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Rapid Communication

Is the High Basal Level of Salicylic Acid Important for Disease Resistance in Potato?[1]

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Potato (*Solanum tuberosum*) plants contain a high basal level of salicylic acid (SA), the role of which in disease resistance is currently unclear. Here we report that, in spite of a drastic reduction in total SA levels in transgenic potato plants expressing the bacterial salicylate hydroxylase gene (*nahG*), there was no significant increase in disease severity when infected by *Phytophthora infestans*. Therefore, the high basal level of SA does not lead to constitutive resistance in healthy potato plants. However, in contrast to control plants, arachidonic acid failed to induce systematic acquired resistance (SAR) in *nahG* plants against *P. infestans*, indicating an essential role of SA in potato SAR. These results suggest that in potato the development of SAR against *P. infestans* may involve increased sensitivity of the plant to SA.

Plants have a variety of active mechanisms for defending themselves against microbial pathogen infection. For most resistant plants an attempted infection by a pathogen results in the induction of defense mechanisms at the sites of infection, which are frequently manifested as necrotic lesions resulting from host cell death (hypersensitive responses). In addition, defense responses are frequently activated in the surrounding and even distal, uninfected parts of the plant, leading to the development of SAR (Klessig and Malamy, 1994; Hammond-Kosack and Jones, 1996; Ryals et al., 1996).

Both the hypersensitive response and SAR are associated with increased expression of a large number of defense or defense-related genes (Klessig and Malamy, 1994). The detailed sequence of molecular events for the initiation and regulation of the hypersensitive response and SAR is unknown. However, considerable progress has been made during the past several years and several components of the signal transduction pathways leading to the activation of disease resistance have been identified (for reviews, see Klessig and Malamy, 1994; Bent 1996; Hammond-Kosack and Jones, 1996; Ryals et al., 1996).

Many studies have indicated that SA is an important signaling factor in the induction of plant disease resistance (Klessig and Malamy, 1994; Chen et al., 1995; Ryals et al., 1996). In an increasing number of plant species elevated levels of SA have been associated with resistance of the infected plant to the invading pathogen (Malamy et al., 1990; Métraux et al., 1990; Rasmussen et al., 1991; Uknes et al., 1993). A large body of evidence suggests that this systemic increase in SA levels is important for the induction of SAR (Klessig and Malamy, 1994; Ryals et al., 1996). In support of this hypothesis, induction of SAR is blocked in transgenic tobacco (*Nicotiana tabacum*) and Arabidopsis plants harboring the bacterial *nahG* gene encoding salicylate hydroxylase, which converts SA to catechol (Gaffney et al., 1993; Delaney et al., 1994). Furthermore, several mutants of Arabidopsis have been identified that are both defective in SA signal transduction and unusually susceptible to pathogen infection (Cao et al., 1994, 1997; Delaney et al., 1995; Shah et al., 1997).

Although the role of SA in disease resistance is well established in tobacco and Arabidopsis, its involvement in activating plant defense mechanisms in other plants is unclear. Studies during the last several years of basal SA levels and the effect of exogenously applied SA have suggested that there might be a substantial difference in SA responsiveness among different plant species. For example, in tobacco and Arabidopsis, the basal SA levels are very low (<50 ng g^{-1} fresh weight) and only a small increase (1.2- to 4-fold, to 60–200 ng g^{-1} fresh weight) is sufficient for the establishment of SAR (Malamy et al., 1990; Enyedi et al., 1992; Vernooij et al., 1994). Furthermore, in certain tobacco hybrids (Yalpani et al., 1993) and Arabidopsis mutants (Bowling et al., 1994; Dietrich et al., 1994; Greenberg et al., 1994; Weyman et al., 1995), stunted growth and the development of necrotic lesions are associated with elevated SA levels. However, in many plant species, including tomato and soybean, basal SA levels far exceed the elevated levels associated with SAR in tobacco and Arabidop-

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Abbreviations: AA, arachidonic acid; SA, salicylic acid; SAR, systemic acquired resistance.

sis (Raskin et al., 1990). In rice the basal SA levels are at least several hundred-fold higher than those found in tobacco and Arabidopsis, without apparent deleterious biological effects (Silverman et al., 1995; Chen et al., 1997).

The role of endogenously produced SA in the induction of potato (*Solanum tuberosum*) disease resistance is currently unclear. It is known that potato plants contain high basal levels of SA (40- to 100-fold higher than those found in tobacco and Arabidopsis; Coquoz et al., 1995) and that application of exogenous SA does not enhance disease resistance against *Phytophthora infestans*, the causal pathogen of potato late blight (Coquoz et al., 1995). Furthermore, although SAR can be induced in potato by certain pathogens (e.g. *P. infestans*; Doke et al., 1987) or pathogen elicitors (e.g. AA; Cohen et al., 1991; Coquoz et al., 1995), no increase in endogenous SA levels has been observed in upper, untreated leaves (Coquoz et al., 1995). The striking differences between these results and those in tobacco or Arabidopsis are evidence that the induction of SAR in potato is either independent of SA or involves a mechanism different from that found in tobacco and Arabidopsis.

To critically evaluate the role of SA in potato disease resistance, we recently constructed transgenic potato plants that express the bacterial *nah*G gene encoding salicylate hydroxylase (You et al., 1991; Gaffney et al., 1993). In this report we demonstrate that, despite a drastic reduction in total SA levels, there is no significant increase in the susceptibility of NahG potato plants to primary infection by *P. infestans*. This suggests that the high basal level of SA in healthy potato plants does not constitutively activate defense mechanisms against *P. infestans*. On the other hand, although SAR against *P. infestans* can be effectively induced by AA in control potato plants, it is not induced in NahG plants. This indicates that SA is an essential component in potato in the AA-induced SAR against *P. infestans*. Thus, although healthy potato plants seem to respond poorly to either exogenously applied or endogenously produced SA, AA treatment renders them responsive to the high basal level of SA, probably by activating certain regulatory components important for SA signal perception and/or transduction.

MATERIALS AND METHODS

Chemicals, Plants, and Pathogen

SA, AA, and other common chemicals were purchased from Sigma. All experiments were done with potato (*Solanum tuberosum*) line FL1067. Plants were propagated in vitro on rooting-inducing medium (Wenzler et al., 1989) and transferred after 10 to 15 d to pots in greenhouses. Plants were grown for 4 to 5 weeks in greenhouses (22°C with a 16-h light period). Isolate 15 of *Phytophthora infestans*, collected in a southern Idaho potato field, was used throughout the study. The fungus was grown on bean agar and the sporangia were harvested by washing the plates with sterilized water, as described previously (Liu et al., 1994).

Construction of Transgenic NahG Potato Plants

Potato plants were transformed by the *Agrobacterium tumefaciens*-mediated leaf disc transformation procedure (Wenzler et al., 1989) using the transformation vector pGA482 (An, 1986) containing a 35S:*nah*G gene construct (Bowling et al., 1994). Transformants were selected for kanamycin (50 μg mL^{-1}) resistance. Several control transgenic lines were also obtained by transforming potato with the vector pGA482 without the *nah*G gene. Independently transformed potato lines were propagated on tissue culture medium.

Determination of SA

Free and conjugated SAs were extracted from potato leaves using the same procedure described for tobacco and Arabidopsis (Bowling et al., 1994). SA separation and quantification were performed by HPLC, as previously described (Bowling et al., 1994).

DNA Preparation and Southern Analysis

Potato genomic DNA was extracted from leaves using a procedure previously described by Dellaporta et al. (1983). For Southern analysis 10 μg of potato genomic DNA was digested with *Bam*HI, separated on a 0.8% agarose gel, and transferred to a nylon membrane using standard procedures (Sambrook et al., 1989). A *Hin*dIII fragment containing the *nah*G gene was labeled with [α-^{32}P]dATP by random priming and used as a probe. Hybridization was carried out in 6× SSC, 5× Denhardt's solution, and 10 μg mL^{-1} denatured salmon sperm DNA at 62°C for 24 h. The filter was washed with 0.5× SSC and 0.25% SDS at 62°C for 2 to 3 h.

RNA Preparation and Northern Analysis

Total RNA from potato leaves was prepared using a procedure previously described by Logemann et al. (1987). For northern analysis, total RNA (20 μg) was separated on an agarose-formaldehyde gel and blotted to a nylon membrane following standard procedures (Sambrook et al., 1989). The blot was hybridized with the [α-^{32}P]dATP-labeled *Hin*dIII *nah*G fragment. Hybridization was carried out in 6× SSC, 5× Denhardt's solution, 50% formamide, 10% dextran sulfate, 50 μg mL^{-1} heparin, and 2 mg mL^{-1} nonfat milk at 65°C for 16 h. The membrane was washed at 65°C for 1 h with 3× SSC and 0.5% SDS, 30 min in 1× SSC and 0.5% SDS, and 40 min in 0.1× SSC and 0.5% SDS.

Treatment and Fungal Infection of Potato Plants

Two transgenic NahG lines (lines 5 and 6) were used throughout the study because they showed the lowest levels of SA from the first SA measurement, although the difference from other lines was very small. To analyze AA-induced SAR in potato, the abaxial surfaces of three lower leaves of 4- to 5-week-old potato plants were sprayed with a sonicated suspension of AA (1500 μg mL^{-1})

using a fine-glass chromatography sprayer as described by Cohen et al. (1991). SA was applied by spraying the plants with 2 mM SA, pH 6.5.

Disease responses of potato plants to *P. infestans* were assessed with a commonly used detached leaflet assay described previously (Liu et al., 1994; Wu et al., 1995). Three leaflets of similar size (25–30 cm) were taken for inoculation by adding 20 μL of sporangia suspension (2×10^5 sporangia mL^{-1}) to the center of the abaxial leaf surface. The inoculated leaflets were kept in Petri dishes with 100% humidity provided by moisturized filter paper. The Petri dishes were incubated at 15°C for 48 h before they were moved to a growth chamber with a 16-h photoperiod at 19°C. Lesion diameters were measured on d 6 after inoculation.

RESULTS

SA Levels in Transgenic NahG Plants

Using *A. tumefaciens*-mediated leaf disc transformation followed by selection for kanamycin resistance (Wenzler et al., 1989), we obtained 11 independently transformed potato NahG lines. Transformation was verified by Southern analysis with a *Hin*dIII fragment containing the 35S:*nah*G chimeric gene as a probe (Bowling et al., 1994). One to three copies of the *nah*G gene were integrated into the genome of these transgenic potato lines (data not shown). Northern analysis indicated that plants from most of these transgenic NahG lines accumulated *nah*G mRNA to very similar levels (Fig. 1).

It has been previously shown that potato, unlike tobacco and Arabidopsis, contains a relatively high level of SA even in the uninfected, healthy plants (Coquoz et al., 1995). Analysis of control potato lines confirmed the presence of high levels of both free and conjugated SA (Table I). In contrast, all 11 NahG lines exhibited a 10- to 20-fold decrease in free SA levels and a 100- to 300-fold decrease in conjugated SA levels compared with control plants (Table I). The similar reduction in SA levels was consistent with the similar level of *nah*G expression observed in these plants from northern analysis (Fig. 1). No morphological differences were observed in these NahG plants as a result of the drastic reduction in SA level. However, during the normal 4- to 7-week period when plants were grown in the greenhouses, flowering of the NahG plants but not the control lines was frequently observed (data not shown).

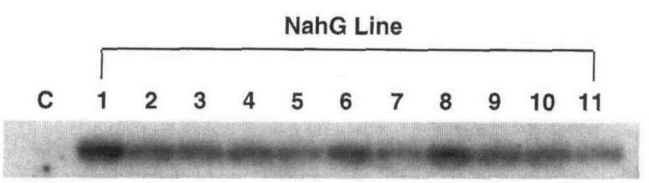

Figure 1. Expression of the *nah*G gene in transformed potato lines. Total RNA was isolated from the leaves of 4-week-old control (lane C) and NahG (lanes 1–11) plants, separated on a formaldehyde-containing argarose gel, and probed with a ^{32}P-labeled *nah*G DNA fragment.

Table I. *SA levels in control and NahG potato plants*

Free and conjugated SA were extracted from leaves of similar age from 4- to 5-week-old control (transformed with PGA482 without the *nah*G gene) and NahG plants. SA extraction, separation, and quantification were performed using the same procedures described for tobacco and Arabidopsis (Bowling et al, 1994). Mean values ± SE from three independent measurements are shown.

Plant Line	SA Level	
	Free SA	Conjugated SA
	ng g^{-1} fresh wt	
Control	141 ± 16	5040 ± 498
NahG-1	4 ± 2	43 ± 10
NahG-2	10 ± 3	65 ± 9
NahG-3	7 ± 1	126 ± 26
NahG-4	9 ± 2	17 ± 2
NahG-5	7 ± 2	46 ± 6
NahG-6	9 ± 2	9 ± 2
NahG-7	5 ± 1	19 ± 3
NahG-8	6 ± 1	13 ± 2
NahG-9	7 ± 2	26 ± 3
NahG-10	7 ± 1	51 ± 11
NahG-11	11 ± 2	38 ± 7

Primary Response to *P. infestans*

It was previously demonstrated that tobacco and Arabidopsis plants expressing the *nah*G gene are impaired not only in the establishment of SAR but also in their responses to primary infection by a variety of viral, fungal, and bacterial pathogens (Delaney et al., 1994). To assess whether the drastically reduced SA levels had any effect on potato disease susceptibility, we first tested the primary response of these NahG plants to infection by *P. infestans*, the fungal pathogen causing late blight. Detached leaves of similar age were taken from NahG and control plants, inoculated with the sporangia of *P. infestans*, and scored for disease severity on d 6 after inoculation. As shown in Table II, the lesions on NahG plants were not significantly larger than those on control plants, indicating that the high basal level of SA in the control plants does not serve to constitutively activate defense mechanisms, at least against *P. infestans*. Furthermore, application of exogenous SA provided little protection against infection by *P. infestans* on either control or NahG plants (Table II). Therefore, unlike tobacco and Arabidopsis, healthy potato plants failed to

Table II. *Effect of nahG expression and application of SA on the response of potato plants to P. infestans*

Three leaves of similar size were detached from control or NahG plants and inoculated by adding 20 μL of sporangia suspension (2×10^5 sporangia mL^{-1}) of *P. infestans*. Lesion sizes were determined 6 d after inoculation and are reported ± SE calculated from three independent experiments.

Plant Line	Lesion Size	
	−SA	+SA
	cm^2	
Control	16.9 ± 3.1	17.1 ± 1.4
NahG-5	17.9 ± 2.3	17.4 ± 1.1
NahG-6	18.1 ± 2.3	18.1 ± 0.7

Table III. *AA-induced SAR to P. infestans in control and NahG potato plants*

Three leaves from each control or NahG plant were sprayed with either water or 1500 μg mL^{-1} AA. Six days after the spray, the leaves immediately above the sprayed leaves were detached and inoculated by adding 20 μL of sporangia suspension (2×10^5 sporangia mL^{-1}) of *P. infestans*. Lesion sizes were determined 6 d after inoculation and are reported ± SE calculated from three independent experiments.

Plant Line	Lesion Size in Upper, Untreated Leaves	
	Water-treated	AA-treated
	cm²	
Control	17.5 ± 2.1	0.8 ± 0.7
NahG-5	18.5 ± 3.2	16.2 ± 1.1
NahG-6	18.8 ± 2.4	16.3 ± 1.2

respond not only to the high basal level of SA found in healthy plants but also to exogenously applied SA.

SAR to *P. infestans*

SA plays a critical role in biologically or chemically induced SAR in tobacco and Arabidopsis (Ryals et al., 1996). In potato, SAR against *P. infestans* can be induced either biologically, by prior exposure of lower leaves to *P. infestans* (Doke et al., 1987), or chemically, by treatment of lower leaves with AA, a natural elicitor produced by *P. infestans* (Cohen et al., 1991; Coquoz et al., 1995). To determine whether SA plays a similar role in potato SAR, we analyzed the upper, untreated leaves of control and NahG plants for lesion formation by *P. infestans* after the lower leaves had been treated with AA. As expected, AA treatment of lower leaves of control plants protected the upper leaves from the challenge infection by *P. infestans* (Table III; Fig. 2). However, AA treatment of lower leaves did not result in a strong reduction in late blight disease severity in the upper leaves of NahG plants (Table III; Fig. 2), suggesting that in potato SA is essential in AA-induced SAR against *P. infestans*.

Biologically or chemically induced SAR in tobacco, Arabidopsis, and cucumber is associated with enhanced SA biosynthesis and, consequently, a systemic increase in SA levels (Malamy et al., 1990; Métraux et al., 1990; Rasmussen et al., 1991; Uknes et al., 1993). We found that the leaves of control potato plants treated with 1500 μg mL^{-1} AA accumulated 2- to 3-fold more free and conjugated SA than did

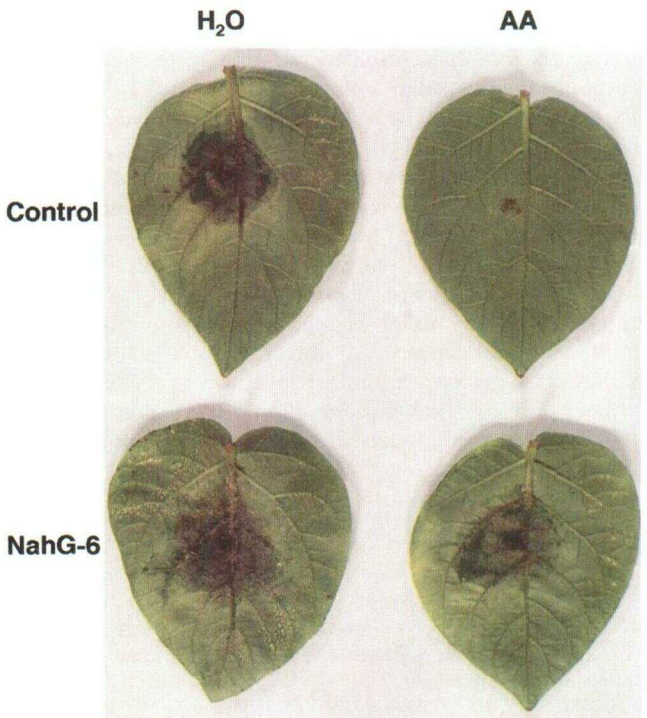

Figure 2. Effects of *nah*G expression on AA-induced SAR in potato. Three leaves from each control or NahG-6 plant were sprayed with either water or 1500 μg mL^{-1} AA. Six days after the spray, the leaves immediately above the sprayed leaves were detached and tested for their disease response to *P. infestans* infection. Upper infected leaves were photographed 5 d after the *P. infestans* inoculation.

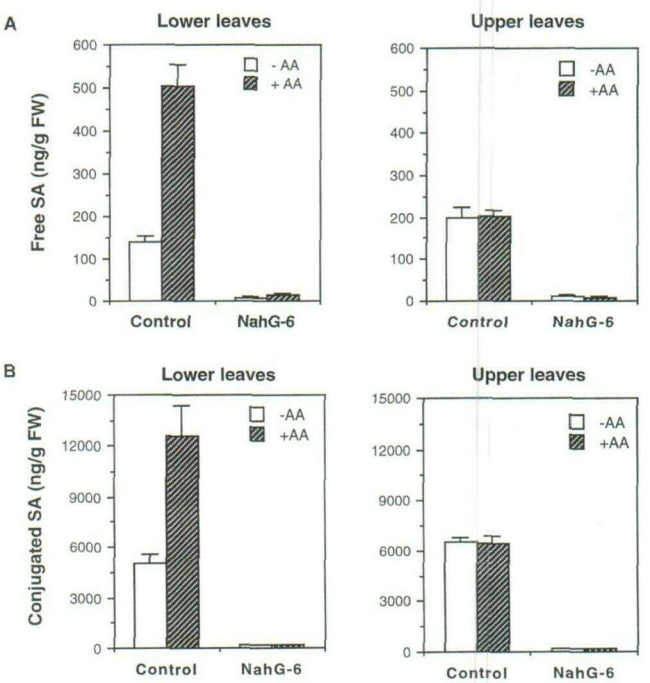

Figure 3. SA levels in AA-treated control and NahG potato plants. Three lower leaves of 4- to 5-week-old potato plants were sprayed with water (−AA) or 1500 μg mL^{-1} AA (+AA). Free (A) and conjugated (B) SA levels in the lower, treated and upper, untreated leaves of control and NahG-6 potato lines were analyzed 6 d after treatment. Mean values ± SE from three independent measurements are reported. FW, Fresh weight.

untreated leaves (Fig. 3). However, AA treatment of lower leaves did not induce any significant increase in either free or conjugated SA levels in upper, untreated leaves (Fig. 3). Similar results have been reported for Bintji, another untransformed potato cultivar (Coquoz et al., 1995).

Therefore, unlike tobacco, cucumber, and Arabidopsis, induction of SAR in potato is not associated with a systemic increase in SA levels. In the NahG plants the basal levels of free and conjugated SA were very low compared with the control plants, and AA treatment had essentially no effect on these levels (Fig. 3). It should be noted that at the concentration used in these experiments (1500 $\mu g\ mL^{-1}$), AA induced similar levels of necrotic lesions on both control and NahG plants. However, at two higher concentrations tested (2000 and 2500 $\mu g\ mL^{-1}$) AA induced significantly more severe necrosis in control plants than in NahG plants (data not shown).

DISCUSSION

Healthy potato plants, unlike tobacco and Arabidopsis, contain a high basal level of SA; however, its role in disease resistance is currently unclear. Since elevated SA levels in tobacco and Arabidopsis are associated with enhanced disease resistance (Yalpani et al., 1993; Bowling et al., 1994; Dietrich et al., 1994; Greenberg et al., 1994; Weyman et al., 1995), it is possible that the high SA level found in healthy potato plants represents a constitutive defense mechanism. If this is the case, a reduction in the SA level should decrease or abolish this constitutively activated defense and cause increased susceptibility to pathogen infection. However, when control and NahG plants were infected with *P. infestans*, no significant difference in disease severity was observed on inoculated leaves, despite the great difference in SA levels (Tables I and II). Therefore, the relatively high SA level in healthy potato plants does not appear to induce constitutive resistance to *P. infestans*.

Since exogenous SA also fails to induce disease resistance in potato (Coquoz et al., 1995; Table II), it appears that healthy potato plants are either unable or only poorly able to respond to SA. Paradoxically, although endogenously produced or exogenously applied SA did not enhance disease resistance in healthy potato plants, a drastic reduction in SA levels in NahG plants reduced the ability of AA to induce SAR to *P. infestans*. These results suggest that at least some aspects of SAR development in potato differ from those found in tobacco and Arabidopsis.

SA-mediated disease resistance results from the activation of a complex set of plant defense mechanisms, including defense or defense-related gene expression (Ryals et al., 1996). As such, SA-mediated defense responses require not only a sufficiently high level of SA but also an effective SA signal perception and transduction mechanism (Fig. 4). In both tobacco and Arabidopsis an effective SA signal perception and transduction mechanism appears to be present, since uninfected plants respond to exogenously applied SA by activating disease resistance mechanisms. However, SA-mediated defense responses are not constitutively expressed in these plants because SA levels are limiting. When plants are infected by a necrotizing pathogen, SA biosynthesis is induced, SA levels increase, and the SA signal transduction pathway(s) is activated, which then leads to enhanced disease resistance (Fig. 4A). By contrast, potato plants have a high constitutive level of SA. Since neither this high level of SA nor the application of exogenous SA enhances resistance against *P. infestans*, potato plants appear to have a

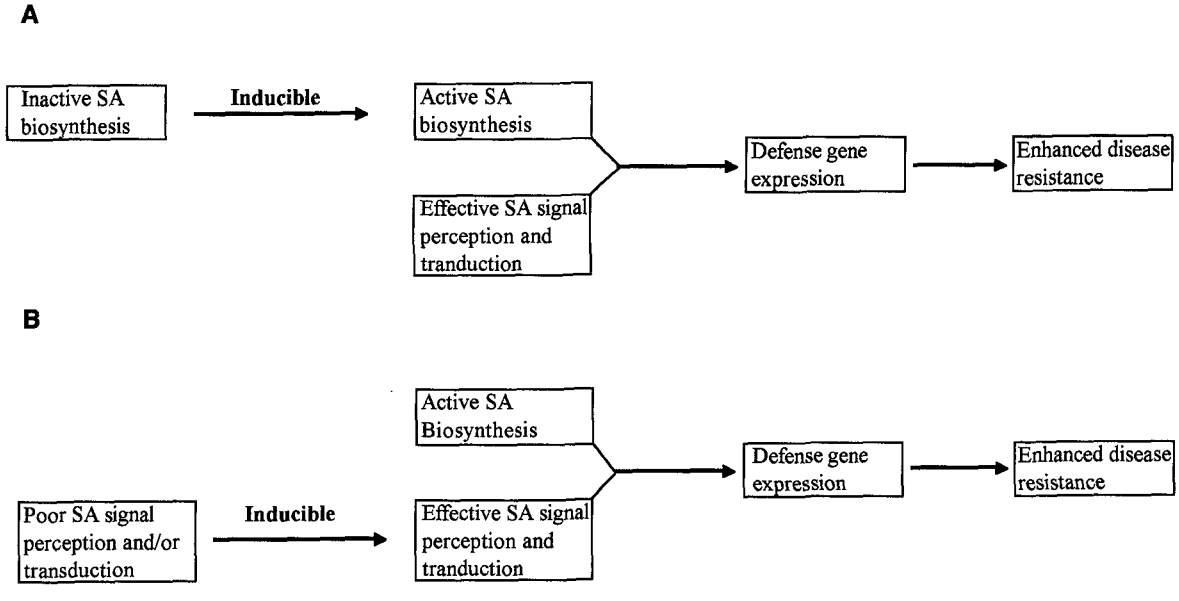

Figure 4. Possible mechanisms of induction of SAR in tobacco and Arabidopsis (A) in comparison with potato (B). Tobacco and Arabidopsis plants have low basal SA levels but possess an effective SA signal perception and transduction mechanism. Induction of SAR in these plants involves inducible activation of SA biosynthesis. Potato plants, on the other hand, have high basal SA levels but possess an ineffective SA signal perception and/or transduction mechanism. Induction of SAR in potato may involve activation of certain molecular mechanisms that enhance the sensitivity of the plant to SA.

poor SA signal perception and/or transduction mechanism. It is interesting that, although AA fails to elevate SA levels in untreated leaves, AA-induced SAR against *P. infestans* still requires SA. Thus, SAR-inducing pathogens or chemicals such as AA may activate or induce a rate-limiting step in SA signal perception and/or transduction in potato rather than stimulating production of the SA signal, as occurs in tobacco and Arabidopsis (Fig. 4B).

How AA treatment enhances the effectiveness of SA perception and/or transduction in potato remains to be determined. It is possible that stimulation of SA biosynthesis in tobacco and Arabidopsis and enhancement of SA signal perception and/or transduction in potato are mediated by different signal transduction mechanisms activated by SAR-inducing pathogens or chemicals. Alternatively, SAR-inducing pathogens or chemicals may activate a common signal transduction pathway with differential impacts on SA biosynthesis and SA signal perception/transduction in different plants. Progress made during the last several years in identifying components involved in SA biosynthesis (Leon et al., 1995) and signal transduction (Cao et al., 1994, 1997; Delaney et al., 1995; Shah et al., 1997) should help future studies to distinguish these possibilities.

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