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Potato Tuber-Inducing Activities of Salicylic Acid and Related Compounds

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Abstract. Salicylic acid (SA) induced potato tuberization in vitro at concentrations greater than 10^{-5} M. A comparison of the tuber-inducing activities of various related compounds suggested that derivatives of benzoic acid with a free carboxyl group and a substituent at the C-2 position of the benzene ring have this activity. Although SA had the strongest activity among the compounds tested, the activity was about one thousandth of that of natural jasmonic acid (*1R,2S*-jasmonic acid) in terms of the threshold concentration for activity. Spraying SA to leaves of plants grown under tuber-noninducing conditions (long days) induced tuberization. However, the natural occurrence of SA was not detected in the leaves of potato plants that had been grown under tuber-inducing conditions (short days) and had begun to form tubers. The results seem to exclude the possibility of the involvement of SA in the natural tuberization of potato plants.

Application of salicylic acid (SA) and of acetylsalicylic acid (aspirin, ASA) to plants has been shown to induce a variety of biological responses, such as floral formation (Kaihara et al. 1981, Khurama and Maheshwari 1978), stomatal closure (Larque-Saavendra 1979), inhibition of ethylene synthesis (Leslie and Romani 1988), resistance to pathogens (Mills and Wood 1984, White 1979), production of pathogenesis-related proteins (Ohashi and Matsuoka 1987), and promotion of colony formation from protoplasts (Carswell et al. 1989).

Tuberization in potato plants is considered to be controlled both by tuberonic acid (TA) and its glucoside, which are formed in leaves under short-day conditions (Koda and Okazawa 1988, Koda et al. 1988). The chemical structure of TA is closely related to that of jasmonic acid (JA) (Yoshihara et al. 1989) and JA also has strong potato tuber-inducing activity (Koda et al. 1991a, 1992). Furthermore, JA seems to be involved in tuberization in yam plants (Koda and Kikuta 1991).

JA is synthesized from linolenic acid via a series of enzyme-catalyzed reactions. The synthesis is initiated by lipoxygenase which catalyzes the incorporation of oxygen into linolenic acid (Vick and Zimmerman 1984). Because TA is one of the products of hydroxylation of JA, it is likely that TA is synthesized via a similar pathway. The biosynthetic pathway to JA resembles that for the synthesis of prostaglandins from arachidonic acid in mammalian cells. The first reaction in the biosynthesis of prostaglandins is the incorporation of oxygen into arachidonic acid, which is catalyzed by cyclooxygenase (Hamberg et al. 1974). It is well known that the anti-inflammatory effect of ASA involves the inhibition of the biosynthesis of prostaglandins, which is brought about by the inhibition of cyclooxygenase (Roth et al. 1975).

The resemblance between the actions of lipoxygenase and cyclooxygenase led us to the hypothesis that ASA and related compounds might inhibit potato tuberization via inhibition of lipoxygenase activity. However, in a preliminary experiment, ASA exhibited slight but significant potato tuber-inducing activity contrary to our expectations. Here we report and discuss the tuber-inducing activities of ASA, SA, and related compounds.

Materials and Methods

Assay for Tuber-Inducing Activity

Tuber-inducing activity was assayed using cultures of single-node segments of potato stems in vitro, as reported previously (Koda and Okazawa 1988). In brief, single-node segments, prepared from etiolated potato shoots (*Solanum tuberosum* L. cv. Irish Cobbler), were sterilized in a 1% solution of sodium hypochlorite for 1 h. Then three segments were planted in a 100-ml Erlenmeyer flask that contained 10 ml of medium (usually White's medium) supplemented with the compound to be tested. Unless otherwise specified, the concentration of sucrose in the

medium was 2%. The medium was adjusted to pH 5.6 and solidified with 0.6% Bacto-agar before autoclaving. The cultures were maintained at 25°C in the dark for 3 weeks, and then the rate of tuberization was calculated as the number of tuberized laterals divided by the total number of laterals that had emerged.

Application of SA to Potato Plants

Potato plants were grown for 2 weeks in a growth cabinet under tuber-noninducing conditions (16-h photoperiod, 26°C), as reported previously (Koda and Okazawa 1988). Then 4-ml aliquots of 10^{-4}, 10^{-3}, and 3×10^{-3} M solutions of SA (pH 5.0) were sprayed onto the plants nine times at intervals of 3 days. The control plants were sprayed with the same volume of distilled water. Four weeks after the beginning of this treatment, the plants were harvested and tuberization in these plants was observed.

An Attempt at Detection of SA in Potato Leaves

Potato leaves (100 g fresh weight) were harvested from plants that had been grown under tuber-inducing conditions (12-h photoperiod, 26°C during the day and 15°C at night) for 4 weeks and had begun to form tubers. The leaves were homogenized with sufficient ethanol to give a final extract in 70% ethanol. The homogenate was kept overnight at 4°C and then filtered. The filtrate was concentrated and the resultant aqueous residue was acidified to pH 3.0. The acidic ethyl acetate fraction was separated from the aqueous residue in the usual way (Koda et al. 1991b) and the fraction was dissolved in a small volume of chloroform. After removal of insoluble compounds by filtration, the soluble fraction was subjected to TLC on a silica gel plate that was developed with a mixture of toluene, ethyl acetate, and acetic acid (30:15:2, vol/vol). Since authentic SA migrated with an Rf of 0.60, the zone from Rf 0.5–0.7 was scraped off and eluted with water-saturated ethyl acetate. The eluate was then fractionated on a Novapak C_{18} column (8 mm i.d. × 100 mm; Radial-pak cartridge, Waters, Milford, Massachusetts, USA) in 50% methanol that contained 0.1% acetic acid at a flow rate of 1 ml min^{-1}. The retention time of authentic SA under these conditions was 7.8 min, and the fractions of the eluate corresponding to SA were collected. The pooled fractions were rechromatographed on the same column in 30% acetonitrile that contained 0.1% acetic acid. The elution profile was monitored at 280 nm. The retention time of authentic SA under these conditions was 7.4 min.

Results

Potato Tuber-Inducing Activities of SA and Related Compounds

We first tested the ability of ASA to inhibit potato tuberization in vitro. Since tuberization can be induced by high concentrations of sucrose (greater than 4%) in medium (Koda and Okazawa 1983), the effect of ASA on tuberization was examined using medium that contained 6% sucrose. ASA did not inhibit the tuberization that was induced by sucrose. By contrast, it stimulated tuberization in the presence of 6% sucrose, the rates of tuberization at concentrations of 0, 10^{-5}, 10^{-4}, and 3×10^{-4} M being 0.46, 0.67, 0.9, and 1.0, respectively.

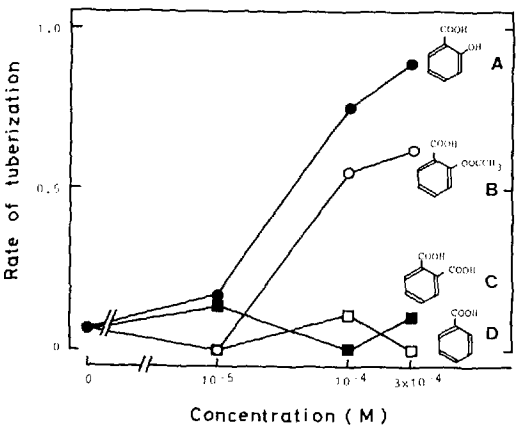

Fig. 1. Comparison of tuber-inducing activities of salicylic acid (A), acetylsalicylic acid (B), phthalic acid (C), and benzoic acid (D). Single-node segments of etiolated potato shoots were cultured aseptically on test media that contained 2% sucrose for 3 weeks.

The above results suggest that ASA and related compounds are capable of inducing potato tuberization. The effects of ASA, SA, benzoic acid, and phthalic acid on tuberization were examined with medium that contained 2% sucrose. Usually tuberization is scarcely observed when this medium is used without any further additions. SA and ASA had tuber-inducing activity when present at concentrations greater than 10^{-5} M (Fig. 1). Figure 2 shows the typical appearance of tubers induced by SA. Just as JA does, SA affected the negative geotropism of the lateral shoots, and the shoots showed a diageotropic growth habit. By contrast, benzoic acid and phthalic acid had almost no activity. Other SA-related compounds, namely, methyl salicylate, saligenine (salicyl alcohol), and salicin, had almost no activity. Gallic acid and 3- and 4-hydroxycinnamic acids also had no activity.

SA (2-hydroxy benzoic acid) has two structural isomers, 3- and 4-hydroxybenzoic acids. Comparison of the activities of these three isomers indicated that SA had the strongest activity, the 3-hydroxy isomer had very low activity and the 4-hydroxy isomer had no activity (Fig. 3).

The above results seem to indicate that derivatives of benzoic acid with a free carboxyl group and a substituent at the C-2 position of the ring have potato tuber-inducing activity. As many inhibitors of the transport of auxin satisfy these requirements (Katekar and Geissler 1977), it seemed probable that such inhibitors have tuber-inducing activity.

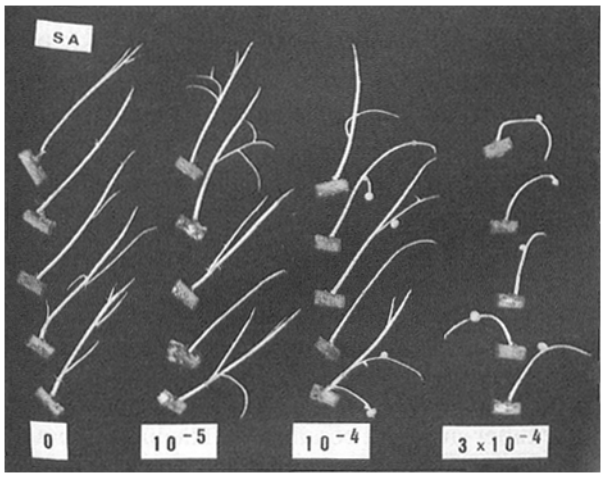

Fig. 2. Typical appearance of tubers induced by salicylic acid. Numbers are molar concentrations used. Sucrose concentration in the medium was 2%.

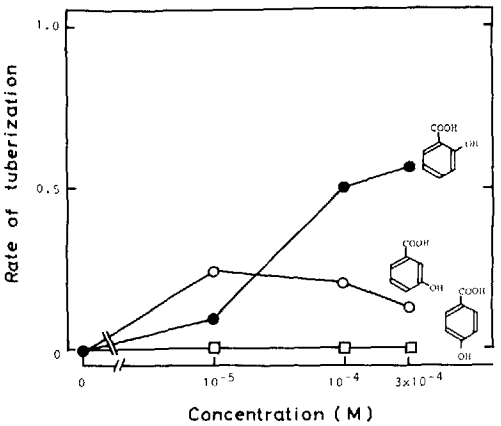

Fig. 3. Comparison of tuber-inducing activities of salicylic acid (2-hydroxy benzoic acid), and 3- and 4-hydroxybenzoic acids.

Figure 4 shows that the tuber-inducing activity of N-1-naphthylphthalamic acid (NPA) was fairly high and that of fluorescein was low. Eosin yellow, rhodamin B, and erythrosin B had similar activities to that of NPA. Rose bengal, in which all the hydrogen atoms in the benzoic acid moiety are replaced by chlorine atoms, had no activity, and 2,3,5-triiodobenzoic acid (TIBA) had very low activity.

Figure 5 shows a comparison of the tuber-inducing activities of SA and natural JA (*1R,2S*-JA) (see Koda et al. 1992). The activity of SA was about one thousandth of that of *1R,2S*-JA when the threshold concentrations for tuber-inducing activity were compared.

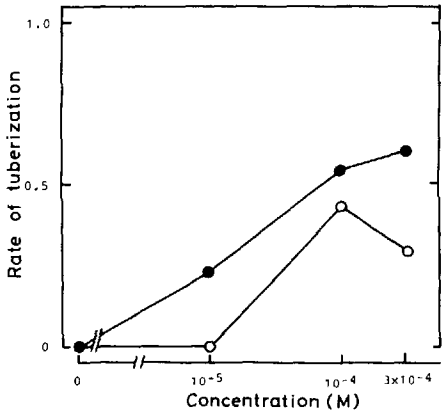

Fig. 4. Tuber-inducing activities of N-1-naphthylphthalamic acid (●) and fluorescein (○).

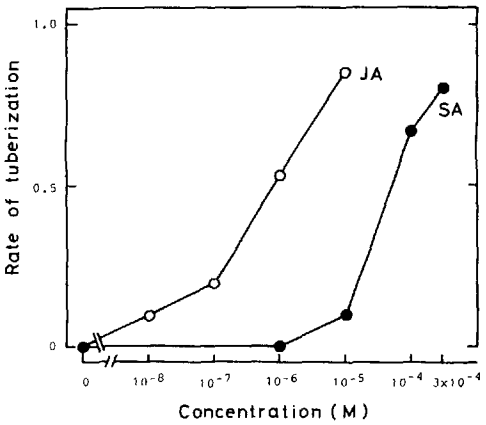

Fig. 5. Comparison of tuber-inducing activities of *1R,2S*-jasmonic acid (JA) and salicylic acid (SA).

Effect of SA on Tuberization in Potato Plants

To examine the ability of SA to induce tuberization in potato plants, solutions of SA were sprayed onto plants which were grown under tuber-noninducing conditions. Four weeks after the beginning of the treatment, tuberization was observed in plants that had been treated with 3×10^{-3} M SA (Fig. 6), while plants that had been treated with lower concentrations of SA, or water, were not tuberized.

An Attempt at Detection of SA in Potato Leaves

SA has been found in several plants and the levels in leaves are known to be increased by stress, for example, by infection (Kurogochi et al. 1978, Malamy et al. 1992, Rasmussen et al. 1991). The ability of SA to induce potato tuberization suggests the possible involvement of SA in the tuberization

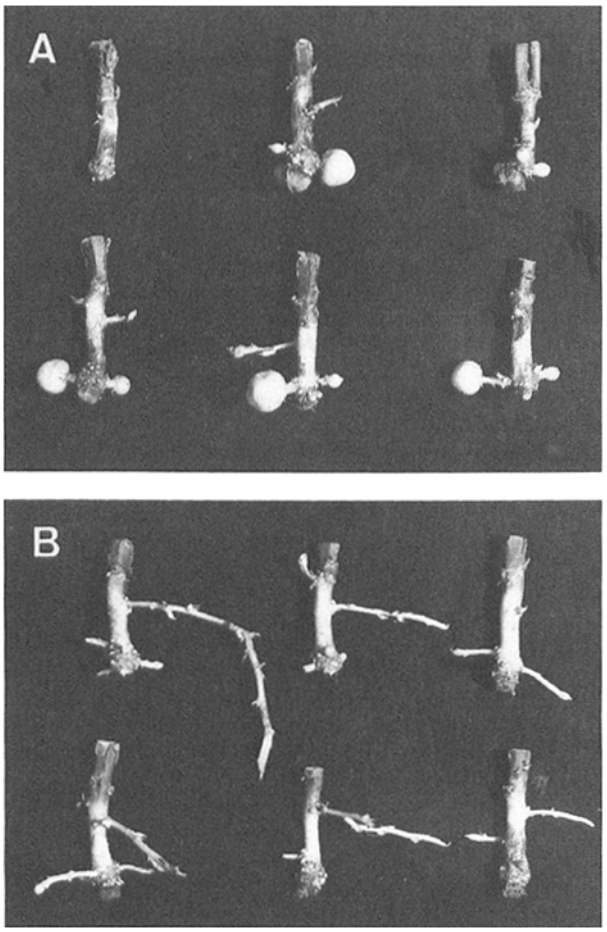

Fig. 6. Appearance of tubers induced by spray application of 3×10^{-3} M salicylic acid to leaves of potato plants. (**A**) Treated with salicylic acid; (**B**) treated with water. To simplify the comparison, roots and mother tubers have been removed.

of potato plants. To examine this possibility, an attempt was made to detect SA in leaves of potato plants that had been grown under tuber-inducing conditions. However, in the elution profile after the second round of HPLC no SA was observed.

Discussion

SA and related compounds had potato tuber-inducing activities, even though the activity of SA was about one thousandth of that of natural JA in terms of the threshold concentration for tuber induction (Fig. 5). A comparison of the tuber-inducing activities of various compounds related to SA suggests that derivatives of benzoic acid with a free carboxyl group and a substituent at the C-2 position of the benzene ring have this activity. It is possible that the active compounds, such as SA, promote the biosynthesis of TA or JA and, as a result, they exhibit tuber-inducing activity. In a preliminary experiment, we compared the tuber-inducing activities in TA- and JA-containing fractions of extracts obtained from single-node segments cultured on medium with or without SA. However, no clear differences were apparent. This result indicates that the tuber-inducing activity of SA is not brought about by the promotion of synthesis of TA and JA, and that SA itself has tuber-inducing activity.

SA was not detected in the extract of 100 g of fresh leaves of potato plants that had been grown under tuber-inducing conditions. The limit of detection of SA with our procedure was 20 ng. Thus, the level of SA in potato leaves was below 10^{-9} mol/kg if SA was present at all. This concentration is about four orders of magnitude lower than the threshold concentration for induction of tuberization. The result excludes the possibility of the involvement of SA in the natural tuberization.

Although the mechanism by which SA and related compounds exert their tuber-inducing activity remains to be elucidated, the results presented herein suggest the possible use of these compounds to control tuberization in field-grown potato plants.

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