

REVIEW

**SALICYLIC ACID: PROPERTIES, BIOSYNTHESIS
AND PHYSIOLOGICAL ROLE**

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Summary. A review is made of recent publications devoted to various aspects of salicylic acid (SA) influence on different physiological processes. The role of SA on plant growth and development, flowering, ion uptake, stomatal regulation and photosynthesis is analysed. SA as a natural inducer of thermogenesis and disease resistance in plants is described. Besides the physiological functions of SA, the general properties, biosynthesis and metabolism of this plant growth regulator are discussed.

Key word: salicylic acid, structure, biosynthesis, physiological functions

Abbreviations: ASA – acetylsalicylic acid; PR – pathogenesis-related; RubisCO – ribulose-1,5-bisphosphate carboxylase/oxygenase; SA – salicylic acid; SAR – systemic acquired resistance; TMT – tobacco mosaic virus

Salicylic acid (SA) or ortho-hydroxybenzoic acid and related compounds belong to a diverse group of plant phenolics. Salicylates from plant sources have been used in medicines since antiquity. In 1828 in Munich was isolated for the first time a small amount of salicin, the glucoside of salicyl alcohol, from willow bar. Ten years latter Raffaele Piria named it SA, from the Latin word Salix for willow tree. The first commercial production of synthetic SA began in Germany in 1874 (see Raskin, 1992 a, b).

Aspirin, a close analog of salicylic acid, was introduced by the Bayer Company in 1898 and rapidly became one of the most popular pharmaceutical preparations in

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the world. During the 19th century many compounds belonging to the group of salicylates were isolated from a variety of plants. The story of salicylates has been summarized by Weissmann (1991).

Aspirin, a trade name for acetylsalicylic acid (ASA), undergoes spontaneous hydrolysis to SA. Exogenously applied it is rapidly converted to SA. Despite the fact that aspirin was not identified as a natural product, it is widely used by many plant scientists in their experiments. The reason is the similarity in their physiological effects.

Phenolics are usually defined as substances that possess an aromatic ring bearing a hydroxyl group or its functional derivative. Free SA is a crystalline powder that melts at 157–159°C. It is moderately soluble in water and very soluble in polar organic solvents. The pH of saturated aqueous SA is 2.4; pKa of SA is 2.98, log Kow = 2.26. SA could be actively transported, metabolized, or conjugated and it could also translocate rapidly from the point of initial application to different plant tissues.

Using modern analytical techniques it was found that salicylates are distributed in many important agricultural plant species. In many plants, such as rice, crabgrass, barley, soybean, the levels of SA has been found to be approximately $1 \mu\text{g g}^{-1}$ fresh weight. A survey of SA in leaves and reproductive structures of non-thermogenic angiosperms confirmed the ubiquitous distribution of this compound in plants. Levels of SA varied substantially in the floral parts of seven non-thermogenic species and in the leaves of 27 non-thermogenic plants (Raskin et al., 1990). The highest levels of SA were determined in the inflorescence with necrotizing pathogens (Raskin, 1992 a).

In the early 1960s it was suggested that in plants SA is synthesized from cinnamic acid by two possible pathways: one involves side-chain decarboxylation of cinnamic acid to benzoic acid followed by 2-hydroxylation to SA. Alternatively, cinnamic acid could be first 2-hydroxylated to a o-coumaric acid and then decarboxylated to SA. These pathways differ in the order of β -oxidation and ortho-hydroxylation reactions and could operate independently in plants. Two key enzymes are involved in SA biosynthesis and metabolism: benzoic acid 2-hydroxylase, which converts benzoic acid to SA, and UDP glucose: SA glucosyltransferase, which catalyzes conversion of SA to SA glucoside. More recent data show that the cinnamic acid \rightarrow benzoic acid \rightarrow SA pathway functions in rice seedlings (Silverman et al., 1995) and tobacco plants (Yalpani et al., 1993). Various microorganisms produce SA primarily via chorismic acid – an important intermediate of shikimic acid pathways, and the rate of SA biosynthesis and excretion can be substantial (Weiss and Edwards, 1980). Microorganisms associated with crop plants are also capable of synthesizing and excreting SA. Large amounts of SA have been found in the soil samples taken from the rhizosphere of *Zea mays* and *Phaseolus aureus* (Pareek and Gaur, 1973).

Many different types of SA conjugates have been found in plant species. They were mainly formed by glucosylation and less frequently by esterification. Large amounts of SA glucosides were detected in *Helianthus annuus*, oat and bean roots

(Yalpani et al., 1992 a, b). Methyl salicylate was found in the leaves of oats, red clover, tobacco and in the volatile fractions of fruits (plum, strawberry, black cherry and tomato). Large amounts of volatile methyl salicylate are released from TMV-inoculated tobacco indicating that this compound may be a major metabolite of SA. It is tempting to speculate that methylsalicylate may function as an airborne signal for both intra- and inter-plant communication.

SA forms conjugates with aminoacids. Salicyloil aspartic acid was identified in wild grapes, but its physiological function is not known (Silvermen et al., 1995).

Physiological functions of salicylates

It is well documented that many phenolic compounds play an essential role in the regulation of different physiological processes, including plant growth and development, ion uptake and photosynthesis. Phenolic molecules produced by plant roots are essential for germination and plant development (Lynn and Chang, 1990). The first indication for a physiological effect of SA was the discovery of flower-inducing action and bud formation in tobacco cell cultures (Eberhard et al., 1989). The stimulatory effect of SA on flowering was latter demonstrated in other plant species and this was ground for suggesting that SA functions as an endogenous regulator of flowering (Cleland and Ajami, 1974). The SA effect was not specific and it promoted flowering in combination with other plant regulators (e.g. gibberellins). Besides flowering, SA also affected multiplication rate, anthocyanin and chlorophyll contents in *Spirodella polyrrhiza*. High concentration of SA (grater than 10^{-6} M) retarded the growth of fronds. SA treated fronds became gibbous with large air-chambers. Maximum gibbosity was observed in the 5×10^{-5} M SA-treated fronds of *Spirodela* (Khurana and Maheshiwari, 1980). The most striking aspect of this study was the observation that SA induced profuse flowering, irrespective of the photoperiodic treatment given. Harper and Balke (1981) found that SA inhibited K^+ absorption in excised oat root tissue. The degree of inhibition was both concentration- and pH-dependent. With decreasing pH, the inhibitory effect of SA increased, suggesting that the protonated form of SA was more active than its charged form. The absorption of SA was also pH dependent. The authors suggest that under proper conditions of pH and concentration, SA could significantly affect mineral absorption by plants in the field. Glass (1973, 1974) reported that SA applied exogenously to barley and oat roots inhibited potassium absorption in a pH- and concentration-dependent manner.

SA caused the collapse of the transmembrane electrochemical potential of mitochondria and the ATP-dependent proton gradient of tonoplast-enriched vesicles (Macri et al., 1986).

It was found that SA produced in the rhizosphere of some plants played the role of an allelopathic chemical and inhibited the growth of the surrounding plants (Schettel and Balke, 1983).

SA is an effective inhibitor of ethylene biosynthesis, the effect being pH-dependent. This effect has been demonstrated with apple disks (Romani et al., 1989). With 10 μM SA maximal inhibition (90%) was achieved in 3 hours. Results with applied SA to pear cell suspension cultures suggest that both compounds acted by blocking the conversion of 1-aminocyclopropane-1-carboxylic acid to ethylene (Leslie and Romani, 1986). Authors described SA as an effective, non-toxic and reversible inhibitor of ethylene biosynthesis at concentrations comparable to those found in some plant tissues.

The ability of certain Arum lilies to generate heat in the inflorescences during blooming has provoked studies on the role of SA in heat production. The heating is believed to be associated with a large increase in the cyanide-insensitive, non-phosphorylating electron transport pathway (Meeuse and Raskin, 1988). A reproducible increase in surface temperature of 0.5–1.0°C was registered with a high-resolution infrared camera. The thermal effect of SA was abolished with inhibitors of the alternative pathway, such as salicylhydroxamic acid and propyl gallate. It was suggested that, as in thermogenic species, SA increased the activity of total respiration and of the cyanide-resistant pathway in tobacco leaves, leading to an elevation in surface temperature (van Straeten et al., 1995). The levels of SA increased during heat production of five aroid species and in male cones of four thermogenic cycads exceeded 1 $\mu\text{g}\cdot\text{g}^{-1}$ fresh weight (Raskin et al., 1990).

For more than 50 years the identity of “calorigen” remained obscure. It was known that it is the agent that triggers high level of heat production in the flowers and inflorescences of some thermogenic plants. Applying very sensitive analytical methods Raskin et al. (1987) showed that SA was calorigen and functioned as an endogenous regulator of heat production. It was also shown that both calorigen extract and SA caused the induction of the alternative oxidase gene and the alternative oxidase protein with mol. mass of 38.9 kDa was isolated and characterized (Elthon and McIntosh, 1987; Rhoads and McIntosh, 1991). The effects of SA on the alternative pathway respiration in slices and isolated mitochondria of dormant and dormancy-breaking potato tubers was compared (Wen and Liang, 1994). It was found that treatment with 20 μM SA increased the capacity of cyanide-resistant respiration in both model systems. The involvement of the alternative pathway was enhanced by SA to a greater extent in dormancy-breaking potato tubers.

Applying SA to oilseed rape plants increased the concentration of glucosinolates in their leaves. Glucosinolates are a group of thioglucosides found in cruciferous plants. When tissues are damaged, glucosinolates are hydrolysed and release various products which are thought to contribute to the plant's defence against microorganisms and pests. Data presented by Kiddle et al. (1994) are among the first demonstrating an increase in secondary metabolite content in response to SA.

SA is an important mediator of the plant defence response to pathogens. The first evidence for this role of SA resulted from the application of an aspirin solution to

tobacco leaves (White, 1979). The plants were found to have enhanced resistance to tobacco mosaic virus (TMV) and reduced number and size of necrotic lesions. Treatment of tobacco genotypes with SA resulted in the coordinate expression of pathogenesis-related (PR) genes. Several lines of evidence suggest that SA is the endogenous signal involved in induction of PR protein synthesis and systemic acquired resistance (SAR) in tobacco and cucumber. Resistance to pathogens and the production of some PR-proteins in plants can be induced by SA or aspirin, even in the absence of pathogenic organisms. Exogenously applied SA induced PR-proteins mainly at the side of application, in contrast to pathogens that induced PR-proteins systemically (for review see Raskin, 1992 a, b). The levels of SA increased dramatically following inoculation of tobacco or cucumber plants with pathogens. Increases in SA levels have been correlated to changes in gene expression. It has been established that during infection by pathogens, a huge number of proteins are induced, including PR-protein families, which are thought to play an active role in defence processes (reviewed in Enyedi et al., 1992). A recent comprehensive study utilizing modern molecular approaches showed that 9 classes of PR-protein mRNAs which are induced during the development of SAR to TMV in tobacco can be induced by SA to a similar degree.

Investigations of the components involved in the SA response revealed the appearance of an SA-binding protein, identified as catalase, an H_2O_2 -degrading enzyme (Chen and Klessing, 1991). It was found that 3 hours after treatment of tobacco leaves with 1 mM SA, the amount of H_2O_2 in the leaves had increased, and the levels continued to rise over the 24-h test period. Direct treatment of leaves with H_2O_2 induced PR-1a gene expression, which makes possible the suggestion that the pathway from pathogen infection to SA accumulation and to PR gene induction could involve the signal of peroxide accumulation.

Some cultivars of tobacco are resistant to TMV and synthesize PR proteins upon infection. In search for the signal or signals that induce resistance of PR genes, Malamy et al. (1990) found that the endogenous SA levels in resistant, not susceptible, cultivars increased 20-fold in infected leaves and 5-fold in uninfected leaves after TMV inoculation. Induction of PR 1 genes followed the rise of SA levels. These findings suggest that SA functions as a natural transduction signal.

Wounding the leaves by chewing insects or other mechanical damage induces the synthesis of defensive proteinase inhibitor proteins in both wounded leaves and distal unwounded leaves. Several chemical signals have been identified in plant leaves that regulate this response such as oligosaccharides (Bishop et al., 1984) or plant growth regulators (ABA, jasmonic acid, auxin), (Farmer and Ryan, 1992; Pena-Cortes et al., 1993). Data of Doares et al. (1995) indicate that SA inhibited the octadenoic pathway that regulates defence signalling in response to predator attacks. The inhibition of synthesis of proteinase inhibitor proteins and mRNAs by SA occurs at a step in the signal transduction pathway, after JA synthesis but preceding transcription of the inhibitor genes.

Plants react to pathogens by inducing an array of defence mechanisms. Pathogen recognition by the plants activates a signal pathway that leads to a rapid oxidative burst. The production of active oxygen species such as superoxide ions is a rapid response that precedes cell death. It has been proposed that oxygen radicals play a direct role in cell death during the hypersensitive response. H_2O_2 formed during the oxidative burst may also trigger cell death and serve as a diffusible signal for induction of defence-related genes in surrounding cells (Levine et al., 1996). Leon et al. (1995) have demonstrated that treatment of tobacco leaves with H_2O_2 induced accumulation of free benzoic acid and SA. They have suggested that H_2O_2 activated SA biosynthesis.

A series of experiments were carried out in order to elucidate whether salicylates fed via petioles to beans could affect transpiration rate. It was found that 10^{-3} M ASA reduced transpiration rate (Larque-Saavedra, 1978). The mechanism of action of ASA in this physiological response is difficult to explain. It could be suggested that ASA might be affecting CO_2 levels within the leaf, thereby inducing stomatal closure. Rai et al. (1986) showed that SA antagonized the ABA-induced stomatal closure of *Comelina* epidermal strips.

Stomatal behaviour and regulation is a very important factor for photosynthetic ability. The established effects of SA on stomatal function, chlorophyll content, transpiration rate and respiratory pathways lead to the assumption that SA might possess another physiological function, most probably involved in regulation of some photosynthetic reactions. In recent investigations Pancheva et al. (1996) demonstrated that long-term treatment (7 days) of barley seedlings with SA decreased the rate of photosynthesis and the activity of RuBP carboxylase, and increased both CO_2 -compensation point and stomatal resistance. The short-term treatment with SA (for minutes to 2 hours) did not affect either the rate of photosynthesis or the capacity of biochemical machinery as compared with untreated control plants. An explanation for these changes could in part be due to stomatal closure and reduced supply of CO_2 . However, the C_i -values were not declined in SA-treated plants. This implies that stomatal closure did not restrict CO_2 entry into the leaf enough to reduce internal CO_2 level and the reduction in photosynthesis probably was nonstomatal. It was suggested that one very possible reason for the observed inhibition of photosynthetic ability and RuBP carboxylase activity could be the effect of SA on protein synthesis, including RubisCO synthesis. Recently, it was found that treatment of barley plants with SA caused decrease in the level of total soluble protein, in particular in the level of RubisCO. In the presence of 1mM SA the level of total soluble protein was about 68% of the control, whereas the level of RubisCO was about 50% that of control plants. The percentage of inhibition on the small subunit was higher, as a result of which the small/large subunit ratio was lower for the experimental variants (Pancheva and Popova, 1997). It was suggested that SA like some other stress factors diminished chloroplast photosynthetic activity as a result of effects on the thylakoid mem-

branes and light-induced reactions connected with them, and in this way it may indirectly participate in regulating the activity of RubisCO.

Although the various physiological and biochemical effects of SA application to plants have been shown, the regulatory mechanisms of endogenous SA is not well understood. Surprisingly, some of the effects of SA in plants resemble to a great extent to those observed in animals. This rises the question if there are any connections between therapeutic effects of salicylates in plants and animals. As it has been shown by Raskin (1992 a,b) some of the effects of SA in plants are also associated with reduction of disease symptoms. All these questions and the possible practical application of salicylates are promising area for future investigations.

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References

- Bishop, P. D., G. Pearce, J. E. Bryant, C. A. Ryan, 1984. Isolation and characterization of proteinase inhibitor-inducing factor from tomato leaves: identify and activity of poly- and ologogalacturonide fragments. *J. Biol. Chem.*, 259, 13172–13177.
- Chen, Z., D. F. Klessing, 1991. Identification of soluble salicylic acid binding protein that may function in signal transduction in the plant disease-resistance response. *Proc. Natl. Acad. Sci. USA.*, 88, 8179–8183.
- Cleland, F. C., A. Ajami, 1974. Identification of the flower-inducing factor isolated from aphid honey dew as being salicylic acid. *Plant Physiol.*, 54, 904–906.
- Doares, S. H., J. Narvaer-Vasquez, A. Conconi, C. A. Ryan, 1995. Salicylic acid inhibits synthesis of proteinase inhibitors in tomato leaves induced by systemin and jasmonic acid. *Plant Physiol.*, 108, 1741–1746.
- Eberhard, S., N. Doubrava, V. Marta, D. Mohnen, A. Southwick, A. Darviell, P. Albersheim, 1989. Pectic cell wall fragments regulate tobacco thin-cell layer explant morphogenesis. *Plant Cell*, 1, 747–755.
- Elthon, T. E., L. McIntosh, 1987. Identification of the alternative terminal oxidase in higher plant mitochondria. *Proc. Natl. Acad. Sci. USA*, 84, 8399–8403.
- Enyedi, A. J., N. Yalpani, P. Silverman, I. Raskin, 1992. Signal molecules in systemic plant resistance to pathogens and pests. *Cell*, 70, 879–886.
- Farmer, E. E., C. A. Ryan, 1992. Octadecanoid precursors of jasmonic acid activate synthesis of wound-inducible proteinase inhibitors. *Plant Cell*, 4, 129–134.
- Glass, A. D., 1973. Influence of phenolic acids on ion uptake. I. Inhibition of phosphate uptake. *Plant Physiol.*, 51, 1037–1041.
- Glass, A. D., 1974. Influence of phenolic acids upon ion uptake. III. Inhibition of potassium absorption. *J. Exp. Bot.*, 25, 1104–1113.

- Harper, J. P., N. E. Balke, 1981. Characterization of the inhibition of K⁺ absorption in oat roots by salicylic acid. *Plant Physiol.*, 68, 1349–1353.
- Kiddle, G. A., K. J. Doughty, R. M. Wallsgrove, 1994. Salicylic acid-induced accumulation of glucosinolates in oilseed rape (*Brassica napus* L.) leaves. *J. Exp. Bot.*, 45, 1343–1346.
- Khurana, J. P., S. C. Maheshwari, 1980. Some effects of salicylic acid on growth and flowering in *Spirodela polyrrhiza* SP20. *Plant Cell Physiol.*, 21, 923–927.
- Larque-Saavedra, A., 1978. The antitranspirant effect of acetylsalicylic acid in *Phaseolus vulgaris*. *Plant Physiol.*, 43, 126–128.
- Leon, J., M. A. Lawton, I. Raskin, 1995. Hydrogen peroxide stimulates salicylic acid biosynthesis in tobacco. *Plant Physiol.*, 108, 1673–1678.
- Leslie, C. A., R. J. Romani, 1986. Salicylic acid: a new inhibitor of ethylene biosynthesis. *Plant Cell Reports*, 5, 144–146.
- Levine, A., R. Tenharen, R. Dixon, C. Lamb, 1994. H₂O₂ from the oxidative burst orchestrates the plant hypersensitive disease resistance response. *Cell*, 79, 583–593.
- Lynn, D. G., M. Chang, 1990. Phenolic signals in cohabitation: implications for plant development. *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 41, 497–526.
- Macri, F., A. Vianello, S. Pennazio, 1986. Salicylate-collapsed membrane potential in pea stem mitochondria. *Physiol. Plant.*, 67, 136–140.
- Malamy, J., J. P. Carr, D. F. Klessing, I. Raskin, 1990. Salicylic acid: a likely endogenous signal in the resistance response of tobacco to viral infection. *Science*, 250, 1002–1004.
- Meeuse, B. J. D., I. Raskin, 1988. Sexual reproduction in the arum lily family, with emphasis on thermogenicity. *Sex. Plant Reprod.*, 1, 3–15.
- Pancheva, T. V., L. P. Popova, 1997. Effect of salicylic acid on the synthesis of ribulose-1,5-bisphosphate carboxylase/oxygenase in barley leaves. *J. Plant Physiol.*, (in press).
- Pancheva, T. V., L. P. Popova, A. N. Uzunova, 1996. Effects of salicylic acid on growth and photosynthesis in barley plants. *J. Plant Physiol.*, 149, 57–63.
- Pareek, R. P., A. C. Gaur, 1973. Organic acids in the rhizosphere of *Zea mays* and *Phaseolus aureus* plants. *Plant Soil*, 39, 445–448.
- Pena-Cortes, A., T. Albrecht, S. Prat, E. W. Weiller, L. Willmitzer, 1993. Aspirin prevents wound-induced gene expression in tobacco leaves by blocking jasmonic acid biosynthesis. *Planta*, 191, 123–128.
- Raskin, I., 1992a. Role of salicylic acid in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 43, 439–463.
- Raskin, I., 1992b. Salicylate, a new plant hormone. *Plant Physiol.*, 99, 799–803.
- Raskin, I., E. A. Ehmann, W. R. Melander, B. J. D. Meeuse, 1987. Salicylic acid: a natural inducer of heat production in Arum lilies. *Science*, 25, 1601–1602.
- Raskin, I., H. Skubatz, W. Tang, B. J. D. Meeuse, 1990. Salicylic acid levels in thermogenic and non-thermogenic plants. *Ann. Bot.*, 66, 369–373.

- Ray, N. K., S. S. Sharma, S. Sharma, 1986. Reversal of ABA-induced stomatal closure by phenolic compounds. *J. Exp. Bot.*, 37, 129–134.
- Rhoads, D. M., L. McIntosh, 1991. Isolation and characterization of a cDNA clone encoding an alternative oxidase protein of *Sauromatum guttatum* (Schott). *Proc. Natl. Acad. Sci. USA*, 88, 2122–2126.
- Romani, R. J., B. M. Hess, C. A. Leslie, 1989. Salicylic acid inhibition of ethylene production by apple disks and other plant tissues. *J. Plant Growth Regul.*, 8, 63–69.
- Schettel, N. L., N. E. Balke, 1983. Plant growth response to several allelopathic chemicals. *Weed Sci.*, 31, 293–298.
- Silverman, P., M. Seskar, D. Kanter, P. Schweizer, J. P. Metraux, I. Raskin, 1995. Salicylic acid in rice. Biosynthesis, conjugation, and possible role. *Plant Physiol.*, 108, 633–639.
- Van der Straeten, D., L. Chaerle, G. Sharkov, H. Lambers, M. van Montagu, 1995. Salicylic acid enhances the activity of the alternative pathway of respiration in tobacco leaves and induces thermogenicity. *Planta*, 196, 412–419.
- Weiss, U., J. M. Edwards, 1980. The biosynthesis of aromatic compounds. New York/Chichester: Willey-Interscience, 728 pp.
- Weissmann, G., 1991. Aspirin. *Sci. Am.*, 264, 84–90.
- Wen, J. Q., H. G. Liang, 1994. Comparison of the effects of salicylic acid on alternative pathway in slices of dormant and dormancy-breaking potato tubers (*Solanum tuberosum*). *Plant Sci.*, 102, 127–131.
- White, R., 1979. Acetylsalicylic acid (aspirin) induces resistance to tobacco mosaic virus in tobacco. *Virology*, 99, 410–412.
- Yalpani, N., J. Leon, M. A. Lawthon, I. Raskin, 1993. Pathway of salicylic acid biosynthesis in healthy and vitus-inoculated tobacco. *Plant Physiol.*, 103, 315–321.
- Yalpani, N., M. Schulz, M. P. Davies, N. E. Balke, 1992 a. Partial purification of an inducible uridine-5'-diphosphate glucose: salicylic acid glucosyltransferase from oat roots. *Plant Physiol.*, 100, 457–463.
- Yalpani, N., N. E. Balke, M. Schulz, 1992 b. Induction of UDP-glucose: salicylic acid glucosyltransferase in oat roots. *Plant Physiol.*, 100, 1104–1119.