



The role of salicylic acid in plants exposed to heavy metals. A review

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Abstract

Salicylic acid (SA) is a very straightforward phenolic compound (C₇H₆O₃, which consists of an aromatic ring, one carboxylic group, and a hydroxyl group), but despite its apparent simplicity, SA plays an important role in a number of plant processes, both under ideal circumstances and in plants that are experiencing challenges. Heavy metal (HM) stress is one of the environmental triggers. A vast collection of evidence has just been shown that SA regulates plant growth and affects communication between plants as well as within them due to the substance's volatile methyl ester form, methyl salicylate. SA interacts with other plant hormones (such as auxin, abscisic acid, and gibberellin) under stress, particularly HM stress, and encourages the production of antioxidant chemicals and enzymes, alerting HM-treated plants and aiding in the counteraction of HM stress. In order to give a complete picture of SA and HM and to guide the direction of future research on this topic, the present literature survey summarizes recent material on the roles of SA in plants experiencing HM stress.

Keywords: Ortho-hydroxybenzoic acid, polyphenols, plant hormone, metal toxicity, and signaling chemical

Introduction

Salicylic acid (SA), also known as ortho-hydroxybenzoic acid, is a phenolic derivative that is found in a wide variety of plants. It is known to regulate a number of physiological and biochemical processes, including thermogenesis, plant signaling or defense, and response to biotic and abiotic stress (Chen et al., 2009) [8]. Salicylic acid is derived from the Latin word *salix*, which means "willow tree." Chemically speaking, SA is a member of a sizable class of plant phenolics and may be extracted in plants in both free and conjugated form. The conjugated form, in instance, results from the aromatic ring being modified by methylation, hydroxylation, and/or glycosylation.

Johan Buchner initially isolated salicin, one of the natural SA derivatives, from the willow tree's (*Salix* sp.) bark in 1828 (Raskin., 1992) [34]. Following this discovery, it was found that almost all willow trees, including *Salix Alba*, *S. purpurea*, *S. fragilis*, and *S. daphnoides*, were particularly rich in this natural compound. The concentration of this compound in plants significantly varies with the seasons, reaching (Foster et al., 1999) [14] values of 3 mg/g of fresh biomass in plants of *S. laponum*.

The Italian chemist Raffaele Piria discovered SA in the late 1838 from the flower and buds of the European species *Spiraea ulmaria*, which was later called *Filipendula ulmaria* (*L.*) Maxim. Piria was the first scientist to recognize this natural substance in species other than *Salix* sp. The revelation that this molecule was not unique to the *Salix* genus opened the door to the research of its biosynthesis, as well as its biochemical and physiological significance in plants, and in 1899 the Bayer Company created a novel medication that is now known as aspirin. (Arif & Aggarwal, 2019) [2].

SA is known to be synthesized through the shikimate pathway via two different metabolic pathways. The enzyme phenylalanine ammonia lyase (PAL) transforms phenylalanine (Phe) to trans-cinnamic acid (t-CA), which is then oxidized to benzoic acid (BA), in the first discovered

route, also known as the phenylalanine route, which takes place in the cytoplasm of the cell. The hydroxylation of the BA aromatic ring by the enzyme benzoic acid 2-hydroxylase (BA2H) results in the synthesis of SA later on. Hydrogen peroxide (H₂O₂) is necessary for the enzymatic conversion of BA into SA by BA2H (Shine et al., 2016) [41]. Ellis and Amrhein (Hayat et al., 2007) [17] provided the first evidence for the first route when they noted that feeding *Gaultheria procumbens* plants labeled 14C-benzoic acid or 14C-cinnamic acid caused the synthesis of labeled SA. Working on rice and tobacco, respectively, (Yalpani et al., 1993) [50] and (Silverman et al., 1995) [42] suggested that trans-cinnamic acid's side chain is decarboxylated to produce BA. The subsequent hydroxylation of BA at site C2 results in the formation of SA. However, recent findings suggested that benzoyl glucose, a conjugated form of BA, is more likely to be the direct precursor of SA.

In the chloroplast, there is a second pathway known as the isochorismate (IC) pathway. Two enzymes—*isochorismate synthase* (ICS) and *isochorismate pyruvate lyase* (IPL)—catalyze the conversion of chorismate to isochorismate in plants, which leads to the production of SA. Recent research on *Arabidopsis thaliana* showed that 90% of the SA involved in defense is synthesized from isochorismate by the plastid-localized *isochorismate synthase1*, whereas 10% comes through the cytosolic PAL route. From a physiological perspective, it is well recognized that SA is crucial for controlling plant development, growth, protection against biotic and abiotic stress, and immunological responses.

For a long time, SA was thought to be merely one of many minor phenolic chemicals produced by plants. The first proof that SA may function as a plant hormone came in 1974, more than a century after its discovery, when it was discovered that SA was a mobile signaling molecule localized in the phloem that stimulated flowering in various plant species. But it wasn't until (Raskin et al., 1990) [35] documented SA's function during *Sauromatum guttatum*'s

thermogenesis that the ultimate proof that SA was a plant hormone was offered. The number of articles focusing on SA as a plant growth regulator, signaling molecule, and plant elicitor shielding plants from biotic and abiotic stress increased exponentially after that point (Dempsey et al. 2016) ^[10]. SA can function alone or in combination with other plant hormones. Recent research has also shown that SA may be essential in defending plants against environmental stressors including heavy metals (HM). In fact, a number of recent manuscripts stated that SA can reduce HM toxicity by influencing both their uptake and/or accumulation in plant organs (Safari et al., 2019) ^[38], as well as scavenging reactive oxygen species (ROS) and/or decreasing their accumulation and/or enhancing the antioxidant defense system, protecting membrane stability and integrity, interacting with plant hormones, up-regulating heme oxygenase, and enhancing the performance of the photosynthetic.

HM Stress and Its Impacts on Plants

A metal or metalloid is described as (HM) if its atomic density is greater than 6 g cm⁻³. This group includes both essential elements, micronutrients that must be consumed in small quantities (such as Cu, Cr, Co, and Zn), as well as non-essential metals like Pb, Cd, and Hg (Adrees et al., 2015) ^[1]. Both essential and non-essential components become harmful to plants and animals when present in greater concentrations. Around the world, heavy metal contamination has become a severe environmental issue. Due to their bio magnification through the food chain, HM released by growing industrialization, uncontrolled population growth, and urbanization affect living biota by compromising soil and water. While increased human activities like mining, painting, and refining have increased their concentration in the biosphere, natural processes like volcano eruptions and rock erosion have increased the release of harmful substances into the environment.

The biotic and abiotic elements of the ecosystem are seriously at risk from soil contamination caused by HM (Keesstra et al. 2018) ^[21]. A larger uptake of HM by plants results from the increased amount of HM in the soil, which can lower plant growth, biomass, photosynthesis, crop production, and quality (Ramzani et al., 2016) ^[33]. The top soil is the biologically most active zone of soil, and it accumulates a lot of hazardous metals, which is quite dangerous for the environment. Plant cell metabolism is adversely impacted by the elevated degree of HM deposition in plant organs (Arshad et al., 2017) ^[4]. A high concentration of hazardous chemicals, such as HM, naturally affects the various physiological activities in plants, including protein metabolism, photosynthesis, respiration, and morphogenesis (Sidhu et al., 2016) ^[43]. For instance, after being exposed to Cd, rice seedlings showed abnormal morphogenesis and reduced root growth. In response to Cd treatment, many plant species, including *Brassica napus*, *Helianthus annuus*, *Thalassia caerulescens*, and *Vigna radiate*, displayed suppression in photosynthesis (Baryla et al., 2001) ^[5]. Recently studied the impact of Pb on the morphology and metabolism of *Sesamum indicum* and discovered that the plant's growth was impacted by the metal's rising concentration. In addition, the plant displayed severe signs of chlorosis, necrosis, and decreased protein and chlorophyll levels at higher Pb dosages.

Due to HM's interference with the photosynthetic process, the main effect of metal toxicity is the peak generation of ROS. In the photosynthesis and respiration processes, ROS such hydroxyl, superoxide, and hydrogen peroxide are formed as a byproduct during the transport of electrons (Kadukova et al., 2011) ^[20]. In plants and other organisms, ROS participate in a complex and well-coordinated regulation network under physiological settings. They also serve a variety of signaling activities. In fact, ROS are part of a sophisticated regulation system in plants that also includes secondary messengers like Ca²⁺, signaling molecules like salicylic acid (SA) and jasmonic acid (JA), and plant hormones like ethylene (ET) and abscisic acid (ABA). The accumulation of ROS, however, can create oxidative stress in the cells, which in turn can cause membrane rupture, DNA damage, ion leakage, and lipid peroxidation in plants when ROS production surpasses physiological levels. For instance, investigated the ultrastructural changes caused by Pb in wheat roots and found that Pb impeded root growth, generated ROS, and damaged mitochondrial and nuclear integrity in the studied plant.

Responses of SA (Salicylic acid) in Plants

Salicylic acid applied to basil and mayoram increased growth and oil generation by improving photosynthesis and nitrogen uptake. Due to SA's increased eugenol synthesis, basil might become a novel dietary source of antioxidant phenolics. A salicylic acid therapy increased total free amino acids, prolines, spermidine, and total polyamines (Fatma Abd El-Lateef Gharib, 2006) ^[13].

Pelargonium graveolens responded better to IAA than IBA when growth regulators were applied exogenously (Kumar et al., 1982) ^[26]. At 200 ppm of ethrol in ginger, a sizable increase in rhizome length and width, bit number, and yield of fresh rhizome was seen. The use of GA₃ and KNO₃ improved the development of tobacco seeds stored in darkness (Sharma and Phukan, 1981) ^[39]. For increasing the size, weight, and quantity of cloves per bulb of garlic, NAA at a concentration of 50 ppm was shown to be the most efficient (Das et al., 1996) ^[9].

Effect of SA to Photosynthesis in Plants Subjected to HM Stress

Numerous physiological and biochemical systems in plants are impacted by the various stressful situations that they undergo. Among these, photosynthesis is typically one of the most affected mechanisms by HM (see a schematization of the effect of HM on chloroplast in HM accumulate in various plant organs and affect the synthesis of photosynthetic pigments, including carotenoids and chlorophylls (Sidhu et al., 2016) ^[44]. HM also alter the chloroplast membrane structure and affect electron transport, impairing light-dependent reactions of photosynthesis. Furthermore, it was shown that exposure time and concentrations are what determine how harmful HM is to PSI and PSII. The results of experiments conducted by showed that PSII is more sensitive to HM stress than PSI, but that both PSI and PSII activity was suppressed at high concentrations. Stomatal conductance and transpiration rate dysfunction also contribute to the photosynthesis inhibition brought on by HM.

To protect the photosynthetic apparatus against harm caused by HM, plants are outfitted with a variety of mechanisms.

SA is a key regulator of photosynthesis that affects plant chlorophyll concentration, stomatal conductance, and photosynthesis-related enzyme activity. Under HM stress, it increases photosynthetic efficiency and strengthens the photosynthetic system. When Cd toxicity was present in *Triticum aestivum*, exogenous application of SA (500 M) improved CO₂ fixation, chlorophyll concentration, and the activities of phosphoenolpyruvate carboxylase and RuBisCO. In addition, after being exposed to 10 Mμ SA, *B. juncea*'s gas exchange parameters and carbonic anhydrase increased under Ni (Ventrella et al., 2009) [48] and Mn (Parashar et al., 2017) stress. By enhancing antioxidant activity in the plants, SA treatment increased the concentration of Chla, Chlb, and carotenoids in barley plants under Pb stress. This enhancement may be caused by the obstruction of Ca channels that aid in the translocation of Pb in roots. Recently, researchers looked into how SA affects the accumulation and reduction of Cd in tomato plants. In tomato plants, exogenous SA treatment also boosted pigment content and photosynthetic performance (Guo et al., 2018) [16]. The regularly reported protective impact of SA for the photosynthetic apparatus may result from increased ROS species detoxification exerted by SA or from the activation of antioxidant apparatus induced by SA.

1. Regulation Mechanism of ROS and Enzymatic Antioxidants Promoted by SA Acid under HM Stress

One of the earliest reactions in plants under HM stress is the production of ROS. According to (Yadav et al. 2010) [49], the Haber-Weiss reaction either causes ROS formation directly or indirectly by interfering with the antioxidant defense system or the electron transport chain. Plants are greatly harmed by ROS (hydrogen peroxide, hydroxyl radicals, and superoxide radicals; H₂O₂, OH, and O₂^{-•}, respectively), which cause the oxidative degradation of cell membranes and big macromolecules. To combat oxidative stress, plants have a robust antioxidant system that includes enzymes like SOD, CAT, APX, and GR as well as nonenzymatic antioxidants like glutathione, ascorbic acid, phenolics, and carotenoids that scavenge and detoxify ROS excess generation in plants.

Lipid peroxidation is the primary kind of oxidative damage caused by HM stress in plants, and SA has been demonstrated to offer stability against HM-induced oxidative damage by enhancing antioxidant machinery in plants (Rivas et al., 2011) [37]. When SA was added to Mn and Cd-treated *B. juncea* and *Cucumis melo*, it was seen that lipid peroxidation, electrolyte leakage, and superoxide ion levels decreased. A few studies suggest that SA can encourage the scavenging of free radicals from HM-induced ROS by controlling the production of specific proteins and molecules that lower H₂O₂ buildup in plants and antioxidant enzymes that are known to reduce ROS. One such protein and molecule is OsWRKY45, which has been identified in rice. By preserving the equilibrium between ROS production and membrane integrity, this helps to avoid membrane rupture. Recent research (Lu et al., 2018) [27] showed that the addition of SA to plants under Cd stress activated antioxidant enzymes like SOD, APX, and other peroxidases, which helped to confer Cd tolerance in plants like *Lemna minor* and *Nymphaea tetragonal*.

2. Regulation of Osmolytes and Polyphenols by SA under HM Stress

To combat the ROS generation caused by HM, plants have developed a variety of defense mechanisms. The equilibrium of ROS in plants under stressful circumstances, such as an excess of HM, is maintained by a variety of antioxidant metabolites, including proline, glycine betaine, polyamines, sugars, and polyphenols. The close relationships between SA and other antioxidant compounds are discussed here in an effort to paint a complete and accurate picture of how SA promotes the regulation of antioxidant molecules in plants exposed to HM.

1.1 Proline

Proline functions as an osmoprotectant, a free radical scavenger, and a cellular structural stabilizer. With the aid of the P5C synthase enzyme, glutamate is transformed into glutamatesemialdehyde and then spontaneously into pyrroline-5-carboxylate (P5C), which is subsequently used to synthesize proline. Later, P5C is reduced to proline with the help of the enzyme P5C reductase. Examples of plants whose proline levels were stimulated by HM stress include *Olea europaea* and *Phoenix dactylifera* (Sharma et al., 2012) [40]. It is unclear, however, whether the accumulation was caused by increased proline synthesis enzyme production, decreased proline oxidation enzyme production, or a combination of the two. Under HM toxicity, SA helps to increase proline levels (Faraz et al., 2019) [15].

According to a report, SA reduced Mn stress by enhancing proline accumulation in *Brassica juncea*, which may have happened as a result of increased proline synthesis enzyme activity. In contrast to stressful situations that can result in osmotic stress, which can happen when plants restrict stomatal conductance in try to limit HM intake, increased proline concentration also keeps water balance in plants. Chinese and Dickman, 2005 Along with acting as an osmoprotectant, proline was also suggested by Chen and Dickman to be a potent ROS scavenger and an essential part of plants' protein pathways (Szabados & Savoure 2010) [45]. However, it was noted that SA pre-treatment reduced proline accumulation in *Zea mays* under Pb stress, which was confirmed by the results in rice plants. This means that the pattern of proline (activation/decrement) might vary depending on the species, the metal, and the amount of HM that the plant species has been exposed.

1.2 Glycine Betaine

According to (Raza et al., 2014) [36], glycine betaine (GB), a quaternary level ammonium molecule that works as an osmoprotectant or compatible solute in plants, accumulates at the cytosolic level in higher plants under stress. In addition to protecting against drought, salinity drought, and HM stress, GB also does this. A very effective way to provide tolerance to HM stress is through exogenous application of GB. We still don't know how SA affects how much GB accumulates in plants that are exposed to metal stress. However, a small number of studies suggested that exogenous therapy of GB in conjunction with SA can reduce HM toxicity. According to recent research (Aldesuquy et al., 2013) [3], GB and SA regulate osmotic pressure and osmolytes concentration in plants that maintain osmotic balance and aid in reducing the negative effects of drought stress on wheat, suggesting a potential cooperative relationship. Additionally, it was claimed that the SA caused

the GB level to increase, aiding in the growth of *Rauwolfia serpentina* plants produced in Na excess due to Cu toxicity. Similar to this, 0.01 M SA helped tomato plants develop and accumulate sugar while also enabling them to tolerate stress and protect themselves against Cu-induced Na toxicity. Similarly, 0.01 M SA enhanced growth and sugar accumulation in tomato plants and provided stress avoidance and tolerance against Na toxicity (Misra & Misra 2012) [29].

1.3 Sugars

The name "sugars" refers to a group of water-soluble carbohydrates that play a role in plant stress tolerance, including the disaccharides sucrose, trehalose, and fructans. Plant growth, development, storage, and signaling all depend on sucrose, a key product of photosynthesis (Keunen et al., 2013) [22]. During transcriptional and post-transcriptional processes, carbohydrates, one of the basic constituents of plants, function as a signaling molecule and a source of energy. Stress-induced accumulation of soluble sugars has been seen in plants, indicating their importance for cellular homeostasis and osmoprotection. Plant growth was aided by the exogenous addition of SA, which increased the amount of sugars and polysaccharides in plants. SA was found to give Cu tolerance in *Helianthus annuus*, according to (El-tayeb et al., 2006) [12]. According to the authors, plants treated with SA have higher levels of soluble carbohydrates, which shield the photosynthetic pigments from Cu toxicity. Similarly, 0.01 M SA offered stress avoidance and Na toxicity tolerance while promoting growth and sugar accumulation in tomato plants.

1.4 Polyamines

Plants' morphological, developmental, and stress responses are significantly regulated by water-soluble compounds called polyamines (PAs) (Nahar et al., 2016) [30]. PA have the ability to control the plant's response to HM toxicity and scavenge HM-triggered ROS. PA function as signaling substances in stressful circumstances and regulate ion homeostasis and ion transport in plants, actively contributing to stress tolerance. According to numerous findings, SA treatment may affect the amount of PA in plants. However, the precise mechanism underlying SA-mediated HM stress tolerance and the link between PA and SA in plants remain unknown. Recently, (Tajti et al., 2018) [46] evaluated the role of putrescine and spermidine on wheat under Cd stress and similarly showed elevated levels of SA in those plants.

1.5 Polyphenols

One of the greatest categories of secondary metabolites are the phenols, which range in complexity from basic aromatic compounds like tannins and lignans to extremely complex molecules. They are produced by the PAL enzyme starting from phenylalanine. Numerous studies have shown that increased phenolic compound synthesis during HM stress can prevent oxidative damage. According to (Chen et al., 2019) [6], increased expression of the phenylpropanoid biosynthesis enzymes including phenylalanine ammonia-lyase, chalcone synthase, shikimate dehydrogenase, cinnamyl alcohol dehydrogenase, and polyphenol oxidase is what mostly causes the accumulation of phenolics. The role of phytohormones in raising the level of some classes of polyphenols, such as anthocyanins, has been shown in

numerous research. Caffeic acid concentrations were shown to be higher as a result of exogenous SA treatment, according to (Dong et al., 2010) [11]. Similar to this, under Ni and Cd stress, SA treatment led to a surge in the activity of PAL in *Matricaria chamomilla* plants.

2. Regulation of Cell Signaling by SA under HM Stress

The SA-induced increase in HM stress tolerance is consistent with the SA's function in stress signaling. According to (Horváth et al., 2007) [19], the mechanism of tolerance is influenced by both the general health of the plants and the concentration and method of SA application. In addition to affecting plant growth and development, abiotic stress controls the DNA replication system. As a result of SA treatment, the topoisomerase gene and chloroplast elongation factor are upregulated, which aids plants in adapting to stressful situations (Hettiarachchi et al., 2005) [18]. Additionally, SA has been shown to in vivo increase the production of TLC1, a family of long terminal repeating retro transposons. This family's expression by SA shows that they play a part in signaling pathways that are mediated by SA since they are transcriptionally active under stressful circumstances. The enhanced activity of AsA-GSH pathway enzymes is another method SA uses to control HM stress plant response (Kovács et al., 2014) [25]. AsA and GSH are both active redox substances that keep the cellular redox equilibrium in plants in check. In addition, SA supplementation enhanced the levels of SOD and POD in *Cannabis sativa* and improved Cd tolerance, which may be connected to higher concentrations of Ca²⁺ (a second messenger) and H₂O₂ that ultimately encourage the activity of antioxidant enzymes that lower the amount of cellular ROS in plants.

3. Crosstalk of SA with Other Plant Growth Regulators

Through interactions with other plant growth regulators or plant hormones, SA controls various plant responses in both optimal and stressful environments (Khan et al., 2013) [23]. Under ideal and stressful conditions, researchers have looked at how SA interacts with other hormones such as auxin, cytokinin, gibberellins, abscisic acid ethylene, and brassinosteroids. Under stressful circumstances, the interaction between SA and hormones may have either a synergistic or antagonistic effect. Recently, (Tamás et al., 2015) [47] examined the SA-regulated relief of Cd-stress via reduction of Cd-induced auxin-mediated ROS generation in barley roots.

According to the scientists, IAA (indole-3-acetic acid) generated stress responses in plants could be reversed by SA treatment, pointing to a potential role for SA in the IAA signaling pathway. It has also been noted that IAA and SA play opposing roles in maize roots. In contrast to SA, which increased total root biomass, the exogenous injection of IAA promoted lateral development while inhibiting main root growth. Increased ethylene production and oxidative stress in plants can result from exposure to a variety of environmental stressors, including HM. either the expression of ethylene-responsive genes or ethylene-related biosynthetic genes reached a peak, which led to the increased ethylene synthesis. By boosting GSH levels, which led to metal detoxification and scavenging ROS brought on by HM-triggered ethylene formation, it has been observed that exogenous SA can reduce Cd stress in wheat. Under Cd stress, SA supplementation led to elevated ABA

levels in wheat seedlings, which was attributed to a de novo ABA production. Furthermore, during HM stress, endogenous ABA regulated the SA-mediated modification of the concentration of dehydrin proteins, illuminating the protective mechanism of SA in wheat plants. Cross-talk between SA and jasmonates is essential for controlling plant growth under abiotic stress conditions. According to Khan et al., (2013) [24], the signaling pathways for SA and jasmonic acid (JA) generally operate antagonistically. The conflict between SA and JA cell signaling is brought about through the Mitogen-Activated Protein Kinase (MAPK) signaling pathway. However, there haven't been any reports of an antagonistic interaction between SA and JA, and it's still not obvious how it works exactly (Per et al., 2018) [32]. More research is needed. For instance, Cu stress caused the manufacture of SA in maize plants, which then triggered JA priming and the production of volatile organic molecules.

Conclusions

One of the main dangers for plants growing in contaminated regions has been recognized as heavy metal stress. Plants have evolved a number of molecular, metabolic, and physiological systems to deal with the negative impacts of heavy metals, enabling them to either avoid stressful situations or adapt to them. Numerous studies have shown that SA, when applied sparingly, is essential for lowering and easing the effects of heavy metal stress on plants. This plant hormone has been shown to benefit plants in both ideal and stressful environments by increasing both its endogenous level and its exogenous application. However, the majority of studies on this subject are primarily concentrated on the function that this molecule performs following an exogenous application, while very few studies, due to the complexity of the cascade effects produced, have revealed the defense mechanisms activated by its endogenous stimulation in response to heavy metals. Therefore, there are still a number of issues that require more research. Additionally, a molecular dissection must have a thorough understanding of the interactions that exist between SA and other phytohormones and/or metabolites as well as the feedback mechanisms that regulate endogenous SA levels in response to heavy metal stress.

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