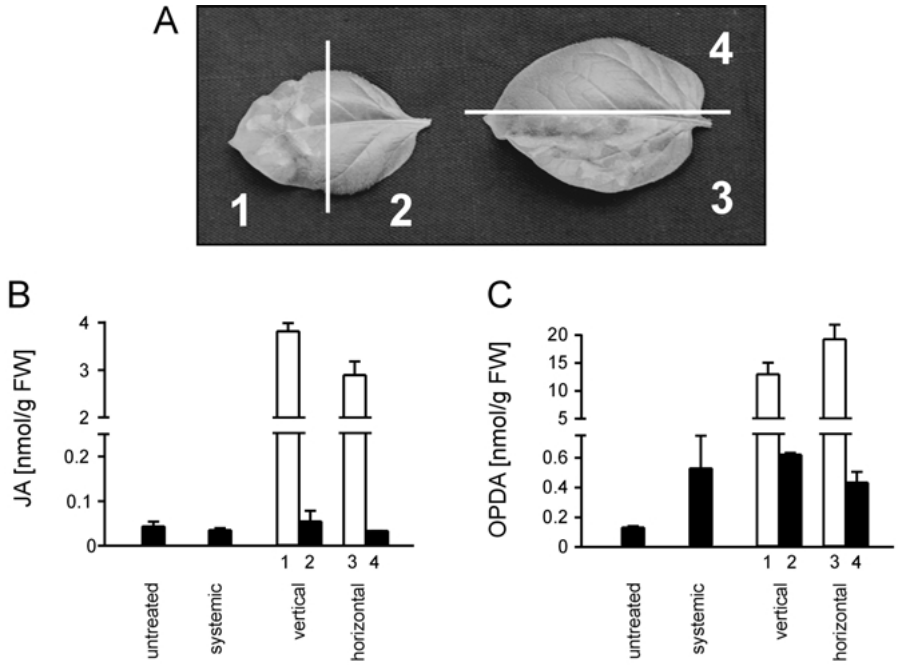

Figure 2. A: Potato leaves 18 h after partial infiltration with *P. syringae* pv. *maculicola*. Numbers mark the areas which were used for determination of JA and OPDA levels (1 and 3: infiltrated, 2 and 4: non-infiltrated halves). B and C: Accumulation of JA (B) and OPDA (C) in potato leaves 18 h after infiltration with *P. syringae* pv. *maculicola*. JA and OPDA levels in leaf areas 1 and 3 from A (white bars) and 2 and 4 (black bars) are shown as well as those from untreated plants and from non-treated upper leaves of bacteria-infiltrated plants ('systemic'). The mean values are from three independent experiments. Error bars indicate standard deviations.

(Leon et al., 2001; Ryan, 2000), the role of octadecanoids in response to pathogen attack in potato is unclear. Therefore, we measured steady-state levels of JA and its precursors LeA and OPDA in potato plants infiltrated with avirulent *P. syringae* pv. *maculicola*.

Lower leaves of five-week-old potato plants (cv. Desirée) grown from sterile plants (16 h light [200 μE], 18 °C, 60% humidity) were infiltrated with *P. syringae* pv. *maculicola* (Kombrink et al., 1996). Both the infiltrated and the upper, non-treated leaves of infiltrated plants were used for analysis of JA, OPDA and LeA (Hause et al., 2000). As controls, leaves were infiltrated either with 10 mM $MgCl_2$ or with the photodynamic substance Eosin which causes necrosis formation upon exposure to light.

We observed a rapid and sustained increase in JA levels in *Pseudomonas*-infiltrated potato leaves starting 6 hpi and reaching maximum levels of approximately 2 nmol/g FW 12 hpi (Figure 1). $MgCl_2$-infiltrated leaves did not contain significantly increased amounts of JA. The slight and late increase in JA levels in Eosin-treated leaves indicates that necrosis formation alone is not responsible for the strong accumulation of JA after infiltration with *Pseudomonas*. No systemic accumulation of JA was observed in bacteria-infiltrated plants or in those treated with $MgCl_2$ or Eosin (Figure 1B).

Compared to JA, OPDA accumulated to about 15 times higher levels (around 30 nmol/g FW) in infiltrated leaves with a transient increase starting 12 hpi (Figure 1C). $MgCl_2$-treated plants did not show accumulation of OPDA, whereas Eosin-treated plants had slightly increased amounts at later time points. Intriguingly, in contrast to JA, OPDA accumulated in infiltrated leaves and also to significant amounts in non-treated leaves of bacteria-infiltrated plants (Figure 1D) starting 6 hpi and lasting over at least 48 h. The highest levels of OPDA, 1.2 nmol/g FW, represented roughly 5% of the amount accumulating locally. Accumulation of OPDA in non-treated leaves of infected plants preceded the systemic accumulation of chitinase A transcripts which started between 12 and 18 hpi (Figure 1H). In infiltrated leaves, chitinase A transcript levels increased before OPDA accumulation

between 6 and 12 hpi (Figure 1G), in accordance with published data (Kombrink et al., 1996).

Linolenic acid, the precursor of OPDA and JA, was present at high amounts of up to 200 nmol/g FW and did not show significant alterations locally or systemically after infiltration of bacteria, $MgCl_2$ or Eosin (Figure 1E and F), suggesting that the amount of free LeA is not limiting in potato leaves.

To analyze the accumulation pattern within the infiltrated leaf, either the tip ('vertical') or one half of the leaf blade ('horizontal') was infiltrated with *P. syringae* pv. *maculicola* as shown in Figure 2A. Local accumulation of JA (Figure 2B) and OPDA (Figure 2C) was similar to the data shown in Figure 1. In the non-infiltrated parts of the leaves, the levels of JA and OPDA were equivalent to the amounts detected in upper non-treated leaves, i.e. OPDA accumulated whereas JA levels were not significantly different from those of untreated control plants. Similar amounts were measured after 'vertical' or 'horizontal' infiltration, indicating an equal distribution of OPDA and JA across the whole leaf.

Pathogen-induced systemic accumulation of OPDA is novel, not only for potato but also for other plants. While JA accumulation is restricted to the area of necroses, similar to *Pseudomonas*-infiltrated tobacco leaves (Kenton et al., 1999), the increase in OPDA levels in upper leaves suggests a role of this JA precursor in induced defense responses in potato. OPDA is a potent inducer of various responses and does not have to be metabolized to JA in order to become active (Blechert et al., 1999; Kramell et al., 2000; Miersch and Wasternack, 2000). In the tendril-coiling system of *Bryonia dioica*, OPDA possesses stronger biological activity than JA (Weiler et al., 1994; Blechert et al., 1999).

The 15-fold higher accumulation of OPDA compared to JA reported here and the similar dose–response relationship for both compounds observed in solanaceous plants (Wasternack et al., 1998) suggests that OPDA might be the preferential signal in potato. Furthermore, the systemic accumulation of only OPDA suggests its role in systemic defense reactions. Two different possibilities can be envisaged on systemic OPDA generation: (i) OPDA might be loaded into the phloem to be transported symplastically as shown for systemin in wound induction (Ryan, 2000), or (ii) OPDA might be generated systemically, analogous to the recent finding in wound-signaling, that systemin activates systemic AOC expression and OPDA/JA formation in tomato (C. Wasternack, personal communication).

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