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Salicylic Acid – A Versatile Plant Growth Regulator

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ISBN 978-3-030-79228-2 ISBN 978-3-030-79229-9 (eBook)
https://doi.org/10.1007/978-3-030-79229-9

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Preface

Salicylic acid, chemically known as ortho-hydroxybenzoic acid, is a phenolic compound. The systemic induced resistance has remained the basic role and identity of salicylic acid till present time. Besides defence responses, salicylic acid and associated derivatives play an important role in the regulation of various physiological and developmental processes in plants, growing under normal and stressful environmental conditions. Plant physiological processes regulated by salicylic acid are seed germination, stomatal movements, photosynthesis, ethylene biosynthesis, enzyme activities, nutrient uptake, flowering, legume nodulation and overall plant growth and development.

Several books from our lab covering various aspects of salicylic acid biology and its role as a phytohormone appeared in 2007, 2011 and 2013. However, in the last 8 years, a lot of development has been noticed in elucidating the specific pathways of salicylic acid signal transduction that leads to a change in the expression of specific genes associated with different developmental programmes. The horizon of salicylic acid-mediated regulation of various physiological processes has also expanded, and various studies enumerating the efficacy of salicylic acid application in practical agriculture have been documented. Therefore, it is a need of the hour to compile the information in the form of a book.

The book comprises of 15 chapters. Chapter 1 of this book presents the role of salicylic acid in regulating bioactive compounds. Chapter 2 deals with the recent data on salicylic acid-mediated increase in absorption and accumulation of macro and micronutrients that contribute to biomass production. The recent progress regarding the role of salicylic acid in reproductive development is covered in Chap. 3. Chapter 4 determines the importance of salicylic acid in pre- and post-harvest attributes in horticulture crops. Chapter 5 provides a review concerning the foliar applications of salicylic acid in improving crop tolerance against drought stress. Interplay between environmental signals and endogenous salicylic acid has been documented in Chap. 6. Chapter 7 deals with the role of salicylic acid in crops to tolerate abiotic stresses. In Chap. 8, interplay between salicylates and jasmonates under stressful conditions is briefly described. Chapter 9 explains the relationship between salicylic acid and polyamines in plants. In Chap. 10, biosynthetic convergence of salicylic acid and melatonin, along with their role in conferring tolerance against plant stress, has been discussed. The crosstalk between GSH and salicylic acid to mitigate environmental stress is presented in Chap. 11. The signal transduction involved in SA-mediated regulation of plant immune system has been discussed in Chap. 12. An insight on the role of various phytohormones including salicylic acid in potato tuber development is described in Chap. 13. Chapter 14 covers the potential short- and long-term effect of salicylic acid in eliminating potato virus. Finally, the role of salicylic acid in mitigation of biotic stress is summarized in Chap. 15.

The aim of this book is to present the collective information regarding salicylic acid which makes it a versatile plant growth regulator. The chapters included provide both theoretical and practical aspects that could be of immense use for future researches through which significant developments are possible. It is envisioned that this book will be a help for students, teachers, and researchers, in understanding the relation to phytohormone and agricultural sciences.

With great pleasure, we extend our sincere thanks to all the contributors for their well-timed response and their contributions. We are thankful to all who helped us by any means throughout the preparation of this volume. We are extremely thankful to Springer Nature for the expeditious acceptance of our proposal and completion of the review process. We express our sincere thanks to the members of our family for all the support they provided and the neglect and loss they suffered during the preparation of this book.

Last but not the least, we are thankful to the Almighty who provided and guided all the channels to work systematically and bring an idea to its final shape in the form of this treatise, *Salicylic Acid: A Versatile Plant Growth Regulator*.

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About the Editors and Contributors

About the Editors

Shamsul Hayat is a professor in the Department of Botany at Aligarh Muslim University, Aligarh, India. He received his Ph.D. degree in botany from Aligarh Muslim University, Aligarh, India. Before joining the Department of Botany as faculty, he worked there as research associate and young scientist. He has also worked as an associate professor at King Saud University, Riyadh, Saudi Arabia; as a BOYSCAST Fellow at National Institute of Agrobiological Sciences, Tsukuba, Japan; and as visiting scientist through INSA-bilateral exchange programme in the Faculty of Biology and Chemistry, Institute of Biology, Department of Plant Biochemistry and Toxicology, University of Bialystok, Poland. His major area of research includes plant hormone, nanoscience and abiotic stress in plants. It has been reported from his group that phytohormone such as brassinosteroids and salicylic acid plays an important role in increasing the photosynthetic efficiency of a plant and regulating the antioxidant system even under abiotic stress. He is also studying the protein profiling in hormone-treated plants under abiotic stress. Dr. Hayat has been awarded Prof. Hira Lal Chakravorty Award by the Indian Science Congress Association, Kolkata, India; Associate of National Academy of Agricultural Sciences, New Delhi, India; BOYSCAST Fellow by the Department of Science & Technology, Government of India, New Delhi; and Young Scientist Award by the Association of the Advancement of Science, Aligarh, India. He has been awarded the Dr. P.S. Khankhoje Gold Medal by Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola. He has been the principal investigator of various projects sanctioned by different agencies, and he has guided seven PhD students and two MPhil students as well as a number of MSc students. Dr. Hayat has published more than one hundred sixty research papers in leading international journals such as *Ecotoxicology and Environmental Safety*, *Environmental & Experimental Botany*, *Plant Physiology & Biochemistry*, *Environmental Pollution*, *Nitric Oxide*, *Protoplasma*, *Plant Signalling & Behaviour*, *Photosynthetica* and *Acta Physiologiae Plantarum* with high impact factor and has also published 12 books by Kluwer Academic, Springer, Wiley-VCH, Science Publisher and Narosa Publishing House. Besides this, he has also published 25 book chapters. Dr. Hayat has presented his work at several national and international conferences in Japan, Brazil, Spain, China, Poland and Saudi Arabia. He is a regular reviewer and is on the panel of editorial boards of national and international journals. Dr. Hayat is also member of important national and international scientific societies. Recently he has been appointed as Indian representative in Asia Association of Plant Scientists.

Husna Siddiqui works in the Department of Botany, Aligarh Muslim University, Aligarh, India. She received her B.Sc. degree from Chaudhary Charan Singh University, Meerut, India, and M.Sc. and Ph.D. degrees in botany from Aligarh Muslim University, Aligarh, India. Her thrust area of research includes the study of plant physiology, nanosciences and plant growth regulator–mediated abiotic stress tolerance in crop plants. She has been awarded the Excellence Award in Science by the Indian Women Scientist Association, Mumbai, India and has also received fellowship under DBT-Builder Programme, by AMU, Aligarh. She has published more than 35 research articles in national as well as international journals of repute with high impact factor, such as *Ecotoxicology and Environmental Safety*, *Environmental & Experimental Botany*, *Plant Physiology & Biochemistry*, *Nitric Oxide*, *Protoplasma*, *Acta Physiologiae Plantarum*, *South African Journal of Botany*, *Carbohydrate Research*, *Bulletin of Environmental Contamination and Toxicology*, and *Carbohydrate Polymers*.

Christos A. Damalas currently serves as an associate professor of agronomy in the Department of Agricultural Development, Democritus University of Thrace, Greece. He holds a B.Sc. in plant science, an M.Sc. in plant physiology and a Ph.D. in weed science. In 2000, he received his diploma in educational studies from the Technical & Vocational Teacher Training Institute of Thessaloniki, Greece. At the B.Sc. level, Dr. Christos Damalas teaches the courses Cereals and Legumes, Industrial Crops, Weed Science, Aromatic Plants, and Pesticide Application Principles, and at the M.Sc. level, he teaches the course Crop Physiology. Before he was appointed as a faculty member in the Department of Agricultural Development, Democritus University of Thrace, in 2010, he spent more than 8 years in leading positions in the Directorate of Agricultural Development, Pieria Prefecture, Greece, as a crop protection specialist. He conducts applied research in the areas of crop physiology, weed science, pesticide safety and farmers' behaviour. He has published more than 100 full scientific papers indexed in Web of Science and currently acts as an editorial board member of well-respected journals in the area of agronomy and plant sciences. His research has attracted attention in the international literature.

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Chapter 1
Elicitation with Salicylic Acid as a Tool for Enhance Bioactive Compounds in Plants

Ana Claudia Pacheco and Pedro Henrique Gorni

Abstract Elicitation is the term used to describe the utilization of molecules that activate plant defense responses, generating increased resistance to biotic and abiotic stresses. Elicitors are defined as natural or synthetic substances that, when applied to plants in small concentrations, initiate or increase the synthesis of secondary compounds related to plant defense. Additionally, elicitors may play an important role in stimulating the biosynthetic pathways for the production of commercially important secondary compounds. In this sense, elicitation has been considered as a technological tool widely tested in order to maximize the production of bioactive compounds, both in medicinal plant species and species categorized as functional foods, bringing significant economic benefits to the pharmaceutical and therapeutic industry (including nutraceuticals). The elicitation can be applied in the culture of cells and tissues and in intact plants. Salicylic acid (SA) is classified as an abiotic elicitor that alters the expression of genes encoding key enzymes of secondary plant metabolism, significantly increasing the production of bioactive compounds such as essential oils, phenolic acids, flavonoids, tannins, alkaloids, tannins, among others. In this chapter we will address the theme "Elicitation of Plants with Salicylic Acid" and adopt the following sequence of topics: (a) definitions of elicitation and elicitors, (b) elicitation as a tool to increase the production of bioactive compounds in plants, (c) SA mechanisms of action as an eliciting molecule and (d) examples of studies about SA elicitation in medicinal plants.

Keywords Salicylic acid · Elicitation · Secondary metabolites

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S. Hayat et al. (eds.), *Salicylic Acid - A Versatile Plant Growth Regulator*,
https://doi.org/10.1007/978-3-030-79229-9_1

1 Introduction

Elicitation is a technique that has been widely studied and used in medicinal plants and consists of the use of substances that promote an increase in the content of bioactive compounds in the plant, which can also be accompanied by an increase in growth, depending on the type of elicitor used. The elicitor molecules have long-lasting effects, are relatively inexpensive and safe for the environment, therefore being acceptable in organic production systems and characterized as a promising technology in terms of sustainable agricultural production (Venegas-Molina et al., 2020). The eliciting substance interacts with a plant membrane receptor activating specific genes, resulting in the stimulation of various organic compounds that are synthesized by plants to ensure their survival, persistence and competitiveness (Namdeo, 2007; Baque et al., 2012). Different elicitor compounds have been tested in agricultural studies, aiming at increasing the plant's tolerance to biotic and abiotic stresses (Venegas-Molina et al., 2020). Plant elicitation is also employed with the aim of increasing the synthesis of compounds from secondary plant metabolism that have pharmacological action and economic importance.

SA is an elicitor compound widely used in medicinal and aromatic plants due to its efficient role in increasing the growth and biosynthesis of bioactive compounds (Gorni & Pacheco, 2016; Gorni et al., 2020). SA induces the expression of genes related to the production of different secondary metabolites in plants (Taguchi et al., 2001), such as terpenes, alkaloids, flavonoids, phenolic compounds and phytoalexins (Silva et al., 2014).

2 Elicitation in Plants

2.1 Concept and Classification

Plant secondary metabolites are important organic composts in plant's interaction into its biotic and abiotic's environment, that reflects on survival and competitiveness (Halder et al., 2019). These secondary metabolites are multifunctional molecules since they can regulate plant development, growth and defense (Erb & Kliebeisnten, 2020). Plant secondary metabolites like alkaloids, terpenes, glycosides, flavonoids, volatile oils, quinones and coumarins among others possesses important biological actions and have great commercial importance in pharmaceutical industries (Rafiee et al., 2016). They are synthesized at a very low concentration from common precursors originated from primary metabolism of the plant and its exploitation is limited by many factors like difficulties in cultivation of the source plant, low productivity and variations in quantity and quality due to seasonal effect and plant cultivation environment (Halder et al., 2019). In this way, the research has been concentrating efforts in an attempt to increase the synthesis and accumulation of secondary metabolites in plants with pharmaceutical importance. Among the plant metabolic engineering strategies to regulate the biosynthesis of these valuable bioactive compounds is the overexpression of pathways genes (homologus and ectopic expression), the suppression of competitive pathways (downregulation of genes) and the use of elicitors (Lu et al., 2016).

Elicitation term is defined as the use of substances that promote the activation or induction of biosynthetic routes of secondary metabolites in plants and can be carried out in cells, tissues and plant organs (Murthy et al., 2014). Elicitation is an attractive strategy for enhancement of secondary metabolite production in plant species in wich the application of metabolic engineering or synthetic biology tools remains difficult due to the lack of proficient transformation methods and genetic information about biosynthetic pathways (Shakya et al., 2019). When performed during the cultivation of the plant, elicitation can also have positive effects on growth, productivity and tolerance to stress, and in many cases the elicitors are called biostimulants (Rafiee et al., 2016).

The elicitors are applied to plants in small concentrations (Li et al., 2016) and are classified based on their nature, and can be natural and synthetic. Natural elicitors are substances of biological origin such as amino acids (aspartate, glutamine, glutamate, tryptophan, phenylalanine, methionine, proline and arginine); nitrogen fixing bacteria (*Azotobacter chroococcum* and *Azospirillum lipoferum*) and rhizobacteria; humic and fulvic acids; yeast extract; seaweed extract and chitosan (Baenas et al., 2014; Naik & Al-khayri, 2016; Rafiee et al., 2016; Pacheco et al., 2019). Synthetic elicitors are plant regulatory compounds (synthetic hormones), phenolic compounds, inorganic salts (phosphites), nutrients (selenium and silicon) and different substances that have stimulatory effects on the plant (Rafiee et al., 2016).

Among the various elicitors, it is important to consider the concentration, elicitation time, the growth stage of the crop at the time of stimulation and also the type of elicitor to be used, because when applied to plants, they induce the expression of genes that encode defense responses of the plant, which may have different effects on the same species (Rodrigues-Brandão et al., 2014; Halder et al., 2019).

2.2 *Elicitation as a Tool to Increase the Production of Bioactive Compounds in Plants*

In medicinal and aromatic plants cultivation, it is vital to associate the biomass production to quality of the raw material. In these species, elicitors may play an important role in stimulating biosynthetic pathways for the production of active compounds of pharmacological and commercial interest (Li et al., 2016), answer that may be associated with the increase in plant growth. The elicitation in medicinal and aromatic plants can be carried out both in vitro and ex vitro conditions.

Plant tissue culture is a widely used technique for large-scale plant production, considered the best method available to produce high quality plants, being free from any disease and pathogens (Nahar & Borna, 2013). One of the applications of in vitro cultivation is the production of biologically active plant compounds and the production of phytopharmaceuticals, guaranteeing the sustainable exploitation of threatened species (Morais et al., 2012). Through in vitro elicitation, it is possible to increase or change the concentration of secondary metabolites (Vieira & Simon, 2000). In this perspective, tissue culture can guarantee greater biomass production of the target species and can be considered a sustainable method for obtaining secondary metabolites (Amaral-Baroli et al., 2016). However, the use of this technique is limited by the difficulty of establishing specific tissue culture protocols for each plant species. Several factors interfere with the development of plants in vitro, including the composition of nutrients in the culture medium, the concentration and combination of plant growth regulators, the culture environment, the behavior of the donor plant and the appropriate choice of explant (Gonçalves & Romano, 2013; Trettel et al., 2018).

Elicitation can also be performed on intact plants (Pérez et al., 2014; Gorni et al., 2021). In the latter case, the elicitors are first tested under controlled conditions to select the most promising compounds, which are then tested under more realistic field conditions, which will make it possible to use this technique in large-scale commercial crops.

3 SA Elicitation in Medicinal Plants

SA is a plant hormone of a phenolic nature, which acts in different physiological processes related to plant growth and development (Qaiser et al., 2010), among them is the photosynthesis, transpiration, stomatal regulation, biosynthesis of chlorophylls and proteins, enzyme activities and nutrients uptake (Idrees et al., 2010; Khan et al., 2015).

Elicitation with SA has been used successfully in different medicinal species, with the objective of promoting concomitant increases in plant biomass and in the content of bioactive compounds. The Food and Drug Administration (FDA) classifies the SA as “a substance generally recognized as safe”, which enables its use in commercial cultivation of medicinal species targeting the market of phytomedicines (Divya et al., 2014).

In *Mentha piperita* plants the application of SA (0.5 and 1.0 mM) resulted in a higher content of phenolic compounds (synapic acid, rutin and naringenin), increasing the antioxidant capacity of tea (Pérez et al., 2014). The 1.0 mM SA concentration in *Achillea millefolium* also resulted in increases in the levels of total polyphenols and flavonoids, and in the antioxidant capacity of leaf infusion, as verified by the DPPH, FRAP, TBARS and NO tests (Gorni et al., 2019).

Serial applications of citric acid (0.4 and 0.7 mM) and SA (0.5 and 1 mM) in basil plants (*Ocimum basilicum* L.), alone or in combination, resulted in 38.3% increases and 25.8% in the essential oil content in the leaves and stems, respectively (Mirzajani et al., 2015). There was a synergism in the joint application of acids in relation to flowering parameters (number of inflorescences per plant and number of flowers in the inflorescence).

The exogenous application of SA resulted in an increase in the content of essential oil and phenolic compounds in fennel (*Foeniculum vulgare*) (Gorni et al., 2017). In *Taxus baccata* callus culture, increases in the diterpenoid taxol (5.1-fold) and total taxanes (3.5-fold) in the SA-pretreated calli cultured on the medium containing 2% glucose, compared to the control, indicated that the two treatments had a significant effect on taxane production (Sarmadi et al., 2018).

In melissa (*Melissa officinalis* L.) plants grown in the field, the foliar application of SA (0.14–14 g L^{-1}) and jasmonic acid (JA) (0.05–0.40 mg L^{-1}) did not effect on amount of essential oil content and dry herbage weight. Whereas, SA and applications changed the percentage of some major and minor constituents of lemon balm (there were highest percentages of β-caryophyllene and caryophyllene). The elicitor and it concentration can be selected due the aimed purpose (Pirbalouti et al., 2019).

In peppermint (*Mentha x piperita* L.) the SA application (225 mg L^{-1}) increased CO_2 assimilation and use, stomatal conductance, water use efficiency and perspiration, which, in turn, resulted in higher production of stem and root biomass (Gonçalves et al., 2020).

In the species *Achillea millefolium*, the elicitation with SA (1.0 mM) in plants grown in the field resulted in increases in the dry matter mass of the plant (leaves and roots), leaf area, chlorophyll, carotenoids, and higher oil content and yield essential, total phenols, total flavonoids and anthocyanins, as well as a greater antioxidant activity evaluated by the DPPH, FRAP, TBARS and NO tests (Gorni et al., 2021).

Moreover, its hormonal effects, the SA acts as a signaling molecule in the modulation of plant defense responses to biotic and abiotic stresses (AHMAD et al., 2018). The exogenous application of SA in cultivated species has proven attenuation action and/or tolerance induction for different types of stressful conditions (Okuma et al., 2014; Silva et al., 2014; Mei et al., 2015; Muthu et al., 2016). In the specific case of medicinal plants, SA has been extensively studied as an abiotic stress-mitigating compound (Pérez et al., 2014; Attia et al., 2018; Abbaszadeh et al., 2020), with the additional effect of increasing the synthesis of secondary compounds of commercial interest. It is interesting to note that in most cases the elicitor effect of SA on the production of secondary compounds in the plant is observed both under normal and stressful conditions (Attia et al., 2018; Ahmad et al., 2018; Mohammadi et al., 2019).

In lemongrass plants (*Cymbopogon flexuosus*), the application of SA neutralized the effect of water stress, resulting in a significant improvement in all attributes studied compared to plants under water stress, including the content and yield of essential oil (Idrees et al., 2010).

Lemon verbena plants (*Lippia citriodora* L.) conducted at 75% field capacity (moderate water stress) and treated with SA (300 mg L^{-1}) achieved the highest essential oil content and yield. SA decreased the adverse effects of drought on physiological parameters and increased the plant acclimation and performance under water deficit condition (Dianat et al., 2016).

There were successful mitigation of the decreased essential oil and its active constituents production in cadmium stressed peppermint plants (*Mentha x piperita* L.) by the exogenous application of SA (10^{-4} M) (Ahmad et al., 2018). SA increased the concentration of menthol but decreased the concentration of menthyl-acetate in peppermint. The authors suggest SA is very useful to the farmers cultivating essential oil crops in the heavy metal polluted soil.

SA exogenous application (1.5 mM) increased the yield and changed the chemical composition and antimicrobial properties of essential oil obtained from leaves and flowers of rue (*Ruta graveolens*) grown under normal and reduced irrigation, under field conditions. The highest oil production was obtained in plants grown under 50% of the field capacity and application of SA (Attia et al., 2018).

In thyme (*Thymus vulgaris* and *Thymus. kotschyanus*), the foliar application of 100 ppm of SA resulted in an increase in essential oil production and thymol content (93.4% in the case of *T. kotschyanus*), both in plants grown under normal irrigation conditions and under water deficiency. In addition, SA exerted a mitigation effect on water stress, reducing lipid peroxidation, increasing the growth of the aerial part and the root and promoting a higher content of carotenoids and proline in plants (Mohammadi et al., 2019).

Abbaszadeh et al. (2020) evaluated the effects of water stress and exogenous application of SA (1 and 2 mM) in rosemary (*Rosmarinus officinalis* L.) grown in the field. Oil percentage, yield and percentage of 1.8-cineol and camphor, along with borneol and verbenone percentage were affected by interactions between drought stress and salicylic acid. The authors state that it is possible to produce plants with high quality and quantity with reduced water consumption.

4 SA Mechanisms of Action as an Eliciting Molecule

Elicitors induce or enhance the biosynthesis of secondary metabolites by induction of defense or stress – induced responses (Ramakrishna & Ravinshankar, 2011). The plant hormones SA, JA and ethylene (ET) are described as the central regulators of induced plant defense responses and these defensive mechanisms are regulated by complex signaling pathways, molecules, and transcriptional regulators (Venegas-Molina et al., 2020). However, plant response to elicitation depends on specific receptor for each elicitor class and different plant species can be responsive to the same elicitor (Vasconsuelo & Boland, 2007).

Signal perception is the initial step of the elicitor signal transduction cascade and, for instance, recognition of different stimuli is central to the ability of plants to respond to elicitation (Rafiee et al., 2016). SA binds to a protein-like receptor on the cell's plasma membrane. From this SA-receptor connection, a cascade of translocable intracellular signals occurs, activating different defense reactions in the plant (Baenas et al., 2014; Rafiee et al., 2016; Halder et al., 2019). This effect can be attributed to the mobility of the SA in the plant, increasing the signaling and the ability to promote complex regulatory actions through interactions between different biochemical and physiological reactions (Halder et al., 2019; Janda et al., 2020).

The initial events triggered in the most of the elicitor–plant cell interactions comprises ion fluxes (K^+ and Cl^-), calcium burst, cytoplasmic acidification, reactive oxygen species (ROS) burst, NADPH oxidase activation, G-protein activation and mitogen–activated protein kinase (MAPK) phosphorylation (Fig. 1.1) (Shakya et al., 2019). ROS control many processes in stressed plants, among them programmed cell death, abiotic stress responses, pathogen defense and systemic signaling (Gill & Tuteja, 2010). SA at suitable concentrations enhances the efficiency of the antioxidant system in plants (Dianat et al., 2016). SA-regulated abiotic stresses in plants are involved in antioxidative responses, suggesting the interaction between SA and ROS signal pathway (Borsani et al., 2001).

Calcium is a ubiquitous signal in plants which mediates the regulation of many cellular processes by different stimuli, among them, elicitation. There is evidence that the action of many elicitors includes changes in the intracellular calcium status (Rafiee et al., 2016). The increase in the concentration of cytosolic Ca^{2+} promotes the synthesis of nitric oxide (NO), another important secondary messenger in intracellular signaling that culminates in the activation of genes related to secondary metabolism (Taiz et al., 2015). Ca^{2+} signals also are linked to other secondary messengers like inositol 1,4,5- triphosphate (IP3) and diacylglycerol (DAG) (Shakya et al., 2019).

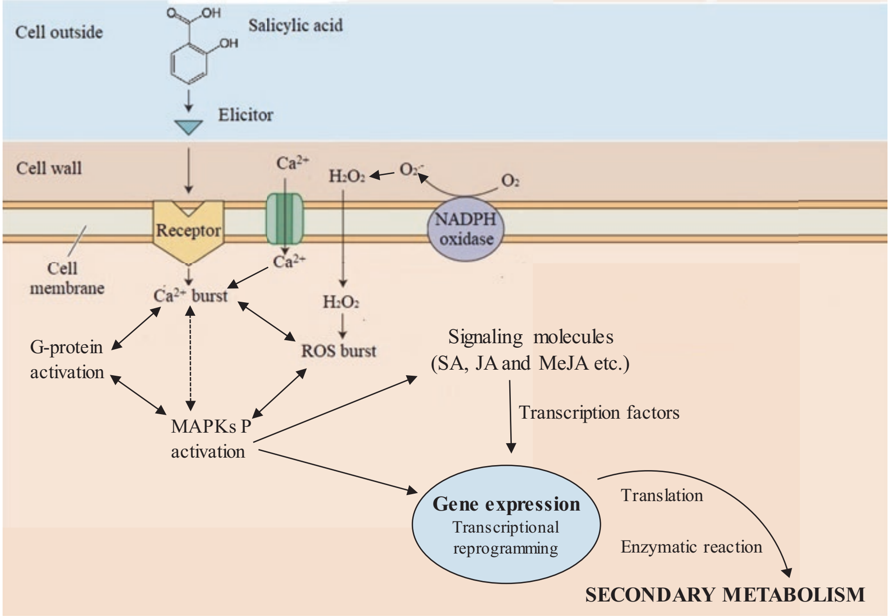

Fig. 1.1 Intracellular cascade events in plant cell response to elicitation. (Adapted from Taiz et al., 2015; Shakya et al., 2019)

It has been demonstrated that mitogen–activated protein kinases (MAPKs) likewise plays a role in plant elicitation (Rafiee et al., 2016). MAPK cascades are major components downstream of receptors that transduce external signals into intracellular responses. MAPKs are phosphorylated and translocate to the nucleus, which results in increased expression of genes coding key enzymes in the biosynthetic pathway of secondary metabolites (Vasconsuelo & Bolan, 2007).

Transcription factors have important regulation functions in the synthesis of bioactive compounds in plants, controlling the transcription of biosynthetic genes of secondary metabolism. Regulating the expression of transcription factors through elicitation or overexpression may constitute important tools to enhance the production of target pharmaceutical compounds (Lu et al., 2016). SA can act in the activation of the transcription factors of the genes involved in the secondary metabolite synthesis (Rodrigues-Brandão et al., 2014; Li et al., 2016).

Secondary metabolites in plants are represented in three major groups named terpenes, phenolics and nitrogen–containing compounds. Despite their important function in plant interaction with biotic and abiotic environments, a new wave of genetic and chemical studies are now demonstrating that secondary metabolites are multifunctional since they can regulate plant development, growth and defense (Erb & Kliebeisnten, 2020). Elicitation activates plant secondary metabolism by modulating the rates of biosynthesis, accumulation and/or vacuolar transport, turnover and degradation (Rafiee et al., 2016).

Studies have shown that SA induces defense responses in the plant through the activation of genes involved in the Shikimic Acid pathway, responsible for the production of different groups of phenolic compounds in plants as flavonoids, tanins and lignin for example (Fig. 1.2). Among the genes activated by the SA are those

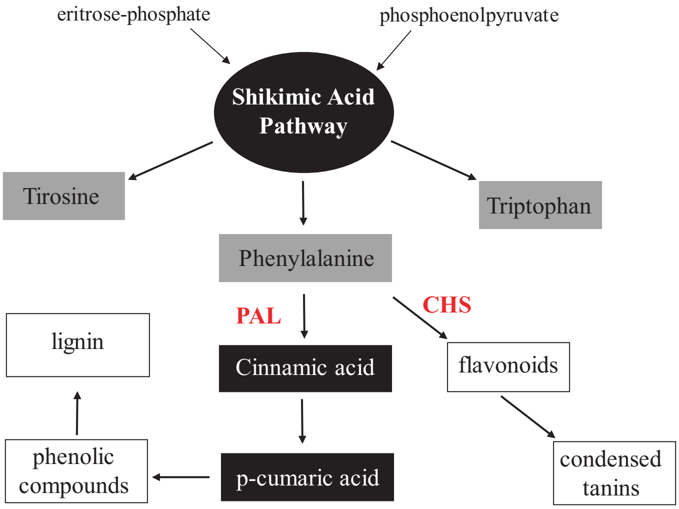

Fig. 1.2 Shikimic Acid Patway in simplified scheme. (*PAL* phenylalanine ammonia lyase, *CHS* chalcone synthase)

encoding the enzyme phenylalanine ammonia-lyase [*PAL*], chalcone isomerase [*CHI*], chalcone synthase [*CHS*], flavonol synthase [*FLS*], anthocyanidin synthase [*ANS*] and production of anthocyanin pigment 1 [*PAP1*] (Li et al., 2016; Gondor et al., 2016). Besides gene transcription, SA also can increase enzyme activity. The accumulation of phenolic compounds (salvianolic acid B and caffeic acid) and the PAL activity were stimulated 8 h after the treatment with SA in *Salvia miltiorrhiza* cell culture, with an positive correlation between PAL activity and phenolic compounds accumulation (Dong et al., 2010). Higher CHS activity with SA application was related in ginger (Ghasemzadeh et al., 2012) and *Centella asiatica* plants (Ibrahim et al., 2017). Foliar application of 1 mM of SA on preharvest of field cultivated *Achillea millefolium* L. resulted in higher PAL and CHS enzymes activity; polyphenols, flavonoids and anthocyanin contents and antioxidant potential in treated plants. These results were extremely consistent in the two consecutive years of the experiment (Gorni et al., 2021).

Recent research suggests that reactive oxygen species (ROS) may act as cellular signalers associated with the gene expression and activity of PAL and CHS, thus, causing the accumulation of phenolic compounds and flavonoids in plants, increasing the plant's tolerance to stress (Ghasemzadeh et al., 2012; Rodrigues-Brandão et al., 2014; Mutlu et al., 2016). The hydroponic treatment with SA in wheat plants resulted in a rapid increase in PAL activity in the leaves and this was confirmed by an increase in gene expression. This may serve as a stress signal, followed by an increase in SA and quercetin, parallel with the synthesis of myricetin and rutin; since these flavonoid are non-enzymatic compounds (Gondor et al., 2016).

Essential oils are secondary metabolites belonging to the group of terpenes, wich are biosynthesized from the basic isoprene units, i.e., isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP) (Sarrou et al., 2015; Fathi et al., 2019). The terpenes are classified depending on the number of isoprene units as monoterpenes (C10), sesquiterpenes (C15), diterpenes (C20), triterpenes (C30), and tetraterpenes (C40). In plants there are two independent pathways to produce IPP and DMAPP (Fig. 1.3). One is the methylerythritol phosphate (MEP) pathway localized in the plastids with seven enzymatic steps. The other pathway is the mevalonic acid (MVA) which is localized to the cytosol, endoplasmic reticulum and peroxisomes and comprises six enzymatic steps (Lu et al., 2016). However, there is a transport from the plastid to the cytosol, known as 'cross talk' between the MEP and MVA pathways (Dudareva et al., 2005; Madji et al., 2015; Elyasi et al., 2016; Fathi et al., 2019).

SA-mediated increment in the plant essential oils might be due to the SA-stimulated vegetative growth, population of leaf oil glands, carbohydrates content, and also due to the beneficial effect of SA on metabolism and enzymes activities responsible for mono or sesqueterpene biosynthesis (Miao et al., 2015). Studies show that the genes of the MVA or MEP pathways were regulated positively with the application of SA. In *Tanacetum parthenium* plants the transcript levels of early pathway (upstream) genes of terpene biosynthesis including 3-hydroxy-3-methylglutaryl-coenzyme A reductase (*HMGR*), 1-deoxy-Dxylulose 5-phosphate reductoisomerase (*DXR*) and hydroxy-2-methyl-2-(E)-butenyl 4-diphosphate

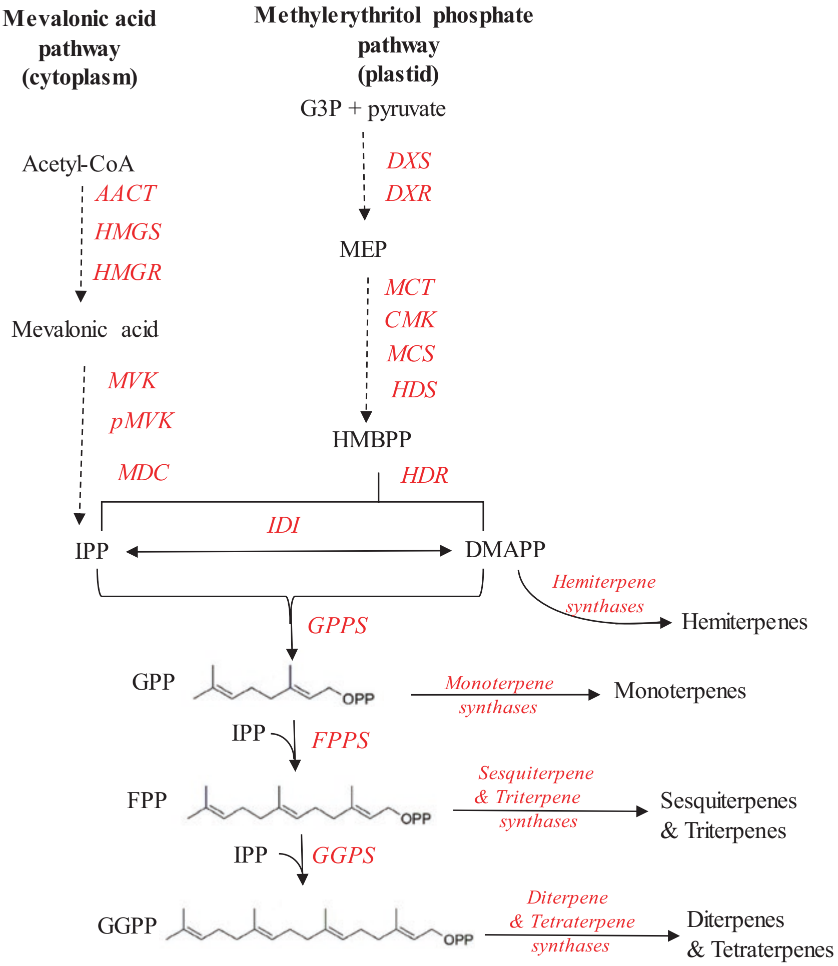

Fig. 1.3 Biosynthethic patway of terpenes. (Adapted from Meena et al. 2017; Abdallah & Quax, 2017. *AACT* acetoacetyl-CoA thiolase, *CMK* 4-(cytidine 5′-diphospho)-2-*C*-methyl-D-erythritol kinase, *DMAPP* dimethylallyl pyrophosphate, *DXR* 1-deoxy-D-xylulose-5-phosphate reductoisomerase, *DXS* 1-deoxy-D-xylulose-5-phosphate synthase, *FPP* farnesyl diphosphate, *G3P* 3-phosphoglyceraldehyde, *GGPP* geranylgeranyl pyrophosphate, *GGPS* geranylgeranyl pyrophosphate synthase, *GPP* geranyl pyrophosphate, *GPS* geranyl pyrophosphate synthase, *HDR* hydroxy-2-methyl-2-(*E*)-butenyl 4-diphosphate reductase, *HDS* hydroxy-2-methyl-2-(*E*)-butenyl 4-diphosphate synthase, *HMGR* 3-hydroxy-3-methyl glutaryl coenzyme A reductase, *HMGS* 3-hydroxy-3 methyl glutaryl coenzyme A synthase, *IDI* isopentenyl diphosphate isomerase, *IPP* isopentenyl diphosphate, *MCT* 2-*C*-methyl-D-erythritol-4-(cytidyl-5-diphosphate) transferase, *MCS* 2-*C*-methyl-D-erythritol-2,4-cyclodiphosphate synthase, *MDC* mevalonate-5-pyrophosphate decarboxylase, *MEP* 2-*C*-methyl-D-erythritol 4-phosphate, *MVK* mevalonate kinase, *pMVK* 5-phosphomevalonate kinase)

reductase (*HDR*) and the biosynthetic genes of parthenolide including germacrene A synthase (*GAS*), germacrene A oxidase (*GAO*), costunolide synthase (*COS*) and parthenolide synthase (*PTS*) were increased by SA treatments (Madji et al., 2015). Similar results were found in plants of thyme (*Thymus vulgaris* L.) where the application of SA resulted in increases in the expression of the biosynthetic genes of thymol and carvacrol (*DXR*, *TvTPS1*, *CYP71D178* and *CYP71D180*) (Madji et al., 2017). In plants of black cumin (*Nigella sativa*), the expression of the genes monoterpene synthase (*MTS*), β-amyrin synthase (*βAS*) and squalene epoxidase (*SQE*) were upregulated by exogenous application of SA at 24 h after treatments (Elyasi et al., 2016).

Alkaloids are secondary metabolites belonging to the group of nitrogen compounds and most of them are derived through the decarboxylation of amino-acid precursors to yield their respective amines, or from anthranilic acid or nicotinic acid. The ability of plants to couple amines to different chemical partners produces a restricted number of versatile chemical backbones (i.e. central intermediates) from which the diversity of alkaloids is produced. Many enzymes from different gene families are involved in alkaloid bioshynthesis and intensive efforts have been made to identify the genes encoding these enzymes (Bunsupa et al., 2012). The elicitation in hairy root cultures of *Papaver orientale* with SA and methyl jasmonato (MeJa) at different time points (6, 12, 24, and 48 h) resulted in the accumulation of the alkaloids thebaine, morphine, and codeine. This effect were directly related to the expression levels of key genes in the morphinan pathway at like *COR*, *SalAT*, *SalR*, *T6ODM*, *CODM* and *Salsyn* (Hashemi & Naghavi, 2016).

Finally, in recent years it has been demonstrated that plant primary metabolism is also affected by elicitation. Molecules of primary metabolism are precursors for biosynthetic pathways of secondary metabolites, and control the transport and storage of secondary metabolites within the cell organelles (Vasconsuelo & Boland, 2007). Regulation of the related primary metabolism of medicinal plants can improve the productivity of target bioactive compounds (Lu et al., 2016). Carbohydrates like sucrose, glucose and D-mannitol are added in cell cultures in addition to elicitors promote higher biomass accumulation and secondary metabolites (Vasilev et al., 2014) and the overexpression of key gene for sucrose hydrolysis can significantly enhance the expression level of genes related to secondary metabolism pathways (Lu et al., 2016). The positive effects of SA elicitation on the physiological processes of *A. millefolium* plants were also reflected in higher carbohydrate, protein and amino acid contents, suggesting that this compound may be associated with the regulation of several essential primary metabolic processes, including photosynthesis and respiration. Thus, it is possible to state that the eliciting action of SA can be indirect, by increasing the concentration of primary metabolites in the treated plants, resulting in a carbon repackaging that favors the biosynthetic pathways of secondary compounds (Gorni et al., 2020).

Acknowledgement We wish to extend our thanks and appreciation to University of Western São Paulo (UNOESTE).

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Chapter 2
Salicylic Acid Increases Root Size That Favours the Absorption and Accumulation of Macro and Micronutrients That Contribute to Biomass Production

C. J. Tucuch-Haas, M. A. Dzib-Ek, S. Vergara-Yoisura, and A. Larqué-Saavedra

Abstract We have demonstrated that applications of low concentrations of salicylic acid (SA) to the shoots of seedlings of plants or to perennial trees significantly increase their growth, development, and productivity. It is proposed that the positive effect of the SA of increasing the size of the roots favours the absorption and accumulation of macro and micronutrients and contribute to biomass and seed production. To test this hypothesis, we run experiments on *Zea mays, Capsicum chinense* and *Solanum lycopersicum* and measure their nutritional absorption after spraying, low concentrations (SA) to the canopy. The results have shown that (SA) significantly increases the length, weight and dry weight of roots, stems, leaves and yield of these species, as well as the levels of nitrogen (N), phosphorus (P) and potassium (K) in the different organs of the plants at the time of harvest. Copper, zinc, manganese, iron, boron, calcium, and magnesium were also increased in most tissues by the effect of SA.

Keywords Growth · Nutrients · Seeds · Yield

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S. Hayat et al. (eds.), *Salicylic Acid - A Versatile Plant Growth Regulator*,
https://doi.org/10.1007/978-3-030-79229-9_2

1 Introduction

We present after almost 50 years of work the results of the effect that salicylic acid (SA) a natural product of easy application and low cost to promote food production and therefore food safety. This makes it a valuable modulating compound of great importance, as an innovator that does not alter the ecosystem in food production, in the immediate future, to serve an ever-larger population. Experiments developed in open field and greenhouse conditions in crops of agricultural interest, with low concentrations of SA (1µM or less), resulted in increased plant size, number of flowers, leaf area and early appearance of flowers for *ornamentals*. (Martin-Méx et al., 2010); while in horticultural crops such as chili (Martín-Mex et al., 2004, 2005) tomato (Javaheri et al., 2012), cucumber (Martin-Mex et al., 2013) and pepper (Elwan & El-Hamahmy, 2009) reported increased yield without altering fruit quality, which has also been observed in fruit species such as papaya (Larqué-Saavedra & Martín-Mex, 2007). Under this same context, for grasses such as wheat (López et al., 1998), rice (Tavares et al., 2014) and maize (Tucuch-Haas et al., 2017a), cereals of great global importance, there are increases of 17%, 60% and 100% respectively in grain yield.

These responses are possible thanks to SA participation in important physiological and biochemical processes in plants (Hayat et al., 2007) like photosynthesis where it regulates the enzymatic activity of Rubisco (Wang et al., 2010) and controls the transport of electrons from photosystem II (Janda et al., 2012; Wang et al., 2010); while in respiration and stomatic conductance (Fahad & Bano, 2012), it intervenes in the control of osmotic and water potentials (Khan et al., 2013) and in the absorption of ions (Fahad & Bano, 2012; Gunes et al., 2007). In addition to these factors, it is also noted the induction of roots, caused by this molecule, which is suggested as a direct responsible for the increased bioproductivity obtained in the species studied.

Since 1975 we have described that acetylsalicylic acid favored rooting of bean shoots. Years later we demonstrated that salicylic acid had a clear effect on the growth of the roots of species of agricultural interest such as soybeans and also ornamental plants, thus initiating a series of experiments that allowed us to ensure that our working hypothesis had a chance in appreciating how flowering and plant productivity increased.

We have postulated that the exogenous supply at concentrations of one µM or less of SA promotes productivity. In both angiosperms and gymnosperms, (Martín-Mex et al., 2013) for its effect on increasing root development, as well as the differentiation of secondary roots which favors better nutrient intake.

2 Effect of Salicylic Acid on Root and Biomass Growth in Cereals

Plant vigor, related to increased root mass and aerial biomass, is mainly subject to genetic and environmental factors, however, studies conducted on a wide variety of plants, of agronomic interest, suggest that SA can contribute significantly to the increase these growth factors. Greater vigor at the seedling level ensures greater adaptation to the environment, which consequently promotes the development and yield of crops. The results of supply SA conducted on wheat and maize seedlings, two cereals of great importance in food worldwide, to study the sensitivity of root and biomass, are described below.

2.1 Wheat

The results of two independent experiments indicate that sprays of 1μM of salicylic acid to the canopy of wheat seedlings (*Triticum aestivum* L.) significantly increases the length and fresh weight of the root, as well as plant height and total fresh biomass.

The seedlings of both experiments grew in agrolite contained in PVC tubes or under open pit conditions, under a completely random experimental design with 8 repetitions per treatment. The seedlings were sprayed for 5 days with 1μM of SA or water as control. Ten days later the measurements were taken and a 34% increase in the length and 30% the weight of the root, were recorded (Tucuch et al., 2015) (Fig. 2.1).

Likewise, the production of total fresh biomass by seedlings reflects that SA significantly increases the biomass formation of seedlings treated with the growth regulator (Fig. 2.2). It is presumed that 1μM of AS is sufficient to impact between 19.3% and 31.7% its biomass. The results obtained of the effect of SA on the estimated variables indicate that the grasses, in this case wheat, respond positively to this growth regulator, mainly affecting the fresh root weight, plant height and total fresh biomass.

2.2 Maize

The results obtained in maize seedlings, from two independent experiments, developed under controlled conditions or open field are presented. In both studies, seeds of a short-life cycle variety, twinned and grown in PVC tubes 4 cm in diameter and 23 cm in length, were used. SA sprayed for five consecutive days to the canopy of seedlings, when they presented the first complete sheet with ligula. Five or ten days

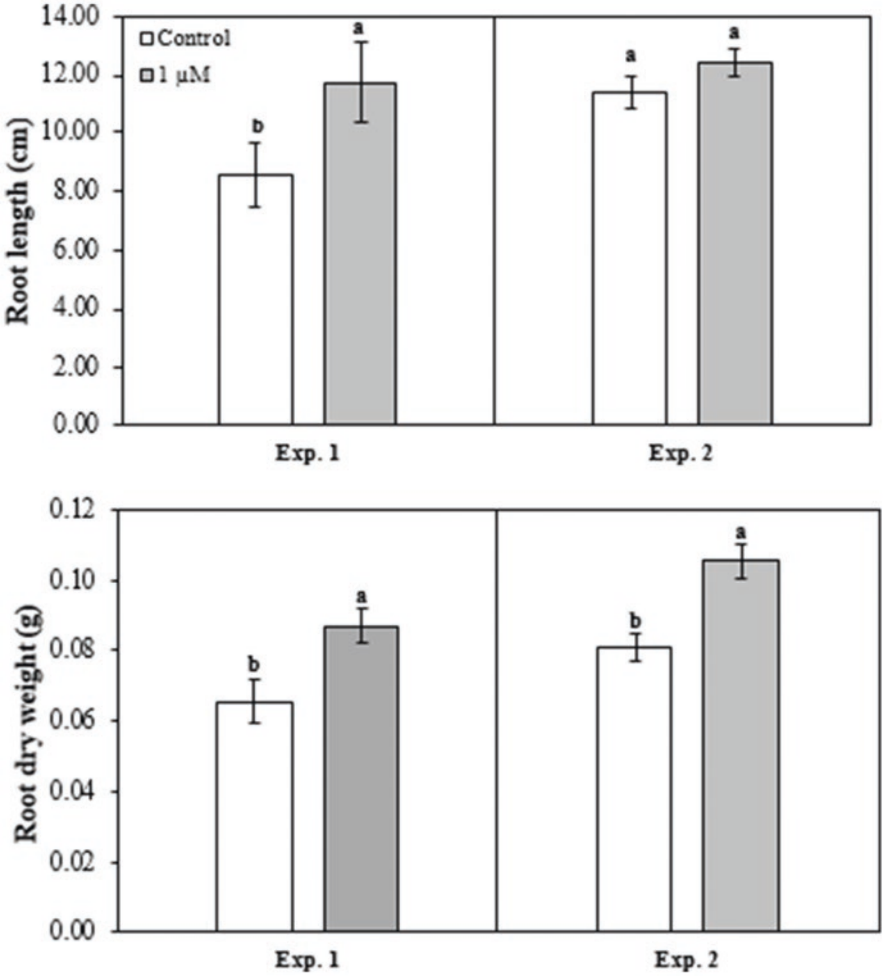

Fig. 2.1 Effect of salicylic acid on wheat seedlings on the length and fresh weight of the root. Each block is the average of 8 samples ± S.E. Means with the same letter are not significantly different

after the last application of SA, seedlings from both tests were harvested for root and stem measured.

Responses from both studies demonstrated the SA ability to influence root development and promote up to 30.6% length, 30% fresh weight and 24.7% volume. Results can be appreciated in Fig. 2.3 (Tucuch-Haas et al., 2016). The highest response expression was determined, with the environmental development conditions and concentration used. Similar benefits occur when plants of the same species are subjected to salinity stress conditions (Khodary, 2004). This behavior has also been documented in crops such as *Glycine max* (L.) (Gutiérrez-Coronado et al.,

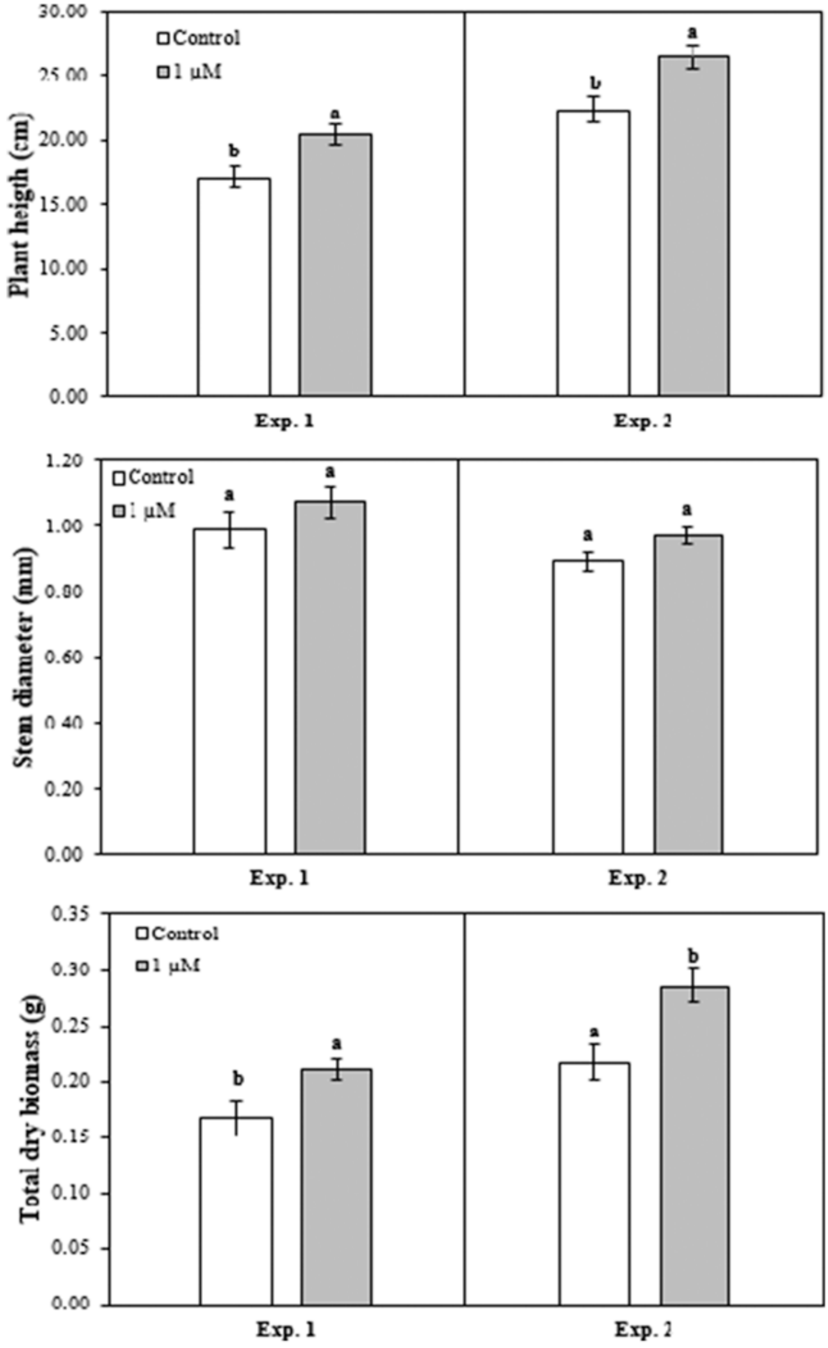

Fig. 2.2 Effect of salicylic acid on the canopy of wheat seedlings on the height, stem diameter and fresh aerial biomass of the plant. Each block is the average of 8 samples ± S.E. Means with the same letter are not significantly different

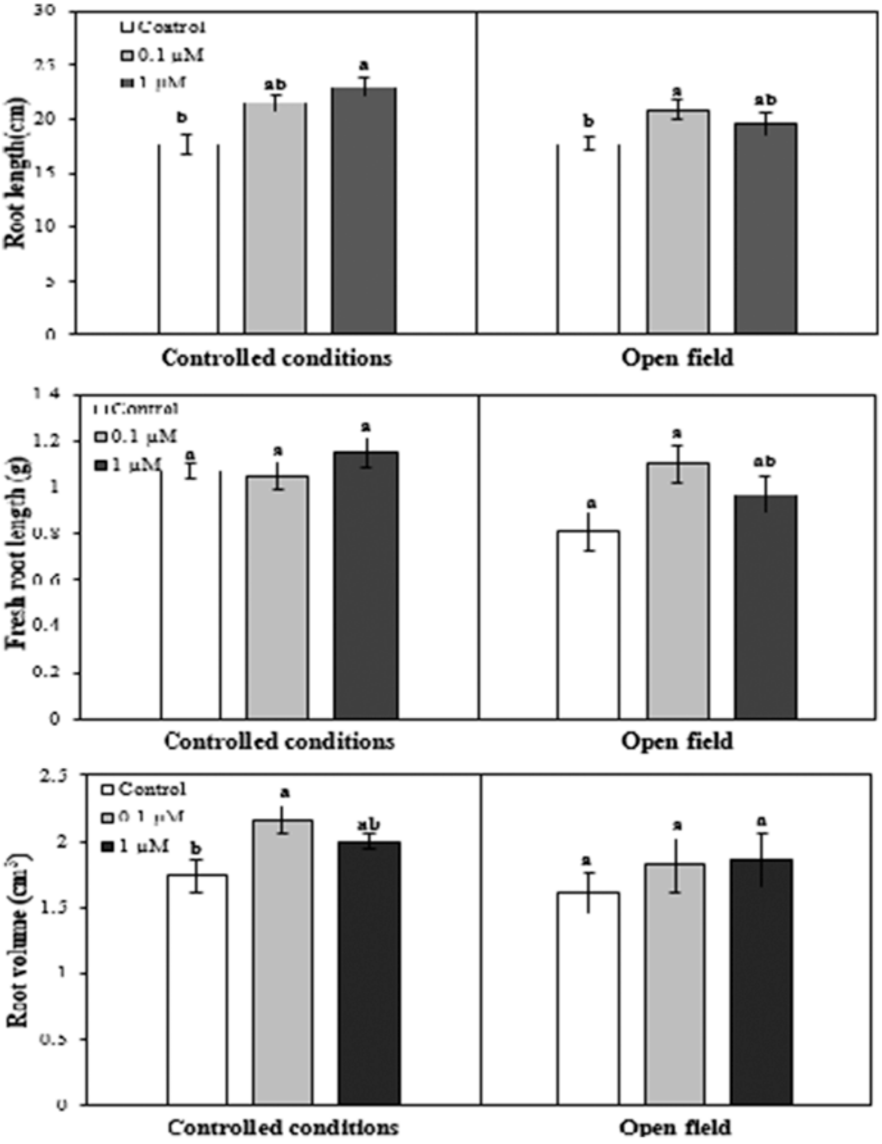

Fig. 2.3 Effect of salicylic acid on corn seedlings on root length, weight, and volume. Each block is the average of 8 samples ± S.E. Means with the same letter are not significantly different

1998), *Lycopersicum esculentum* (Larqué-Saavedra et al., 2010), *Triticum aestivum* (Arfan et al., 2007), *Catharanthus roseus* (Echeverría-Machado et al., 2007) and *Chrysanthemum morifolium* (Villanueva-Couoh et al., 2009).

Other variables estimated, in the two experiments outlined in previous paragraphs are presented in Table 2.1 (Tucuch-Haas et al., 2016). The height of the plant

Table 2.1 Effect of different concentrations of salicylic acid, in the diameter, height and total fresh biomass of corn seedlings, grown in growth room (CC) or in the open (CA)

	Plant height (cm)		Stem diameter (mm)		Total fresh biomass (g) (root and aerial part)	
Treatments	C. C	C. A	C. C	C. A	C. C	C. A
Control	13.7^{a}	32.0^{a}	2.34^{a}	1.94^{a}	1.70^{ab}	1.83^{b}
1µM	12.9^{a}	39.1^{a}	2.31^{a}	2.18^{a}	1.77^{a}	2.27^{ab}
0.1µM	14.5^{a}	39.4^{a}	2.26^{a}	2.31^{a}	1.58^{ab}	2.60^{a}

The means with the same letter within the same column are not significantly different (Tukey, ≤0.05)

increases by 22.1% with the treatment of 1.0µM of SA, while with 0.1µM of SA this increase was 23% compared whit the control. Similarly, the diameter of the stem was stimulated at 12.3% and 19% compared to control with SA treatments 1.0 and 0.1µM, respectively. Unlike the above variables, the fresh biomass weight of seedlings showed a significant increase highlighting the 0.1µM concentration of SA with an increase of up to 42% in the experiment developed under field conditions.

Other variables estimated in the canopy of seedlings, in the two experiments outlined in previous paragraphs are presented in Table 2.1. The height of the plant increases by 22.1% with the treatment of 1.0µM of AS, while with 0.1µM of SA this increase was 23% compared to the control. Similarly, with SA, the diameter of the stem was stimulated at 12.3% and 19% compared to control. Unlike the above variables, the fresh biomass weight of seedlings showed a significant increase highlighting the 0.1µM concentration of SA with an increase of up to 42% in the experiment developed under field conditions.

3 Effect of Different Concentrations of Salicylic Acid on Germination and Quality of Tomato Seedlings

In addition to the foliar application of SA to induce significant responses in the development of the root and vastago of plants, the inbibition is another of the methods to test the effect of this compound. Particularly in tomato plants when the seeds underwent a process of imbibition of salicylic acid for 24 h in 0.0, 1.0, 0.01 and 0.0001µM and cultivated for 7 days in these concentrations or distilled water. It was found that this compound does not inhibit germination and it significantly promotes the root length of seedlings and the formation of secondary roots (Figs. 2.4, 2.5, and 2.6).

Based on the findings of Shakirova et al. (2003) and Echevarría-Machado et al. (2007) it is suggested that the mechanism of action, of the SA to stimulate root development, occurs by promoting the cell division of the apical meristem of the root cap, the increase in the size of the root cap and the appearance of lateral roots; that exert positive changes in the morphology, length, area, volume, perimeter and fresh and dry weight of the root, as reported in plants of *Glycine max* L. (Gutiérrez-Coronado et al., 1998), *Pinus patula* (San-Miguel et al., 2003), *Crysanthemum*

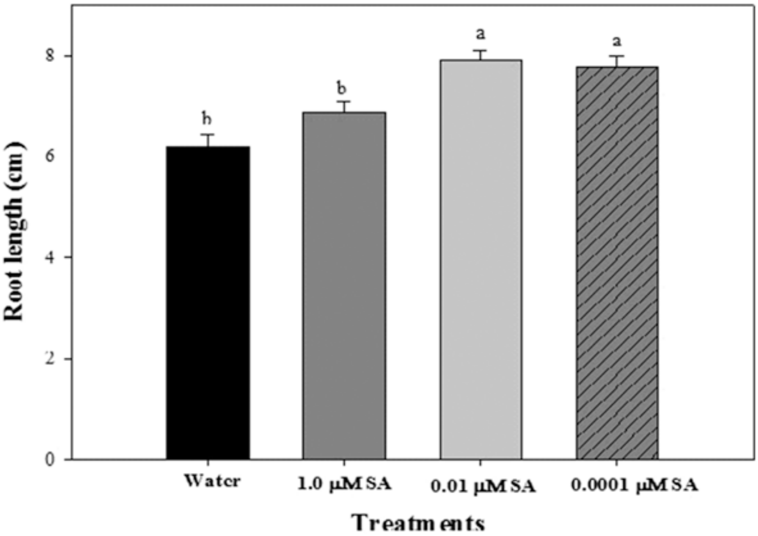

Fig. 2.4 Root length of tomato seedlings grown for 7 days in distilled water. These seedlings came from seeds embedded for 24 h in one of the different concentrations of Salicylic Acid indicated in the graph or from seeds embedded in distilled water Different letters indicate significant statistical difference (Tukey, P ≤ 0.05). The data is average ± standard error (n × 10)

morifolium (Villanueva-Cohuo et al., 2009), *Catharanthus roseus* (Echevarria-Machado et al., 2007) and *Lycopersicon esculentus* (Larqué-Saavedra et al., 2010), when the SA is supplied in a range of 10 fM to 1µm, which demonstrate the sensitivity of the root to this compound.

The results of this study and those related to the effect on root length and volume, an organ of vital importance to any plant organism, support the proposal that foliar applications of SA, in the seedling stage, would favor greater absorption of soil ions because the area of exploration of the plant is greater, an approach that was published by Larqué-Saavedra and Martín-Mex (2007), who also noted that one of the mechanisms by which bioproductivity with SA is increased through root modification.

4 Impact of Salicylic Acid on the Nutrimental Status of Maize and Habanero Chili

Existing evidence of work conducted to study the impact of the significant effect on root morphology and anatomy, by salicylic acid action, on nutritional status of plants, a clear contribution of this effect in the accumulation of macro and micro nutriments in the different organs of plants. Bioassays carried out, in the open field, in maize plants, known locally as Xmejen-naal (short cycle), indicate that

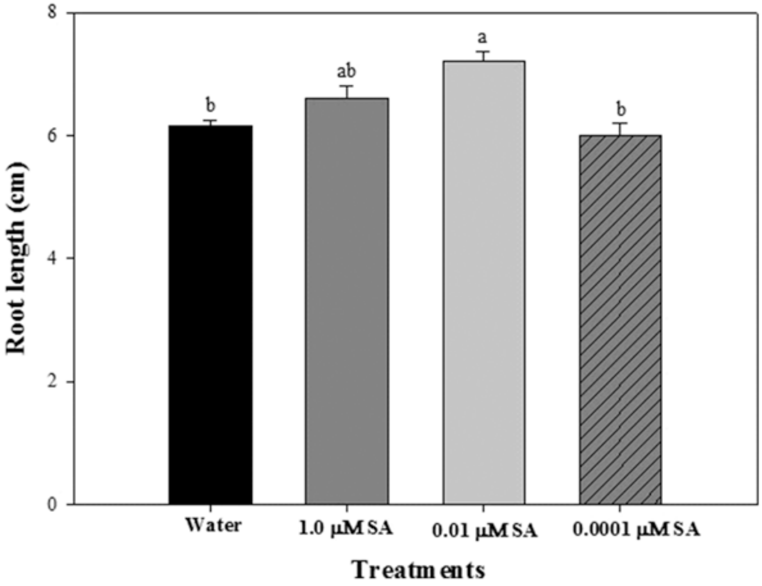

Fig. 2.5 Root length of Rio Grande variety tomato seedlings grown for 7 days in one of the different concentrations of Salicylic Acid indicated in graphica or distilled water. Different letters indicate significant statistical difference (Tukey, $P \leq 0.05$). The data is average ± standard error (n × 10)

contractions of 0.1–1µM of AS, sprayed in the canopy, in seedling stage exert a maximum stimulation effect on the absorption and accumulation of essential elements (Table 2.2). Such responses were reported, in commercial hybrid maize plants, sprayed with 1µM of acetylsalicylic acid (compound analogous to SA), using the same methodology, as in landrace maize plants (Table 2.3).

On the other hand, *in Capsicum chinense* the supply of 1µm of salicylic acid, sprayed to seedlings 17–30 days of age, in 5-day intervals, in which the behavior of the nutritional content in the different organs of the plants was studied. It was shown that it induced an increase in the accumulation of nutrients in root, stem, leaves and fruits. Highlighting the fruits and roots with the largest and least accumulation respectively, for most of the elements studied (Tucuch-Haas et al., 2019). The case of nutritious fruit is an indication of higher quality since it gives it a high nutritional value (Table 2.4).

Unlike calcium in which there is no major impact on the responses obtained, in the species studied, most of the elements are favored in their absorption and accumulation by this compound. These results and those of other authors such as Gunes et al. (2007), El Tayeb and Ahmed (2010), Sánchez-Chávez et al. (2011), and Fahad and Bano (2012) who have reported increases in the content of essential ions in the tissues of plants treated with SA support the hypothesis suggested, that the perceived impact on root development of plants, when SA is supplied at low contractions, which consequently increases the area of soil exploration, has a significant

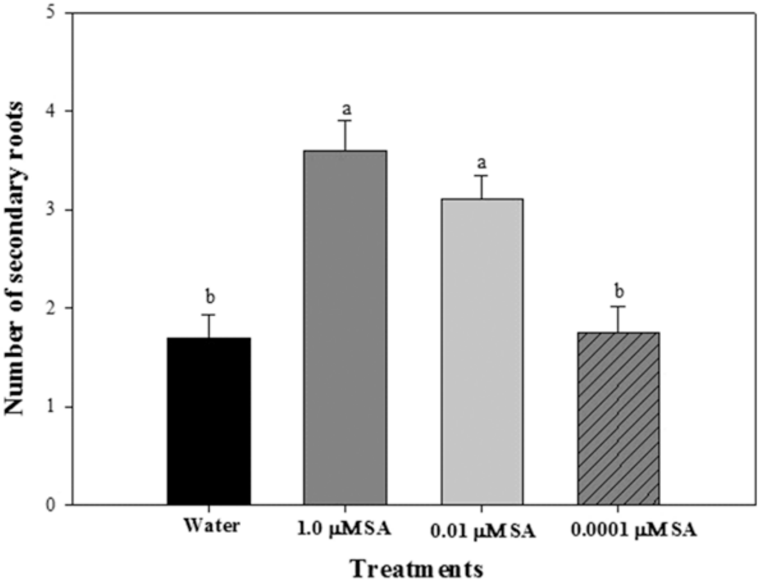

Fig. 2.6 Number of secondary roots of 7 days old Rio Grande variety tomato seedlings grown on different concentrations of Salicylic Acid or distilled water. Different letters indicate significant statistical difference (Tukey, $P \leq 0.05$). The data are averages ± standard error (n × 10)

Table 2.2 The increase in nutritional minerals in tissue and grain of maize (Xmejen-naal), sprayed with salicylic acid to seedlings

	Increases in comparison with water control (%)	
Nutriment	Tissue	Grain
Nitrogen (N)	72	44
Phosphorus (P)	83	114
Potassium (K)	57	106
Calcium (Ca)	0	95
Magnesium (Mg)	45	117
Sulfur (S)	60	81
Iron (Fe)	40	48
Covers (Cu)	133	105
Zinc (Zn)	93	64
Manganese (Mn)	138	56
Boron (B)	100	27
Sodium (Na)	73	86

Table 2.3 Salicylic acid sprayed on the canopy of hybrid maize seedlings stimulates the increase in nutritional mineral content in biomass and grain

	Increases in comparison with water control (%)	
Nutriment	Tissue	Grain
Nitrogen (N)	48	42
Phosphorus (P)	42	53
Potassium (K)	19	32

Table 2.4 Spray of salicylic acid to seedlings stimulates the increase of mineral content in fruits, leaves, stems and root, in habanero chili

	Increases in comparison with water control (%)			
Nutriment	Fruit	Leaf	Stem	Root
Nitrogen (N)	116	45	5	52
Phosphorus (P)	110	39	39	17
Potassium (K)	97	29	28	29
Calcium (Ca)	2	47	40	2
Magnesium (Mg)	77	67	67	9
Sulfur (S)	nf	nf	nf	nf
Iron (Fe)	99	55	100	0
Covers (Cu)	43	19	0	0
Zinc (Zn)	98	78	0	6
Manganese (Mn)	71	81	24	0
Boron (B)	100	34	14	30

nf not found

impact on the absorption of water and nutrients from the soil (Larque-Saavedra & Martín-Mex, 2007).

5 Effect of Salicylic Acid on Aerial Biomass

The increase in root mass, in conjunction with its absorption capacity of root nutrition, by effect of SA, as already noted in previous paragraphs, significantly influences the biomass and yield of the crops. The work carried out to study the benefit of SA in biomass, in the cultivation of corn, when sprayed 1μM in the canopy of seven-day-old seedlings, for five consecutive days and evaluated at harvest time increased up to 68% in comparison with the control treatment. In conjunction with the total biomass it favors the height and diameter of the stem (Tucuch-Haas et al., 2017a; Table 2.3). Similar effects are reflected, when plants are subjected to salinity stress (Keshavarz et al., 2019) or drought (Ghazi, 2017). These results are also consistent with what was reported in ornamental plants such *as Petunia hibirda* and *Crysanthemum morifolium,* in which it favored the foliar area, the production of dry matter, the plant height, the diameter of the stem and the number of flowers

Table 2.5 Effect of the application of salicylic acid to the canopy of maize plants (var. Xmejennal), in plant height, stem diameter and aerial dry biomass from two experiments at 140 days after planting

	Treatment	PH (cm)	SD (mm)	TDB (g plant^{-1})
Exp. 1	Control	213.50 b	16.03 b	218.4 b
	1.0μM	244.00 a	22.65 a	368.50 a
Exp. 2	Control	206.22 a	19.98 a	229.03 b
	1.0μM	218.40 a	20.28 a	347.49 a

PH plant height, *SD* stem diameter, *TDB* total dry biomass. Means with the same letter in each column for each experiment, are not significantly different (Tukey, ≤ 0.05)

(Villanueva-Cohuo et al., 2009; Martín-Méx et al., 2010) as in *Carica papaya,* where it stimulated the height and diameter of the stem (Martín-Mex et al., 2012) (Table 2.5).

6 Effect of Salicylic Acid on Grain Yield and Quality

The exogenous supply of salicylic acid is a technology that allows to increase the root mass and mineral absorption of plants, associated with the achievement of high yields and better-quality characteristics of the harvested product. Attention has now been paid to this compound, searching for alternatives to meet global food demand.

Work carried out on a variety of crops, underpins its participation as a plant growth regulator with the ability to significantly impact yield. For example, the exogenous supply of 1μm SA to the canopy of cultivars of maize plants Xmejen-nal, reflected an increase of 100% above the control in grain yield, as well as benefits in the length, diameter andweight of the cob in two experiments (Tucuch-Haas et al., 2017a; Table 2.4); under the same context, but in the cultivation of habanero chili, similar responses were documented (Tucuch-Haas et al., 2017b; Figs. 2.4 and 2.7, Table 2.6).

In a work carried out by Tucuch and collaborators in an experimental plot in Sinaola México, with a hybrid variety of commercial corn, sprayed with 1μM of ASA (acetylsalicylic acid), an increase of 35% in the weight of the seed was documented, which that favored an increase in yield of 957 kg ha^{-1}. Which also demonstrates the beneficial potential of SA analog compounds (Fig. 2.8).

Regarding quality, it has been documented that SA when sprayed in early stages, the quality of the grain is improved, reflected as an increase in the content of nitrogen (N), phosphorus (P) and potassium (K) (Tables 2.1 and 2.2), moreover lipids, carbohydrates, and proteins (Farouk et al., 2018). Similarly, larger flower diameters are reported in ornamental plants (Villanueva-Cohuo et al., 2009) and increases in color, firmness, total soluble solids, vitamins C, lycopene and brix grades in fruits and vegetables (Elwan & El-Hamahmy, 2009; Karlidag et al., 2009; Javaheri et al.,

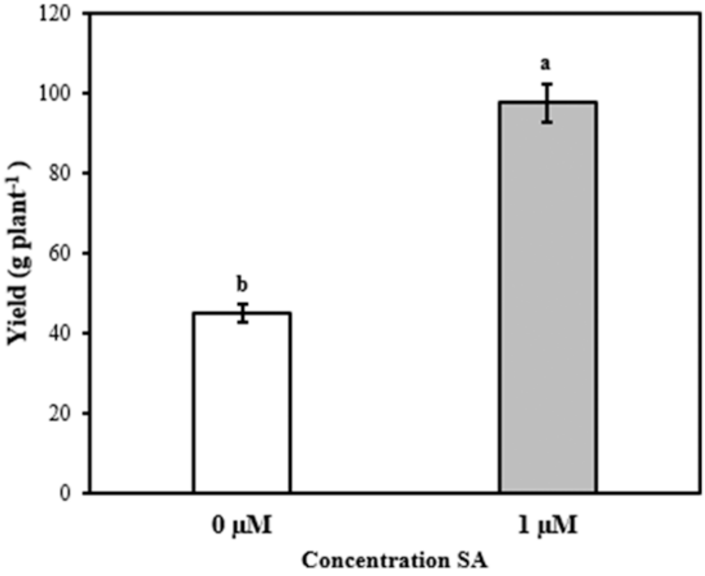

Fig. 2.7 Effect of 1µm of salicylic acid, sprayed in the canopy of seedlings, on the yield per plant (218 days after the last application) on habanero chili. Bars with the same letter are not significantly different Tukey's test at a p. ≤ 0.05. Each value is the average of 5 individuals

Table 2.6 Effect of salicylic acid on some parameters of cob and grain of maize (Xmejen-nal) in the seedling stage of two experiments

	Treatment	CL (cm)	CD (mm)	CW (g)	NG (#)	Gp (g plant^{-1})
Exp. 1	Control	13.0 b	36.4 b	79.4 b	240.0 b	57.5 b
	1µM	18.0 a	44.9 a	164.5 a	374.5 a	120.2 a
Exp. 2	Control	14.6 b	38.6 b	106.9 b	134.5 b	62. 6 b
	1µM	17.5 a	45.0 a	185.9 a	349.3 a	140. 1 a

CL cob length, *CD* cob diameter, *CW* cob weight, *NG* number of grains, *GP* Grain performance per cob. Means with the same letter in each column for each experiment are not significantly different (Tukey, ≤ 0.05)

2012). Moreover, the SA has a high potential in controlling post-harvest losses of horticultural crops (Asghari & Aghdam, 2010). Evidence of lower losses, greater firmness and color, reduction in ethylene production and reduced fungal damage, in strawberry (Babalar et al., 2007; Shafiee et al., 2010); or the decrease in internal darkening in pineapple (Lu et al., 2011), in post-harvest work, record the participation of SA in a longer shelf life.

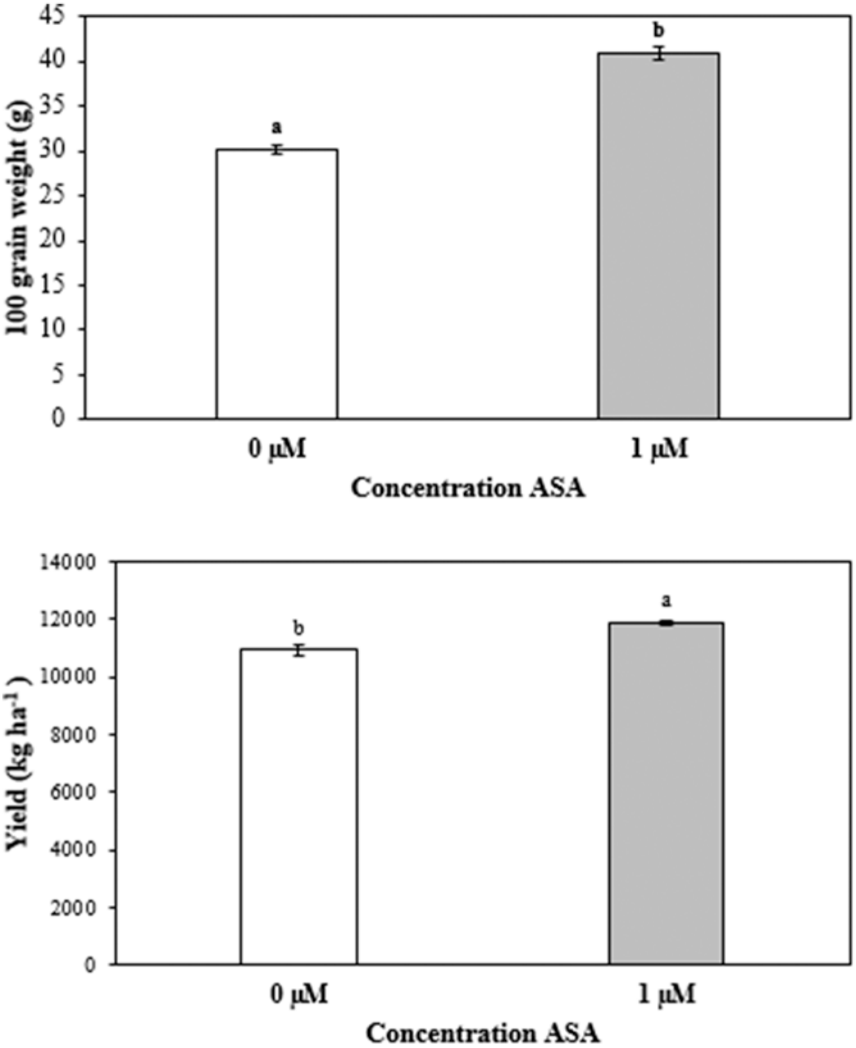

Fig. 2.8 Effect of spraying in seedling stage different concentrations of aspirin on grain weight and yield kilograms per ha. Averages of n × 5± standard error are displayed; Identical letters on the bars indicate no significant difference (Tukey, α × 0.05)

7 Conclusion

The results have shown that 1µM of SA, sprayed to the canopy of seedlings, significantly increases the length and dry weight of the root, the height and dry biomass of the plant and the yield in these species, as well as the levels of nitrogen (N), phosphorus (P) and potassium (K) in different organs of the plants at the time of harvest. Magnesium (Mg) copper (Cu), zinc (Zn), manganese (Mn) and boron (B) also increased in most tissues by the SA effect. It is proposed that the positive effect of the SA of increasing the size of the roots favors the absorption and accumulation of macro and micro nutriments and contributes to the production of seeds and fruits.

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Chapter 3

The Role of Salicylic Acid in Plant Reproductive Development

Aslıhan Çetinbaş-Genç and Filiz Vardar

Abstract Salicylic acid (SA) is an essential phytohormone that regulates plant growth, development, and defense during stress conditions. SA, in low amounts, participates in the coordination of physiological processes such as closure of stomata, uptake of nutrients, synthesis of chlorophyll pigments, protein synthesis, homeostasis of phytohormones, transpiration and photosynthesis in plants. It also plays an important signaling molecule in local and systemic disease resistance response of plants after pathogen attack. Similarly, SA-dependent signaling pathways regulate plant responses to abiotic stress factors altering antioxidant enzyme activities. Besides the vegetative development, SA induces flowering by increasing cell metabolic rate, increasing flower life, and retarding senescence. Although there are several studies on the effects of SA during vegetative development, few studies on the relation in reproductive organ development is available. Some of them concerns pollen viability and pollen tube growth. In a case study, different concentrations of SA (0.005, 0.025, 0.05 and 0.25 mM) improved the pollen germination in kiwifruit (*Actinidia deliciosa*). In recent years SA has been the focus of intensive research due to its physiological functions during growth-development and stress defense. The discovery of its targets and the understanding of its molecular mechanisms in developmental and physiological processes could help to clear the complex SA signaling network, confirming its critical role in plant growth, development, and defense. In the present chapter, we aimed to focus the role of SA during plant reproductive development under regular and/or stress conditions.

Keywords Anther · Flowering · Germination · Pistil · Pollen

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S. Hayat et al. (eds.), *Salicylic Acid - A Versatile Plant Growth Regulator*,
https://doi.org/10.1007/978-3-030-79229-9_3

1 Introduction

Salicylic acid (SA; 2-hydroxybenzoic acid) is a phenolic compound. It exists naturally in plants as a hormone-like substance and plays an important role for the regulation of plant growth and development at low concentrations (Guo et al., 2007; Rivas-san & Plasencia, 2011; Feng et al., 2018). It has been also known that SA has an important role in improving the plant defense system during biotic and abiotic stress conditions (Tateda et al., 2014; Trost et al., 2014).

SA triggers local and systemic resistance response in plants after pathogen attack (Zhou et al., 2009). In the presence of pathogens (fungi, fungal-like organisms, bacteria, phytoplasmas, viruses, viroids, nematodes and parasitic plants), plant recognize the invading pathogen and govern by direct or indirect interaction between the products of plant resistance genes (*R*) and pathogen avirulence genes (*Avr*) (Keen, 1990; Kachroo et al., 2003). One of the common responses to pathogens is the formation of necrotic lesions at the infection sites known as hypersensitive response (HR) (Greenberg et al., 1994; Dangl et al., 1996). HR limits spreading of the pathogen to the adjacent cells. It has been realized that concurrent with HR development, SA levels increase in the inoculated leaves (Hammond-Kosack & Jones, 1996; Yang et al., 1997; Kachroo et al., 2003). Subsequent to these events, the uninoculated parts of the plant develops a long-lasting and broad-based resistance to pathogen infection. This reaction known as systemic acquired resistance (SAR). It has been known that endogenous SA increases, and it is one of the key signaling regulators required for activation of *PR* (pathogen related) gene expression and resistance to certain pathogens (Dempsey et al., 1999). It has been reported that plants unable to synthesize enough SA, fail to express *PR* genes and become hyper-susceptible to pathogen infection. Studies on various SA accumulating and non-accumulating *Arabidopsis* mutants have confirmed a correlation between SA levels and disease resistance (Weymann et al., 1995; Ryals et al., 1996; Bowling et al., 1997; Clarke et al., 1998; Rate et al., 1999; Shah et al., 1999, 2001; Shirani et al., 2012). Researchers also suggest that programmed cell death (PCD) has a close relationship with the accumulation of SA. It controls the timing and degree of PCD during HR (Greenberg et al. 1994; Alvarez, 2000; Kovacs et al., 2016; Liu et al., 2019).

SA also have an important alleviating role in abiotic stress, including drought, salt, UV, low-high temperature, and heavy metal (Guo et al., 2007; Hua et al., 2008; Bideshki & Arvin, 2010; Azooz & Youssef, 2010; Liu et al., 2012; Khan et al., 2013; Wang et al., 2016; Yalçın & Vardar, 2016; Feng et al., 2018). SA enhances plant capacity and resistance to different stresses like a defensive compound such as betaine, glycine, and proline (Sakhabutdinova et al., 2003; Kolupaev et al., 2011). SA also stimulates the antioxidant enzymes which are scavengers of free radicals (Sağlam et al., 2011; Al-Rawi et al., 2018).

Beside the alleviation of stress conditions, both endogenous and exogenous SA also ameliorates the growth and development of plants under normal conditions. It ameliorates and regulates seed germination, crop/fruit yield, enzyme activities, ion uptake and transport, glycolysis, water relations, stomatal regulation, and phytohormone synthesis (Khan et al., 2003; Noreen et al., 2009; Zamaninejad et al., 2013; Yanik et al., 2018). It has been claimed that plant metabolism is regulated and protected by SA changing the effects of ethylene, abscisic acid, gibberellic acid and cytokinins (Canakci, 2011). Several researchers revealed that effects of exogenously applied SA differ due to the species, developmental stage, tissue/organ, the type of application and the concentration of SA (Shirani et al., 2012; Zamaninejad et al., 2013).

Greenhouse management practices attract attention and agronomic techniques to improve the quality of crops are widely used recently (Scarano et al., 2018; Preciado-Rangel et al., 2019). Growth and development are promoted by a variety of compounds that are exogenously applied. One of the widely used compounds is SA as a growth promoting signal molecule (Chen et al., 2009). SA prolongs fruit life and increases the biosynthesis of seconder metabolites (Lee et al., 2010; Fugate et al., 2013; Flores-Lopez et al., 2016).

Whereas there are several researches concerning the role of SA during vegetative development and ameliorating effects of SA during stress conditions, a few studies are subjected the role of SA during reproductive development. Among these the best known is the ameliorating effect of SA during the flowering.

2 Effect of SA on Flowering

SA is suggested in connection with plant flowering (Angiosperms) as an endogenous signaling molecule. Whereas the effects of SA in flowering were demonstrated in several plant species, the molecular mechanism is still unclear (Hayat et al., 2007; Pacheco et al., 2013; Liu et al., 2019). Lee and Skoog (1965) first concerned that 4 μM SA stimulated flower bud formation in tobacco callus. Afterwards, Khurana and Cleland (1992) reported that 3–10 μM SA promoted flowering in several members of Lemnaceae family. Further researches revealed that endogenous SA levels were very high during initiation or transition to flowering in non-thermogenic plants, and in inflorescence of thermogenic plants (Raskin et al., 1990; Aberau & Munné-Bosch, 2009; Vicente & Plasencia, 2011). Besides, Bayat et al. (2012) reported that exogenous application of SA (1, 2 mM) provided an early flowering and high number of floral buds per plant in *Calendula officinalis*. Moreover, SA-deficient *Arabidopsis* plants represents late flowering suggesting an interaction of SA with photoperiod and autonomous pathway (Martinéz et al., 2004). *LD, FVE* and *FCA* genes are the regulators of autonomous pathway and SA stimulated transition of flowering depending on these genes. It has been reported that exogenously applied 100 μM SA alleviated the delayed flowering in *ld-1, fve-3* and *fca-9* mutants (Vicente & Plasencia, 2011).

Genetic analyses revealed that SA have effects on expressions of some genes related to flowering. Shi et al. (2013) indicated that SA controls early flower development by *NONEXPRESSOR OF PR3 (NPR3)* gene in *Arabidopsis*. Besides

Yamada and Takeno (2014) reported that SA induces the expression of *FLOWERING LOCUS T 2 (FT2)* gene during the regulation of the flowering in *Pharbitis nil* (Japanese morning glory). Recent discoveries also demonstrated that SA is involved in regulating transcription of *CONSTANS* (*CO*-regulator of photoperiod pathway), *FLOWERING LOCUS C* (*FLC*-flowering repressor integrating autonomous and vernalization pathway) and a small number of integrators (*FLOWERING LOCUS T, SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1*) (Martinéz et al., 2004; Vicente & Plasencia, 2011).

Furthermore, there are some studies concerning the relation of SA and female flower development. Liu et al. (2019) studied female flower development along with stamen abortion in tung tree (*Vernicia fordii*), which is a monoecious and diclinous species with male and female flowers on the same inflorescence. Their cytological results suggested that the abortion of stamens in female flowers abort with PCD which was caused by tapetum degeneration before meiosis occurs. Analyses of phytohormone levels and co-expression networks also suggested that SA accumulation could trigger PCD and inhibit the development of stamens in female flowers in tung tree. Similarly, Xu et al. (2020) investigated flowers of *Litsea cubeba* (Lour.) Pers. which is a dioecious plant with degenerative reproductive organs in both male and female individuals. According to HPLC-MS/MS analysis, the researchers revealed an increase in SA content and a decrease in gibberellin content in the abortion processes in female flowers during pre-meiosis in female flowers of *L. cubeba*. These studies provide new insights into the role of SA in regulating the abortion of stamens in female flowers; whether this process does not work sterile ovules will be produced. Yet, the mechanism of degeneration in the organs and SA effect during development of male and female flowers is poorly understood.

Whereas there are several genetic approaches concerning flowering and SA relation, more researches are needed to explain the signaling pathway, interaction of SA with other plant growth regulators. Besides, the action mechanism of SA should be clarified during formation of male or female flower formation.

3 Effects of SA on Anther Development

One of the essential steps of the sexual reproduction of plants is the production of viable pollen in anther lobes. The sporophytic layer which gives pollen grains after meiosis is coated by four layers: the tapetum, the middle layer, the endothecium, and the epidermis from interior to exterior. The tapetum plays a crucial role for the development of microspores to mature and fertile pollen grains (Goldberg & Sanders, 1993; Ma et al., 2005; Vardar & Ünal, 2012). It has been widely known that a mutation or defect in tapetal cells cause inviable or infertile pollen production (Niu et al., 2013; Vardar & Ünal, 2012). After anther dehiscence and pollination, a fertile pollen grain germinates on stigma and elongates the pollen tube containing sperm cells to the ovule via style.

As our knowledge, there are rare studies concerning direct relation with SA and pollen maturation or pollen tube growth. It has been suggested that SA can induce mRNA accumulation of callose synthase 5 (Gsl5) for deposition of callose in microspore tetrads (Ostergaard et al., 2002). Shivashankara et al. (2019) compared pollen contents of three mango cultivars (Amrapali, Alphonso and Totapuri). In three cultivars the pollen grains were rich for free sugars and amino acids. However, phytohormones like IAA, IBA, ABA, GA, zeatin, jasmonic acid and SA were significantly different in low fruit setting cultivars (Alphonso and Totapuri) compared to high fruit setting cultivar (Amrapali). Pollen viability percentage was significantly higher in Amrapali (high fruit setting cultivar) which may be related to higher SA concentration.

As a case study we aimed to evaluate the effect of SA treatment on pollen performance of kiwifruit (*Actinidia deliciosa*). Pollen grains were collected from male flowers and germinated for 6 h at room temperature. Brewbaker and Kwack medium containing 12% sucrose was used for germination assays and medium was supplemented by different concentration of SA (0.005 mM, 0.025 mM, 0.05 mM or 0.25 mM). SA-free medium was used for control treatment. According to results, SA at all concentrations alleviated the pollen germination. Also, 0.005 mM, 0.025 mM, and 0.05 mM SA increased the pollen tube length, while 0.25 mM SA did not create a significant change. The most effective concentration on pollen germination and tube elongation was found to be 0.025 mM and 0.05 mM SA. Pollen tube abnormality rate increased concomitant with the increased SA concentration (Table 3.1). Excessive swelling in the tube tips was evident, especially in 0.05 mM and 0.25 mM. Also, SA treatments affected tip localized callose. Intense callose accumulation on the tube tip indicating the decrease of pollen performance was quite evident, especially in 0.05 mM and 0.25 mM (Fig. 3.1). Although the most effective concentration on pollen germination and tube elongation was found to be 0.025 mM and 0.05 mM SA, high tube abnormality rate, and intense callose accumulation at tube tip observed in 0.05 mM. Therefore, the best concentration that increases the pollen performance was found to be 0.025 mM.

Rong et al. (2016) revealed that the inter-convertible SA and methylated SA regulate *Arabidopsis* pollen tip growth independent of known NPR3/NPR4 SA receptor-mediated signaling pathways. These findings suggest that SA as a signal molecule can regulate pollen tube growth.

Plants are particularly more sensitive to abiotic stress during the meiosis and young microspore stage which result in reduced pollen fertility (Parish et al., 2012;

Table 3.1 The effect of SA treatment on pollen performance of kiwifruit

	Control	0.005 mM SA	0.025 mM SA	0.05 mM SA	0.25 mM SA
Germination (%)	38.94 ±2.06^a	54.65 ±2.20^b	58.93 ±6.73^c	59.14 ±4.89^c	45.63 ±8.79^b
Tube length (µm)	83.83 ±56.85^a	115.49 ±64.53^b	126.19 ±56.46^b	120.36 ±70.94^b	87.77 ±58.03^a
Abnormality rate (%)	3.33 ±0.94^a	5.33 ±0.94^a	7.33 ±0.94^a	12.66 ±2.49^b	15.33 ±2.49^b

Different letters in table indicate significant differences ($P < 0.05$)

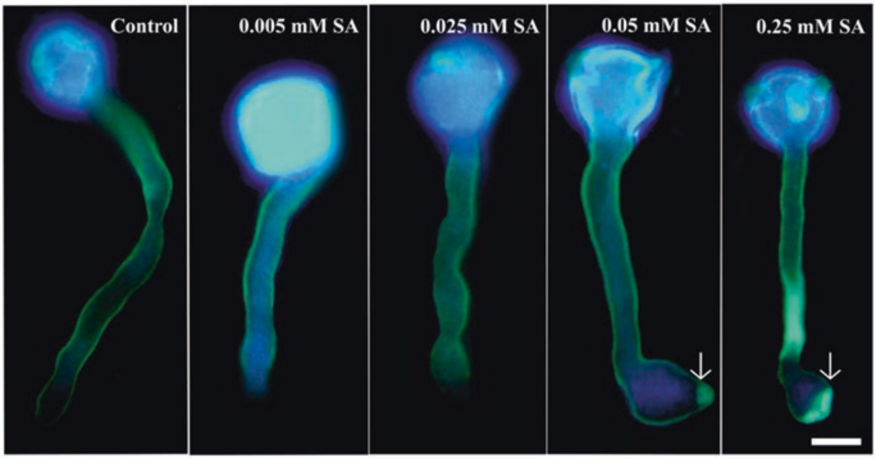

Fig. 3.1 The effect of SA treatment on callose accumulation. Arrow indicates callose in the tips of pollen tubes. Bar: 10 µm

Sage et al., 2015; Smith & Zhao, 2016). It has been known that extremely high temperatures are becoming an increasingly severe to plants and SA can enhance the stress tolerance. Feng et al. (2018) treated a susceptible rice cultivar (Changyou1) at the pollen mother cell (PMC) stage with SA and then subjected to heat stress. According to their results SA reduced the accumulation of reactive oxygen species in anthers to prevent tapetal PCD and degradation giving rise to higher pollen viability. Researchers concluded that SA could protect plants from heat stress by alleviating the damage during the floret differentiation stage of rice (Fu et al., 2015; Zhang et al., 2017; Feng et al., 2018). Moreover, it can be suggested that SA is involved in pollen development, reduction of pollen sterility and pollen tip growth (Rong et al., 2016; Zhao et al., 2018). However more detailed researches are needed.

4 Effect of SA on Pistil Development

The female organ gynoecium has one or more pistils. Pistil located in the center of a flower and consists of stigma, style, and ovary. The ovary carries one or more ovules. Ovules have a crucial role during the plant life cycle in seed plants (Gymnosperm and Angiosperms) and contain female gametophyte (embryo sac). After fertilization ovule develops into seeds which constitute a new sporophyte. Seeds contain embryo and endosperm and are also of high economic importance for human and animal nutrition. Thereafter mature ovule generates seed in the fruit. Therefore, understanding the molecular mechanisms that control ovule initiation and development especially by plant growth regulators not only for scientific aspects but also for an agricultural and economic point of view (Khan et al., 2019).

Barro-Trastoy et al. (2020) summarized the current knowledge of the hormonal regulation of ovule development. According to the review auxins promote ovule primordia initiation from placenta and regulate the spacing between integuments. Cytokinins regulate ovule number positively. Proper ovule pattern establishment is regulated by auxins and cytokinins. Brassinosteriods also regulate ovule number positively and outer integument growth. However gibberellic acids regulate ovule number negatively and proper integument development (Bencivenga et al., 2012; Huang et al., 2013; Galbiati et al., 2013; Larsson et al., 2017; Cucinotta et al., 2018; Gomez et al., 2018; Jia et al., 2020). The hormonal regulation of ovule development is clarified in detail, however there is no evidence that SA has negative or positive effect on ovule development.

Moreover, several researchers reported that exogenously applied SA increased the growth, yield and nutraceutical quality in seeds and fruit. Al-Rawi et al. (2018) reported that 37.5, 75, 112.5 mg/L SA increased the protein content and grain yield of wheat seeds dose dependently. Similarly, Zamaninejad et al. (2013) alleviated the drought stress with SA spraying and revealed increase in corn yield. Preciado-Rangel et al. (2019) also advised to use average concentration (0.15 mM) of SA to obtain a higher content of bioactive compounds without affecting the yield and commercial quality of cucumber fruits. Baba et al. (2017) also revealed that exogenous usage of SA increased floral growth characters, fruiting, and yield of strawberry dose dependently.

5 Conclusion

The data concerning the role of SA in flower development in plants is insufficient. New discoveries to understand the effects and molecular mechanism of SA in reproductive organ development under regular and stress conditions are needed.

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Chapter 4
Role of Salicylic Acid in Pre- and Post-Harvest Attributes in Horticulture

Barket Ali

Abstract Salicylic acid (SA) is the seventh class of phytohormones after the worldwide acceptance of brassinosteroids as the sixth and five other classical ones. It is a beta hydroxy phenolic acid and is represented by the analogues; salicylic acid, acetyl salicylic acid and methyl salicylic acid. It is basically a defence related hormone and is primarily responsible for the development of systemic resistance against pathogens and tolerance to abiotic stress in plants. Under normal growth conditions, SA regulates several physiological responses such as stomatal movements, pigment accumulation, photosynthesis, ethylene biosynthesis, secondary metabolite production, heat production, enzyme activities, abscission reversal, nutrient uptake, flower induction and overall growth and development of the plant. Exogenous application of SA also affects the growth, physiology, yield, and quality of the produce in several fruit crops, grown under different environmental conditions. Besides this, SA also affects the post-harvest attributes of the fruits; maintenance of quality, prevention of fruit decay and pathogen attack, and increase in the shelf life of the fruit. All this information is comprehensively presented in this chapter, besides an outline of the scheme of SA biosynthesis.

Keywords Acetyl salicylic acid · Fruit yield · Fruit quality · Horticulture · Salicylic acid

1 Introduction

The word salicylic acid (SA) is derived from Latin *Salix*, which means “a willow tree”. Although the use of willow bark (as a source of SA) was practiced for the cure of fever, aches and pain relief, since centuries ago. It was Johann Buchner who for the first time isolated a salicyl alcohol glucoside called salcin, in the

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S. Hayat et al. (eds.), *Salicylic Acid - A Versatile Plant Growth Regulator*,
https://doi.org/10.1007/978-3-030-79229-9_4

year 1828. Another derivative namely acetyl salicylic acid is also available at commercial level under the name "Aspirin" since 1874 (Weissmann, 1991). SA and the related derivatives were exclusively used for pharmaceutical purposes. Presently, SA and other salicylates are extracted from various plant species and are represented by some active analogues or derivatives; salicylic acid, acetyl salicylic acid, methyl salicylic acid and dihydroxy benzoic acid (Sahu, 2013; Fig. 4.1).

In plants, aspirin was successfully used against tobacco mosaic virus and SA derivatives were recognised for their defensive responses in plants (White, 1979). The systemic induced resistance has remained the basic role and identity of SA till present time (Jin et al., 2017). Besides a key role in defence responses, SA and related derivatives also play important role in the regulation of various physiological and developmental processes in plants, growing under normal and stressful environmental conditions (Sahu, 2013). The physiological processes affected by SA are seed germination, stomatal movements, pigment accumulation, photosynthesis, ethylene biosynthesis, heat production, enzyme activities, abscission reversal, nutrient uptake, flower induction, membrane functions, legume nodulation and overall plant growth and development (Hayat et al., 2007; Sahu, 2013). Moreover, they also play an important role in the elicitation of secondary metabolite production in plants (Ali, 2021). SA also protects the plants from different stress conditions such as salt stress (Yusuf et al., 2008), heavy metal stress (Ali, 2017; Hayat et al., 2010), drought stress (Sedaghata et al., 2020), ultraviolet radiation, ozone and temperature stresses (Khan et al., 2015). In addition to this, SA also favours the yield of various agricultural crops (Hayat et al., 2005, 2007). Moreover, SA and related derivatives also show a substantial impact on the growth and development, flowering, fruiting, and fruit quantity and quality of different crops of horticultural importance. All these studies are compiled here to present the detailed information regarding the role of SA in the yield and quality attributes in different fruit crops.

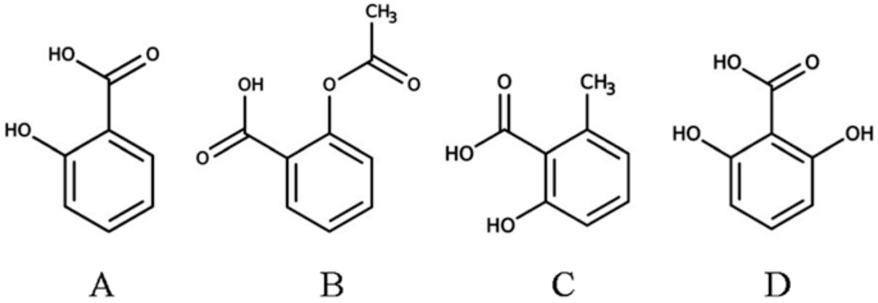

Fig. 4.1 Chemical structure of salicylic acid derivatives; (**a**) Salicylic acid, (**b**) Acetyl salicylic acid, (**c**) Methyl salicylic acid and (**d**) Dihydroxy benzoic acid

2 Outline Biosynthesis and Transport

In plants, there are two different pathways of SA biosynthesis from phenylalanine precursor. (a) Enzyme phenylalanine ammonia-lyase converts phenylalanine to cinnamic acid, which is hydroxylated to ortho-coumaric acid followed by oxidation of side chain to convert into SA. (b) Cinnamic acid formed by the catalytic action of the enzyme phenylalanine ammonia-lyase (PAL), followed by its oxidation to give rise benzoic acid. In the next step, benzoic acid 2-hydroxylase enzyme catalyses the hydroxylation of benzoic acid at ortho-position to synthesise salicylic acid (Raskin, 1992, Hayat et al., 2007, Sahu, 2013; Fig. 4.2). However, according to Chen et al. (2009) SA biosynthesis is not as simple as previously thought. These workers suggested that it is synthesised from isochorismate, which is synthesised from chorismate in two steps catalysed by isochorismate synthase (ICS) and isochorismate pyruvate lyase (IPL), respectively. Two genes have been identified for ICS in *Arabidopsis*, but none for IPL which has made the pathway of biosynthesis quite ambiguous (Chen et al., 2009; Fig. 4.2).

SA and related analogues play a fundamental role in systemic acquired resistance (Tripathi et al., 2019). Therefore, their long distance transport is essential. It is well established that these signal molecules are transported in conjugate form and the conjugation with glucose and/or methyl groups takes place immediately after their synthesis (Sahu, 2013). The transport takes place through apoplast, which is accomplished by changes in pH gradient and SA deprotonation. However, in cuticle defective plants, SA en routes cuticle wax instead of apoplast, which is facilitated by increased transpiration and decreased water potential (Lim et al., 2020).

3 Role of Salicylic Acid in Horticulture

The productivity of a horticultural crop is determined by a large number of factors which include overall growth and development of the crop, flowering and attachment of the flower to the parent plant, fruit setting, fruit maturation and post-harvest storage. Salicylic acid has been found to affect these parameters favourably, besides preventing the post-harvest decay of the crop caused by various pathogens, thus retaining the post-harvest quality and increasing the shelf life of the fruit. The effect of SA on various attributes related to the horticulture is presented plant wise in the text below and Table 4.1.

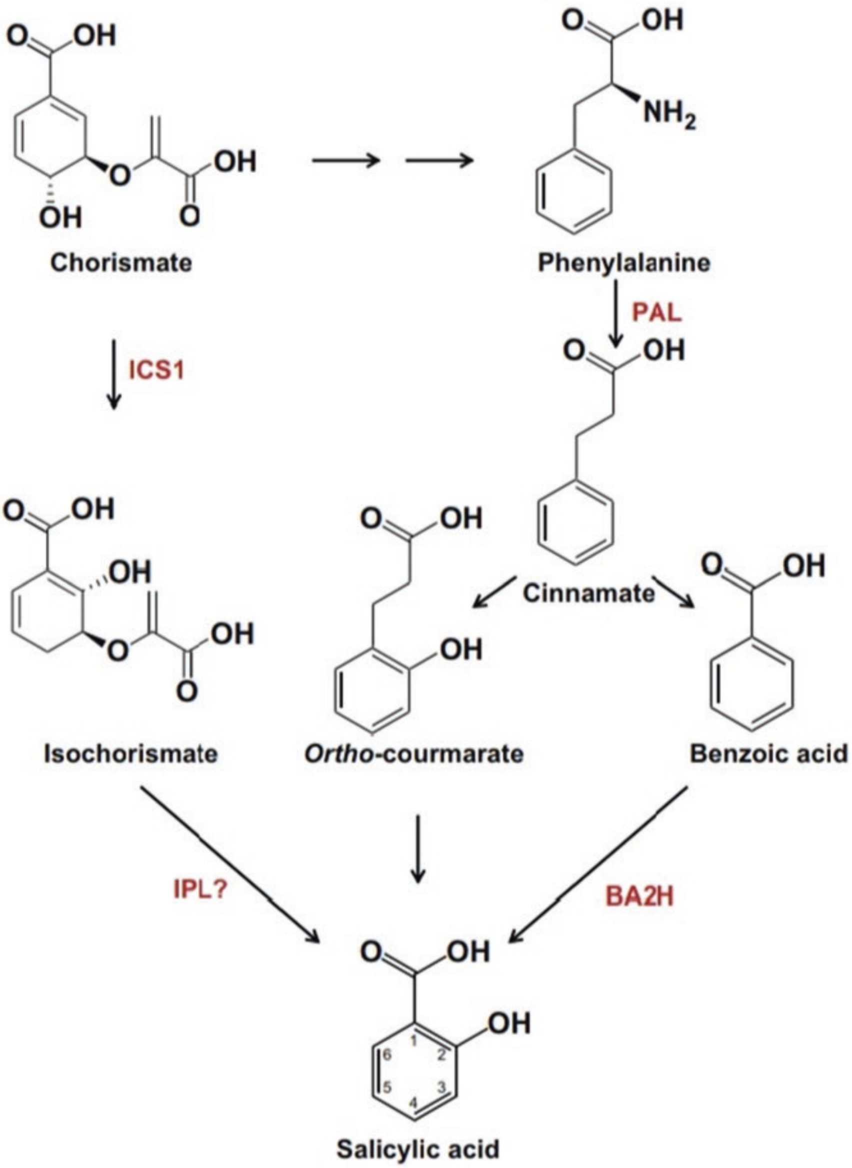

Fig. 4.2 An outline of the SA biosynthesis. (Adopted from Jin et al., 2017)

Table 4.1 Effect of salicylic acid on the pre- and post-harvest responses in some fruit crops

Plant species	SA dose	Response	References
Dragonfruit (*Hylocereus polyrhizus*)	0.1, 1.0, 2.0 and 5.0 mM	Enhanced antioxidant potential as well as prolonged the cold storage of the fruits and prevented the chilling injury.	Mustafa et al. (2018a)
Guava (*Psidium guajava*)	1 and 2 mM	Reduced weight loss and colour change, retained fruit firmness, total phenolics and vitamin-C content.	Madhav et al. (2018)
Jamun (*Syzygium cumini*)	1.0 or 1.5 mM	Preserved fruit quality, delayed senescence and retained the quality attributes; total anthocyanins, total phenolics, total flavonoids, total antioxidant capacity, and radical scavenging activity.	Saurabh et al. (2019)
Jujube (*Zizyphus jujube*)	2.0 and 4.0 mM	Extended post-harvest shelf life together with the maintenance of quality of the fruits such as fruit firmness, titratable acidity, total phenolic content, antioxidant activity, ascorbic acid and antioxidant potential.	Zeraatgar et al. (2018)
Jujube (*Zizyphus jujube*)	2.0 mM	Significantly enhanced the resistance to post-harvest diseases caused by *Monillinia fructicola* and *Alternaria alternata* and prevented the losses.	Cao et al. (2013)
Mangosteen (*Garcinia mangostana*)	0.1, 1.0, 2.0 and 5.0 mM	Delayed pericarp hardening and improved the quality attributes; phenolic content, polyphenol oxidase activity and antioxidant potential.	Mustafa et al. (2018)
Muskmelon (*Cucumis melo*)	0.1, 0.25, 0.50 or 1.0 mM (ASA)	Either of the mode of acetyl salicylic acid (ASA) application (pre-sowing seed soaking or foliar spray) improved the growth and protected the seedlings from the drought stress.	Korkmaz et al. (2007)
Saffron (*Crocus sativus*)	0.5, 1.0, and 2.0 mM	2.0 mM concentration induced maximum flowering and membrane stability index and reduced the electrolyte leakage. Corolla dry weight and stigma dry weight also responded favourably to the SA treatment.	Khayyat et al. (2018)
Papaya (*Carica papaya*)	1.0 and 2.0 mM	SA treatment enhanced the shelf life, antioxidant capacity and total phenol content in fruits. However, other chemicals (especially calcium chloride) used in the study were more effective than SA, in controlling the post-harvest disease incidence and the other quality attributes.	Lata et al. (2018)

(continued)

Table 4.1 (continued)

Plant species	SA dose	Response	References
Plum (*Prunus salicina*)	0.5, 1.0, 1.5, 2.0, 2.5 mM	Reduced the chilling injury of the fruits during cold storage that was accompanied with a decline in the respiration rate and ethylene production. Moreover, SA also declined the electrolyte leakage, malondialdehyde content, reduced the activities of polyphenol oxidase and peroxidase, and enhanced polyamine accumulation.	Luo et al. (2011)
Strawberry (*Fragaria* × *Ananassa*)	2 mM	Post-harvest SA treatment reduced weight loss and fruit decay and increased the fruit firmness together with the retention of fruit quality attributes.	Shafiee et al. (2010)
Watermelon (*Citrullus lanatus*)	0.5, 1.0, 2.0, 3.0 and 4.0 m mol/L	SA treatment enhanced the activities of antioxidant enzymes; guaiacol peroxidase, ascorbate peroxidase, catalase, superoxide dismutase and glutathione reductase. The enhanced antioxidant system conferred cold resistance. 1.0 m mol/L concentration was the most effective whereas the higher concentrations proved counter-productive.	Jing-Hua et al. (2008)
Wax apple (*Syzygium samarangense*)	0.5 and 1.0 mM	Pre-harvest application maintained the fruit firmness and enhanced the antioxidant capacity, free radical scavenging activity, total phenols and total flavonoid contents. However, weight loss, fruit colour other quality attributes remained unaffected.	Supapvanich et al. (2017)

3.1 Effect on Apple

The growth and yield of apple is adversely affected by several pathogens. However, exogenous application of SA has been found as a successful preventive measure to reduce the losses inflicted by these pathogens. The pathogens include *Venturia inaequalis*, *Botryosphaeria obtuse*, *Diplocarpon mali*, *Glomerella cingulata*, and *Penicillium expansum*. SA treatment enhanced the efficacy of *Cryptococcus laurentii* yeast in antagonising the blue mold disease caused by *Penicillium expansum*. However, SA alone treatment was effective *in vitro* rather than *in vivo*. The antagonistic response was concentration dependent, where 10 μg ml^{-1} was the most effective dose (Yu & Zheng, 2006). *Venturia inaequalis* and *Botryosphaeria obtuse* are the causal agents of apple scab and frog eye leaf spot or black rot diseases, respectively. Foliar application or trunk injection of salicylic acid (2-hydroxybenzoic acid) or its analogue, benzothidiazole or actigard, at tight cluster stage of flower bud development and at late pink bloom stage significantly reduced the infection caused by *Venturia inaequalis* and *Botryosphaeria obtuse* in Honeycrisp and Cortland cultivars (Abbasi et al., 2019). However, some studies suggested that apple response to scab is SA-independent. Here apple plants are proposed to adopt an alternate defence strategy mediated by strengthening of the cell wall integrity and the fungal degradation. In addition to the physical barriers, some chemicals e.g. phenols and depriving the pathogen of nutrients in resistant cultivars seems the additive strategy against *V. inaequalis* (Cova et al., 2017). In another study, the pre-treatment of Gala cultivar with SA (0.1 to 1.0 mM) induced the expression of five pathogenesis-related genes; *PR1*, *PR5*, *PR8*, *Chitinase* and *β-1,3-glucanase* and alo enhanced the activities of antioxidant and defence related enzymes. These elevated activities strongly induced the resistance against *Glomerella* leaf spot disease caused by *Glomerella cingulata*. The induced resistance was manifested in the reduction in lesion numbers and disease index (Zhang et al., 2016). Similarly, Sun et al. (2018) reported the elevation of endogenous levels of SA that was synchronous with the over-expression of *chitinase*, *β-1,3-glucanase* (SA-related marker gene) and *MdATG18a* gene. The enhanced expression of these genes induced the resistance to *Diplocarpon mali*, the causal agent of marssonina apple blotch disease, thus minimizing the impact of this disease on apple production.

3.2 Effect on Apricot

Immersing the fruits of apricot (*Prunus armeniaca*) in salicylic acid (1.0, 2.0, or 3.0 mM) for 10 min duration helped in maintaining the post-harvest quantitative and qualitative characteristics. Fruit treatment with 3.0 mM dose stimulated the highest accumulation of phenolics. Moreover, SA treatment also slowed down the ripening, softening and senescence processes, thus prolonged the shelf-life as well as retained the valuable marketing characteristics of the fruits (Hajilou & Fakhimrezaei, 2013). Similarly, treatment of the fruits with SA under cold storage decreased the chilling injury, fruit decay, and electrolyte leakage and enhanced the phenolic and carotenoid contents together with the retention of antioxidant capacity and ascorbic acid concentration (Ezzat et al., 2017). The application of SA to the *Prunus armeniaca* also delayed the fruit softening, colouring, decay, and chilling injury, thus increasing the post-harvest shelf life of the fruits. SA also improved the quality characteristics; phenolics and antioxidants. Moreover, the response generated by SA was more pronounced when applied in combination with chitosan oligochitosan (Cui et al., 2020).

3.3 Effect on Banana

In banana (*Musa* sp.), post-harvest dipping in SA (1 and 2 mM) decreased the weight loss and total soluble solids and retained the firmness and green colour leading to an increase in the post-harvest storage shelf life. The higher dose (2 mM) was more effective. However, an inconsistency was noticed regarding the total phenols and flavonoids concentrations in peel and pulp and vitamin C in pulp. Moreover, SA generated a better response when given in combination with gum Arabic (Alali et al., 2018). A similar pattern of response was recorded by Khademi et al. (2019) when post-harvest banana were exposed to SA and/or ultrasound. The combined treatment favoured the retention of the phenol content, antioxidant capacity and prevented the chilling injury and electrolyte leakage, prolonging the cold storage shelf life of the fruits. In another study, SA-induced chilling tolerance was attributed to the accumulation of disaccharides, unsaturated fatty acids and amino acids such as proline (Chen et al., 2020a).

3.4 Effect on Cherry

Treatment of Cristalina and Prime Giant cultivars of sweet cherry with 1 mM of salicylic acid, acetyl salicylic acid or oxalic acid at commercial ripening stage delayed the post-harvest ripening process and maintained the firmness. The treatments also retained the quality characters, total phenolics, anthocyanins and antioxidants under cold storage (Valero et al., 2011). SA treatment also favoured the post-harvest antioxidant potential of cornelian cherry fruits. The treated fruits also possessed the elevated concentrations of total phenols, flavonoids, anthocyanins and ascorbic acid, and enhanced activity of phenylalanine ammonia-lyase (Dokhanieha et al., 2013).

3.5 Effect on Citrus

Citrus sp. affected by the exogenous application of SA include lemon, kinnow and orange. In *Citrus limon* exogenous application of SA, in combination with methyl jasmonates enhanced the tolerance to the chilling injury mediated via the increase in phenylalanine ammonia-lyase activity and the subsequent accumulation of phenolics as well as a decrease in the peroxidase activity (Siboza et al., 2014). SA treatment also decreased the level of reactive oxygen species and enhanced the activities of enzymes associated with antioxidant system, such as catalase, ascorbate peroxidase, and glutathione reductase, as well as heat shock proteins in lemon flavedo, thereby increasing the tolerance to post harvest storage chilling injury (Siboza et al., 2017). In addition to protection against chilling injury, SA facilitated the citrus canker disease management that was evident from the reduced lesion number per leaf and disease severity, in Mexican lime (*Citrus aurantifolia*). However, SA treatment generated a better response when given in combination with *Pseudomonas fluorescens* (Al-Saleh et al., 2016). Similarly, in Kinnow mandarin (*Citrus nobilis*), SA application prevented the weight loss and spoilage, retained the firmness and juice content and other quality attributes; soluble solids, titratable acidity, pectin, total carotenoids and ascorbic acid contents, thus helped the extension of cold storage life together with the maintenance of quality of the fruits (Baswal et al., 2020). Moreover, treatment of the fruit also prevented the kinnow mandarin from fungal infection, in addition to the retention of antioxidant capacity and phenolic content as well as minimising the fruit decay (Haider et al., 2020).

Post-harvest treatment of Cara cara cultivar of navel orange (*Citrus sinensis*) with SA retained the antioxidant capacity; superoxide dismutase, catalase, glutathione reductase, dehydroascorbate reductase, and ascorbate peroxidase as well as non-enzyme components such as ascorbate, dehydroascorbate, glutathione and oxidized glutathione. SA treatment also reduced the lipid peroxidation and favoured the fruit storage (Huang et al., 2008). Similarly, fruit immersion in SA enhanced the resistance against chilling injury in navel orange together with the maintenance of the quality attributes when given alone or in combination with *Aloe vera* gel (Rasouli et al., 2019). SA also enhanced the activities of the enzymes related to phenylpropanoid pathway resulting in the accumulation of phenolic acids and their subsequent metabolite lignin that facilitated the resistance against blue and green molds, which was evident from decrease in disease incidence and lesion diameter (Zhou et al., 2018). Action of SA against devastating fungal pathogen *Penicillium digitatum* that causes green mould disease of blood orange was also demonstrated. Here SA treatment increased the shelf life of the fruits and prevented the spoilage as well as retained the firmness and other quality attributes of the fruits (Aminifard et al., 2013). Wang and Liu (2012) reported an increased resistance against citrus canker caused by *Xanthomonas axonopodis* in navel orange that was mediated via the increase in the endogenous free and bound SA levels as well as the enhanced expression of some pathogenesis related genes. In satsuma mandarin (*Citrus unshiu*) SA also enhanced the contents of H_2O_2 and some defense-related metabolites, such as ornithine and threonine resulting in the prevention of post-harvest decay of the fruits and retention of the quality attributes (Zhu et al., 2016).

3.6 Effect on Grapes

Pre-harvest spray of ‘El-Bayadi’ table grapes with SA and GA improved the weight and length of the clusters, berry firmness and also enhanced the quality attributes; titratable acidity, vitamin C, mineral concentration, and antioxidant capacity. However, GA treatment generated a response better than SA (Alrashdi et al., 2017). Post-harvest application of SA to the grape berries enhanced the expression of phenylalanine ammonia-lyase and subsequently the accumulation of phenylpropanoids (Chen et al., 2006). Activation of phenylalanine ammonia-lyase and the accumulation of phenolics also occurred under high temperature in the berries (Wen et al., 2008). Similarly, exogenous SA treatment also induced the expression of *Vitis vinifera C-repeat binding factor 4* (*VvCBF4*) genes, enhancing the tolerance to the cold stress (Aazami & Mahna, 2017). SA treatment also favoured the post-harvest attributes of ‘Superior seedless’ cultivar. SA reduced weight loss, enhanced berry firmness, separation force as well as total phenolic content, maintaining the quality attributes during the storage period (Léay, 2017). Likewise, the post-harvest treatment of ‘Bidaneh Ghermez’ cultivar improved the berry appearance as well as retained the fruit quality and prevented the fruit deterioration (Ranjbaran et al., 2011). Moreover, in combination with chitosan, SA treatment effectively controlled the green mold caused by *Penicillium digitatum* that was evident from the reduced lesion diameter and disease incidence. The treatment also retained the quality attributes together with the activation of enzymes associated with phenolic biosynthesis and their subsequent accumulation leading to increase in storage shelf life of the fruit (Shi et al., 2018). In addition to the stimulation of phenolic accumulation, disease control and retention of quality attributes, SA also enhanced the essential oil concentration as well as its composition in the young shoots and the berry peels. Young shoots exhibited sabinene, limonene and terpinen-4-ol as the major constituents of the oil. Moreover, limonene, β-myrcene and octanal were the major compounds in peel oil (Khalid et al., 2018).

3.7 Effect on Litchi and Longan

Salicylic acid in combination with chitosan reduced the weight loss, pericarp browning and decay loss, and also retained the fruit quality; concentrations of anthocyanins, phenolics, flavonoids, ascorbic acid as well as antioxidant capacity in litchi (*Litchi chinensis*) (Kumari et al., 2015). Likewise, dipping of longan (*Dimocarpus longan*) fruits in SA and subsequent exposure to UV-C also reduced the skin browning as well as retained the free radical scavenging activity under cold storage (Promyou & Supapvanich, 2020). In addition to the maintenance of the fruit quality under cold storage, SA also countered the disease incidence by *Phomopsis longanae* Chi, mediated via maintaining the energy level as well as decreasing the respiration in longan (Chen et al., 2020b).

3.8 Effect on Mango

Supplementation of SA with waste water favoured the growth, yield and fruit quality of two cultvars ‘Keitt’ and ‘Ewais’ of mango (*Mangifera indica*). Moreover, the treatment prevented the malformation, inhibited the pre-mature fruit abscission and maintained the antioxidant potential of the fruits (Helaly et al., 2018). Pre-treatment of the fruits with SA differentially regulated the expression of some important genes associated with fruit ripening, affecting the quality attributes of the fruits. SA treatment maintained the fruit firmness, total soluble solids, titratable acidity, and vitamin C as well as appropriately regulated the level of ethylene (Hong et al., 2014). Lokesh et al. (2020) also recorded an increase in the shelf life, marketable fruits percentage, firmness and reduction in the weight loss during storage in response to the foliar treatment of SA in Alphonso cultivar.

3.9 Effect on Peach

Exogenous application of SA to the Elberta cultivar of peach (*Prunus persica*), at swollen bud stage significantly favoured the reproductive attributes; per cent flowering, fruit weight and yield (Mohamadi & Pakkish, 2014). Foliar treatment of SA in combination with some minerals; calcium, potassium, magnesium and boron improved the overall tree health as well as fruit yield of Sandvliet cultivar (Hendricks et al., 2015). Similarly, SA also interacted synergistically with aminoethoxyvinylglycine and nitric oxide in 'Oz Delight' cultivar to manipulate the ethylene production and respiration rate, which favourably affected the fruit quality and reduced the fruit softening, mediated via the decrease in the oxidative stress. These manipulations ultimately favoured the post-harvest storage of the fruit (Tareen et al., 2017). Post-harvest dipping of the fruits of Beijing cultivar reduced the chilling injury, enhanced the fruit firmness, induced the accumulation of heat shock proteins, and retained other quality attributes including the antioxidant capacity of the fruits (Wang et al., 2006). A similar pattern of response was recorded in the Flordaking cultivar (Tareen et al., 2012). Besides reducing the flesh browning and increasing the shelf life of the fruits during cold storage, SA treatment also triggered the expression of the genes associated with volatile ester biosynthesis pathway. The elevated expression of these genes helped maintain the flavour quality during the cold storage (Yang et al., 2020). In addition to the maintenance of the quality attributes, endogenous and exogenous SA also decreases the disease incidence in peach. Application of SA to the foliage of Sandvliet cultivar controlled the *Xanthomonas* infection on both leaves and the fruit. However, the SA treatment showed more efficiency when applied in combination with the Ca, Mg and B supplementations (Hendricks et al., 2015). Likewise, *Pichia guilliermondii*-induced accumulation of endogenous SA decreased infection caused by *Rhizopus stolonifer* and *Penicillium expansum*. The infection was controlled via the increase in the activities of SA synthase and defence-related enzymes as well as the expression of pathogenesis-related genes (Zhao et al., 2020).

3.10 Effect on Pineapple

Post-harvest dipping of pineapple (*Ananas comosus*) in varied concentrations of SA decreased the internal browning and increased the shelf life of the fruits together with the retention of the quality attributes. The extension of the shelf life was accompanied with a reduction in the activities of enzymes, peroxidase, polyphenol oxidase, and phenylalanine ammonia-lyase. However, the activities of superoxide dismutase and ascorbate peroxidase exhibited an increasing trend in response to the SA treatment (Lu et al., 2010). SA treatment through peduncle infiltration also generated a similar pattern of response. In addition to a decline in internal browning and other quality attributes, SA also decreased the lipid peroxidation and enhanced the antioxidant capacity of the fruits (Youryon et al., 2019).

3.11 Effect on Pomegranate

Treating the fruits of pomegranate (*Punica granatum*) by dipping in different concentrations of SA effectively reduced the chilling injury. The resistance to the chilling injury was accompanied with a decrease in the electrolyte leakage as well as decline in the degradation of ascorbic acid (Sayyari et al., 2009). Another analogue of SA namely acetyl salicylic acid also enhanced the resistance to chilling injury that was accompanied with the retention of nutritional quality, bioactive compounds as well as the total antioxidant capacity of the fruits (Sayyari et al., 2011).

3.12 Effect on Strawberry

Foliar treatment of strawberry (*Fragaria* × *ananassa*) cv. Pajaro with SA in combination with nickel sulphate favoured the plant growth/weight as well as enhanced the fruit yield and fruit quality characters; concentration of anthocyanins, polyphenolics and vitamin C of the fruit (Jamali et al., 2013). SA also interacted synergistically with humic acid in Camarosa cultivar to enhance the yield, size and quality attributes (vitamin C, soluble solids, minerals and titratable acidity) of the fruits (Aghaeifard et al., 2016). Sabrosa cultivar also responded positively to the combined treatment of SA and methyl jasmonate. The treatments showed a favourable effect on the post-harvest shelf life of the fruits together with the retention of fruit quality and antioxidant capacity, under cold storage (Asghari & Hasanlooe, 2015). Pre-harvest application of SA also favoured the plant growth, fruit set and fruit yield in Chandler cultivar (Kumar & Kaur, 2019). In addition to a positive impact on the growth and productivity of different cultivars of strawberry, SA treatment also antagonises the deleterious impact of salinity on these parameters. Foliar spray of Camarosa cultivar subjected to salt stress exhibited an improvement in growth and attributes related to the photosynthesis that were reduced by the stress. However, the antioxidant system in response to the stress was further strengthened by the SA treatment (Faghih et al., 2017). Similarly, foliar application of Selva cultivar with SA enhanced the mineral uptake as well as vigour of the plants under salt stress (Jamali & Eshghi, 2015). Furthermore, SA treatment also invoked the resistance against *Podosphaera aphanis* fungus, the causal agent of powdery mildew. The disease resistance was mediated via the expression of a large number of genes including those associated with phenylpropanoid biosynthesis, plant hormone signal transduction pathways and pathogenesis. The expression of the genes enhanced the accumulation of proanthocyanidins and pathogenesis-related proteins, which helped in conferring the disease resistance in Benihoppe cultivar of octoploid strawberry (Feng et al., 2020).

4 Conclusion

Salicylic acid is a phytohormone primarily associated with the defence responses in plants against various pathogens as well as abiotic stress factors such as salinity, drought, temperature, UV radiations and heavy metals. SA also improves the plant growth and development under normal growth conditions. The improved plant growth and development, both under normal and stressful situations significantly favours the yield of different crops including the horticultural crops. The increase in the fruit yield is facilitated via the increased flowering, prevention of premature flower abscission and improvement in the overall physiology of the crop. SA also prevents the premature fruit fall and appropriately regulates the ethylene biosynthesis and fruit ripening, which is mediated via the expression of specific genes. In addition to these favourable responses SA also helps in the retention of post-harvest quantitative and qualitative attributes of the fruits. Post-harvest treatment of the fruits reduces the fruit softening, maintains the fruit firmness and fresh weight and retains the moisture, minerals, vitamins and antioxidant potential of the fruits. Different fruits crops are inflicted with huge losses by different pathogens. These pathogens are also countered efficiently by the post-harvest SA treatment, thus reducing the losses inflicted by fruit decay.

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Chapter 5
Foliar Applications of Salicylic Acid for Improving Crop Tolerance to Drought Stress: A Review

Christos A. Damalas and Spyridon D. Koutroubas

Abstract Salicylic acid (SA) is a multifaceted plant growth modulator that is reported to engage in plant reaction to stresses. Drought is a common environmental constraint of crop growth, influencing global agricultural productivity. Foliar applications of SA have been recorded to improve growth under drought stress in major crops such as cereals (wheat, barley, maize, and rice), oilseed crops (sunflower, safflower, sesame, and flax), legumes (beans, mungbeans, and crown vetch), vegetable crops (cucumber, okra, Chinese cabbage, fennel, and strawberry), medicinal plants (basil and black cumin), some ornamental and non-woody fruit plants, along with other herbs. Foliar applications of SA can reduce the toxic effects of oxidative stress provoked by drought through different mechanisms. First, SA may act by boosting osmolytes, including total soluble sugars and proline, thus maintaining the water status of plants under drought stress. Second, SA may act by improving the enzymatic activity of peroxidase, superoxide dismutase, and catalase (common antioxidant enzymes) often manifested with lower levels of lipid peroxidation, lipoxygenase activity, and H_2O_2 production. Third, SA may act by maintaining the total chlorophyll content of plants, thus preserving the photosynthetic apparatus of plants. Low concentrations of SA are generally beneficial, but high concentrations can be either detrimental or of no benefit. Recent reports on the improvement of crop tolerance to drought stress with foliar or applications of SA and proposed metabolic pathways for induced tolerance by SA treatments are reviewed.

Keywords Application dose · Crop growth · Exogenous application · Growth stage

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S. Hayat et al. (eds.), *Salicylic Acid - A Versatile Plant Growth Regulator*,
https://doi.org/10.1007/978-3-030-79229-9_5

1 Introduction

Drought refers to the inadequacy of water availability during crop growth, which restricts the yield potential of a crop (Singh et al., 2018). Occurring at any stage of the crop growth, the impact of drought on plant growth varies in intensity and duration according to the circumstancess (Lu et al., 2020). Accordingly, different drought patterns can lead to the preference of different resistance strategies by plants. Nowadays, drought is a major environmental constraint affecting global agricultural productivity. Therefore, tolerant crops to drought are required to safeguard food production for an ever-increasing world population.

Plants cope with drought stress following different mechanisms. Successful plant reproduction prior to the occurence of drought stress is a common escape strategy (Chaves et al., 2003). Drought escape reflects the capacity of plants to complete their biological cycle before the occurrence of water deficit. Drought resistance reflects the capacity of plants to withstand limited yield loss under drought compared with the maximum yield potential under a normal (non-stressfull) environment, often linked to the ability of a plant to reach and absorb water from the soil, even under conditions of water deficit (Blum, 2005; Richards et al., 2010). Under high water potential, drought resistance involves several adjustments of plant growth such as increase in stomatal resistance, decrease in leaf area, and preservation of water absorption capacity by expansion of root density and depth. Under low water potential, drought resistance involves maintainance of turgor pressure through osmotic adjustment. Essential mechanisms of plant adaptation to water deficit stress are regulated by several genes, but biochemical and molecular pathways explaining plant adaptation to drought are not well understood for improving plant resistance to drought.

Plant bioregulators are biochemical compounds that can stimulate plant growth and improve productivity when applied at appropriate plant growth stages, even in small quantities (Pasala et al., 2016). Various plant hormones (phytohormones) are involved in the control of exogenous and endogenous signals in plant growth. Apart from auxins, gibberellins, cytokinins, ethylene, and abscisic acid, common phytohormones also include brassinosteroids, jasmonates, and salicylic acid (SA) (Stamm et al., 2011). These molecules are being tested for enhancing crop productivity in agriculture under stress. SA (Fig. 5.1) is an endogenous signal hormone involved in numerous plant processes in response to biotic and abiotic stressful conditions (Nazar et al., 2015). SA was found to behave as a chemical messenger in regulating

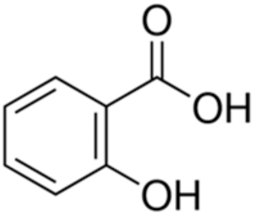

Fig. 5.1 Salicylic acid chemical structure

biological processes at relatively low concentrations (Pasternak et al., 2019). In fact, SA has been reported to lessen the toxic effects of abiotic stress at low concentrations, while it often induces oxidative stress at higher concentrations (Rivas-San Vicente & Plasencia, 2011; Miura & Tada, 2014). This chapter summarizes literature reports on the effect of foliar applications of SA for improving crop tolerance to drought.

2 Literature Identification – Data Collection

A systematic review of the literature was conducted searching SCOPUS database to identify relevant publications on the impact of foliar application of SA on drought tolerance of crops. Searching and selecting relevant publications were based on keywords such as drought-SA-foliar, drought-SA-exogenous, and drought-SA-tolerance. The references of each selected publication were checked to ensure that any other data of vital importance were finally included. Our search in SCOPUS database resulted in 43 articles published from 2002 to 2020 dealing with SA use and drought (Fig. 5.2). Publications on the topic are mainly from 2012, reaching maximum numbers in 2019 and 2020.

2.1 Cereals

Pretreatment with 0.5 mM SA hydroponically improved growth of wheat seedlings under drought stress, expressed in increased dry biomass of shoots and roots (Kang et al., 2012). Moreover, wheat seedlings tolerance induced by SA addition was linked with proteins involved in several processes, including photosynthesis and energy production (Kang et al., 2012). Similarly, wheat seedlings that received 0.5 mM SA hydroponically showed less restricted growth by drought stress, in terms of plant height, root length, and lipid peroxidation (Kang et al., 2013). Furthermore, increased levels of ascorbate and glutathione under drought stress were noted with the addition of SA, implying alleviation of the toxic impact of drought stress on the growth of wheat seedlings by SA via the glutathione-ascorbate cycle (Kang et al., 2013). Other research also confirmed that foliar spray of SA at 200 mg per L under drought stress sustained or enhanced various physiological parameters of wheat, including biological yield, SPAD values of chlorophyll, and concentration of amino acids (Noreen et al., 2017). For example, drought conditions reduced the biological yield of the wheat variety MH-97 by 72.3%, but SA spraying mitigated this reduction in biomass by 20.1% (Noreen et al., 2017). In addition, SA spraying improved the SPAD values of chlorophyll and the concentration of amino acids by 12.5% and 17.3%, respectively compared with the untreated control. Finally, SA spraying improved 100-grains weight by 9.4% compared with the untreated control. Other research reported that the treatment with SA improved

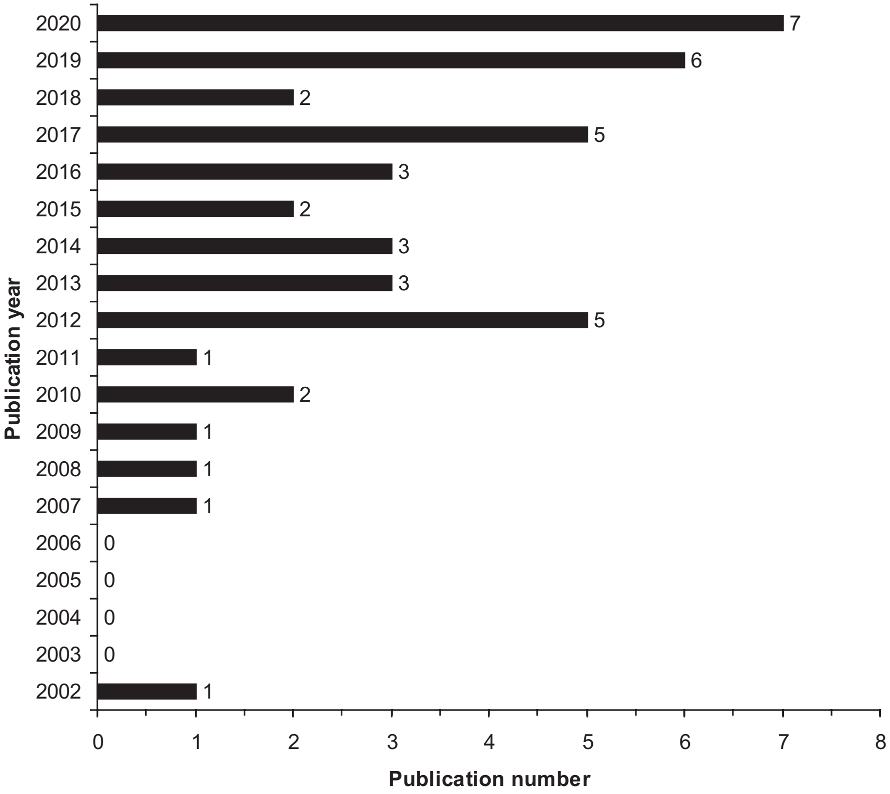

Fig. 5.2 Publications related to foliar applications of salicylic acid for promoting crop tolerance to drought

wheat biomass and yield components under drought, while SA treatment was involved in the up-regulation of the CBF14 gene (Kareem et al., 2019). Contrary to the above, wheat seedlings of the cv. Chinese Spring that were treated with 0.5 mM SA hydroponically and subsequently subjected to drought stress showed reduced tolerance to drought, although an escalation in the activity of guaiacol peroxidase and ascorbate peroxidase was noted (Horváth et al., 2007).

Barley plants grown under drought stress showed reduced levels of dry weight and net CO_2 assimilation rate, but the addition of SA increased these attributes (Habibi, 2012). Under drought conditions, SA treatment improved photosynthesis of barley plants, which was associated with an increase in stomatal conductance, whereas photosystem II (PSII) quantum yield was not altered (Habibi, 2012). Drought stress negatively affected shoot growth, photosynthetic pigments, and K^+ content in barley leaves, while altered the contents of soluble carbohydrates, organic acids, and total phenolic compounds, also including antioxidant activity and Na^+ content (Fayez & Bazaid, 2014). Application of 50 μM SA improved these attributes under water stress, indicating an ameliorative effect of SA on stressed barley plants by maintaining low malondialdehyde content and decreased Na^+/K^+ ratio in the leaves (Fayez & Bazaid, 2014).

In maize, application of 0.5 mM SA increased putrescine content, but spermidine content was increased only when SA addition was followed by low temperature. The treatment of maize palnts with 0.5 mM SA and 15% polyethylene glycol (PEG) increased the electrolyte leakage and decreased photosynthesis (Németh et al., 2002). Maize plants that were treated with SA (100 ppm) and L-tryptophan (15 ppm) showed improved relative water content, stability of leaf membranes, chlorophyll, and K^+ content compared with control plants, suggesting that foliar application of SA and L-tryptophan are involved in maize tolerance to drought (Rao et al., 2012). In addition, exogenous use of SA minimized water loss and prevented leaf rolling in two maize cultivars over control, while lipid peroxidation because of drought stress was also prevented by SA use. Pretreatment of maize plants with SA induced all antioxidant enzymes activity during drought compared with control. SA also reduced the ascorbate and glutathione content in maize plants (Saruhan et al., 2012). Exogenous use of SA in a drought-stressed environment increased the hydraulic conductivity of maize root and the relative water content of the leaf, compared with drought stress alone (Shan & Wang, 2017). Similarly, the photosynthetic pigments, relative water content, root and shoot length, and K^+ accumulation were higher in the maize hybrid SC 705 treated with humic acid and SA compared with the maize hybrid SC 260 (Bijanzadeh et al., 2019). Other research showed that the applications of methyl jasmonate or SA did not improve maize tolerance to drought, while the simultaneous application of methyl jasmonate plus SA alleviated the toxic effects of oxidative stress by drought, as expressed in lower levels of lipid peroxidation, lipoxygenase activity, and H_2O_2 (Tayyab et al., 2020). Application of methyl jasmonate plus SA also preserved plant water status in a drought-stressed environment by increasing osmolytes. In addition, increased activity of catalase, peroxidase, and superoxide dismutase by the applications of methyl jasmonate plus SA was noted (Tayyab et al., 2020).

In rice, SA application at 50, 100, and 150 mg per L improved the performance of rice irrespective of stress. Foliar application of SA with 100 mg per L was the best treatment to improve rice performance irrespective of stress compared with control (Farooq et al., 2009). Moreover, a large reduction in attributes such as gas exchange and water relation by drought stress was noted. Although water deficit promoted H_2O_2, malondialdehyde, and relative membrane permeability, foliar application of SA improved rice growth in terms of maintenance of water status, metabolites synthesis, and carbon assimilation (Farooq et al., 2010). Application of H_2O_2 or SA under drought reduced oxidative damage in rice seedlings through the regulation of antioxidant enzymes. However, augmentation of plant-water status and proline level were significant paremeters in drought tolerance. The addition of H_2O_2 or SA also protected photosynthetic pigments maintaining normal photosynthesis under drought (Sohag et al., 2020).

2.2 Oilseed Crops

Athough drought stress decreased achene and oil yield in sunflower plants, SA application improved these attributes in a drought-stressed environment. Drought stress also increased proline in sunflower leaves, which was further enhanced by SA application. However, SA application did not improve achene oil content under water stress conditions (Hussain et al., 2008). Other research showed that integrative use of plant growth promoting bacteria with SA was an effective strategy for the improvement of plant growth in sunflower under drought stress (Khan et al., 2019). Similar research (Siddique et al., 2020) showed that seed inoculation with KS42 and KS7 rhizobacteria supplemented with foliar spray of glycine betaine or seed inoculation with KS42 rhizobacteria combined with SA application provided the maximum improvement in selected plant traits and water use efficiency in sunflower. Using biological and chemical means together is a novel approach for marginal areas where water deficit conditions limit sunflower production (Siddique et al., 2020).

Safflower plants grown under different irrigation levels (water volumes 25% and 100% of the field capacity) with SA application (250 μM) showed an increased production of osmolytes and non-enzymatic scavengers such as proline (Chavoushi et al., 2019).

In sesame, water deficit stress decreased leaf area index, stomatal conductance, total chlorophyll content, and net photosynthetic rate, which were manifested in reduced plant dry biomass and seed yield, despite elevated levels of carotenoid content and activity of antioxidant enxymes. SA ameliorated all the above traits under drought, supporting SA use for mitigating the toxic effects of drought on sesame (Yousefzadeh Najafabadi & Ehsanzadeh, 2017). Similarly, sesame plants under water deficit stress showed a decrease in most growth parameters studied (Pourghasemian et al., 2020). Moreover, drought stress stimulated osmolytes and antioxidant enzymes activity, while foliar application of SA, beeswax waste, and licorice extract mitigated the oxidative damage and increased water use efficiency under drought stress. Sesame leaf treated with each substance (i.e. SA, beeswax waste, and licorice extract) showed better activity of the antioxidant enzymes than leaf treated with water. Beeswax waste showed more pronounced alleviating effect on sesame growth under drought than that of licorice extract and SA (Pourghasemian et al., 2020).

SA application at 25, 50, and 75 mg per L improved biomass production in flax varieties when exposed to drought stress compared with untreated plants. SA application increased the concentrations of photosynthetic pigments, total soluble sugars, and free amino acids. In addition, decreased lipid peroxidation in terms of malonaldhyde content was noted compared with the untreated control. Furthermore, SA application improved flax yield and several yield paremeters such as plant height, number of fruiting branches and capsules per plant, and 1000-seeds weight compared with the untreated control (Bakry et al., 2012).

2.3 Legumes

Chickpea cultivars were susceptible to terminal drought stress, regarding crop growth and yield, but SA application increased plant biomass under stress. SA spraying promoted proline content of chickpea leaves under complete drought stress conditions. Moreover, foliar application of SA reduced the electrolyte leakage in chickpea leaves under both normal and water deficit conditions (Farjam et al., 2014). Other research showed that lack of irrigation at flowering and pod formation of mung bean affected growth and yield, but application of SA mitigated the toxic effects of drought stress. Under water deficit, foliar application of SA at 100 ppm was useful in lessening the negative effects of water stress on mung bean (Majeed et al., 2016). Other research showed that foliar application of SA promoted growth, productivity, and product quality of snap bean plants as well as some physiological parameters of the plants grown under drought stress. In addition, total chlorophyll content and relative water content were enhanced in plants treated with SA when subjected to drought stress (El-Tohamy et al., 2018). However, SA had no significant effects on the parameters of gas exchange and photosynthetic pigments of common bean plants, although it helped to regulate the levels of H_2O_2 in plants through the antioxidant enzymes activity (Lopes et al., 2019). In crown vetch (*Coronilla varia*), seedlings treated with 1.0 mmol SA per L showed higher dry biomass under drought stress. Furthermore, treatment with 0.5–1.0 mmol SA per L increased proline, soluble protein content, and antoxidant enzymes activity, with the rate of 1.0 mmol SA per L producing the best results. Nevertheless, no mitigation effect was noted at SA concentration above 2.0 mmol per L on growth of seedlings under drought stress (Ma et al., 2017).

2.4 Vegetable Crops

Cucumber seedlings that were pretreated with SA showed improved levels of biomass accumulation as well as chlorophyll content and chlorophyll fluorescence ratio under drought stress (Baninasab, 2010). Moreover, the beneficial effect of SA was manifested with increased proline contents in the shoot tissues and prevention of electrolyte leakage from the leaves. The seed-soaking method for SA treatment was more effective than the foliar spray (Baninasab, 2010). Other research showed that application of 1 mM SA twice (2 + 4 leaf stage) as well as three times (2 + 4 leaf stage + flowering) in okra was equally effective in improving all morphological traits, except root length. However, in terms of physiological traits and yield, three applications of SA (2 + 4 leaf stage + flowering) were superior (Munir et al., 2016). Chinese cabbage seedlings that were pretreated three or six times with SA (10^{-4} M) showed tolerance to drought. Pretreating seedlings six times was found to be more efficacious than pretreating three times (Cha et al., 2020). In fennel, SA improved the activitiy of antioxidant enzymes, increased water potential, and maintained relative water content under drought. Similarly, improved levels of leaf osmolytes, chlorophyll content, carotenoids, and seed essential oil content were noted when fennel was grown under drought (Askari & Ehsanzadeh, 2015). In strawberry cv. Kurdistan, SA application increased root dry weight, leaf number, total dry weight of plant, yield, and fruit weight under drought. Moreover, SA application increased catalase and peroxidase activity in two strawberry cultivars under drought (Ghaderi et al., 2015).

2.5 Medicinal Plants and Other Crops

In basil, water deficit provoked lower plant height and biomass production compared with non-stressed plants. The same trend was also observed in the level of Chl-a and Chl-b, but foliar application of SA improved basil growth under water shortage (Damalas, 2019). In black cumin, SA application through the root increased plant tolerance to drought, so that SA pre-treated plants exhibited only slight injury symptoms (Kabiri et al., 2014).

Drought stress reduced the number of developed leaves, relative water content, chlorophyll content, and biomass production in busy Lizzie (*Impatiens walleriana*). Drought stress increased water loss from leaves, provoking proline, H_2O_2, and malondialdehyde accumulation. Application of SA mitigated the effects of drought stress on most plant parameters, except of proline accumulation. SA application secured photosynthetic pigments and membranes from oxidative damage (Antonić et al., 2016). Water deficit damaged plant growth of milk thistle (*Silybum marianum*), including relative water content, seed yield, and oil content. However, foliar application of 1 mM SA had beneficial effects on seed yield and oil content compared to foliar application of 0.5 mM SA. Applying 1 mM SA increased total soluble carbohydrates, total phenolic compounds, and proline content under both normal and water deficit conditions (Estaji & Niknam, 2020). Low concentration of SA improved resistance to drought in red spider lily (*Lycoris radiata*), while higher concentrations showed poisonous effects. The application of 2 mmol SA per L was highly beneficial in omotic regulation, membrane system, and antioxidant enzymes activity for promoting the drought resistance of *Lycoris radiata* (Jiang et al., 2012). Treatment with SA (10^{-6} M) of never never plant (*Ctenanthe setosa*) blocked water loss and delayed leaf rolling under drought. SA application enhanced the activity of all antioxidant enzymes during drought. SA treatment promoted the levels of ascorbate, glutathione, α-tocopherol, carotenoid, and endogenous SA (Kadioglu et al., 2011). Drought stress decreased growth parameters and photosynthetic pigments content of wavyleaf mullein (*Verbascum sinuatum*). However, SA increased the tolerance of *Verbascum sinuatum* to water stress through the promotion of enzymatic and non-enzymatic antioxidant defense systems (Karamian et al., 2020). SA application alleviated drought damage by reducing electrolyte leakage and increasing relative water content in the leaves of creeping bentgrass (*Agrostis stolonifera*) treated plants. SA application enhanced amino acids accumulation (proline, serine, threonine, and alanine) as well as carbohydrates accumulation (glucose, mannose, fructose, and cellobiose) (Li et al., 2017). Drought stress negatively affected growth of toothpick plant (*Ammi visnaga*) plants and fruits yield, whereas increased two major γ-pyrones: khellin and visnagin in most organs (Osama et al., 2019). The detrimental effects of drought stress on growth parameters were reduced by foliar spray of 2 mM SA. SA application significantly increased the polyphenolic content and the radical scavenging activity of plants under drought (Osama et al., 2019).

3 Conclusions

SA plays a major role in the control of the abiotic stress responses in several crops. As reviewed herein, foliar applications improved growth under drought stress in major crops such as cereals, oilseed crops, legumes, vegetable crops, medicinal plants, some ornamental and non-woody fruit plants, along with other herbs. First, SA may act by boosting osmolytes, including total soluble sugars and proline, thus maintaining the water status of plants under drought stress. Second, SA may act by improving the enzymatic activity of peroxidase, superoxide dismutase, and catalase (common antioxidant enzymes) often manifested with lower levels of lipid peroxidation, lipoxygenase activity, and H_2O_2 production. Third, SA may act by maintaining the total chlorophyll content of plants, thus preserving the photosynthetic apparatus of plants. Generally, low concentrations of SA promote plant tolerance to drought, while high concentrations or continual application induce inhibitory effects on plant growth and reduce tolerance. The specific role of SA in abiotic stresses remains unclear. Such knowledge is necessary for clarifying the specificity of plant responses to common abiotic stresses.

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Chapter 6
Interplay Between Environmental Signals and Endogenous Salicylic Acid

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Abstract Salicylic acid (SA), a naturally occurring growth regulator and signaling molecule plays critical roles in the growth and development of plants along with stress tolerance. Most of the synthesized SA is glucosylated and methylated in plants. A number of studies reveal the role of SA in modulating metabolic responses and redox balance, which in turn induces tolerance mechanisms in plants vulnerable to abiotic stresses. Although much of the events associated with SA's role on stress tolerance are studied at physiological and metabolic levels, the understanding of SA-regulated genes/proteins and signaling pathways might provide more solid background for developing abiotic stress tolerance in plants. Since abiotic stress is the most significant constraint in crop production, the eco-friendly approaches to induce tolerance in plants to cope with stresses is highly desirable. In this chapter, we will discuss the recent progress on the physiological and molecular roles of SA in response to abiotic stresses in plants.

Keywords Antioxidants · Biosynthesis · Metal toxicity · Osmotic stress

1 Introduction

Salicylic acid (SA), a phenolic compound, belongs to the secondary metabolites in plants (Hadacek et al., 2011). Recently, SA has earned its recognition as a crucial endogenous plant hormone owing to its well established regulatory roles in plant growth, development, disease resistance and thermogenesis (Vlot et al., 2009). SA can be produced within the plant via the phenylalanine pathway, where the phenylalanine ammonia lyase enzyme converts phenylalanine into trans-cinnamic acid, which is oxidized into benzoic acid. Benzoic-acid-2-hydroxylase catalyzes the hydroxylation of BA aromatic ring and commences SA production (Shine et al.,

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S. Hayat et al. (eds.), *Salicylic Acid - A Versatile Plant Growth Regulator*,
https://doi.org/10.1007/978-3-030-79229-9_6

2016; Zhang et al., 2016). Another way is isochorismate route, where chorismate converts into SA, a reaction in which two enzymes, isochorismate synthase and isochorismate pyruvate lyase, are catalyzed (Métraux, 2002; Garcion et al., 2008; Rekhter et al., 2019). The involvement of endogenous SA as a signaling molecule in response to adverse environmental conditions is widely studied in numerous plant species. SA is known to play a pivotal role in plant growth control, protection against biotic and abiotic stress and plant immune responses (Wei et al., 2018; Hartmann & Zeier, 2019; El-Shazoly et al., 2019; Luo et al., 2019; Pasternak et al., 2019). SA raises plant resistance to nearly all forms of abiotic stresses drought (Fayez & Bazaid, 2014), salinity (Khan et al., 2014; Nazar et al., 2015), osmotic (Naser Alavi et al., 2014), heavy metals (Zhang et al., 2015). In recent years, some reviews have been accomplished to summarize that SA can alleviate heavy metal toxicity influencing both their uptake and/or accumulation in plant organs (Shi et al., 2009; Wang et al., 2011; Dalvi & Bhalerao, 2013; Kohli et al., 2018; Safari et al., 2019) along with the scavenging and/or reducing their aggregation of reactive oxygen species (ROS) and/or strengthening the antioxidant protection mechanism (Malik et al., 2018; Mohamed & Hassan, 2019; Mostofa et al., 2019; Wang et al., 2019) protecting membrane stability and integrity (Belkadhi et al., 2015), interacting with plant hormones (Tamás et al., 2015) and improving the performance of the photosynthetic machinery (Tahjib-Ul-Arif et al., 2018; Yin et al., 2018; Wang et al., 2019). However, the positive impact of exogenous SA on heavy metals has been reported by many studies which often cite the important role of exogenous SA in terms of increasing antioxidant activities in scavenging ROS (Belkadhi et al., 2015; Tamás et al., 2015; Mostofa et al., 2019; Wang et al., 2019). In the literature, we also found salicylic acid increases the drought tolerance in plants (Hayat et al., 2008; Kadioglu et al., 2011). SA, in addition, accelerates the expression of most salt-responsive genes and signaling pathways that are responsible for salt stress cell apoptosis. In addition to controlling different plant metabolic processes, in both favorable and stressed environments, SA interacts with mineral nutrients to control different plant responses. It can control the absorption and metabolism of many mineral components and enhance the development and growth of plants under abiotic stress (Chen et al., 2011; Khokon et al., 2011; Wang et al., 2011; Tufail et al., 2013; Nazar et al., 2015). Thus, literature shows SA increases plant tolerance capacity to different environmental signals by regulating various levels of metabolites, antioxidative enzymes, metal nutrients, and osmolytes. Owing to the importance of SA in abiotic stress management, further researches needs to be focused on the identification of molecular and signaling regulation of SA in abiotic stress tolerance in the plants to better understand the underlying mechanisms and development of innovative strategies for crop improvement. In particular, the present chapter focuses on broadening our understanding of the area of abiotic stress tolerance regulation through physiological mechanisms and molecular tools, with particular emphasis on SA involvement.

2 Biosynthesis of SA in Plants

Despite years of diverse efforts, the SA biosynthetic pathway is yet to be fully defined. In plants, two plausible SA biosynthetic pathways, namely the isochorismate synthase (ICS) pathway and the phenylalanine ammonia-lyase (PAL) pathway, have been revealed (Lefevere et al., 2020; Dempsey et al., 2011) till now (Fig. 6.1). Chorismate originated from the shikimate phenylpropanoid pathway in the plastid (Dempsey et al., 2011), is the precursor for either pathway (Dempsey et al., 2011; Lefevere et al., 2020).

The ICS pathway: Study of Arabidopsis mutant *SA* induction-deficient 2 *(sid2)*, a mutation to a gene *ICS1* encoding isochorismate synthase (ICS) that converts chorismate into isochorismate (Zhang & Li, 2019). Lefevere et al., (2020) demonstrated the pathogen-induced synthesis of SA by ICS pathway (Zhang et al., 2019) that was previously described in bacteria (Dempsey et al., 2011). Two *ICS* genes, namely *ICS1* and *ICS2* was identified in the Arabidopsis genome (Zhang et al., 2019), the model plant widely accepted for the investigation of SA biosynthesis and signaling pathways (Zhang & Li, 2019). It is worth mentioning that both ICS1 and ICS2 are localized within the chloroplast (Strawn et al., 2007; Garcion et al., 2008; Dempseya et al., 2011; Rekhter et al., 2019). SA accumulation was comparable in pathogen-induced *ICS1* but not *ICS2* single mutant and uninduced wild-type plants (Zhang et al., 2019), suggesting that the basal SA level is maintained by ICS2, whereas induced SA hyperaccumulation is contributed by ICS1. Interestingly, *ICS1* and *ICS2* double mutant plants still accumulate SA, although the level is even lower (Zhang et al., 2019), indicating the existence of an ICS-independent pathway (Garcion et al., 2008; Dempsey et al., 2011). The subsequent step that is the conversion of isochorismate into SA is catalyzed by isochorismate pyruvate lyase (IPL), an enzyme identified only in bacteria (Mercado-Blanco et al., 2001; Lefevere et al., 2020) but not in plants. In fact, the bacterial counterpart of *IPL* gene has not been found in the Arabidopsis genome (Chen et al., 2009; Dempsey et al., 2011). Recently, a cytosolic amidotransferase (Rekhter et al., 2019; Lefevere et al., 2020), avrPphB susceptible3 (PBS3), characterized only in Arabidopsis, assumed to conjugate glutamate to isochorismate producing isochorismate-9-glutamate (Rekhter et al., 2019; Torrens-Spence et al., 2019). The conjugation product in turn, is proposed to be converted enzymatically (Torrens-Spence et al., 2019; Lefevere et al., 2020) or through non-enzymatic spontaneous decomposition into SA and 2-hydroxy-acryloyl-N-glutamate (Rekhter et al., 2019; Lefevere et al., 2020). Nevertheless, *PBS3* (Lee et al., 1995; Lefevere et al., 2020) or *EPS1* (Torrens-Spence et al., 2019; Lefevere et al., 2020) knockout mutants unlike *ICS1* mutant, do not abolish pathogen-induced SA accumulation, indicating the possible existence of a PBS3/EPS1 independent pathway. Enhanced Disease Susceptibility 5 (EDS5) protein, a homolog of the bacterial multidrug and toxin extrusion proteins (MATE), localized at the chloroplast envelope was identified through the analysis of another *sid1/eds5* mutant (Zhang et al., 2019). EDS5 was proposed to export SA or one of its precursor isochorismate from the plastid to the cytosol (Zhang et al., 2019). Therefore,

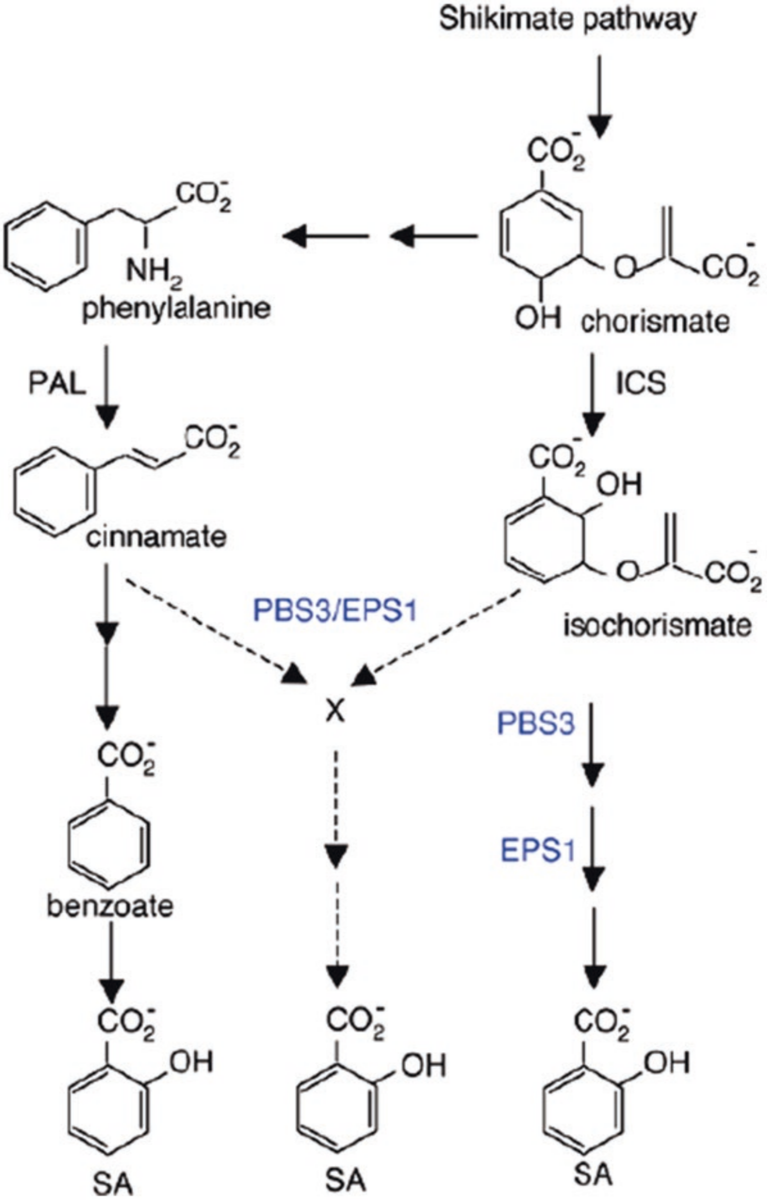

Fig. 6.1 Biosynthesis pathways of SA in plants. (Chen et al., 2009)

EDS5 perhaps acts as a bridge between stromal ICS and cytosolic PBS3 to complete the ICS pathway.

The PAL pathway: SA biosynthesis was initially described from phenylalanine (Phe) via PAL pathway (Zhang et al., 2019), whereas the extensively studied ICS pathway is rather newly elucidated. The precursor chorismate enters into the PAL pathway by way of Phe through prephenate. Chorismate mutase (CM), identified in several copies in most plant genome (Lefevere et al., 2020), an upstream enzyme of this pathway catalyze the conversion of chorismate to prephenate (Lefevere et al., 2020). However, the enzyme PAL, that catalyzes the conversion of Phe to trans-cinnamic acid (*t*-CA) via a non-oxidative deamination reaction (Zhang et al., 2019) is considered to be the entry point of this pathway, as the upstream metabolites are diverted into various biosynthetic routes (Lefevere et al., 2020). Interestingly, PAL that is present in many plant species (Cass et al., 2015; Lefevere et al., 2020), also provide precursors for diverse defense-related phenolic compounds in addition to SA (Lefevere et al., 2020). Four PAL genes were identified in Arabidopsis genome (Cochrane et al., 2004) and the quadruple PAL mutants accumulate 25% and 50% of the basal and pathogen-induced SA levels of that of wild-type varieties, respectively (Zhang et al., 2019). Therefore the PAL pathway definitely has an important role to play in SA biosynthesis (Zhang et al., 2019), which is further supported by the ability of *ICS1* and *ICS2* double mutant plants to accumulate lower levels of SA (Zhang et al., 2019). Prior feeding experiments with radio-labeled metabolites, including Phe, *t*-CA, or BA revealed that the *t*-CA derived from Phe is converted to SA through *ortho*-coumaric acid or BA (Dempsey et al., 2011). Depending on the plant species, *t*-CA can take incompletely defined three possible biosynthetic routes to BA, namely a β-oxidation route from cinnamoyl-CoA, a non-oxidative route from cinnamoyl-CoA, and a non-oxidative route from *t*-CA to BA (Dempsey et al., 2011). Alternatively, BA might also be converted to SA through glucose-conjugated ester of BA and SA glucose ester (Ruuhola & Julkunen-Titto, 2003; Dempsey et al., 2011). *Abnormal inflorescence meristem1* (*AIM1*) mutant analysis in Arabidopsis has led to the identification of another key enzyme of PAL pathway, which was later identified in rice as well (Lefevere et al., 2020). In addition to its major role in fatty acid and amino acid oxidation, the β-oxidation enzyme, AIM1, has been reported to catalyze the conversion of *t*-CA into benzoic acid (BA) (Bussell et al., 2014; Lefevere et al., 2020). The final step that is the conversion of BA to SA is proposed to be catalyzed by an inducible BA 2-hydroxylase activity (Dempsey et al., 2011). Although a wide variety of plant enzymes can execute the task, the exact enzyme carrying out the BA2H activity has not been identified yet.

Interestingly, the contribution of these SA biosynthetic pathways is not uniform for the entire plant kingdom instead their significance has been reported to vary among plant species. The ICS pathway plays a major part in Arabidopsis (Lefevere et al., 2020), whereas in rice the PAL pathway appears to be predominant (Duan et al., 2014). Furthermore, the ICS and PAL pathways act cooperatively and contribute equally in the case of soybeans (Zhang et al., 2019). Additionally, the basal levels of SA have been reported to differ between rice shoots and roots (Duan et al., 2014; Lefevere et al., 2020), indicating that the activity of these pathways is not uniform even within the same species. Therefore, SA biosynthesis should not be generalized among and within plant species.

3 Role of SA in Regulating Mineral Deficiency and Metal Toxicity

By regulating physiological and biochemical processes, SA acts as an endogenous natural signal molecule and plays a pivotal role in defense mechanisms (Joseph et al., 2010; Gunes et al., 2007). However, the role of SA in mineral nutrition deficiency responses is little studied compared to other phytohormones. Kong et al., (2014) showed that co-treatment of SA and NO effectively alleviated chlorosis induced by Fe deficiency by elevating Fe uptake to the above-ground plant parts and also by limiting Fe deficiency-induced oxidative stress. In plants, two significant Fe deficiency regulators, the expression levels of bHLH38 and bHLH39, are dramatically induced by the SA-inducible transcription factor following the application of SA (Kang et al., 2003). In Arabidopsis, due to Fe deficiency, increased SA accumulation and elevated endogenous SA level reduced chlorosis induced by Fe-deficiency (Shen et al., 2016). In another study, Shen et al. (2016) demonstrated that the accumulation of SA in the Fe-deficiency responses is required in Arabidopsis (Fig. 6.2).

The crosstalk between signaling molecules and pathways is of great importance in elucidating metalloid toxicity tolerance in plants. Although the role of SA in response to metal toxicity is relatively known, however, reports revealing their interactive role in governing toxicity tolerance in plants are very limited to a few types of metal. In a recent study, the application of SA has shown to be effective in mitigating selenium toxicity, predominantly by the induction of antioxidant enzymes accompanied by the improvement in photosynthetic efficiency in maize (Naseem

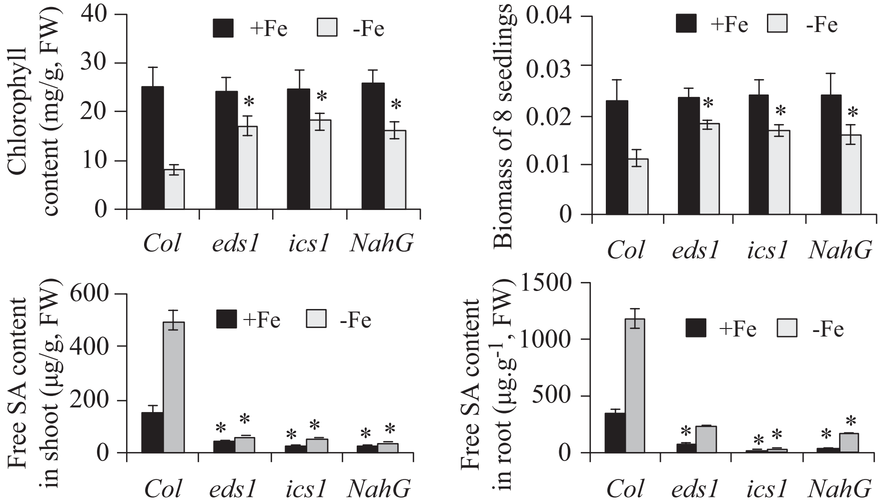

Fig. 6.2 Chlorophyll content, SA content and biomass in Arabidopsis wild-type and mutant plants

et al., 2020). This is in accordance with the elevation of reactive oxygen species (ROS) in Se-toxic plants (Chen et al., 2014). Kaya et al. (2019) also demonstrated the effectiveness of the combined application of SA and nitric oxide in restoring oxidative injury mediated by the ascorbate-glutathione cycle. Meanwhile, Under Cd toxicity, SA-fed tomato plants showed increased chlorophyll content and photosynthetic efficiency (Guo et al., 2018). Also, it is reported that SA supplementation under Pb overdose elevated chlorophyll and carotenoid content on maize (Munné-Bosch et al., 2007; Najafian et al., 2009) and barley leaves through lifting ROS detoxification activities triggered by reduced transportation of Pb to root system (Arshad et al., 2017). Moreover, gas exchange parameters and carbonic anhydrase raised in *B. juncea* when exposed to SA treatment under Mn stress (Parashar et al., 2014). SA plays a vital role in regulating photosynthesis, influencing the content of chlorophyll, stomatal conductivity, and enzyme-related photosynthesis activities in plants and in particular, the protective role of SA may be related to the activation of the photosynthetic apparatus and activation of ROS-scavenging enzymes or metabolites under stress in plants (Rivas-San Vicente & Plasencia, 2011). Free radical scavenging antioxidants allow plants to avoid or lessen oxidative burst and to survive different abiotic stresses (Eraslan et al., 2008; Gratão et al., 2015; Alves et al., 2017). To counteract oxidative stress, plants have a powerful antioxidant apparatus that includes various enzymes (SOD, CAT, APX, GR) and non-enzymatic antioxidants (e.g., glutathione, ascorbic acid, phenolics, carotenoids) that scavenge and detoxify the overproduction of ROS in plants (Sharma et al., 2012). The first oxidative injury to plants due to metal stress is lipid peroxidation. The SA has been shown to provide stability against heavy metal-induced oxidative damage by triggering antioxidant machinery in plants (Rivas-San Vicente et al., 2011). Mn and Cd-stressed *B. juncea* and *Cucumis melo* treated with external SA exhibited pronounced reduction in lipid peroxidation, electrolyte leakage, and superoxide ion (Parashar et al., 2014; Zhang et al., 2015). SA and NO together intensified the GSH content and glyoxalase pathway enzymes in safflower under Zn toxicity (Namdjoyan et al., 2017). These findings were supported by SA-induced GSH accumulation leading to reduced oxidative stress in *Brassica juncea* (Nazar et al., 2015). The involvement of SA in minimizing lead-induced oxidative stress was also suggested by Wang et al. (2011) by regulating the uptake of several plant-beneficial elements such as Mn, Ca, Cu, Fe, P, and Zn. Similarly, Gunes et al. (2005) reported SA enhanced plant growth and stimulated mineral nutrient concentrations such as P, K, Mg, and Mn under boron stress in *Z. mays*. Besides, the enhancement in enzymatic antioxidants (SOD, APOX, MDHAR, DHAR, and GR) and non-enzymatic antioxidants (GSH and ASA) upon SA addition was reported to reduce O_2 and H_2O_2 production rate and MDA content in Ni-stressed corn plants (Wang et al., 2009). Additionally, SA was found to improve free radical scavenging activities by elevating antioxidative enzymes in *Brassica napus* (Ali et al., 2015), *Iris hexagona* (Han et al., 2015), *Oryza sativa* (Guo et al., 2007, 2009), *Zea mays* L. (krantev et al., 2008), and *Cannabis sativa* L. (Shi et al., 2009) in response to Cd toxicity, which helped to confer Cd tolerance in plants. SA implementation retained mineral status by regulating the functions of mineral transporters and increasing the absorption of mineral nutrients, resulting in a decrease in Cd root-to-shoot translocation and an increase in antioxidant metabolism (Wang et al., 2011). Al-induced decline in CAT activity was enhanced due to SA surplus in rice, which significantly suppressed H_2O_2 accumulation and lipid peroxidation (Pandey et al., 2013). Although a bulk of studies documented that SA affects the action of antioxidant enzymes under metal stress (Guo et al., 2007, 2009; Parashar et al., 2014; Zhang et al., 2015), some have stated a reduction in CAT activity under metal stress (Krantev et al., 2008; Guo et al., 2013). In some cases, SA-mediated stress responses are raised in H_2O_2 formation by repressing CAT activity (Krantev et al., 2008). However, some experiments have shown that SA, an iron-chelating molecule, functions as an antioxidant since it can specifically scavenge hydroxyl radicals, which can minimize the rise in antioxidant enzyme activity caused by metals (Ahmad et al., 2011).

Thus, it appears that the synergistic effects of SA may be the paramount status in triggering cellular mechanisms to counteract the adverse impact of metal toxicity in plants. However, molecular characterization would further advance our understanding of the crosstalk between SA and environmental stress controlling the endogenous levels of SA with other associated phytohormones in response to metal stress in plants. Uncovering particulars of SA signaling and their relationship in metal stress tolerance would be fascinating.

4 Role of SA in Regulating Osmotic Stress Tolerance

Plants have emerged various mechanisms to counteract osmotic stresses. Evidence regarding exogenously utilized SA can promote plant growth in response to drought stress is well documented. Stomatal movements control the rate of water loss through transpiration for regulating drought tolerance ability in plants (Neill et al., 2008). Abscisic acid (ABA) is a phytohormone that induces stomatal closure through the processing of ROS under inadequate water (Acharya & Assmann, 2009). The exogenous application of SA also triggers the aggregation of ROS, H_2O_2 and Ca^{2+}, contributing to stomatal closure (Dong et al., 2001; Mori et al., 2001; He et al., 2007). Assmann 2010 observed that the addition of SA under drought stress boosted endogenous levels of both SA and ABA, which later functions to overcome water stress by advancing stomatal closure. Although both ABA and SA have an interaction network with stomatal closure, ABA regulates SA-dependent defensive response antagonistically (Fujita et al., 2006). The basic mechanisms for the interplay between SA and ABA on the regulation of water-deficit responses are poorly studied. We hypothesize that SA improves ABA accumulation in tissue along with ROS generation, which eventually induces stomatal closure. In *Arabidopsis thaliana*, the expression pattern of *SIZ1* gene-directed to the endogenous increase of SA inside the plant which induced stomatal closure under drought conditions (Miura et al., 2013). Furthermore, in drought-exposed wheat, SA contributes to the expression of over 76 proteins. These proteins play a major role in photosynthesis, metabolism of sugars, metabolism of proteins, stress and defense, energy generation, signal transduction and metabolism of toxins (Kang et al., 2012). Moreover, another study reported SA promoted the cytosolic Ca concentration through ROS production, which contributed to stomatal closure in *Vicia faba* (Mori et al., 2001). However, SA supplementation plays a pivotal role in the modulation of enzymatic and non-enzymatic components and antioxidant metabolites to overcome the oxidative stress of drought-exposed plants (Alam et al., 2013). Plants keep osmotic stability through osmoregulation directed by amines, soluble sugars, glycine betaine, prolines, and many more metabolites. Glycine betaine which accumulates as cytosolic solute, serves as an osmoprotectant in higher plants (Raza et al., 2014). It is asserted that GB and SA efficiently maintained osmotic balance to limit drought stress in wheat (Aldesuquy et al., 2013). Likewise, Ashraf and Foolad (2007) opined that glycine betaine in stressed plants adjusts the osmotic stability within cell, improving membrane integrity and plant growth. Proline modifies antioxidant activities and also membrane integrity by hindering the overproduction of ROS, which leads to osmotic balance in plants (Iqbal et al., 2014). Several studies reported that salicylic acid plays a significant role in intensifying the proline metabolism under abiotic stresses such as drought (Misra & Saxena, 2009; Khan et al., 2013). On the other hand, Shan and Wang (2017) mentioned SA triggered NO level in *Zea mays* resulting in root growth under drought stress, unveiling the interaction between NO and SA in plant development. Furthermore, many reports showed that SA accelerated the germination rate in plants under NaCl excess (Lee et al., 2010; Torabian 2010; Asadi et al., 2013; Boukraâ et al., 2013) while also enhanced the growth of barley (El-Tayeb 2005), wheat (Tammam et al., 2008), sunflower (Noreen & Ashraf, 2008), mungbean (Khan et al., 2010) and maize (Tufail et al., 2013) subjected to salt stress. Further, SA is appeared to improve photosynthetic impairments in leguminous plants such as *Vigna radiata* (Nazar et al., 2011) and *Medicago sativa* (Palma et al., 2013) under salinity. Besides, SA prevented the accumulation of Na^+ and Cl^- in salt-stressed *Z. mays* and *Brassica* (Gunes et al., 2007). Similar findings were documented in *Ocimum Basilicucm* (Delavari et al., 2011) and in broad bean (Azooz et al., 2011). Perhaps, SA provides some sort of restraint in accumulating ions in plant organs to reduce stress conditions. Meantime, Jayakannan et al. (2013) reported that SA dosing extended the K^+ content and checked Na^+ supply to the aerial parts of Arabidopsis and similarly, Khan et al. (2010) detected that the applying of exogenous SA diminished Na^+ and enhanced N, P, K and Ca content in mungbean which intensified salt tolerance capacity. This outcome means that the SA application can be responsible for mitigating salinity stress and improving the growth, yield and nutrient content of plants. By activating several antioxidant enzymes required for plant safety against osmotic and salt stress, the introduction of SA induced antioxidant defense responses (Noreen et al., 2009; Patel & Hemantaranjan, 2012). SA minimizes lipid peroxidation and membrane permeability (Horváth et al., 2007). It is in agreement with the reports on a significant increase of antioxidant metabolism IN SA-fed plants under salt stress (Palma et al., 2013). Exogenous use of SA in *T. aestivum* following salinity stress upregulated the transcription rate of antioxidant genes for example, *GPX1, GPX2, DHAR, GR, GST1, GST2, MDHAR,* and *GS* (Li et al., 2013). Moreover, SA influenced the activity of enzymes required in proline biosyntheses, such as γ-glutamyl kinase and pyrroline-5-carboxylate reductase under salinity stress. It was also reported that the SA induced the rise in glycine betaine level inside the plant, which improved plant growth and biomass of *Rauwolfia serpentina* grown under Na excess (Misra & Misra, 2012). These findings explicitly show that by modifying the physiological process and antioxidant defense function, SA decreased the stress condition within the plant.

5 Conclusion

Environmental signals or fluctuations are closely with the changes of endogenous SA levels in plants. However, it is likely that the changes of SA are not directly induced by the particular stress itself rather there are of number of intermediate molecules that govern the level of SA. Despite the route of SA induction, it plays critical roles in plant adaptations in response to different abiotic stresses. The interactions of SA and environmental signals can be exploited by manipulating the reaction of plants to the changing climate and the sustainable development of agriculture.

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Chapter 7
The Role of Salicylic Acid in Crops to Tolerate Abiotic Stresses

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Abstract Abiotic pressures including drought, high temperature, flooding and salinity influence the development and productivity of plants. Also, global climate change will increase the incidence and magnitude of abiotic stress, indicating that a variety of growth with improved stress tolerance is crucial to future sustainable crop production. Salicylic acid (SA) is a phenolic compound formed by a varied variety of plant species to varying degrees and it considers a naturally occurring plant hormone that serves as an essential signaling agent that contributes to abiotic stress tolerance. This endogenous driver of plant growth engages in various physiological and metabolic reactions and picking up of ions and movement. Also, involved in endogenous signaling is salicylic acid which activates plant defense against abiotic stresses. SA helps plants react to abiotic stresses including heavy metal toxicity,

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S. Hayat et al. (eds.), *Salicylic Acid - A Versatile Plant Growth Regulator*,
https://doi.org/10.1007/978-3-030-79229-9_7

temperature changing, UV light and osmotic force pressures. SA influence often depends on many variables, such as implementation style, concentration, environmental circumstances, plant species and organs. SA synthesis occurs through two pathways, isochorismate (IC) and the ammonia-lyase phenylalanine (PAL) pathway. It consists of genes that encode chaperones, heat shock proteins, antioxidants and secondary metabolite biosynthesis genes including sinapyl alcohol dehydrogenase, cinnamyl alcohol dehydrogenase and cytochrome. One significant and notable general belief is low concentrations of SA increase plant antioxidant capacity, but high of it induce cell death or abiotic stress susceptibility. This chapter presents an overview of the historical and background of the role of the SA, chemical composition, synthesis and metabolism of SA, transport of SA in plants, the response of plant stages to SA, the role of SA response in plants under abiotic stresses and the role of biotechnology for reducing the abiotic stress.

Keywords Abiotic stresses · Crops loss · Phytohormones · Salicylic acid · Stress tolerance mechanisms

1 Introduction

Salicylic acid (SA; 2-hydroxybenzoic acid) in plants is a very significant phenolic phytohormone that plays a role in plant defense as a key signaling molecule (Janda et al., 2014). In 1979, the first mention of SA playing its role in plant protection was (White, 1979). In 1828, salicin was the earliest compound to be separated from the bark of the willow tree and was subsequently called salicylic acid (Popova et al., 2012). Salicylic acid is one of the essential regulators of plant growth that has gained focus because of its critical function in both physiological aspects and responses to plant defense. Numerous researches have shed light on its role in controlling different biochemical pathways, additional demonstrating its influence over plant morphology and physiology. By affecting various processes, it is involved in granting immunity to plants against both biotic and abiotic stresses. It interacts with transcript factors and thus adjusts gene expression (Pieterse et al., 2012).

Salicylic acid is typically present in plants, either in the free state or in the form of glycosylated, methylated, glucose-ester or amino acid conjugates, in amounts of a few µg/g of fresh mass or less (Lee et al., 1995; Raskin et al., 1990). Thermogenic flowers can be detected in the greatest amounts while flowering, or after pathogenic infection (Raskin, 1992). Salicylic acid plays a key role in controlling biochemical and physiological processes such as cell formation, respiration, stomatal opening, senescence, seed germination, seedling production and plant lifetime thermotolerance (Boatwright & Pajerowska-Mukhtar, 2013). Because of its numerous functions in regulating physiological processes in plants, SA has acquired plant hormone status. It affects the growth and development of plants via cell elongation, division of cells and differentiation of cells. It also manages various enzymatic activities, multiprotein biosynthesis and photosynthesis and helps to improve antioxidant ability

(Blokhina et al., 2003; El-Tayeb et al., 2006). Its role in seed development, respiration, senescence, nodulation and yield has also been identified (Vlot et al., 2009). Via its impact on their biosynthesis or their signaling mechanisms, these SA effects can be directly or indirectly due to interaction with other hormones (Yusuf et al., 2012). SA has a possible function like a non-enzymatic antioxidant due to its phenolic existence (Arfan, 2009).

In plants, SA accumulation is directly associated with stress tolerance, where less SA accumulation is responsible for susceptibility and a plant is immune with high accumulation. Gaffney et al., (1993) and Delaney et al., (1994) reported that plants with decreased SA levels were highly susceptible to stress because they were inefficient in triggering defensive responses due to no expression of the SA-hydroxylase gene. Also, SA has been proven to be a favorable compound for growing plant production, in addition to its function in protecting against pathogen attacks, especially under unfavorable environmental conditions. After the exogenous use of SA, the higher grain yields were believed to be due to membrane stabilization and increased antioxidant activity, which could help sustain higher rates of photosynthesis even at low temperatures. This could lead to more grains per cob and greater grain weight, contributing eventually to higher grain yields (Szalai et al., 2016). It was also shown that the amount of superoxide dismutase activity in young seedling leaves was increased by soaking maize seeds in SA solution (Krantev et al., 2008) and the number of grains per cob, grain yield and harvest index parameters was also significantly increased after both optimum and late sowing dates (Rehman et al., 2015).

SA foliar application under greenhouse conditions supported red amaranth growth and yield. It also improved chlorophyll biosynthesis and increased the levels of some bioactive compounds, such as betacyanins and total polyphenol, and increased the antioxidant activity (Khandaker et al., 2011). Similarly, by stimulating the antioxidant metabolism and inducing the activity of nitrate reductase and ATP-sulfurylase enzymes, spraying with SA alleviated the salt-induced decrease in photosynthetic activity in mung bean (Nazar et al., 2011). As reported in the bean, tomato (Senaratna et al., 2000) and wheat (Hamada, 2001), the application of a low SA concentration can increase drought tolerance, while a high concentration normally decreases it (Miura & Tada, 2014). Besides water stress, SA has been found to increase the tolerance of plants to many other forms of stress (Khan et al., 2015). These stressors included high salinity (Szepesi, 2011), high temperatures (Dat et al., 1998) and low temperatures (Sasheva et al., 2013).

Salicylic acid (SA) is a plant phenolic hormone that is commonly distributed in plants, although the basal levels vary between species. In regulating a multitude of physiological processes such as seed germination, vegetative development, photosynthesis, respiration, thermogenesis, flower formation, production of seeds, or senescence, it plays an important role. SA effect on these processes can be direct or indirect, as SA is also involved in the regulation of the synthesis and signaling of other plant hormones. SA is, however, primarily known for its central role in the interaction of plant stress, and hundreds of papers have been published over the last two decades on its implications for the plant response to abiotic stress. By inducing multiple signaling cascades, proteins, and interference with gene expressions, SA has the potential to activate both resistances in plants. The goal of this chapter is to increase our understanding of the recent advances in SA physiological functions as well as the underlying signaling mechanisms leading to plant defense.

1.1 Historical Background

Salicylic acid (SA) derives its name from the word Salix, which is the scientific name of the willow tree. The American Indians and the ancient Greeks understood that as a painkiller and antipyretic, the leaves and bark of willow trees could be used. The unique effects of willow bark extract on fever, pain and inflammation were recognized. The use of salicylic tea to reduce fevers around 400 BC and willow bark preparations were part of the pharmacopeia of Western medicine in classical antiquity and the Middle Ages. The ancient Sumerians and Egyptians started to apply bark and leaves from willow, myrtle, poplar and meadowsweet plants as early as the fourth century BC to alleviate pain caused by eye disease, rheumatism, childbirth and fever. The first clinical trial in the seventeenth century was carried out by Edward Stone using powders isolated from willow bark and successfully healed most fever patients (Norn et al., 2009). Salicylic acid was not isolated and was determined to be the active ingredient of these traditional herbal remedies until the nineteenth century. In 1828, by the German pharmacologist Johann Andreas Buchner (Fischer & Ganellin, 2010), salicin (an alcoholic β-glucoside with the salicylic group) was isolated from willow bark. In the nineteenth century, pharmacists were experimenting with and prescribing a variety of chemicals related to salicin, the active component of willow extract, which was marketed in 1879 by the company Bayer with the trade name Aspirin (Sneader, 2000). It was one of the best-known antistress compounds used by humans is acetylsalicylic acid, commercially known as aspirin. Salicylic acid (SA) has been identified as being a phytohormone while his role in flowering induction was found to be on the lily of voodoo. Later, during the 1970s, it was understood that the application of salicylic acid to tobacco was plants induce the expression of defensive genes and increases resistance to viruses. SA is best known as a defense-related hormone (Ryals et al., 1994; Fu & Dong, 2013). Even so, SA only position on plant resistance was in 1990. In 1979, Raymond F. White published the first findings that SA was involved in plant immunity, explaining that the application of (acetyl-SA) to virus-susceptible conferred resistance to tobacco mosaic virus (TMV) (White, 1979), increasing reports indicate that SA is indeed a key plant hormone that controls plant immunity. Plant defenses evolved highly under biotic and abiotic stress conditions. Fine-tuning of complex phytohormones enables the balanced response of plants to developmental and environmental cues, thus minimizing defense-associated fitness costs. Harmony of phytohormones, such as jasmonic acid (JA), abscisic acid (ABA) and salicylic acid (SA), result in specific responses to specific stimuli (Koo et al., 2020).

1.2 *Definition and Chemical Structure*

Salicylic acid chemically ortho-hydroxybenzoic acid belongs to the plant phenolic group. SA is a very simple phenolic compound (a $C_7H_6O_3$ compound composed of an aromatic ring, one carboxylic, and a hydroxyl group) and this simplicity contrasts with its high versatility and the involvement of SA in several plant processes either in optimal conditions or in plants facing environmental cues, including heavy metal stress. Also, it is a regulator of many physiological and biochemical processes such as thermogenesis, plant signaling or plant protection and biotic and abiotic stress response (Chen et al., 2009, Wani et al., 2017). SA belongs to a wide community of plant phenolics from a chemical point of view. SA can be isolated in both free and conjugated forms in plants. In particular, the conjugated form is the product of aromatic ring methylation, hydroxylation, and/or glycosylation (Lovelock et la., 2016; Maruri-López et al., 2019). Salicin, one of the natural SA derivatives, was first isolated by Johan Büchner from the bark of the willow tree (*Salix* spp.) in 1828 (Raskin, 1992; Muthulakshmi & Lingakumar, 2017). The concentration of SA in plants fluctuates significantly during the various seasons (highest content in spring and summer, lowest content in autumn and winter, reaching 3 mg/g of fresh biomass in *Salix lapponum* plants) (Petrek et al., 2007).

SA biosynthesis begins with phenylalanine and follows one of two established synthesis routes, one of which includes trans-cinnamic acid and benzoic acid hydroxylation (BA). Feeding tobacco leaf tissue with putative precursors showed that only BA was able to increase SA tissue levels, indicating that BA in tobacco is a direct precursor of SA (Sharma et al., 1996). Later results, however, suggested that the immediate precursor is more likely to be benzoyl glucose, a conjugated form of BA (Chong et al., 2001). In cucumber, potato and rice, SA cinnamic acid-derived synthesis also takes place. The other potential route is the formation of trans-cinnamic acid ortho-coumaric acid (ortho hydroxy-cinnamic acid), accompanied by a chain-shortening reaction leading to SA.

Salicylic acid is a mono-hydroxybenzoic acid that is benzoic acid with a hydroxy group at the ortho position. The molecular formula of SA is ($C_7H_6O_3$ or HOC_6H_4COOH) and molecular weight (138.12 g/mol). Synonyms of salicylic acid are (2-Hydroxybenzenecarboxylic acid, Orthohydroxybenzoic acid, and 2-Carboxyphenol). The chemical structure depiction showed in (Fig. 7.1).

2 Synthesis and Metabolism of Salicylic Acid

2.1 *Biosynthesis of Salicylic Acid*

The nature of SA is lipophilic and the essential compound for it is beta-hydroxy phenolic. Plant phenolics are mainly manufactured via the shikimic acid pathway (Khan et al., 2015). It is commonly recognized that plants have two pathways for

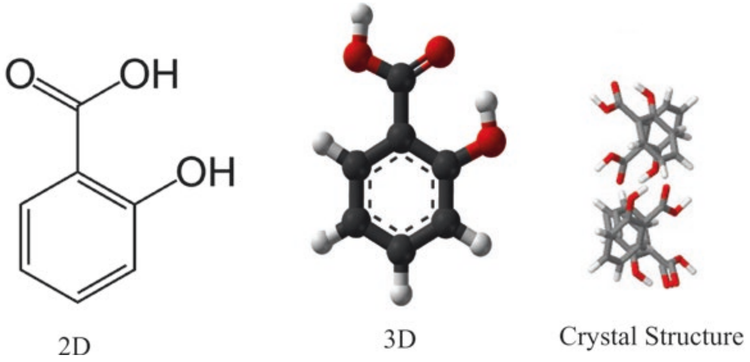

Fig. 7.1 Salicylic acid chemical and interactive chemical model and crystal structure depiction

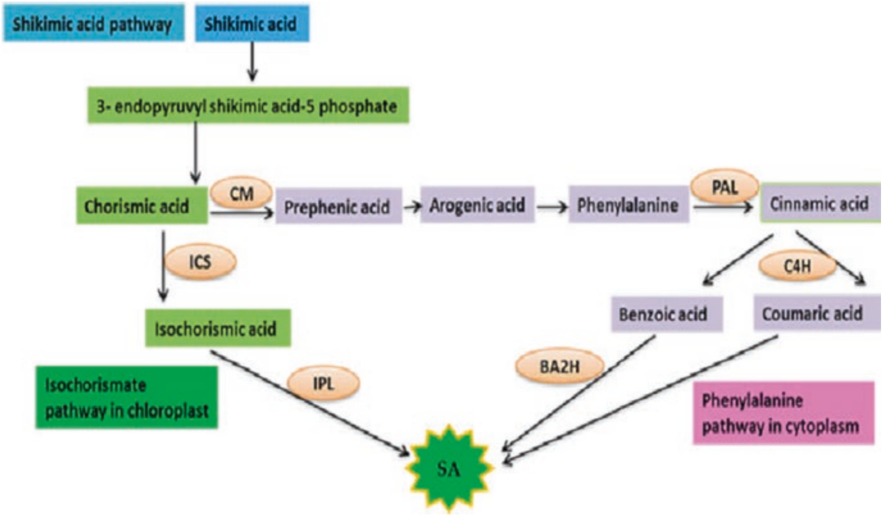

Fig. 7.2 Schematic illustration of salicylic acid biosynthesis in plants. (Source: Lone et al., 2020)

referring to a set synthase the isochorismate synthase (ICS) in chloroplasts and phenylalanine (Phe) ammonia-lyase (PAL) in the cytoplasm to metabolize SA (Jayakannan et al., 2015), both coming from turning carbohydrate forerunner into amino aromatic acids such as phenylalanine (Lefevere et al., 2020) (Fig. 7.2).

The pathways for SA biosynthesis differ for different plant species. These two pathways may be just the same importance in soybeans. One of these paths may be more important than the other. In rice, the PAL pathway appears to be more significant, unlike (Arabidopsis) the ICS pathway is relevant (Lefevere et al., 2020). On the other hand, SA has a role phytohormone and it is omnipresent in the plant (Khan et al., 2015) despite its levels differ between plant parts. For example, in rice, the shoots are superior to their SA content over the roots (Duan et al., 2014; Silverman et al., 1995) (Figs. 7.3 and 7.4).

The first pathway is ICS which was discovered in Pseudomonas species, SA is synthesized to generate the pseudomonas siderophore. In this biosynthesis, the PmsCEAB gene cluster plays a crucial role (Table 7.1). High sequence similarity with ICS in *E. coli* is demonstrated by PmsC, where this enzyme is found. The conversion of chorismate to IC catalyzes. An isochorismate pyruvate lyase (IPL) gene encodes the PmsB gene, which transforms IC to SA. In a two-step process in Pseudomonas, this suggests that SA is synthesized from chorismate (Huang et al., 2020). Another unfunctional enzyme, isochorismate pyruvate lyase, transforms IC into SA and pyruvate (Mercado-Blanco et al., 2001; Serino et al., 1995). Otherwise, A single, bifunctional enzyme controls SA composition in *Yersinia enterocolitica* and *Mycobacterium tuberculosis*. SA synthase (SAS) converts chorismate by intermediate isochorismate directly to SA (Harrison et al., 2006; Kerbarh et al., 2005; Pelludat et al., 2003). Garcion et al., (2008) explained that the genome of Arabidopsis comprises two ICS homologs, AtICS1 and AtICS2 and the induced mutants ics1 and ics1ics2 by UV showed a decrease in SA accumulation about 90% in Arabidopsis leaves (Lefevere et al., 2020).

While in the phenylalanine pathway, phenylalanine is used by a nonoxidative deamination reaction to produce cinnamic acid and this conversion is catalyzed by the PAL enzyme. The conversion of cinnamic acid to benzoic acid can then occur along a non-oxidative route or the conversion can be related to fatty acid β-oxidation (Horváth et al., 2007; Mustafa et al., 2009; Verberne et al., 1999). By the action of benzoic acid 2-hydroxylase, SA is then derived from benzoic acid (BA2H).

SA can be changed in various ways, the active molecule in the planta is assumed to be free SA. Most SA is found in a glucose conjugated inactive state in plants. Inhibiting the conversion of SA to SA-O-b-D-glucoside contributes to improved disease tolerance (Lebeis et al., 2015). SA can be methylated by a benzoic acid/SA

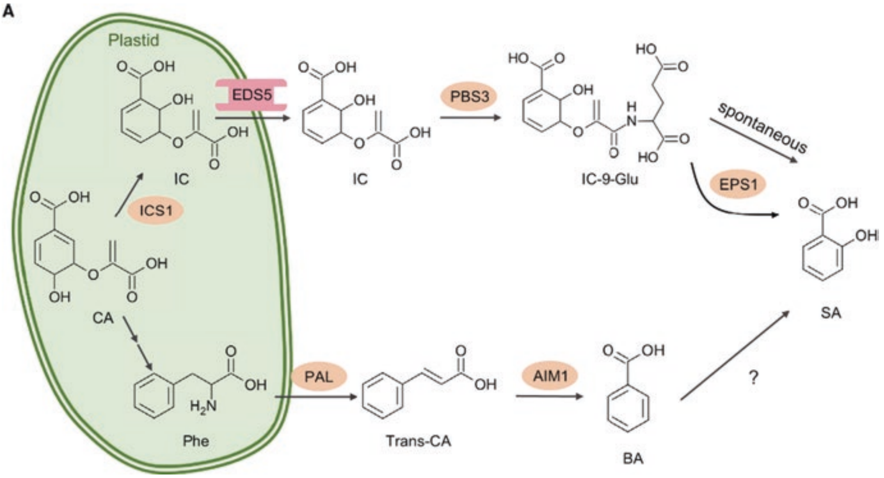

Fig. 7.3 The Biosynthetic pathways for salicylic acid (SA) and N-Hydroxypipecolic acid (NHP). (Source: Huang et al., 2020)

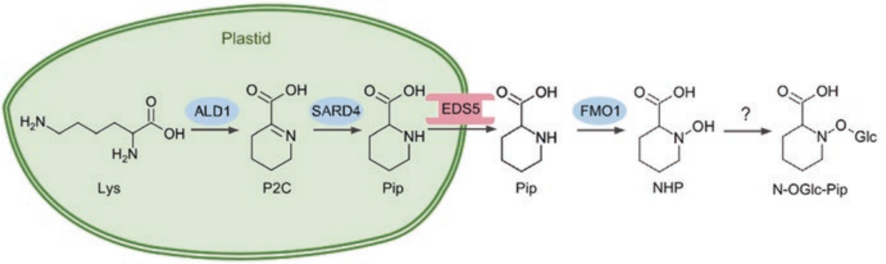

Fig. 7.4 The biosynthetic pathways for salicylic acid (SA) and N-Hydroxypipecolic Acid (NHP). The isochorismate synthase (ICS) pathway. (Source: Huang et al., 2020)

Table 7.1 The effect of salicylic acid on *Sinningia speciosa* 'Ultra' growth (gloxinia)

Treatments	Number of Leaves	Leaf Area (cm^2)
Control	13 ± 0.6a	456.4 ± 3.0c
10^{-10} M AS	13 ± 0.4a	496.2 ± 2.3bc
10^{-8} M AS	13 ± 0.6a	680.9 ± 1.2a
10^{-6} M AS	14 ± 0.4a	565.2 ± 1.6b

Means with the same letter are not significantly different at $P \geq 0.05$, Tukey's. Source: Larqué-Saavedra and Martin-Mex (2007).

carboxyl methyltransferase to form methyl SA (Chen et al., 2003). Also, the GH3.5 protein can integrate SA with aspartic acid to form SA-Asp (Chen et al., 2013; Mackelprang et al., 2017). Latest experiments have shown that SA is converted into 2,3-hydroxyl benzoic acid and 2,5-hydroxyl benzoic acid by two SA hydroxylases, salicylic acid 3-hydroxylase (S3H/DLO1) and salicylic acid 5-hydroxylase (S5H/DMR6) (Zhang et al., 2013b; Zhang et al., 2017). UGT76D11 can be further glycosylated by 2,3-hydroxyl benzoic acid and 2,5-hydroxyl benzoic acid (Huang et al., 2018).

2.2 Microbial Production of Salicylic Acid

2.2.1 Metabolism of Salicylic Acid

SA concentrations can have many levels in the plant and it can be changed through impregnable rejoinders. SA can be transformed into inactive or storage forms by metabolism to intone its activity, with increased permeability, with fine-tuning levels (Dempsey et al., 2011).

SA can be combined with glucose or methyl to form two compounds, the first, SA 2-O-β-D-glucoside (SAG) is a detoxified and vacuole storage form of SA by three Arabidopsis UDP-glucosyltransferases can be shifted SA to SAG (Dean & Delaney, 2008; Lim et al., 2002; Song, 2006). Two mutants (UGT74F1 or UGT76b1) reduced SAG levels and increased SA accumulation, leading to enhanced disease resistance in Arabidopsis (Noutoshi et al., 2012). The second compound MethylSA (MeSA)which gives permeability and dispersal for membrane and plain releasing from plants, with potentiality, MeSA can be converted back to SA by SA-binding protein 2 in tobacco (SABP2) (Park et al., 2007).

SA metabolism is primarily regulated at the transcriptional level 7.5. The genes engaged in the synthesis of salicylic acid and alteration were revealed at too few levels in the absence of stress or hormone induction. A feedforward amplification loop is likely to be present as exogenous SA induces the expression of genes associated with robust SA accumulation, including ICS1, EDS5, PBS3, SGT1 and UGT74F1. SA-mediated activation of NPR1 leads to feedback inhibition of ICS1 expression once adequate amounts of SA have been produced, thus preventing runaway SA accumulation (Dempsey et al., 2011). Goda et al., (2008) found that the transcriptional regulation by the hormones IAA, JA and ET (but not SA) of SA altering genes such as BSMT1 and GH3.5 which as well have a function in controlling cellular SA concentrations.

3 Transport of Salicylic acid in Plants

Plants must control their growth and development in response to various internal and external stimuli (Khan et al., 2020; Wolters & Jurgens, 2009). Phytohormones, which are several signaling molecules found in small quantities in cells, mediate these responses. Its pivotal roles have been identified in promoting plant adaptation to ever-changing environments by mediating growth and development, source/basin changes and nutrient allocation (Fahad et al., 2015). While plant reaction to abiotic stress depends on different factors, plant hormones are the most essential endogenous substances for the modification of physiological and molecular responses, as sessile organisms, a crucial necessity for plant survival (Fahad et al., 2015). Plant hormones function either at their synthesis site or in plants elsewhere after they have been transported (Peleg & Blumwald, 2011). In plant production and plastic growth, plant hormones are of prime significance. These include auxin (IAA), cytokinin (CK), salicylic acid (SA), abscisic acid (ABA), ethylene (ET), gibberellin (GA), brassinosteroid (BR) and jasmone (JAs). A relatively new plant hormone is Stregolactone (SL). The chemical compositions of the main plant hormones are shown in Fig. 7.5.

SA was listed within the plant hormone group two decades ago (Raskin, 1992a, 1992b). A hormone is an organic molecule that works locally and/or at a distance from its synthesis site at very low concentrations, according to a well-recognized concept. Given its small concentrations in plant tissues, its presence in the phloem sap and its change in concentration in reaction to pathogen attack, SA fulfills these requirements (Malamy et al., 1990; Metraux et al., 1990). Many aspects of SA features have been analyzed in-depth since these early findings. SA accumulation is facilitated by abiotic stress such as UVB and ozone. SA synthesis happens in the

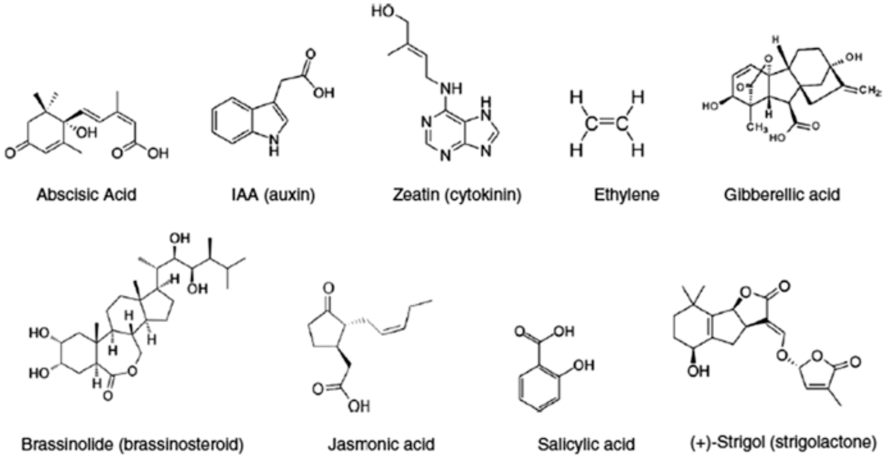

Fig. 7.5 Major phytohormone groups involved in the reaction and tolerance to abiotic stress in plants. (Source: Wani et al., 2016)

cytosol or isochorismate pathway in chloroplasts through the phenylalanine pathway (Wildermuth et al., 2001).

3.1 *Intracellular Transport*

Several studies have examined the role of SA as a phytohormone including its role in the resistance and tolerance of many abiotic stresses, including ozone, UV radiation, heat, cold, metal and salinity stresses (Dempsey & Klessig, 2017; Horváth et al., 2007; Rivas-San Vicente & Plasencia, 2011; Yuan & Lin, 2008). SA can be converted into several metabolites within the cytoplasm, such as 2-O-ß-D-glucoside (SAG) salicylic acid, methyl salicylate (MeSA), 2-O-ß-D-glucoside methyl salicylate (Me SAG), glucose-salicylic acid ester (SGE) and its derivatives. In the greatest instances, SAG tends to be the primary metabolite. It is processed in the vacuole, including the conjugation of glucose from other hormones (Dean & Mills, 2004). Figure 7.6 summarizes an overview of these pathways of synthesis and metabolism (van Butselaar & Van den Ackerveken, 2020).

The diffusion of SAG through the phospholipid layers should be very low because of its physical and chemical properties and this indicates that there is another mechanism involved in SAG splitting inside the cell. There are, indeed, two successful mechanisms in the vacuole that regulate the accumulation of SAG (Fig. 7.7). Comparative vacuolar absorption occurs in soybean cells through the ATP-linked transport mechanism of the cassette (Dean & Mills, 2004), while it occurs in tobacco cells via the H^+-antiport process triggered by the proton gradient generated by proton-tonoplast pumps (Dean et al., 2005).

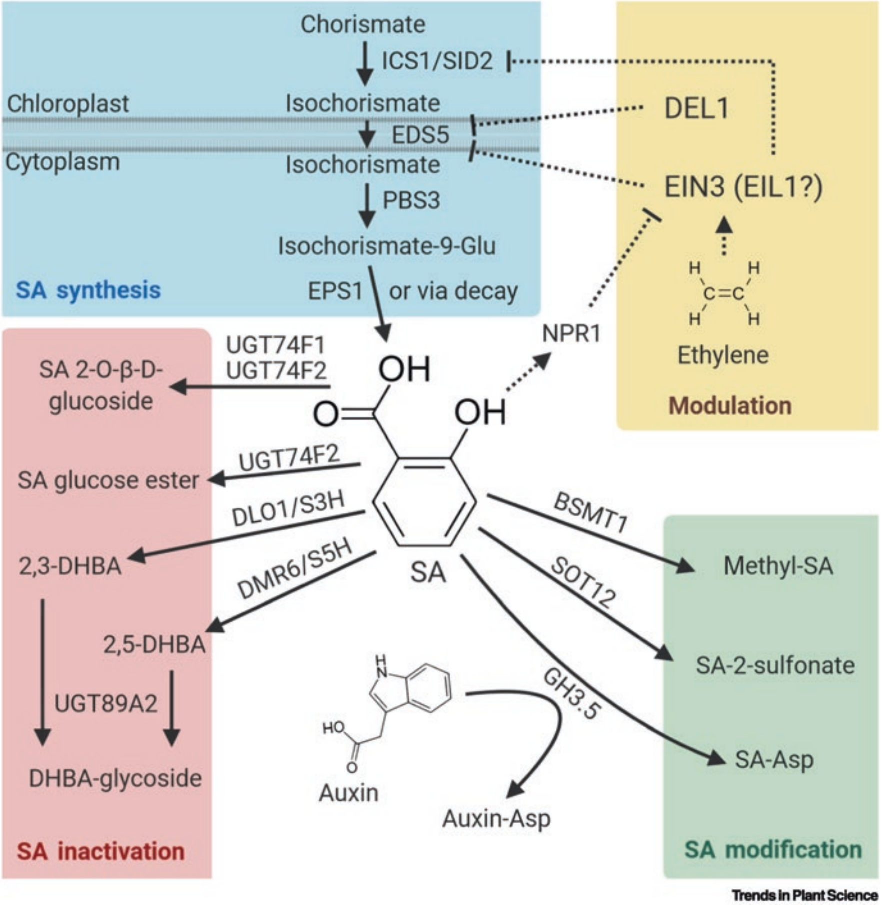

Fig. 7.6 Levels of Bioactive Salicylic Acid (SA) are highly coordinated and growth-affected. (Source: van Butselaar & Van den Ackerveken 2020)

Via the plasmodesmata (symplastic pathway) or the apoplastic pathway, free endogenous SA can enter neighboring cells (cell wall). In the above case, the hormone must first cross the donor cells plasma membrane, then the recipient cells plasma membrane. The physical and chemical properties of SA are known to make it well suited for rapid diffusion, especially in its inseparable form, through the plasma membrane of plant cells. Via the ion trap process, SA will accumulate in its anionic form in the cytosol (pH 7.3) from a stroke chamber (pH 4.5–5.5) (Belt et al., 2017; Rocher et al., 2009; Yalpani et al., 1991). However, there is a sluggish flow of SA (Chen et al., 2001). SA is consumed in rapidly suspended tobacco cells at 20 or 200 lM. Cells treated with 20 and 200 lM excreted 50 and 85% of the SA absorbed over time (5 h), respectively. EGTA strongly inhibits SA secretion and the inhibition can be reversed by adding Ca^{2+} at 200 lM but not at 20 lM. Likewise, in the 200 lM

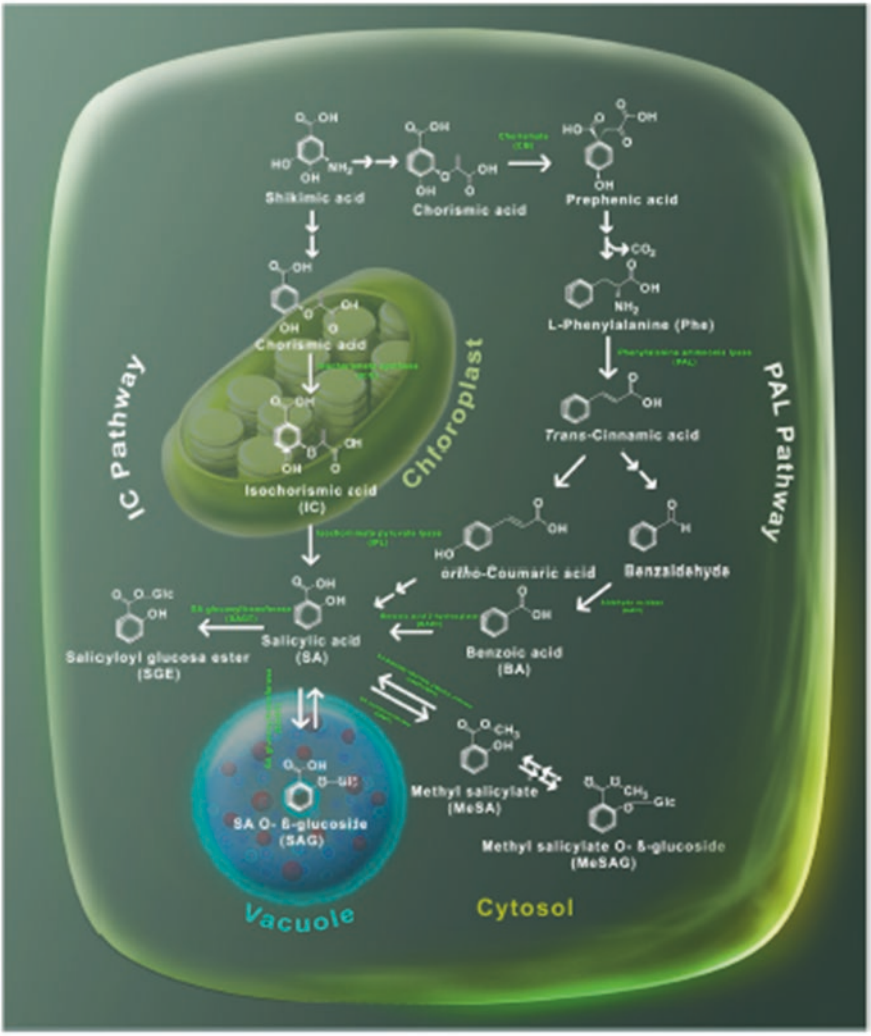

Fig. 7.7 SA metabolism. To generate SA, phenylalanine ammonia-lyase (PAL) and isochorismate, plants use two pathways (IC). Shikimic acid functions in both pathways as a precursor. The PAL process is carried out in the cytosol, where through the action of the chorionic mutase enzyme, the shikimic acid is converted to chorismic acid and then to phenylalanine. Subsequently, to generate trans-cinnamic acid, the enzyme PAL uses phenylalanine, which in turn transforms into ortho-coumaric acid and benzaldehyde. Benzaldehyde is then converted by the enzyme aldehyde oxidase (AAO) to benzoic acid (BA). A catalytic reaction by the enzyme benzoic acid hydroxylase (BA2H) transforms BA to SA. In chloroplasts, the IC pathway occurs, SA is synthesized by isochorismate synthase to produce isochorismate, which is then converted by the action of isochromic pyruvate lyase enzymes to SA. Glucosyltransferase (SAGT) is formed by salicylic acid glucosides (SAG and SGE), while methylation of SA is carried out by the methyltransferase enzyme. From SA substrates and derivatives, the chemical structures and their cellular localization are seen. (Source: Maruri-López et al., 2019)

pretreatment, cycloheximide just inhibited SA secretion. According to the authors, this could suggest, among other possibilities, the probability that, in addition to the basic current flow vector, the SA stream carrier of a catalyst was involved under recent experimental conditions (Belt et al., 2017; Chen & Kuc, 1999; Chen et al., 2001; Kawano et al., 2004; Maruri-López et al., 2019; Per et al., 2017; Verma and Agrawal 2017).

SAG accumulation in the vacuole is regulated by two active mechanisms (Fig. 7.7). The conjugate is taken into the vacuoles of soybean cells via an ATP-binding cassette transporter mechanism (Dean & Mills, 2004), whereas it is taken into the vacuoles of tobacco cells via an H^+-antiport mechanism energized by the proton gradient generated by the action of the tonoplast proton pumps (Dean et al., 2005). SA may enter cells either through the plasmodesmata (symplastic pathway) or through the cell wall (apoplastic pathway). In the above case, the hormone must first cross the donor cells plasma membrane, then the receiving cells plasma membrane. The physicochemical properties of SA, especially in its undissociated form, make it well suited for rapid diffusion through the plasma membrane of animal and plant cells. SA will accumulate in the cytosol (pH = 7.3) under its anionic form through the ion-trap mechanism from the apoplastic compartment (pH = 4.5–5.5) (Yalpani et al., 1991). Nonetheless, there is a sluggish SA efflux (Chen et al., 2001). When 20 or 200 lM SA is applied to tobacco cells in suspension, it is quickly absorbed (within 5 min). The cells treated with 20 and 200 lM, respectively excrete 50 and 85% of the absorbed SA after 5 hours. EGTA inhibits SA excretion greatly, and the inhibition can be reversed by adding Ca^{2+} in the 200 lM treatment but not in the 20 lM treatment. Similarly, only the 200 lM treatment of cycloheximide prevents SA excretion. According to the scientists, this may point to the presence of an inducible SA efflux carrier in addition to a constitutively current efflux carrier under the latter experimental conditions (Chen et al., 2001; Kawano et al., 2004).

4 The Response of Plant Stages to Salicylic Acid

Because of its function as an endogenous signal mediating local and systemic plant defense responses against pathogens, salicylic acid (SA) has been the subject of intense research in recent years. Also, SA is involved in the plant response to abiotic stresses including drought, chilling, heavy metal toxicity, heat and osmotic stress. In this way, SA tends to be an important therapeutic agent for plants, just as it is for mammals. Apart from its role in biotic and abiotic stress, SA is important for the control of physiological and biochemical processes throughout the plant lifespan. The identification of its targets and knowledge of its molecular mechanisms of action in physiological processes may aid in the dissection of the complex SA signaling network, confirming its significance in plant health and disease. The evidence for the function of SA in plant growth and development is examined here by comparing exogenous SA application experiments with genotype analysis of genotypes affected by SA levels and/or perception.

Under stress conditions, SA regulates essential plant physiological processes such as photosynthesis, nitrogen metabolism, proline (Pro) metabolism, glycine betaine (GB) development, antioxidant protection system, and plant-water ties, protecting plants from abiotic stresses (Khan et al., 2010, 2012a,b,c, 2013b, 2014; Miura & Tada, 2014; Nazar et al., 2011). In addition to its participation in the induction of defense-related genes and stress resistance in biotic stressed plants (Kumar, 2014), SA has been shown to enhance plant tolerance to severe abiotic stresses such as metal (Zhang et al., 2015), salinity (Khan et al., 2014, Nazar et al., 2015), osmotic (Alavi et al., 2014), drought (Fayez and Bazaid 2014) and heat stress (Khan et al., 2013b).

4.1 Germination Stage

Exogenous SA has an impact on seed germination. Crop germination is regulated by environmental factors as well as interactions between plant hormones such as abscisic acid (ABA), jasmonic acid (JA), gibberellins (GAs), ethylene (ET), brassinosteroids (BRs), auxins (AUXs) and cytokinins (CKs). The role of SA in seed germination has been a source of debate, with contradictory reports claiming that it can either inhibit or increase seed vigor. The identified inconsistencies can be attributed to the SA concentrations used. SA concentrations >1 mM delay or even prevent germination in *A. thaliana* (Rajjou et al., 2006). In barley, SA doses ranging from 3 to 5 mM completely inhibit seed germination (Xie et al., 2007), while SA doses ranging from 3 to 5 mM completely inhibit seed germination in maize (Guan & Scandalios, 1995). SA negative seed germination regulator effect is most likely due to oxidative stress induced by SA. Because of increased activity of Cu, Zn superoxide dismutase and inactivation of H_2O_2 degrading enzymes catalase and ascorbate peroxidase, H_2O_2 levels in Arabidopsis plants treated with SA (1–5 mM) increase up to three-fold (Rao et al., 1997). Seed germination is aided by SA in the presence of abiotic stress. SA substantially enhances Arabidopsis seed germination and seedling establishment under various abiotic stress conditions when applied exogenously (Alonso-Ramrez et al., 2009; Rajjou et al., 2006).

Just 50% of Arabidopsis seeds germinate under salt stress (100–150 mM NaCl), but seed germination rises to 80% in the presence of SA (0.05–0.5 mM). Alonso-Ramrez et al., (2009) found that exogenous application of SA partially reverses the inhibitory effects of oxidative (0.5 mM paraquat) and heat stress (50 °C for 3 h) on seed germination. These findings are consistent with the Arabidopsis sid2 mutant delayed germination phenotype under high salinity (Alonso-Ramrez et al., 2009). Because of a mutation in the isochorismate synthase gene, this mutant has low SA levels (Table 7.1).

While NahG transgenic lines expressing bacterial salicylate hydroxylase have lower SA levels than wild-type plants, salinity does not affect germination (Borsani et al., 2001). The apparent discrepancy is due to the catechol antioxidant influence, which is a consequence of the salicylate hydroxylase that accumulates in NahG seeds and seedlings (Lee et al., 2010). Thus, oxidative harm reduces the germination promotion effect of SA under high salinity conditions. Furthermore, proteomic analyses revealed that SA activates two superoxide dismutases in Arabidopsis germinating seeds, suggesting that SA boosts antioxidant ability (Rajjou et al., 2006). Also, SA treatment (0.5 mM for 24 h) results in a substantial up-regulation of translation initiation and elongation factors, proteases and two subunits of the 20S proteasome, supporting the hypothesis that SA enhances seed germination by encouraging the synthesis of important germination proteins and the mobilization or degradation of seed proteins accumulated during seed maturation. Also, the biosynthesis of several enzymes involved in metabolic pathways such as the glyoxylate cycle, the pentose phosphate pathway, glycolysis and gluconeogenesis is strongly activated by SA, suggesting that SA promotes the release from a quiescence state to the establishment of a vigorous seedling (Rajjo et al., 2006).

In hormone signaling integration, the ubiquitin-proteasome system (UPS) pathway is essential. Since GAs and ABA play opposing roles in germination control, SA could serve as a rheostat for both hormones. The UPS appears to be a mechanism that balances the antagonistic regulation of seed germination between ABA and GAs, according to recent biochemical data (Piskurewics et al., 2008; Zentella et al., 2007). Multiple hormone signaling pathways converge in the UPS, affecting many aspects of plant growth and development (Santner & Estelle, 2009, 2010; Vierstra, 2009).

NPR1, a central transducer of SA signaling in plant defense responses, has recently been discovered to interact with Cullin3-based E3 ligases and other components of the COP9 signalosome, which regulates proteasomal degradation, in the nucleus. Furthermore, SA-induced phosphorylation of Ser11 and Ser15 residues promotes NPR1 proteasome-mediated turnover (Spoel et al., 2009). The transcriptional co-activator NPR1 is predominantly sequestered in the cytoplasm as an oligomer, but in pathogen-infected cells, SA accumulation promotes the partial reduction of the NPR1 oligomer to a monomer, which is targeted to the nucleus by a bipartite nuclear localization sequence (Mou et al., 2003). When basal SA levels are low and no infection is present, NPR1 reaches the nucleus and it has been proposed that it regulates additional genes. If this scenario is confirmed, it will be important to investigate the role of these genes in regulation.

4.2 Vegetative Stage

Salicylic acid (SA) naturally occurs in plants in very low amounts and participates in the regulation of physiological processes in the plant such as stomata closure, nutrient uptake, chlorophyll synthesis and protein synthesis (Khan et al., 2003; Piatelli et al., 1969; Shakirova et al., 2003). Salicylic acid is a dependent signaling pathway that regulates plant responses to both abiotic and biotic stress factors (Rao et al., 2000). It has shown many important functions in the plant and can change the physiological behavior of the plant. Foliar application with relatively low concentrations of salicylic acid also promoted and influenced the growth, development, differentiation of cells and tissues of plants and enhanced the plant growth parameters (Helgi-Öpik & Rolfe, 2005). Salicylic acid promotes some physiological processes and inhibiting others depending on its concentration, plant species, development stages and environmental conditions (Senaratna et al., 2000). The antioxidant capacity and phenolic content of tomato plants were increased at 10–4 M salicylic acid treatment (Khan et al., 2003). This salicylic acid could be expected to influence the growth and yield of lupine plants.

4.2.1 Effect of Salicylates in Root System

Increasing the total soil volume explored by the plant root is one of the most effective ways to boost yield since the plants can absorb more water and nutrients. Increased initiation of secondary roots results in increased rooting density, which is a second significant feature. Both aspects (root length and density) were found to be affected in experiments with salicylic acid. While tolerance to water flows into the root was not included in these studies, it is predicted that a positive relationship would be found. Additional assays were performed to determine the impact of SA on root growth and development after it was discovered that SA influenced rooting. In a 1996 study, it was discovered that foliar application of SA to the shoots of soybean plants had a major effect on the root scale (Gutierrez-Coronado et al., 1998; Fig. 7.8). Similar results were obtained with *Tagetus erecta* using SA solutions at concentrations as low as 10^{-10} M (Sandoval-Yepiz, 2004; Fig. 7.9).

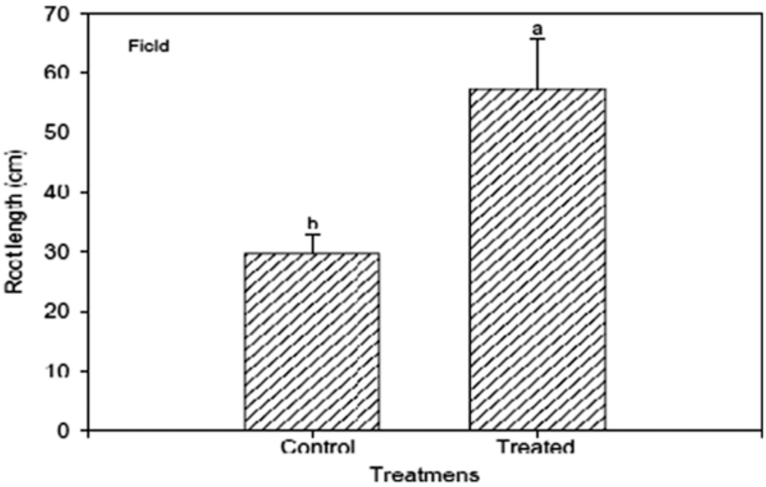

Fig. 7.8 Effect of salicylic acid (8–10 M) applied to the shoot on root length of *Glycine max* seven days after treatment in field conditions. (Source: Larqué-Saavedra and Martin-Mex, 2007)

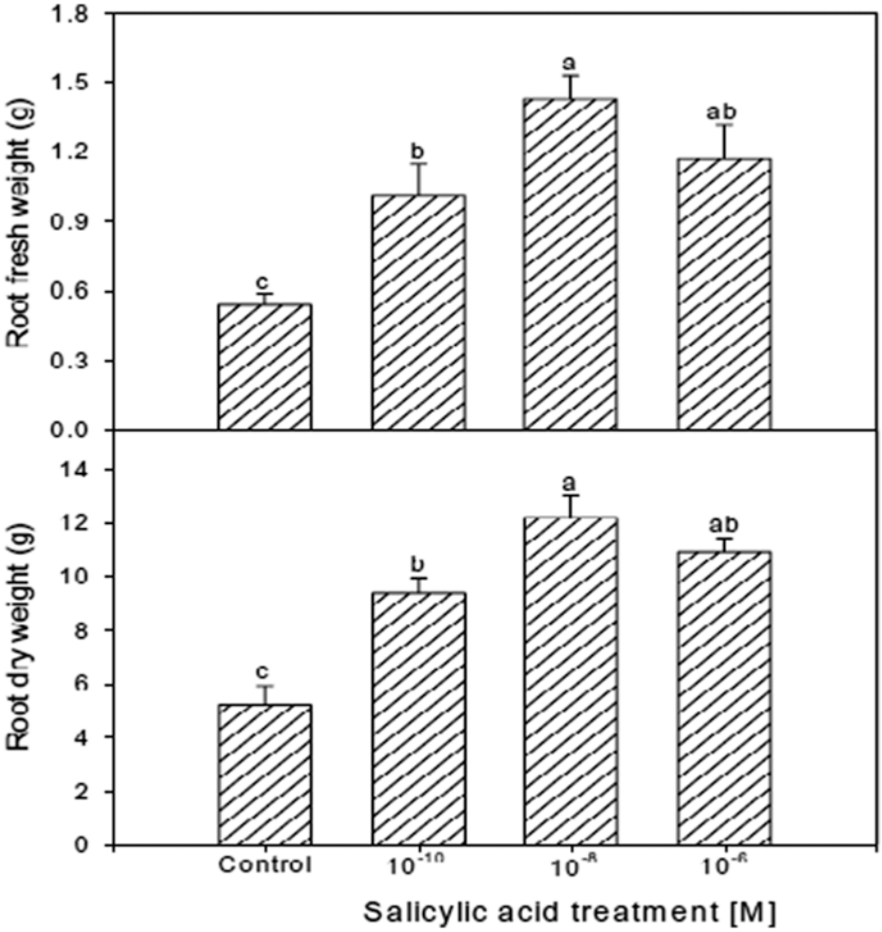

Fig. 7.9 The effect of salicylic acid applications on *Tagetes erecta* fresh and dry mass. (Source: Larqué-Saavedra and Martin-Mex, 2007)

4.2.2 Effect of Salicylates on Shoot Growth

Salicylates increased shoot growth in a variety of plant species, including Clitoria, where biomass production is important as forage for animal feed (Martin-Mex & Larque-Saavedra, 2001). Similarly, as it was applied to ornamental plants, it often increased biomass. In response to SA care, data showed that stem diameter, number of leaves, and fresh and dry mass of the shoot were all higher. SA increased the number of leaves produced in ornamental plants like gloxinia and violet, and the leaf area was over 10% higher than the control. The diameter of the rosette plant was measured at similar amounts (Table 7.1 and Table 7.2).

Table 7.2 The effect of salicylic acid on *Saintpaulia ionantha* growth (violet)

Treatments	Number Leaves	Rosette Diameter (cm)
Control	16 ± 1b	139 ± 16b
10^{-10} M AS	19 ± 2a	177 ± 12a
10^{-8} M AS	18 ± 3ab	152 ± 11ab
10^{-6} M AS	18 ± 2ab	156 ± 10a

Means with the same letter are not significantly different at $P \geq 0.05$, Tukey's. Source: Larqué-Saavedra and Martin-Mex (2007)

4.2.3 Impact of Salicylates on Horticultural Plant Yield

Plants treated with salicylates yielded more in the species, whether grown in a greenhouse or the open. SA caused larger tubers in carrots (60%), beet (16%) and radish (200%) (Aristeo-Cortes, 1998). These results from his preliminary research indicated that SA could play an important role in plant bio-productivity, which could be related to the previously described effect of promoting plant root length. Commercial plantations of *Carica papaya* pre-treated with SA have shown a substantial increase in fruit production in recent years as compared to the monitor. The experiment lasted over 2 years and enabled the plant to complete its life cycle by cultivating the Maradol variety, which is widely planted in the Caribbean. SA is one of the growth regulators that producers are requesting to increase papaya production by about 20%. (Herrera-Tuz, 2004; Martin-Mex et al., 2005). Furthermore, in commercial plantations of tomato grown in the greenhouse and open conditions, lower SA concentrations (6–10 M) increased fruit yield from 90 to 120 ton/ha without affecting fruit quality. This effect was replicated in a cucumber plantation, where the same dose of SA increased yield from 139 to 170 ton/ha while maintaining fruit quality (Larque-Saavedra and Martin-Mex, unpublished results, Fig. 7.10). As a result, the application of SA to horticultural plants may potentially increase their bio-productivity. For better output, lower concentrations of this natural plant growth hormone could be used on other economically important crops.

4.2.4 Photosynthesis

SA effects on leaf and chloroplast structure and RuBisCO activity. Also, SA affects leaf and chloroplast structure (Uzunova and Popova 2000), stomatal closure (Mateo et al., 2004; Melotto et al., 2006), chlorophyll and carotenoid contents (Chandra and Bhatt 1998; Fariduddin et al., 2003; Rao et al., 1997), and the activity of enzymes like RuBisCO (ribulose-1,5-bisphosphate carboxylase/oxygenase) and carbonic anhydrase (Pancheva and Popova 1998; Slaymaker et al., 2002). Exogenous SA has been shown to have different effects on photosynthesis parameters depending on the dosage and plant species studied. High SA concentrations (1–5 mM) reduce photosynthetic rate (PN) and RuBisCO activity in barley plants, as well as reduced

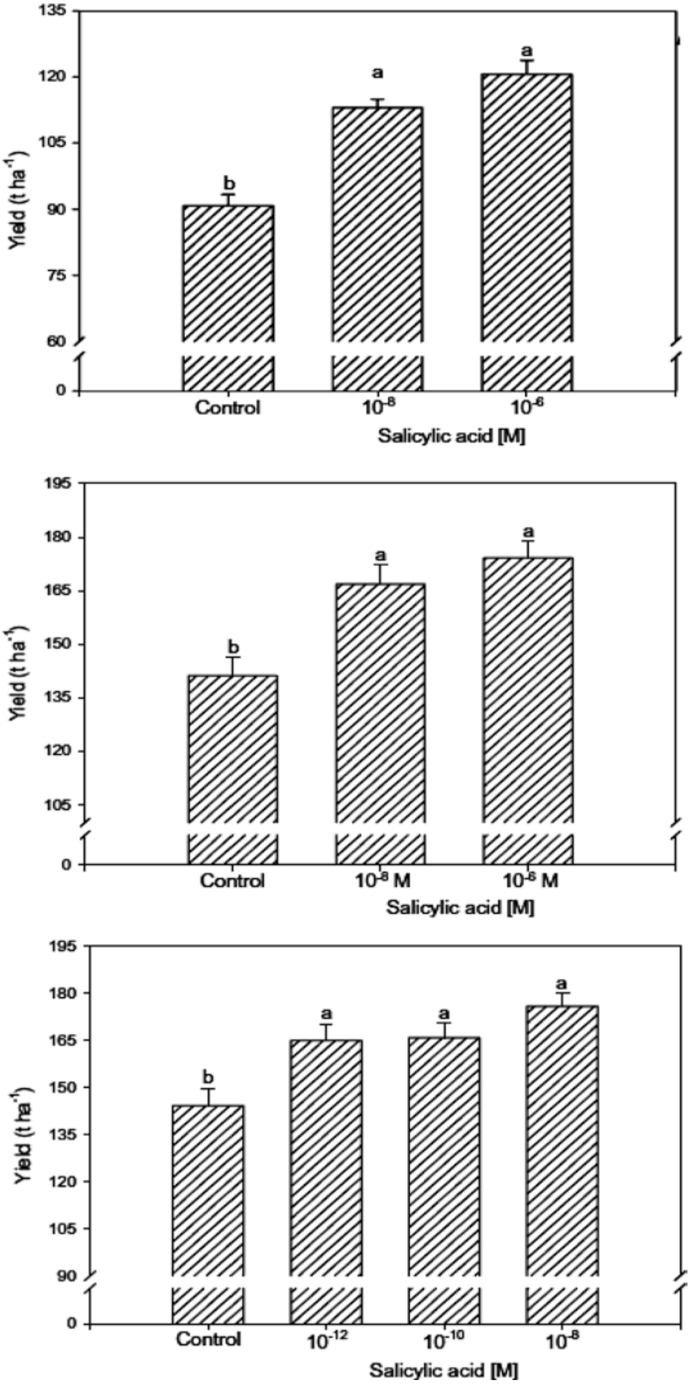

Fig. 7.10 Effects of salicylic acid treatments on *Lycopersicon esculentum* yield (A), *Cucumis sativum* (B) and *Carica papaya* (C). (Source: Larqué-Saavedra and Martin-Mex, 2007)

chlorophyll content in cowpea, wheat, and Arabidopsis (Chandra and Bhatt 1998; Moharekar et al., 2003; Pancheva et al., 1996; Rao et al., 1997). Pancheva and Popova (1998) attributed the decline in RuBisCO activity to a 50% reduction in protein levels relative to non-treated plants, while total soluble protein decreased by 68%. Exogenous SA causes a reduction in the width of the adaxial and abaxial epidermis, as well as the mesophyll tissue, in the leaf anatomy. Ultrastructurally, such modifications are associated with an increase in chloroplast length, swelling of grana thylakoids, and stroma coagulation (Uzunova and Popova 2000). Thus, SA effects on thylakoid membranes and light-induced reactions linked to them cause reduced photosynthetic activity at high concentrations.

In mustard seedlings, a lower concentration of SA (10 lM) increases photosynthetic net CO_2 assimilation. Carboxylation performance, chlorophyll content, and carbonic anhydrase and nitrate reductase activities are all up-regulated as PN rises (Fariduddin et al., 2003). Since elevated AUX levels increase PN and nitrate reductase activity, it was suggested that the beneficial effects of this low dose of SA in photosynthesis may be linked to the prevention of AUX oxidation by SA (Ahmad et al., 2001).

4.2.5 Respiration

The alternative oxidase (AOX) pathway is regulated by SA. SA regulates the AOX pathway by inducing its gene expression in both thermogenic and non-thermogenic plants (Kapulnik et al., 1992; Rhoads & McIntosh, 1992). The addition of 2–20 lM SA to tobacco cell suspension culture induces an increase in cyanide resistant O_2 absorption within 2 h, as well as a 60% increase in the rate of heat evolution from cells, as measured by calorimetry (Kapulnik et al., 1992). Furthermore, SA treatment causes NtAOX1 gene expression to increase in a concentration-dependent manner, which is linked to protein abundance. After 4 hours of SA therapy, the abundance of NtAOX1 transcripts increases 2–six-fold, then decreases. AOX combines ubiquinol oxidation with molecular oxygen reduction to produce water in a reaction that is unaffected by cytochrome oxidase inhibitors. AOX is a possible target of SA for plant growth regulation because it is a non-proton-driven carrier that allows versatile control of ATP synthesis to maintain growth rate homeostasis (Moore et al., 2002). Also, AOX is thought to inhibit the formation of reactive oxygen species (ROS) in mitochondria. Overexpression of AOX causes a 57% reduction in ROS abundance in cultured tobacco cells, while antisense suppression of AOX causes a five-fold increase in ROS levels relative to wild-type cells. A second oxidase downstream of the ubiquinone (UQ) pool has been proposed as a way to keep upstream electron transport components more oxidized, lowering ROS generation by the respiratory chain (Maxwell et al., 1999).

Mitochondrial electron transport is inhibited. SA can regulate electron transport and oxidative phosphorylation in plant mitochondria, in addition to inducing the alternative respiration pathway, which is dependent on the expression of the AOX gene (Norman et al., 2004; Xie and Chen 1999). In tobacco cell cultures, SA inhibits both ATP synthesis and respiratory O_2 uptake within minutes of incubation, even at concentrations as low as 20 lM. Within the first 30 minutes of incubation, treatment with 500 lM SA reduces ATP levels by 50%, during which they begin to fall to as low as 15% of control levels (Xie & Chen, 1999).

4.2.6 Nutrients Uptake

Experiments were carried out to see whether the possible beneficial impact of SA on root growth could be linked to an improvement in macronutrient and micronutrient uptake. This was accomplished by measuring these elements in the plant tissues of treated plants. The results revealed that plants treated with SA had substantially higher levels of macronutrients in their tissues. In contrast, to control plants, nitrogen, phosphorus and potassium accumulation was higher in fruits (116, 110, and 97%), leaves (45.5, 39.4 and 29.1%), roots (52.6, 17 and 29.4%), and stems (5.0, 39.4 and 28.3%). The majority of the plant tissues treated with SA had higher levels of other nutrients including copper, zinc, manganese, boron, calcium, magnesium, and iron (Table 7.3; Fig. 7.11 and Fig. 7.12).

4.3 Flowering Stage

The effect of salicylates on the flowering process was assessed since it is a parameter that is closely related to productivity. In this respect, it has been reported that SA is a flower-inducing factor in *Lemna* (Cleland & Ajami, 1974). They discovered that this photoperiod-sensitive plant flowering phase was aided by an endogenous amount of SA. SA capacity to cause flowering. For a long time, scientists have known that SA plays a role in flowering control. Initially, it was discovered that 4

Table 7.3 The effect of spraying habanero pepper plantlets (*Capsicum chinense*) with 1 M salicylic acid on the content of macronutrients in different organs was harvested 128 days later

		N	P	K	Ca	Mg
Tissue	Treatment	Mg Planta $^{-1}$				
Fruit	Control	150.18b	12.41b	101.12b	33.81a	11.22b
	1µM de AS	325.72a	26.13 a	199.37 a	34.83 a	19.85a
Leaf	Control	440.93b	26.01b	108.79 b	417.10b	78.44b
	1µM de AS	641.94	36.27a	140.46a	616.33a	131.27a
Stem	Control	512.14b	16.09b	142.62b	311.55b	111.95a
	1µM de AS	561.04a	20.97a	183.12a	437.62a	191.36b
Root	Control	1353.53b	90.04b	475.03b	1819.98a	303.65a
	1µM de AS	2065.70a	105.88a	615.12a	1861.48a	382.28a

Values with the same letter are not significant (Tukey $P < 0.05$). Each value is the mean of five individuals (Data adapted from preliminary research work to be published). Source: Tucuch-Haas et al., (2017)

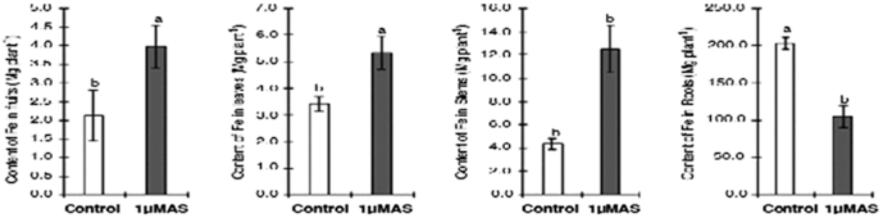

Fig. 7.11 The effect of a 1 M salicylic acid spray on the iron content of the root and leaf. Stem and fruit of habanero pepper plants. Values that begin with the same letter are unimportant. Each value is the mean of five individuals ± s.e. (Data adapted from preliminary research work to be published). (Source: Tucuch-Haas et al., 2017)

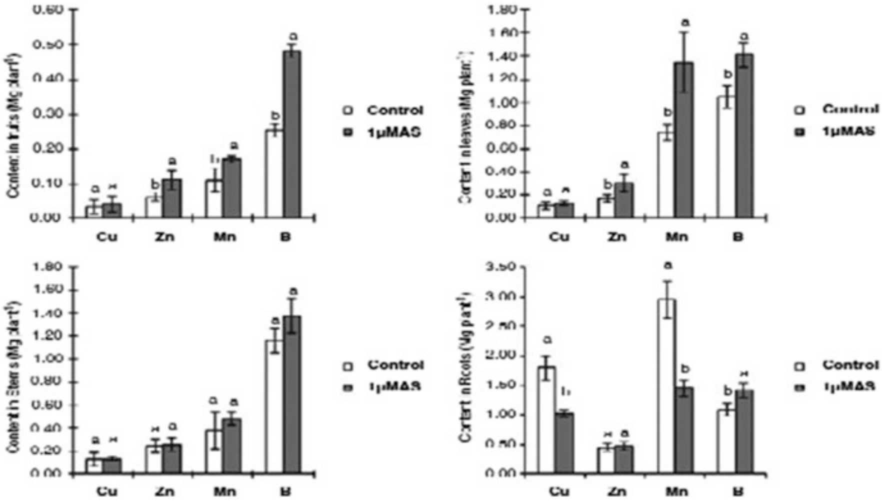

Fig. 7.12 Copper (Cu), zinc (Zn), manganese (Mn), and boron (B) concentrations in the root, leaf, stem, and fruit of habanero pepper plants sprayed with 1 M salicylic acid. Values with the same letter are not significant (Tukey $p = 0.05$). Each value is the mean of five individuals (Data adapted from preliminary research work to be published). (Source: Tucuch-Haas et al., 2017)

lM SA promotes the development of flower buds from tobacco callus (Lee & Skoog, 1965). SA was later established as the phloem-transmissible factor secreted in aphid honeydew that causes flowering in *Lemna gibba* plants grown in non-photoinductive light (Cleland & Ajami, 1974). SA (3–10 lM) promotes flowering in some *Lemnaceae genera*, including long-day (LD), short-day (SD) and photoperiod-insensitive varieties (Khurana & Cleland, 1992). Flowering is caused by inadequate nutrition stress in the SD species *Pharbitis nil*. Treatment with amino-oxyacetic acid, a phenylalanine ammonia-lyase inhibitor, prevented flowering under these conditions, but SA application restored it. Only under stress is this behavior observed; thus, it appears that SA is needed but not sufficient to induce flowering (Wada & Takeno, 2010; Wada et al., 2010).

Further research has shown that thermogenic plants have high endogenous SA levels in their inflorescences (Raskin et al., 1990) and that SA levels in non-thermogenic plants like tobacco and Arabidopsis increase 5 and two fold in their leaves at the onset of or during the transition to flowering, respectively (Abreu & Munne-Bosch, 2009; Yalpani et al., 1993). According to these results, SA-deficient Arabidopsis plants (NahG, sid1/eds5 and sid2) flower late under both SD (8 h light and 16 h dark) and LD (16 h light, 8 h dark) conditions, suggesting that SA interacts with photoperiod and autonomous pathways (Martinez et al., 2004). A crucial connection between SA and flowering has recently been discovered in sunflowers. The HD-Zip II transcription factor HAHB10 induces flowering in Arabidopsis by upregulating unique flowering transition genes and repressing genes linked to biotic stress when expressed constitutively. Interestingly, HAHB10 expression is caused by SA treatment and *P. syringae* infection (Dezar et al., 2011).

SA and photoperiod interaction, as well as autonomous pathways. In Arabidopsis, flowering is regulated by an interconnected network of many pathways and the roles of several genes have been identified. CONSTANS (CO) is a central regulator of the photoperiod pathway, FLOWERING LOCUS C (FLC) is a flowering repressor that integrates the autonomous and vernalization pathways, and these pathways converge on a small number of integrators such as flowering locus T (FT) and suppressor of overexpression of constant 1 (SOC1) and these pathways converge on a small number of integrators such as flowering locus T (FT (SOC1). The SOC1 gene, which encodes a MADS-box protein that is regulated by CO and repressed by FLC, binds the photoperiod and autonomous pathways (Mouradov et al., 2002). SA has recently been discovered to play a role in controlling the transcription of these genes (Martinez et al., 2004).

Under either SD or LD conditions, the late-flowering phenotype of SA-deficient plants is associated with 2- to three-fold higher expression of the floral repressor gene FLC and decreased levels of the FT transcript relative to wild-type plants. Furthermore, exogenous application of 100 lM SA to Arabidopsis wild-type plants lowers FLC transcript levels, and UV-C light irradiation, which triggers SA accumulation, activates FT expression.

Interestingly, even though SA appears to be a repressor of FLC expression, FLC-3 NahG mutant transgenic lines flower at the same time as their parental plants grown under LD and SD conditions, indicating that this gene is not needed for the late-flowering phenotype of SA-deficient plants. Similarly, the expression of other genes in SA-deficient plants, such as CO and SOC1, differs between SD and LD conditions. In LD-grown SA-deficient plants, levels of CO and SOC1 transcripts decrease; 50% when compared with wild-type plants, but in SD-grown SA-deficient plants the transcript levels of CO increase 2 to three fold and SOC1 expression does not change, compared with wild-type plants. Under LD conditions, exogenous SA (100 lM) could revert the late-flowering phenotype of the co-1 mutant, but not of the soc1 mutant, according to genetic analysis of SA interactions with these components of the photoperiod pathway. As a result, this evidence indicates that SA controls flowering by communicating with a CO-independent branch of the photoperiod-dependent pathway (Martinez et al., 2004).

Arabidopsis SIZ1 is a key flowering regulator through the control of SA-mediated floral promotion. Loss-of-function siz1 mutants have an early flowering phenotype under SDs that correlates with high SA levels. Early flowering is suppressed in these mutants when NahG is overexpressed. FLC expression is positively regulated by SIZ1 through sumoylation of FLD (FLOWERING LOCUS D), a plant orthologue of human histone demethylase 1. (Jin et al., 2008). Additional evidence from genetic approaches has shown that the SA-induced transition to flowering is also dependent on the autonomous pathway genes LD, FVE, and FCA.

The delayed flowering phenotype of ld-1, fve-3, and fca-9 mutants is unaffected by 100 lM SA. Furthermore, under SD conditions, fve-3 NahG and fca-9 NahG plants flower later than their parental plants, but only fca-9 NahG plants flower after fca-9. It has been proposed that SA regulates flowering time in LD-grown plants through an FCA-independent pathway that may be regulated by FVE, whereas under LD conditions, SA may regulate integrator genes such as FT and SOC1 in parallel to both branches of the autonomous pathway. Since NahG-overexpressing plants are completely sensitive to cold temperatures, exogenous GAs, or constitutive activation of the GA signaling pathway in the spy-3 mutant context for flower growth, the vernalization and GA pathways do not appear to be affected by SA (Martinez et al., 2004). More research is needed to determine how the SA signaling pathway interacts with other hormones involved in Arabidopsis flowering time control, the mediators of this crosstalk (MAPKs, transcriptional regulators, or transcription factors), and whether SA regulates flowering through NPR1.

4.4 Reproductive Stages

4.4.1 Growth

In contrast to other plant hormones, the role of SA in plant growth has received little attention. Most reviews on this subject either ignore SA or give only a cursory description of its function (Santner & Estelle, 2009; Santner et al., 2009; Wolters & Jurgens, 2009). Exogenous SA effects on vegetative development. Exogenous SA has a different impact on plant growth depending on the plant species, developmental stage and SA concentrations measured. SA has been shown to promote growth in soybean (Gutierrez-Coronado et al., 1998), wheat (Shakirova et al., 2003), maize (Gunes et al., 2007) and chamomile (Kovacik et al., 2009). Shoot and root growth increased in soybean plants treated with 10 nM, 100 lM and up to 10 mM SA. Shoot and root growth increase 20 and 45% in soybean plants treated with 10 nM, 100 lM, and up to 10 mM SA 7 days after application. Wheat seedlings treated with 50 lM SA produce larger ears and the apical meristem of seedling roots shows increased cell division (Shakirova et al., 2003). Similarly, 50 lM SA stimulates the growth of chamomile plant leaf rosettes and roots by 32 and 66%, respectively, but higher concentrations (250 lM) have the opposite effect (Kovacik et al., 2009). It has been proposed that SA growth-promoting effects are linked to improvements in hormonal status (Abreu & Munne-Bosch, 2009; Shakirova et al., 2003) or the improvement of transpiration, photosynthesis and stomatal conductance (Stevens et al., 2006). Exogenous SA (100 lM and 1 mM) inhibits trichome production in *A. thaliana* by reducing trichome density and number (Traw & Bergelson, 2003). Although the biochemical events involved in SA regulation of cell division and development are unclear, these findings are consistent with the acetylated derivative antiproliferative properties in mammalian tumor cell lines (Dihlmann et al., 2001; Ruschoff et al., 1998).

The SA signaling pathway and Arabidopsis growth rate are related. The characterization of Arabidopsis mutant or transgenic plants affected in the SA signaling pathway provides more clear evidence supporting the key function of endogenous SA in the regulation of plant cell development. Arabidopsis plants that overexpress the SA-inducible DOF (DNA binding with one finger) transcription factor OBP3 have slowed growth in both their roots and aerial portions, leading to death in the worst cases (Kang & Singh, 2000). Arabidopsis mutants with constitutively elevated levels of SA, such as cpr5 (constitutive expression of PR5; Bowling et al., 1997), acd6–1 (accelerated cell death; Rate et al., 1999) and agd2 (accelerated cell death; Rate et al., 1999), display this dwarf phenotype (aberrant growth and death; Rate & Greenberg, 2001). When compared to wild-type Arabidopsis NahG transgenic plants, the SA depleted Arabidopsis NahG transgenic plants have a faster growth rate (Abreu & Munne-Bosch, 2009; Du et al., 2009), which is expressed in a 1.7-fold increase in leaf biomass (Abreu & Munne-Bosch, 2009).

Another intriguing result is that at HL intensities, the growth inhibitory effect of high SA levels in many Arabidopsis CPR mutants is partially overcome. Under HL conditions, the dwarf phenotypes of cpr6–1, cpr5–1, and dnd1–1 are partially reverted, while cpr1–1 reverts to almost normal development. This mutant growth retardation is caused by reduced photosynthetic activity, and they can increase PSII operating efficiency during acclamatory responses to HL (Mateo et al., 2006). SA appears to be a key molecule in maintaining a proper balance between photosynthesis and development, even though the exact mechanisms are unknown.

4.4.2 Effect of Fruit Formation

After demonstrating the impact of SA on the flowering phase, the fructification of plants treated with this growth regulator was assessed. The results show that the plants sprayed with 1μM SA produced 342 fruits, compared to only 244 for the control plants. In other words, compared to the monitor, the treated plants produced 40% more fruits. Fruit type measurements revealed no significant differences between the apical diameter and the medial zone (Table 7.4).

Table 7.4 Effects of spraying habanero pepper plantlets (*Capsicum chinense*) with 1µM salicylic acid on various fruit estimators. The mean of 20 repetitions ± s.e. is presented

	Treatments	
Variable	Witness	AS_1X10–6
NFP	244.8 ± 28.6b	342 ± 52.9a
AWF	3.6 ± 0.34a	3.7 ± 0.20a
WFP	770.0 ± 45.1b	1032.7 ± 100.2a

NFP Number of fruits per plant, AWF Average weight per fruit, WFP Average weight of fruits per plant. Means with the same letter between each line are statistically equal (Tukey, a = 0.05 for NFP, PPF; Duncan, a = 0.05 for PFP) (Data adapted from preliminary research work to be published). Source: Tucuch-Haas et al. (2017)

5 Role of Salicylic Acid Response in Plants under Abiotic Stresses

Different abiotic stresses are still critical trouble affecting crop yield worldwide (Minhas et al., 2017) (Fig. 7.13). Koo et al., (2020) declared that research results up to date confirmed that SA could adjust various plant reactions which make plants able to tolerate different abiotic stresses. As well as Fayez and Bazaid (2014) mentioned before that plant ability to tolerant most of the abiotic stresses was developed by SA. Applying SA to abiotically stressed plants was an important factor to stimulate different mechanisms of tolerance (Khan & Nafees, 2014), as it plays an efficient role in controlling the vital growth process and strengthen plant defense reactions cultivated under abiotic stressed conditions (Miura and Tada 2014). Chilling, heat, salinity, toxic metals and drought, harm plant yield, salicylic acid emphasizes plants growth under various abiotic stresses (Bali et al., 2017), but according to Horvàth et al., (2007), SA can positively affect specific enzymes or defense system genes in plants to tolerate stresses (Fig. 7.14).

The quality and productivity of the main crops such as wheat, maize, rice, and barley are diminished mainly due to water shortage (Sabagh et al., 2020), since it affects the anthesis time, ending with poor quality small seeds (Fahad et al., 2017). Results of Ghilavizadeh et al., (2019) indicated the usage of that SA increased grains productivity when fennel plants subjected to drought at half flowering and budding phase. Ilyas et al., (2017) referred to the SA function in controlling different crop reactions under drought. Agami et al., (2019) referred to the role of salicylic acid in improving wheat tolerance to drought stress. The negative impact of drought on different plants was mitigated when applying SA according to many scientists (Jini and Joseph 2017). Agami et al., (2019) concluded that SA can improve the productivity and growth process of the drought-stressed plants to be close to them in un-stressed plants. According to Kang et al., (2012), tolerance of wheat seedlings that were pre-treated with SA was improved under low water stress**.** Canales et al., (2019) referred to SA role in modifying oat plant responses to a shortage of water. Also, Sánchez-Martín et al., (2015) mentioned that the usage of SA in a specific dose reduced the negative impact of water lowering on oats plants. Plant

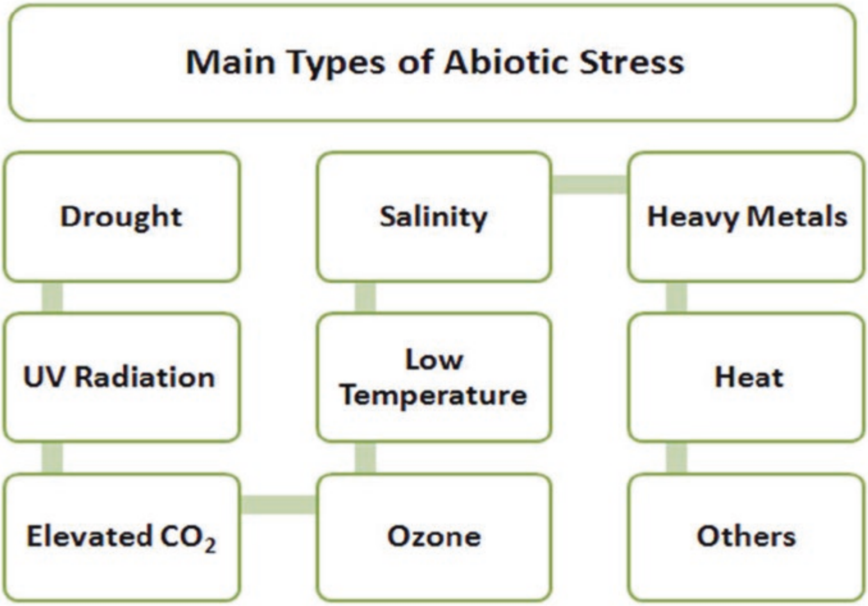

Fig. 7.13 Main types of abiotic stresses in the world. (This diagram was constructed by MM Saleh)

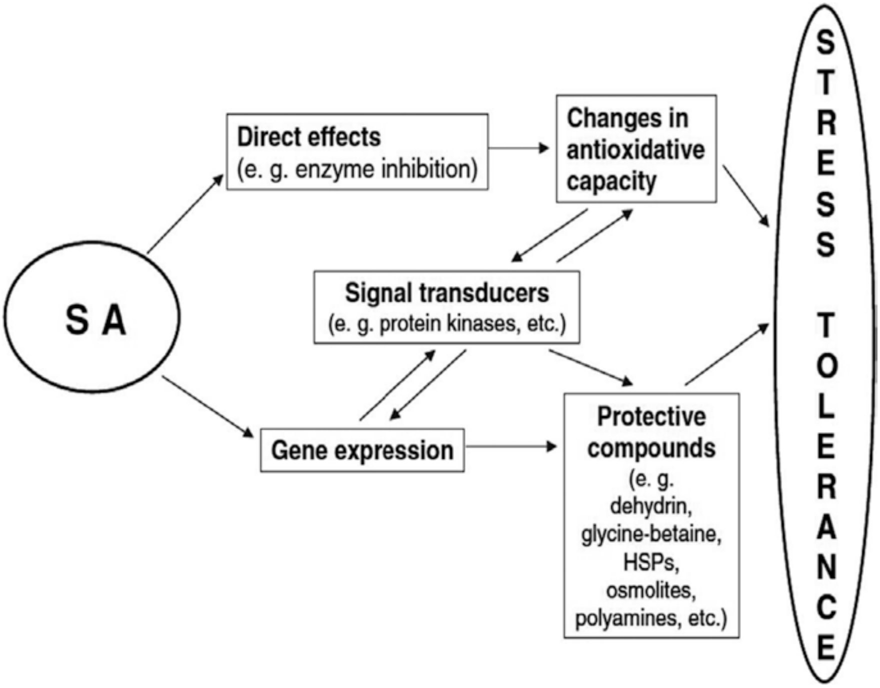

Fig. 7.14 Diagram of the SA role in stimulation the tolerance of abiotic stresses. (Source: Horvàth et al., 2007)

growth stage, ecological circumstances, plants nature and SA usage dose are all limiting factors of SA role in mitigating drought stress impact on crops Miura and Tada (2014). Hara et al., (2012) found that high doses of salicylic acid can lead to plant death or at least to make plants unable to resist the abiotic stresses, while the low doses can improve the defense system in different species of plants. It was found that SA enhanced yield traits and growth process in roots, as well as oil percentage in seeds of the spice fennel plants cultivated under different circumstances of water (Askari and Ehsanzadeh 2015). The results of Damalas (2019) referred to SA ability to diminishing many negative effects of drought on sweet basil yield and plant growth. El-Esawi et al., (2017) reported that drought stress did not affect rosemary plants when they applied SA. Similar results were concluded concerning main crops in the world like barley (Pirasteh-Anosheh et al., 2015), and cotton (El-Beltagi et al., 2017), in addition to vegetables such as lettuce (Khalifa et al., 2016), *Brassica juncea* (Kannaujia & Vaipeyi, 2018), okra (Munir et al., 2016) and radish (Raza & Shafiq, 2013), the spicy plants like peppermint (Figueroa Pérez et al., 2014) and fruits like strawberry (Eshghi & Jamali, 2012). Bandursk (2013) mentioned that the destructive impact of drought on plants could be diminished by SA which encouraged defense processes via various mechanisms (Fig. 7.15).

5.1 Salinity Stress

Wang et al., (2019) referred to the damage role of soil salinity on plants worldwide. Misra et al., (2020) declared that salinity affects almost 13% of lands globally. Metabolic processes are negatively affected by salt stress (Emilia et al., 2020) (Fig. 7.16).

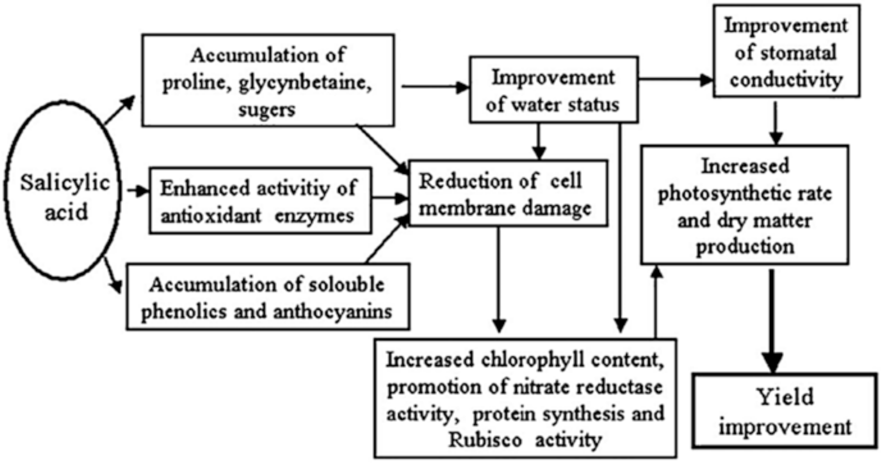

Fig. 7.15 Diagram of SA action in emphasizing plant resistance to drought stress. (Source: Bandursk 2013)

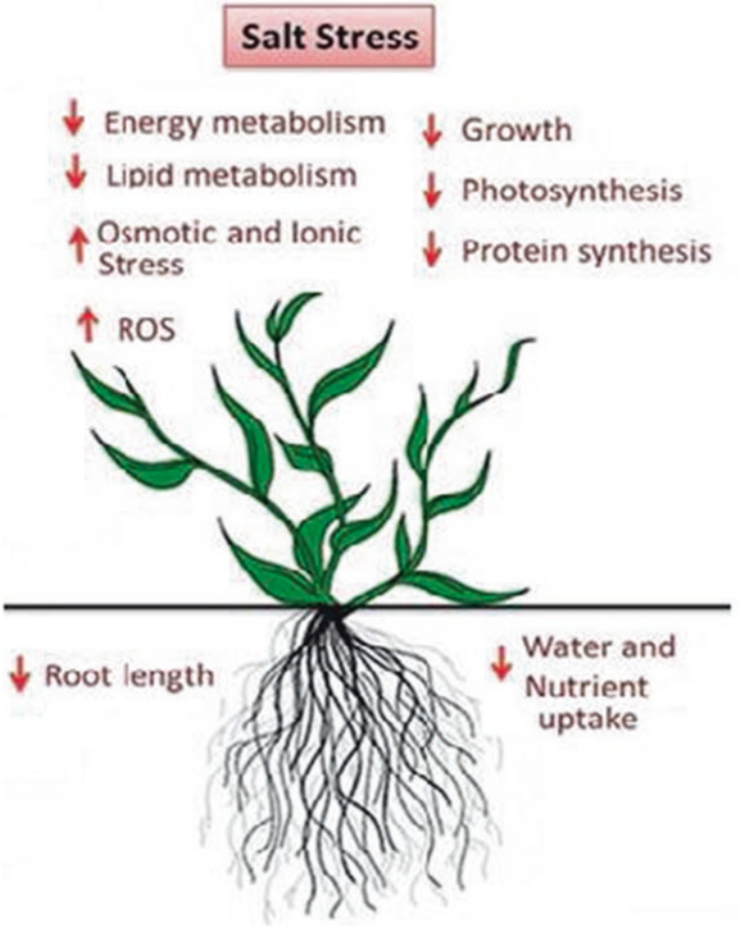

Fig. 7.16 Diagram of the negative impact of salt stress on the plant. (Source: Adapted from Emilia et al., 2020)

Many scientists affirmed the role of salicylic acid in improving crops tolerance under salt stress like *Brassica juncea* (Nazar et al., 2015) and *Medicago sativa* (Palma et al., 2013). SA controls plant growth process cultivated under salt stress, cooperates with other hormones, antioxidants and reacts with reactive oxygen species ROS, enhances nutrition which all ensuing salinity tolerance (Fig. 7.17) (Hoque et al., 2020). Results of Bastam et al., (2013) indicated that the usage of SA will enhance the growth parameters of plants cultivated under salt stress. SA stimulates salinity tolerance and rises the final biomass in salt-stressed *Torreya grandis* plants (Li et al., 2014). According to Lee et al., (2010), seed germination in different salt-stressed plants is emphasized after the external usage of SA. The harmful impact of salinity can be diminished by SA (Pirasteh-Anosheh et al., 2014). Miura and Tada (2014) clarified the role of SA in controlling the defense system and metabolism

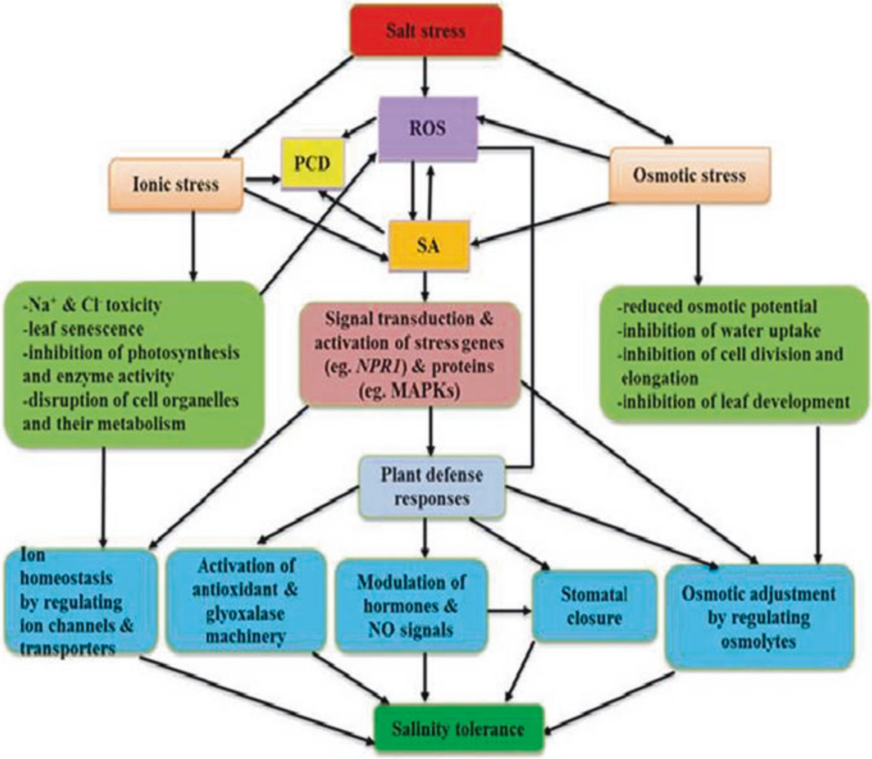

Fig. 7.17 A diagram represents the role of SA in plant tolerance to salinity. (Source: Hoque et al., 2020)

process in crops under salt stress (Wang et al., 2012). SA was reported as a significant tool in alleviating plant tolerance to salinity especially in *Vicia* (Khan et al., 2014), they referred to the positive effect of SA in mitigating the salinity impact in barley plants (Khan et al., 2014). Li et al., (2013) concluded that the usage of SA increased salinity tolerance in bread wheat plants. The various damages effect of salinity on eggplants could be reduced when using SA (Csiszàr et al., 2014). Nazar et al., (2015) reported that SA shrinks the impact of salinity and they concluded that SA increases absorption of sulfur and nitrogen which are both related to the tolerant plants under salt stress. Tufail et al., (2013) said that SA sustained various plant processes relating to growth under salinity. Results of El-Beltagi et al., (2017) indicated that the usage of SA in treating *Gossypium barbadense* crop improved productivity traits and raised components in leaves under salinity stress. The different negative outcomes of salt stress on beans can be diminished by the usage of SA (Khan and Nafees 2014). Jayakannan et al., (2013) concluded that usage of SA increased the weight of salt-stressed Arabidopsis seedlings. Kalaivani et al., (2016) explained that the pretreatment with SA leads to significant raise in germination and increasing plant weight in rice subjected to salt stress since SA play role in providing high absorption of water and nutrients to plant.

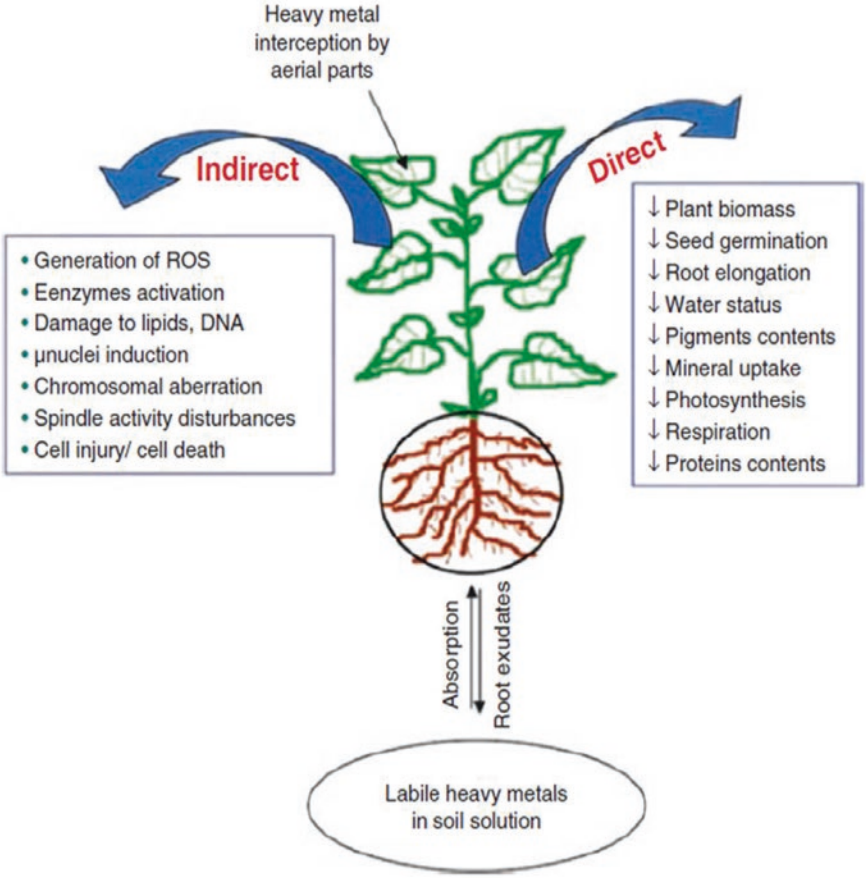

Fig. 7.18 Poisonous impact of the up-taken heavy metals on plants. (Source: Iqbal et al., 2020)

5.2 *Heavy Metals Stress*

The expression of heavy metals according to Iqbal et al., (2020), is used for all metals that have large density causing poisonous and leads to decreasing the productivity of the crop because of the reduction in growth traits (Fig. 7.18). Chapagain et al., (2017) mentioned that heavy metals have many negative effects on the quantity and quality of plants. According to Adrees et al., (2015), the density of heavy metals is bigger than 6 g cm^{-3} (Pinto et al., 2015). Heavy metals turn into critical abiotic stress all over the world, since there are absorbed by crops in high amounts, which causes a reduction in yields and quality of crops (Ramzani et al., 2016). The raised concentration of essential or nonessential metals in soil, causes toxicity for all living organisms including plants (Kumar et al., 2019), while Akpor et al., (2014) said that even the little concentrations of heavy metals are toxic. According to Penna and Nikalje (2018), many metals like iron, zinc, manganese and copper are essential for plant growth, but the high concentration of these metals is harmful. Metals like chromium, selenium, antimony, nickel and arsenic are considered heavy metals (Yadav 2010). Arshad et al., (2017) reported the negative impact of the accumulated heavy metals in crops. Sidhu et al., (2017) explained how many plant growth processes and metabolism were influenced because of heavy metal stress (Sidhu et al., 2017). Plants cultivated under abiotic stresses depend on their survival on the regulation compounds in their defense system like SA (Wani et al., 2017). Zengin (2015) affirmed the role of SA in controlling both respiration and the photosynthesis process, in addition to emphasizing crop defense systems under heavy metal stress. Ghani et al., (2015) resulted that treating the plant with SA consider as the guarantee to keep the plant safe under heavy stress. Faraz et al., (2017) found that treating plants with SA is efficient in stability-enhancing of plant membrane and emphasizing the defense system. The external usage of SA in *B. campestris* plants increases productivity and lowers the negative impact of lead Pb stress (Hasanuzzaman et al., 2019). The growth of *Helianthus annuus* was enhanced when using SA (Saidi et al., 2017). Under chromium Crstress, the growth of *Brassica napus* L. was improved after usage of SA (Gill et al., 2016). Moravcová et al., (2018) results indicated that SA promoted growth parameters concerning leaves and roots of maize crop planted subjected to copper Cu stress. As well as Zaid et al., (2019) results affirmed that SA could recover growth of *Brassica juncea* L. under nickel (NI) stress. Huda et al., (2016) declared that SA improved protein% and most of the growth traits and fixed the stability of plant membrane in rice subjected to chromium Cr stress. Moreover, Gill et al., (2016) affirmed that under chromium Cr stress, rising of plant growth and improvement of all activities of the plant-scavenging system are induced by SA.

5.3 Heat Stress

Out of many abiotic stresses, heat stress considers as the main stress of world concern since it negatively affects productivity and growth of the main crops (Nazar et al., 2017). Pál et al., (2013) defined heat stress as the increase of temperatures degrees for a limited time to the point that negatively affects plant growth stages. Heat hastens the initiation of different growth stages of many crops like maize, sugarcane, cotton and sunflower (Ahmad et al., 2016, 2017; Abbas et al., 2017; Tariq et al., 2018). Heat is the most damaging stress in tropical and subtropical regions, particularly when it is combined with a lack of water, which results in lower yields (Maibam et al., 2020). Sita et al., (2017) said that the prediction rate of increasing heat per decade is 0.2 Celsius degree. Lamaoui et al., (2018) confirmed the damaging impact of the accomplished heat and drought stress. Heat stress leads to necrosis in some crops like sugarcane and considers the main reason for lowering the net assimilation rate (Srivastava et al., 2012) and having dried and rolled leaves (Omae et al., 2012). The operation of the plant membrane is altered by heat stress (Hemantaranjan et al., 2014). In general, plants developed many mechanisms to tolerate heat stress and to outdo its negative impact (Nazar et al., 2017; Rasmussen et al., 2013) (Fig. 7.19).

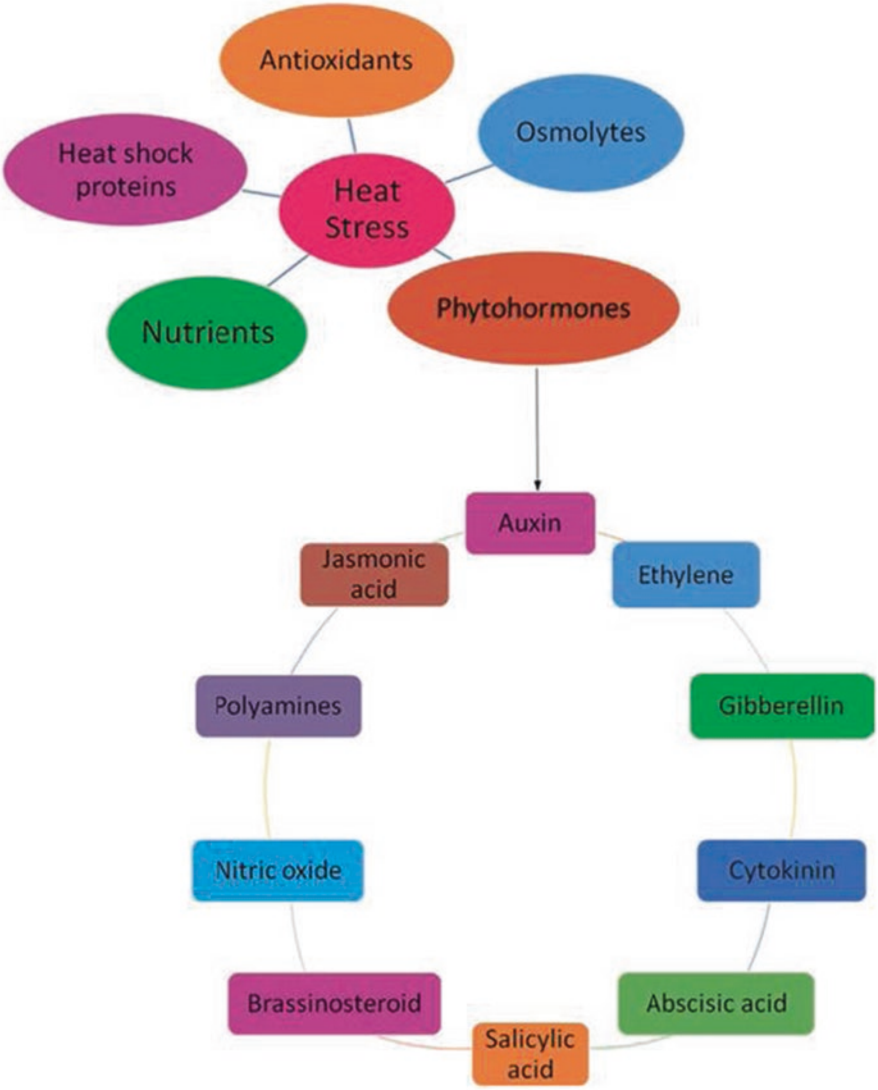

Fig. 7.19 Illustration represents the main strategies in the plant to tolerate heat stress. (Source: Nazar et al., 2017)

Many scientists have confirmed the function of SA in improving heat stress tolerance in crops (Khan et al., 2013a). Lubovská et al., (2014) and Song et al., (2013) mentioned that SA considers the main factor in diminishing the harmful impact of heat stress via controlling most growth stages in plants (Asensi-Fabado et al., 2013). In another study, Galani et al. (2016) recommended applying SA on cotton seedlings to emphasize their tolerance and to decrease the negative effects of heat stress.

Shi et al., (2006) found that using SA in *Cucumis sativa* was efficient in enhancing yield under heat stress. The usage of SA restricts ethylene creation and improves tolerance in bread wheat subjected to heat stress (Khan et al., 2013b). Pál et al., (2013) clarified that SA is accompanied by most crop reactions for overcoming heat stress. Wang and Li (2006) found when they sprayed SA on grape leaves, that plants were tolerant for heat stress. Kaur et al., (2009) mentioned that applying SA on some plants leads to emphasizing the role of heat-tolerant proteins. Potato tolerance to high temperatures was increased for the long term by using SA, according to López--Delgado et al., (2004). In Arabidopsis plants, Larkindale and Huang (2004) found that the vegetative usage of SA will diminish the negative effects of heat stress. Shi et al., (2006) declared that one of SA derived compounds improved the cucumber's ability to tolerant high-temperature stress.

5.4 Low-Temperature Stress

One of the essential limits of crop improvement is the low temperature (Wang et al., 2020). Both Huang et al., (2014) and Trischuk et al., (2014) defined two types of low-temperature stress which causes remarkable damages, chilling when temperatures are above zero and freezing when temperatures are below zero. Different species of crops and plants are exposed to either chilling or low-temperature stress (Kazemi-Shahandashti et al., 2014). Chilling restricts main maize production (Pál et al., 2020). Complicated regulation techniques were improved by low-temperature stressed plants (Hincha & Zuther, 2014). In plants subjected to chilling stress, the accumulation of SA is caused (Pál et al., 2020). The external usage of SA on wheat leaves hindered the harmful effects of chilling stress (Wang et al., 2018). According to Ding et al., (2016), SA diminished the freezing damage index in tomatoes. Yang et al., (2012) found that treating peach with SA discarded chilling damages. Kosova et al., (2012) declared that SA regulates low-temperature reactions in plants. Rodas-Junco et al., (2020) confirmed the role of SA in raising crop ability to tolerate low temperatures stress. In lemon, Siboza et al., (2014) noticed that SA emphasized the accumulation of phenols and increased activities of growth under low-temperature stress. Many types of research included that the external usage of SA in barley suffered from low-temperature stress, improved plant activities (Mutlu et al., 2013), Also enhanced *Musa acuminate* plants to tolerant freezing stress (Kang et al., 2012). Takatsuji and Jiang (2014) referred to the main function of SA in chilling stress response. Szalai et al., (2016) mentioned that the usage of SA on maize at the first spring planting season in Hungary was efficient since plants in this time of year may be exposed to chilling stress. Sasheva et al., (2013) reported that the crop tolerance of cold stress is emphasized by SA. Janda et al., (2017) mentioned that in general, the external usage of SA increases the final grain productivity as a result of a sustained rate of photosynthesis under cold stress. Miura and Tada (2014) explained how SA controls the defense system, metabolism and other process relating to water in crops subjected to low temperatures (Wang et al., 2012). Under low temperatures, the soaked pea seeds with SA can improve yield (Szalai et al., 2011). The harmful impact of low-temperature stress on crop development was diminished after using SA (Kurepin et al., 2013). Chilling stress impact was alleviated in cucumber after treating seedling with SA (Lei et al., 2010).

5.5 Ultraviolet Radiation (UV) Stress

According to Gill et al., (2015), the different harmful results of the raised ultraviolet radiation on plants increased the necessity for precautions procedures since it reduces plant growth and crop productivity via diminishing photosynthesis (Mohammed and Tarpley 2011). Horvàth et al., (2002) reported that salicylic acid was accumulated in plants that were exposed to ultraviolet radiation. Ranceliene and Vyšniauskiene (2012) declared that SA could diminish the negative effects of ultraviolet at the root level. Mohammed and Tarpley (2013) referred to SA role in improving growth in rice plants-exposed to ultraviolet radiation. SA treatment helped increase photosynthesis for UV- exposed plants (Li et al., 2014).

Bandurska (2013) declared that SA can decrease the destructive impact of the combined ultraviolet radiation stress and drought. The attenuation of the damaging effect of ultraviolet radiation in soybean was achieved by the treatment of seedlings with SA (Zhang & Li, 2012). Mahdavian et al., (2008) clarified that SA induced the antioxidant enzymes to act moderately in leaves of paper subjected to ultraviolet radiation stress. Also, Ervin et al., (2004) mentioned that SA reduces ultraviolet radiation harmful effects.

5.6 Ozone Stress

Ozone is one of the abiotic stresses that threaten crop yields (Connor 2002), and plants and trees in the forests (Wang et al., 2007). The photochemical responses among ultraviolet light and nitrogen-oxides lead to the formation of ozone (Mauzerall & Busconi, 2001), which consider as a poisonous factor for its ability to oxidize plant internal systems and to create un-favorable compounds in plants (Evans et al., 2005). According to Wang et al., (2020), ozone leads to different injuries on crops. Avnery et al., (2011) mentioned that the reduction in crop productivity caused by ozone stress was more than seventy-nine million metric tons in the world. Castagna and Ranieri (2009) declared that ozone effects leave cells harmfully in plants, as well as motivates the automatic death of affected cells (Overmyer et al., 2005). Plant resistance to ozone stress can be enhanced by SA (Song et al., 2014). Ozone stress according to Tosti et al., (2006) promoted the synthesis of SA in *Arabidopsis* plants and Rao and Davis (2001) concluded that a high concentration of SA prompted oxidization activities in *Arabidopsis* plants under ozone stress. Many Scientists confirmed SA role in adjusting the negative effects of ozone stress on plants (Pál et al., 2013; Yoshida et al., 2009). Tamoki (2008) reported that SA in plants regulates the modifications caused by ozone stress. As well, Yoshida et al., (2009) referred to the possibility of supporting the growth process in plants exposed to ozone stress via salicylic acid. An and Mou (2011) mentioned that SA had an essential effect in protecting plants of tomato and tobacco under ozone stress. Sharma et al., (1996) clarified that SA played an important role in reducing the bad effects resulted from ozone stress in *A. thaliana* plants and other scientists agreed when they mentioned that adjusting plant reaction under ozone stress can be achieved when using SA (Rao & Davies, 1999). Extraordinary doses of ozone hinder crop growth, motivate automated cell death and loss of quality (Nagatoshi et al., 2016).

5.7 Elevated CO_2 Stress

Ainsworth et al., (2007) mentioned that the daily raise of CO_2 concentration in the earth's atmosphere has a considerable effect on plants, especially the crop yields in the future (Lobell & Gourdji, 2012). For instance, in rice, the morphology of leaves and thickness of stoma will be changed because of increasing CO_2 (Pedersen et al., 2009), in addition to modifications in growth stages (Gray and Brady 2016). Moreover, the linoleic acid diminished, while α-linolenic acid raised Goufo et al., (2014). The raised concentration of carbon dioxide ends in some plants with a reduction of the endogenous salicylic acid which weakens the plant's ability to resist biotic stresses (Li et al., 2011)**.** In tobacco, the entire amount of SA in the leaf was improved with the elevated CO_2 (Matros et al., 2006).

5.8 Flooding Stress

For various crops, flooding considers important abiotic stress in drained areas that resulted from heavy rainfalls (Jackson and Colmer 2005). Flooding has many negative impacts on plants since it reduces the growth of both roots and shoots (Hashiguchi et al., 2009), diminishes plant ability to obtain light (Vervuren et al., 2003), affect plant respiration (Hossain et al., 2009) and declines plant gas exchange with the surrounding environment (Armstrong & Drew, 2002). The role of SA in mitigating flooding stress is not well documented, but according to Kamal and Komatsu (2016), salicylate (which is a compound derived from salicylic acid) with Jasmonate were both positively involved in soybean root growth under flooding stress.

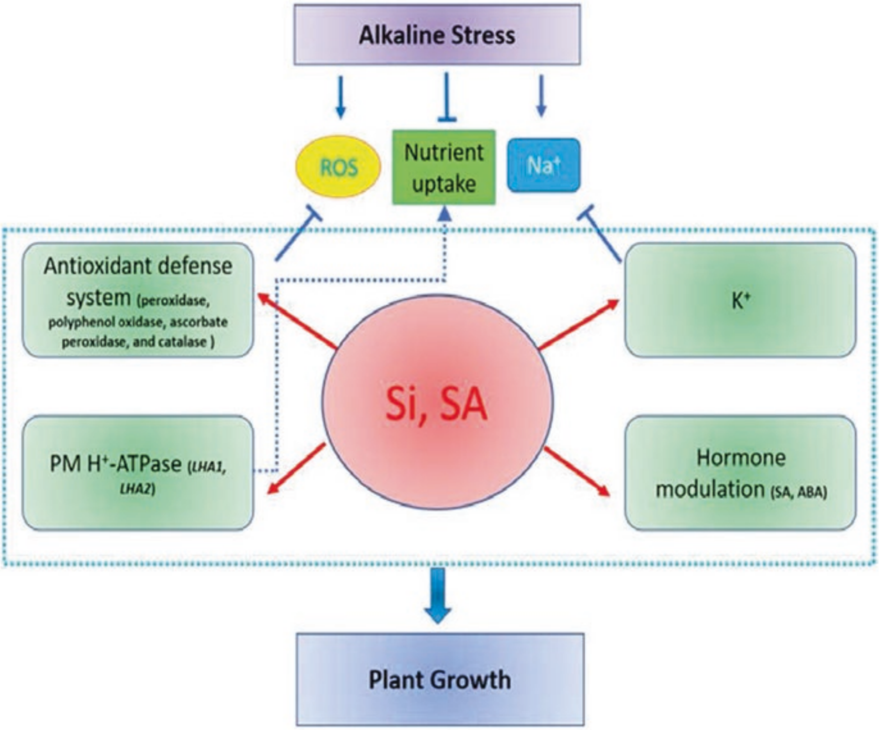

Fig. 7.20 Illustration of how Si and SA improve tomato plants tolerance to alkaline stress. (Source: Khan et al., 2019)

5.9 Alkaline Stress

Alkaline is recognized stress in soils or environments that have high pH and consider as a real danger for the growth and yields of all plants (Fig. 7.20) (Khan et al., 2019). Scientists did not concentrate on various methods of plant tolerance to alkaline stress compared to salinity stress (Yang et al., 2008). Alkalinity emphasizes the production of toxic compounds like some radicals (Mohsenian and Roosta 2015) and reactive oxygen species ROS which consider as the main reason for harsh damages for protein, lipids and plant cells (Apel and Hirt 2004). Mir et al., (2018) reported that alkaline stress diminished the content of chlorophyll in crops (Abdel Latef and Tran 2016).

Khan et al., (2019) studied the effect of using both silicon (Si) with SA on tomato tolerance to alkaline stress (pH = 9), their results showed that the treatment of Si with SA enhanced most of the growth parameters in roots and shoots, raised chlorophyll content, encouraged defense system which was reported also by (Eraslan et al., 2008), controlled the oxidative injuries and diminished the number of accumulated ROS, which referred to the role of Si with SA in the mitigation of alkaline stress.

6 Role of Biotechnology

Due to abiotic stresses, crop production varies greatly from year to year. As a result, finding genotypes that are tolerant to abiotic stress is a prerequisite for crop improvement (Börner et al., 2010; Eltaher et al., 2018, 2021; Ghazy et al., 2020; Saleh et al., 2020; Mourad et al., 2019; Nawara et al., 2017; Salem, 2004; Salem & Matter, 2014; Salem et al., 2007; Salem et al., 2021; Worland & Snape, 2001). From a reproductive point of view, in the presence of abiotic stresses, stress tolerance can be defined as the capacity to maintain a consistently high output (yield). Advances in the genetic and molecular understanding of abiotic stress responses have resulted in the discovery of many genes/QTLs linked to abiotic stress tolerance in many crops i.e., wheat, rice and maize as sites of quantitative traits (QTLs) (Laurie et al., 1994; Quarrie et al., 1994; Salem et al., 2007; Snape et al., 1996, 1997). The expression of certain candidate genes related to abiotic stress was directly related to stress tolerance genes/QTLs, suggesting that these genes/QTLs could be the molecular basis for abiotic stress tolerance (Cattivelli et al., 2002). Hence, identification of genetic components of abiotic stress tolerance is important to ensure further advanced breeding (Cattivelli et al., 2002). In response to abiotic stress events, various hormonal signals are strengthened. These modulate the expression of genes and influence distinct physiological responses of plants. The major signaling molecules of salicylic acid, ethylene, jasmone and abscisic acid are thought to regulate plant defenses. Of particular note is ethylene, a volatile plant hormone that is involved in the control of plant responses to a variety of biotic and abiotic stresses and its role in systemic resistance acquired (SAR) (Sticher et al., 1997). A lot of evidence suggests that salicylic acid plays a major role in stimulating gene expression for plant defense.

In various wheat recombinant inbred lines (RILs) grown under drought conditions, the stable QTL of yield efficiency was put on chromosome 6A (Snape et al., 2007). Therefore, there is a potential association between endogenous synthesis of the phytohormones ABA, ethylene, jasmine and salicylic acid (Cattivelli et al., 2002; Karafylidis & Turner 2002; Glazebrook et al., 2003; Liechti & Farmer 2002; Sharp 2002; Quarrie et al., 1994; Zhu 2002). Pathways of stress signaling are not independent. A, E, S and J are produced by stressed plants and they induce several genes/QTLs that respond to abiotic stresses when applied externally (Tocho et al., 2014; Yamaguchi-Shinosaki & Shinosaki, 2006). The function of hormones, however, is not evident (Nakashima & Yamaguchi-Shinosaki, 2005). Both abiotic stress factors and exogenously applied hormones may be caused by certain genes/QTLs. Some genes are only caused by hormones or abiotic conditions (Yamaguchi-Shinosaki & Shinosaki, 2006). In the doubled haploid lines (DHLs) plants carrying aphid resistance genes, chromosome positions were identified in nine stress hormone-induced QTLs closely linked to growth traits on the arm of chromosome 6AS.

Morphological, physiological, and molecular genetic studies have revealed the fundamental roles of different genes/proteins in SA-induced abiotic stress

Table 7.5 SA-regulated genes have been documented in higher plants under abiotic stresses

Gene names	Abiotic stress	Crop	References
PR	Salt	Arabidopsis	Seo et al., (2008)
	Salt	Peanut	Jain et al., (2012)
	Salt and drought	Pepper	Hong and Hwang (2005)
	Salt and drought	Bean	Hanafy et al., (2013)
	Salt, drought and heat	Arabidopsis	Kwon et al., (2007)
	Salt and heavy metals (cu and cd)	Tobacco	de las Mercedes Dana et al., (2006)
	Drought	Arabidopsis	Miura et al., (2013)
	Cold	Tomato	Ding et al., (2002)
	Cold	Arabidopsis	Liu et al., (2013)
	Heavy metals (Cd and Hg)	Tobacco	Sarowar et al., (2005)
GST, GPX, GR, DHAR, MDHAR, and GSH	Cold	Eggplant	Chen et al., (2011)
	Salt	Wheat	Li et al., (2013)
	Drought	Wheat	Kang et al., (2013)
HSP	Cold	Peach	Wang et al., (2006)
	Heat	Tomato	Snyman and Cronje´ (2008)
AOX	Cold	Cucumber	Lei et al., (2010)
	Cold	Tobacco	Fung et al., (2006)
APX	Cold	Tomato	Duan et al., (2012)
	Salt, drought and cold	Rice	Zhang et al., (2013a)
CAT	Cold	Tomato	Ding et al., (2002)
DHAR	Drought and ozone	Tobacco	Eltayeb et al., (2007)
MDHAR	Salt	Rice	Sultana et al., (2012)

AOX alternative oxidase, *APX* ascorbate peroxidase, *CAT* catalase, *DHAR* dehydroascorbate reductase, *GPX* glutathione peroxidase, *GR* glutathione reductase, *GSH* glutathione synthetase, *GST* glutathione-S-transferase, *HSP* heat shock protein, *MDHAR* monodehydroascorbate reductase, *PR* pathogenesis-related protein. Source: Kang et al., (2014)

tolerance, and several important genes encoding PR proteins, antioxidant enzymes, heat shock proteins (HSPs), and alternative oxidases (AOXs) have been identified (Table 7.5).

7 Conclusion and Prospects

In the regulation of growth and physiology with the abiotic stress responses of plants, salicylic acid plays an important role. Depending on plant species, the SA was found to be efficient in the various forms of application of foliar spray/incorporation with rising media. The low SA concentration had a beneficial effect on plant abiotic stress tolerance. The high SA concentration, by comparison, showed toxic effects. To achieve its best effect on various plant species, both the concentration and application method of SA are therefore important. Some unknown steps and enzymes should be discovered in the biosynthesis route of SA. Among the most important problems in the plant, breeding is population growth and the resulting increased nutritional requirements, along with abiotic pressures. The main obstacles affecting plant growth are pre and post-flowering stresses. The most critical climate change that draws great interest from plant breeders worldwide is global climate change such as drought, salinity, ultraviolet rays, frost, or warming of temperature. Major research has already been done worldwide on the role of SA in tolerating abiotic stress due to the great economic importance of plants due to their use as food, feed, seeds and industrial uses. Both plant breeders and crop producers have an interest in producing crops that, with the least possible damage, can withstand environmental changes. It is of fundamental significance to understand the mechanisms by which plants interpret environmental signals and transmit signals to the cellular system to trigger adaptive responses. It appears to be reluctant to use fundamental research outcomes in practice. It is maybe no exaggeration to suggest that, without being tested in reality, a significant portion of the fundamental science results lie in scientific articles.

The only way to translate fundamental scientific discoveries into practical agriculture is for molecular biologists, plant physiologists and geneticists on the one hand and plant producers and breeders on the other, to work together globally. This may lead to the production of environmentally sustainable crop yield improvement methods under different environmental conditions the outdoor application technique of naturally occurring bioactive compounds to crops and vegetables to more effectively meet local and foreign market demand. Outputs and outcomes in the long term can help improve the resilience of crop production in more complex conditions and eventually contribute to the protection of yields. Also, they will help optimize the use of water and reduce nutrient losses in agricultural systems, lowering the impact of agricultural activities on the environment, particularly in terms of water quality.

Also, because classical breeding methods are laborious and time-consuming, the introduction of new alleles by hybridizing plants from different plant genetic resources, such as modern varieties with locally adapted varieties, increases the genetic diversity of the plant and pre-selects important characteristics needed to ensure significant natural diversity at the phenotype level. Further research is needed to better understand the complex regulation of stress hormone tolerance and its relation to abiotic tolerance. A lot of QTL mapping has been done in many crops using different kinds of DNA markers. Applying QTL mapping to various characteristics will accelerate the tolerance of abiotic stress by helping breeders to (a) recognize certain trait-controlling genes, (b) understand the effect of trait-controlling genes/QTLs, (c) locate the gene/QTLs, and 4) study the association of interest between different genes/QTLs. All these points assist in pyramiding multiple target genes into a single genotype, considering the complexity of the studied germplasm.

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Chapter 8
Interplay Between Salicylates and Jasmonates Under Stress

Yamshi Arif, Priyanka Singh, Husna Siddiqui, and Shamsul Hayat

Abstract Phytohormones like salicylic acid (SA) and jasmonic acid (JA) plays a significant role in providing plant defence against several stresses. These low molecular weight signaling molecules plays the key role in improving plant tolerance against several pathogens through the manifold signaling pathways. SA triggers the systemic acquired response (SAR) whereas JA augment induced systemic response (ISR). Nonetheless, salicylates and jasmonates works antagonistically whereas contrary to this they also work synergistically to each other and facilitate plants immune and further provide resistance against stress. Interaction of salicylate and jasmonate is interceded by several molecular players which participates in the signaling pathway and promotes plant sustenance during different biotic and abiotic circumstances. This chapter highlights the recent advances and present knowledge on role of gene in interaction of SA and JA along with their role in modulating developmental and physio-biochemical processes in plant against several stresses.

Keywords Abiotic stress · Antioxidant defense system · Biotic stress · Germination · Photosynthesis · ROS

1 Introduction

Stresses are recognised as the major agricultural threat for sustainable agricultural practices. Plant responds to several biotic (pathogen, herbivory, parasitic) and abiotic (salinity, drought, heavy metal, heat, cold) stresses to a great extent, coordinated by producing several signals and phytohormones (Wang et al., 2020). Plant hormone play chief role in attenuating stress and enhance plant tolerance by increasing plants morphological, physio-biochemical and yield traits. Additionally, osmoprotectant like proline content is increased, and antioxidant enzymatic and

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S. Hayat et al. (eds.), *Salicylic Acid - A Versatile Plant Growth Regulator*,
https://doi.org/10.1007/978-3-030-79229-9_8

non-enzymatic machinery is triggered which scavenges reactive oxygen species (ROS) and reduce malondialdehyde (MDA) content (Faghih et al., 2017; Ilyas et al., 2017; Moharramnejad et al., 2019). In particular, salicylates and jasmonates serves as the elicitors that facilitate production of important secondary metabolites and signaling biomolecules, which participates in signaling process and can modulate responses related to plant defence against several biotic and abiotic stresses (Liu et al., 2017; Wang et al., 2017; Moharramnejad et al., 2019). Thus, SA and JA mutually serve as important tool in conferring stress tolerance in plants.

SA serves as beneficial signaling molecule and the multifaceted plant hormone which participates in vast array of plant growth, metabolic, biochemical, molecular and defence responses under normal and stressful condition (Arif et al., 2020a). SA also shows positive regulate photosynthesis, respiration, stomatal conductance, relative water content, antioxidant system and osmolyte content in mitigating biotic and abiotic stresses (Arif et al., 2020a). JA and methyl jasmonates (MeJA) altogether known as jasmonates; produced by via lipid- derived bioactive compounds via oxylipin biosynthetic pathway, which plays potent role in providing plant tolerance against necrotrophic pathogen, herbivore, mechanical wounding and several environmental stress (Lemos et al., 2016). JA modulates several cellular and developmental processes from seed germination to fruit ripening and then to senescence (Khan & Khan, 2013; Ruan et al., 2019). Moreover, JA modulates plants physiology and activates several signaling pathway and antioxidant defence machinery which are closely associated to provide plant tolerance against stress and boost up plants immunity (Wang et al., 2020).

With the exponential rise in global population, the demand for food is also augmenting and to meet these needs it is necessary to facilitate the crop productivity. However, alongside plants encounter several biotic and abiotic stresses which impair plants growth, development and productivity of crop. Thus, SA and JA are plant hormones that are biochemically linked and are involved in plant growth, development and cellular processes. Recent studies reported that they both mutually are implicated as signaling agents in response to both biotic and abiotic stresses (Moharramnejad et al., 2019; Ghassemi-Golezani et al., 2020). Several investigations are available showing antagonistic effects of SA and JA to each other, but the recent researches focuses on the interplay between these two hormones in modulation of physio- biochemical metabolic pathways under stress is still in its infancy (Khan & Khan, 2013; Santisree et al., 2020). Biotic and abiotic stress induced ROS and MDA content is which is ameliorated by biochemically linked SA and JA pathway; they both synergistically activate plants enzymatic and non-enzymatic antioxidant machinery. Antioxidant enzymes which are up-regulated by SA and JA mutually include catalase (CAT), peroxidase (POX), superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione peroxidase (GPX) and glutathione reductase (GR); whereas glutathione, ascorbate, phenolic compound, flavonoid, α- tocopherol, soluble sugar and amino acid are non-enzymatic antioxidant which are produced by SA and JA together during stress for scavenging ROS (Wang et al., 2017, 2020; Moharramnejad et al., 2019).

This chapter mainly focuses on the interplay of SA and JA under biotic and abiotic stress including salinity, drought, heavy metal, heat and cold. Furthermore, association of SA and JA in ROS detoxification via enzymatic and non- enzymatic defence system is also briefly discussed. Present chapter also highlights interconnection between salicylate and jasmonate biosynthesis regulation and signaling pathway in plant defence response. Additionally, crosstalk of salicylates and jasmonates in plants physio-biochemistry is appraised.

2 Salicylate and Jasmonate Role in Plants Defense

SA is the phenolic signaling molecule and important phytohormone involved in defense against biotrophic pathogens and plays remarkable role in local and systemic acquired resistance. (Sendon et al., 2011; Lemos et al., 2016). Furthermore, SA plays pivotal role in inducing plant defense against several abiotic stress (Arif et al., 2020a). SA is synthesized via isochorismate or phenylpropanoid pathway and is metabolized to form SA methyl salicylate (MeSA) via methylation, and glucose-ester and SA-glucoside via glucosylation (Arif et al., 2020a). SA accumulation inside plant results in activation of defensive gene i.e. pathogenesis-related1 (PR1) genes (Lemos et al., 2016). Several SA-binding proteins (SABP2) plays chief role in plant defense against stress (Arif et al., 2020a, b). MeSA plays chief role in activation of systemic acquired resistance (SAR). Furthermore in *Nicotiana* species SABP2 converts MeSA to SA in distal tissues and activates SAR for activating defense system (Robert-Seilaniantz et al., 2011). A key player in plant defense is NPR1 (NONEXPRESSOR of PR GENES1), a positive regulator of SA defense pathway. In absence of SA, NPR1 is present in cytoplasm in multimeric form; however during stress SA level increases in plant and it leads to redox changes and through S-nitrosylation and thioredoxin activity causes dissociation of NPR1 complex protein and NPR1 migrates towards nucleus. In particular, NPR1 binds to TGA transcription factor and increase SA mediated defense system (Arif et al., 2020a). SA increases activation of several antioxidant enzymes which reduces ROS and induces plants defense mechanism during stress (Arif et al., 2020a).

Jasmonates comprises of JA, its derivatives and its precursor 12-oxo- phytodienoic acid (OPDA) are synthesized via lipid- derived bioactive compounds such as through oxylipin biosynthetic pathway which plays key role in providing plant defense against necrotrophic pathogen, herbivore, mechanical wounding and several climatic stress (Lemos et al., 2016). Formation of JA conjugates like JA- isoleucine, jasmonyl-L- isoleucine (JA-Ile) followed by the binding of endogenous active ligand to the F- box protein i.e. coronate insensitive1 (COI1) causes ubiquitination, additionally this leads to repressor protein jasmonate zim (JAZ) 26S proteosome degradation (Sheard et al., 2010). In particular, degradation leads to disruption in JAZ protein and transcriptional activator interaction, which causes activation of JA-responsive genes via de-repression of JA biosynthesis and signaling (Gonzalez-Cabanelas et al., 2015). In *Arabidopsis thaliana*, JA defense signaling pathway is divides into two broad antagonistically controlled level, they are the basic helix-loop-helix lucine zipper TF MYC2, which is enhanced by herbivores, insects and other stressors and activate the MYC-2 branch marker gene i.e. *vegetative storage protein 2* (VSP2) and other pathway is induced by necrotrophic pathogens which enhances ethylene response factor 1 (ERF1)-branch marker gene i.e. *plant defensin 1.2* (PDF 1.2) (Lemos et al., 2016). Ethylene plays synergistic and antagonistic role in JA pathway, which increases ERF1 branch and inhibits MYC2 branch (Lemos et al., 2016).

3 Biosynthesis of SA and JA and Its Regulation in Plants Defense Response

Biosynthesis of SA takes place via isochorismate (IC) and phenylanine ammonia lyase pathway (PAL). IC synthase (ICS) and PAL are the chief enzyme that participates in SA synthesis (Dempsey et al., 2011). PAL is the most common pathway for SA production after hydroxylation of benzoic acid via benzoic acid 2-hydroxylase enzyme (Khan et al., 2012; Arif et al., 2020a). Benzoic acid is produced starting from cinnamic acid through β-oxidation of fatty acid or via non-oxidative pathway, followed by production of trans-cinnamic acid from phenylaline takes via PAL enzyme (Dempsey et al., 2011; Khan et al., 2012). However, in IC pathway, ICS converts chorismate into isochorismate which is latterly converted into SA via action of IC pyruvate lyase (Khan et al., 2012; Arif et al., 2020a). In Arabidopsis during pathogen infection, ICS mutants cause loss of SA accumulation. Furthermore, there is only 10% reduction in PAL activity which reduces SA biosynthesis under pathogen attack (Arif et al., 2020a). ICS is involved in calcium signaling with help of calcium ions (Ca^{2+}) and calmodulin. Transcription factor (TF) CBP (Calmodulin Binding Protein 60 g) and its homolog which is SARD1 (Systemic Acquired Resistance Deficient 1) plays key role in ICS regulation, which is followed by modulation in SA biosynthesis. In particular, during pathogenic infection, at early stages CBP60g plays chief role whereas at late stages SARD1pays key role in SA accumulation and boosting plant immunity (Arif et al., 2020a). SA biosynthesis is increased via WRKY28, which interacts with ICS1 promoter and induce plan tolerance against stress. Furthermore, Calmodulin Binding Transcription Activator 3 (CAMTA3) or Signal Responsive Gene1 (SR1) binds to Enhanced Disease Susceptibility1 (EDS1) and restrain SA biosynthesis and make plant susceptible to disease; however CAMTA3/SR1 mutants increases SA production and boost plant immunity (Kim et al., 2013; Arif et al., 2020a).

JA biosynthesis takes place via α-linolenic acid (Vick & Zimmerman, 1983). From plasma membrane linolenic acid (18:3) is released and is converted into 13-hydroperoxylinoleic acid (13-HPOT) via enzyme 13-lipoxygenase, followed by its conversion into 12,13-epoxyoctadecatrienoic acid via allene oxide synthase (AOS), which is latterly acted on by allene oxide cyclase (AOC) (Ruan et al., 2019).

AOC helps in steroconfigurtion and synthesize (9S, 13S)-12-oxo-phytodienoic acid (OPDA); sn-1 of the chloroplast lipid monogalactosyldiacylglycerol contains OPDA as substituent. It causes OPDA release by lipase and induces JA biosynthesis. 9S, 13S OPDA is reduced into 3-oxo-2(2- pentenyl) cyclopentane-1-octanoic acid (OPC- 8:0) via OPDA reductase, which is subsequently converted into JA via three cycles of β- oxidation inside peroxisome (Khan et al., 2012). During stress, JA is synthesized, subsequently JA resistant1 (JAR1) converts JA into active JA- L- isoleucine (JA- Ile). In particular, JA-Ile perception via its receptor coronatine insensitive1 (COI) facilitates degradation of JAZ repressors leading to release of TF such as MYC, WRKY, NAC, MYB and ERF, which help in triggering plant immunity and conferring stress tolerance (Ruan et al., 2019).

4 Interplay Between Salicylate and Jasmonate Signaling Pathway in Plants Defense Response

SA and JA both interact with each other in regulation of plant defense against several stresses. Jasmonates and its derivative are effective against necrotrophic pathogen, insect and herbivore whereas salicylate mediates defense against biotrophic pathogen and other environmental stress (Fig. 8.1) (Koornneef & Pieterse, 2008). SA and JA signaling interact and cross communicate at multiple regulatory points; JA negatively regulates SA- responsive gene expression. Thus, SA and JA work antagonistic of each other (Lemos et al., 2016). In Arabidopsis, SA carboxyl methyltransferase (AtBSMT1) plays chief role in SA and JA interaction. JA enhances AtBSMT1 expression and depletes SA by converting it to MeSA, which participates in an antagonistic effect on SA signal transduction (Koo et al., 2007). NPR1 protein and WRKY TF plays key role in SA signal transduction and regulates SA-mediate defense suppressing JA response. JA responsive genes include *lipoxygenase2* (LOX2), PDF 1.2 and VSP2. Furthermore, glutaredoxin have negative impact on small portion of JA-responsive genes which are sensitive to SA- linked suppression and MAP kinase (MPK), which has negative impact on SA signal transduction contrary to it JA signaling is positive regulator (Lemos et al., 2016). SA acts antagonistically to JA responsive gene expression LOX, PDF 1.2 and VSP2 (Spoel et al., 2003). *Arabidopsis* npr1 mutant however showed increased JA accumulation via induced JA-responsive gene expression. Thus, this suggests that NPR1 has inhibitory effect on JA responsive gene expression (Spoel et al., 2003; Sendon et al., 2011). SA and JA have antagonistic relationship in several plant species (Thaler et al., 2012). SA and JA functions antagonistically to regulate defense system against pathogen, SA and JA works independent of Apetala2/ethylene response factor (AP2/ERF) TF ORA59, which manages JA pathway. Furthermore, WRKY75 control signaling of both SA and JA and enhance plants immunity against stress (Schmiesing et al., 2016).

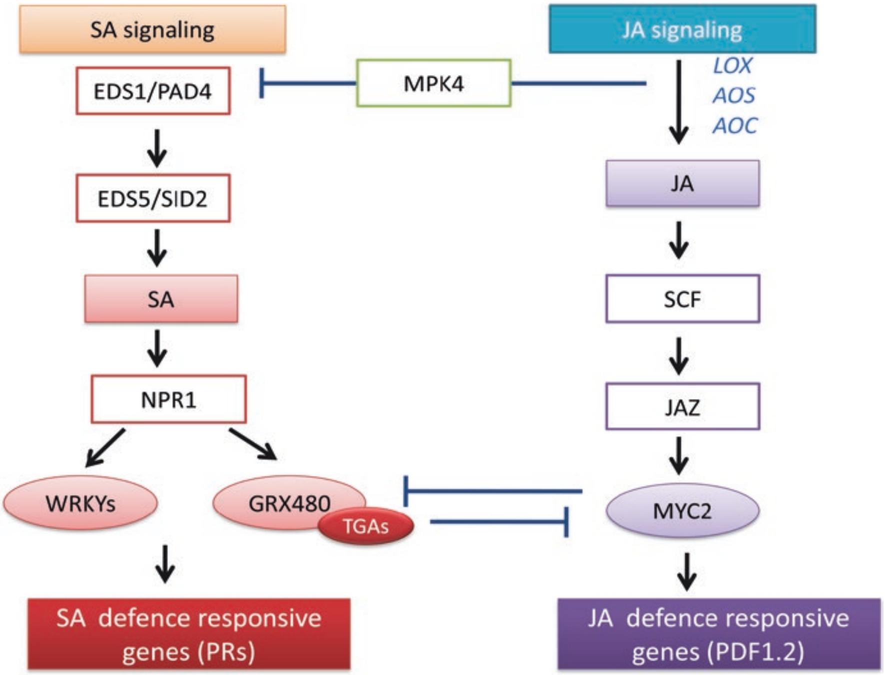

Fig. 8.1 Illustration of SA and JA signaling that activates several defense responsive genes. **Abbreviation:** *SA* salicylic acid, *JA* jasmonic acid, *EDS1* enhanced disease resistance, *PAD4* phytoalexin deficient4, *NPR1* non expresser of pathogenesis related1, *PRs* pathogenesis related genes, *SCF* SKP1/Cullin/F-box, *LOX* lipoxygenase, *AOS* allene oxide synthase, *AOC* allene oxide cyclase, *PDF1.2* plant defensin1.2

Recently it was reported that apart from having antagonistic relationship SA and JA share common pathway of defense mechanism. In Arabidopsis, JA and SA both contributed in boosted up immunity and provided tolerance against biotrophic and necrotrophic pathogens. In rice, SA and JA together up-regulated several defense responsive gene in combating biotic stress. Furthermore, SA and JA activate the common defense system against pathogen defense response (Fig. 8.1) (Tamaoki et al., 2013). Still the mechanism is unclear.

5 Interplay Between Salicylate and Jasmonate Role in Plant Physiology and Biochemistry

SA and JA plays key role in improving physio-biochemical traits such as growth, biomass relative water content, chlorophyll content, photosynthesis and yield traits (Ghassemi-Golezani & Farhangi-Abriz, 2018a). In wheat, two plant hormones SA and JA synergistically plays key role in enhancing germination trait like germination percentage, germination index, promptness index and seedling vigor index. Furthermore, they also increases shoot and root height and biomass, leaf area water potential, membrane stability and chlorophyll content. In particular, level of proline, soluble sugars and amino acid was also augmented (Ilyas et al., 2017). SA and JA treatment in buckwheat increased germination and improved phenolic content (gallic acid, caffeic acid, catechin, rutin chlorogenic acid and epicatachin) and organic acid content (Park et al., 2019). Mendoza et al. (2018) reported that SA and MeJA modulated biochemical traits in *Thevetia peruviana*. In particular, SA and JA together increased antioxidant, phenolic and flavonoid content; they both serve as signaling molecule and catalyst that induce plant physio-biochemical traits under healthy and stressful environment (Mendoza et al., 2018). In *Bidens pilosa*, SA and JA application increased metabolite content such as caffeoylquinic acid, tartaric acid esters (chicoric acid and caftaric acid), chalcones (okanins), and flavonoids (quercetin, kaempferol) (Ramabulana et al., 2020). Salicylates and jasmonates increases essential oil content in peppermint and improve plant cellular processes (del Rosario Cappellari et al., 2019). Furthermore, in *Ziziphora persica*, SA and JA play remarkable role in increasing growth parameters, chlorophyll and carotenoid content; they also increased volatile compound and secondary metabolite in shoot culture and nodal segment (Zare-Hassani et al., 2019). Faghih et al. (2017) reported that in strawberry SA and JA synergistically induced plant morphology, photosynthetic traits, gas exchange parameter, stomatal conductance and antioxidant enzymatic defense system. In *Brachypodium distachyon,* salicylate and methyl jasmonate plays crucial role in modulating cell wall structure (Napoleão et al., 2017). SA and JA increased quality and quantity of protein in soybean seeds by increasing amino acid content (Farhangi-Abriz & Ghassemi-Golezani, 2016). In cotton, *GhWIN2* gene regulates the cuticle biosynthesis pathway and also takes part in positively influencing JA biosynthesis and negatively affecting SA biosynthesis (Li et al., 2019). Combined pre-harvest application of salicylate and jasmonate play potent role in improving fruit quality (Mirdehghan et al., 2012). Furthermore, modulation in physio-biochemical after SA and JA application suggests that SA and JA are overlapped to certain extent and have cross-talk between signaling and metabolic pathway activation (Ramabulana et al., 2020).

6 Interplay Between Salicylate and Jasmonate in ROS Detoxification

Reactive oxygen species (ROS) serves as the secondary messenger in several signaling pathway and play crucial role in plants defense mechanism during biotic and abiotic stresses (Arif et al., 2020a, b). Thus, ROS accumulation is interconnected with the SA and JA mediated signaling pathway (Liu et al., 2017; Faghih et al., 2017). It was reported that SA, JA and ROS level is correlated, and found that during stress SA and JA works antagonistically of ROS (Moharramnejad et al., 2019).

To counteract with ROS induced oxidative damage, SA and JA application triggers plants antioxidant defense system, it includes enzymatic and non-enzymatic antioxidant. Enzymatic antioxidant such as CAT, POX, SOD, APX and GR; whereas non-enzymatic antioxidant includes glutathione, ascorbate, phenolic compound, flavonoid, α- tocopherol, soluble sugar and amino acid (Faghih et al., 2017; Ilyas et al., 2017; Moharramnejad et al., 2019). In *Rauvolfia serpentine in vitro* cultures, salicylate and jasmonate synergistically activated antioxidant enzymatic defense system (CAT, POX, SOD) which helps in scavenging ROS and induce production of reserpine and ajmalicine; and further induce plant growth, development and regeneration (Dey et al., 2020). In rosemary and soybean SA and JA induced oxidative stress resistance by activating antioxidant enzyme, soluble sugar and amino acid content. Furthermore, MDA content was also reduced. In particular, SA and JA are associated with each other in ameliorating stress induced ROS and MDA by activating several signaling and biochemical pathways (Farhangi-Abriz & Ghassemi-Golezani, 2018b; Nahrjoo & Sedaghathoor, 2018).

7 Interplay Between Salicylate and Jasmonate in Biotic Stress Tolerance

SA and JA serve as the potent elicitors and triggers production of secondary metabolites and signaling biomolecules participates in signaling process and can modulate responses related to plant defence against several biotic stress (Fig. 8.2) (Liu et al., 2017; Moharramnejad et al., 2019). JA signaling pathway is activated by SA receptors such as NPR3 and NPR4 at early stage of effector triggered immunity (ETI). Furthermore, it was investigated that JA plays critical role in ETI and their lies mutual cooperation between two defence hormones SA and JA; they provide plant immunity against biotrophic and necrotrophic pathogen; here plants can increase resistance to biotrophic pathogen by activating programmed cell death (PCD) defence mechanism (Liu et al., 2016). Insects and pests attack plant and impair plant growth and development; and inhibits several cellular and metabolic processes (Fig. 8.2). Furthermore, they reduces plant yield and productivity (Stella de Freitas et al., 2019) SA and JA application plays potent role in inducing plant resistance to pathogen and in inducing pest management in several useful crops. SA and JA application on wheat made plant less attractive to aphids such as *Sitobion avenae Fabricius*, 1775 (Hemiptera: Aphididae) and enhance plant immunity and induce proper growth and development. In groundnut, SA and JA treatment negatively affected growth of *Helicoverpa armigera Hübner*, 1809 (Lepidoptera: Noctuidae), by inducing antioxidant defence system. Additionally SA and JA treatment in rice provide immunity against rice stink bug (RSB), *Oebalus pugnax Fabricius*, 1775 (Hemiptera: Pentatomidae), and further induced rice growth, development and yield attributes (Stella de Freitas et al., 2019). In rice, the brown planthopper (BPH; Homoptera: Delphacidae) is a typical phloem-sucking herbivore and

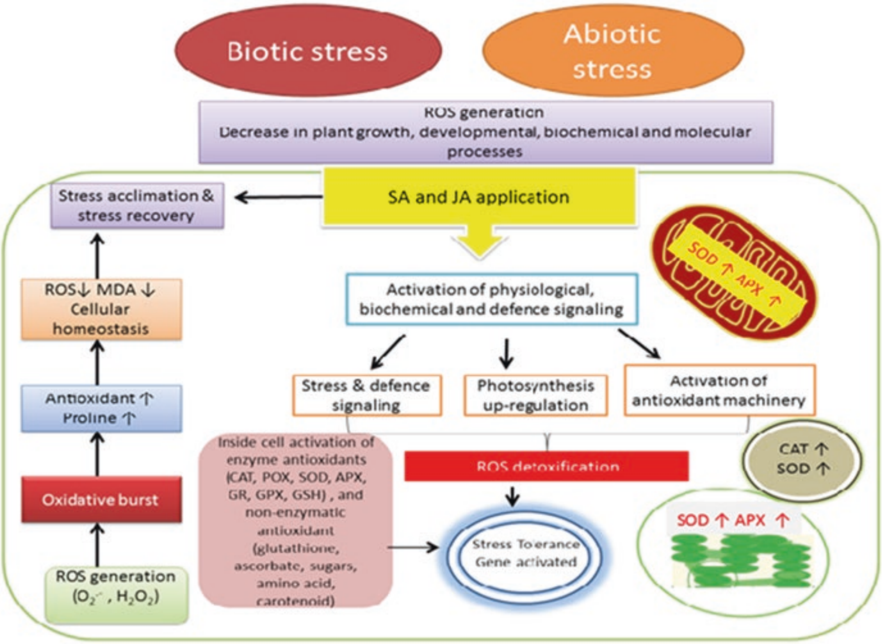

Fig. 8.2 A model of SA and JA interplay in ameliorating biotic and abiotic stress via several defense signaling pathway and antioxidant defense system which modulates several physiological and biochemical traits

is one of the most serious and destructive insect pests that cause greater decline in rice productivity. However, SA and JA play crucial role in activating defence signaling pathway and activating expression of defence related genes, which increases rice tolerance to BPH and triggers plant yield and productivity (Pan et al., 2018).

Plants have evolved a sophisticated defence system to respond to various biotic stresses. Fungal infection is one of the most deleterious biotic stresses that inhibits plant growth and development and causes plant death. SA and JA plays key role in activating plant self- protecting system (Luo et al., 2019). SA and JA in poplar reduced fungal infection by increasing growth and inducing signalling pathway and TFs that mitigate fungal infection (Luo et al., 2019). In tomato, gray mold disease caused by *Botrytis cinerea* hampers tomato growth, development and productivity. Furthermore, *Clonostachys rosea* is a fungus which is applied on tomato which induces JA and SA signaling pathway and help in provide tomato resistance to gray mold disease. SA and JA regulated activity of enzyme such as CAT, PAL and polyphenol oxidase (PPO); they also increase activity of mitogen-activated protein kinase (MAPK), WRKY, Lexyl2, and atpA and decreases ROS content (Wang et al., 2019). In *Solanum lycopersicum*, SA and JA modulated defensive pathways in Trichoderma-induced resistance to the *Meloidogyne incognita* (root knot nematode) (Martínez-Medina et al., 2017). Zhang et al. (2017) found that in *Arabidopsis thaliana-Botrytis cinerea* pathosystem, SA and JA together plays crucial role in boosting *Arabidopsis* immunity and inducing resistance against *Botrytis*. Furthermore, SA and JA together activated defence signalling that function to constrain virulence in pathogen population; further underlying transcriptional responses were highly plastic. Indeterminate domain (IDD)/BIRD proteins belong to a highly conserved plant-specific group of TFs which modulate plant cellular, physiological, biochemical and development responses. Therefore, during biotic stress (such as *Pseudomonas syringae* and *Botrytis cinerea*) IDD modulates SA and JA homeostasis inside plant and activate defence signal transduction. Furthermore, hypomorphic idd4SRDX line increases growth and activates wide range defence related genes and enhances plant tolerance against pathogen. Thus, salicylates and jasmontaes together play in conferring biotic stress tolerance and increasing plant innate immunity (Volz et al., 2019). SA and JA together play crucial role in mitigating *Meloidogyne incognita* pathogenicity by activating defence pathway and inducing expression of defence related genes and proteins such as serine/threonine-protein kinase, LRR receptor-like serine/threonine-protein kinase, and disease resistance proteins; they also increased activity of CAT, POX and SOD and help in enhancing disease resistance (Fig. 8.2) (Du et al., 2020). SA and JA serve in boosting plant immunity in endemic Turkish yarrow species *Achillea gypsicola* by acting as potent elicitors that increase the cell growth, development, production of camphor and phenolic compound (Açikgöz et al., 2019). SA and JA induce expression of TF WRKY, which regulate plants defence against several biotic stresses. WRKY70 overexpression increased resistance to the bacterial necrotroph *Erwinia carotovora*, the fungal biotroph *Erysiphe cichoracearum* and the hemibiotrophic *Pseudomonas syringae*. SA and JA up-regulates expression of NtWRKY50-OE2 which helps in mitigating pathogenic bacterium *Ralstonia solanacearum* (Liu et al., 2017).

Moreover, there also lie antagonistic interactions between SA and JA-dependent signaling pathways during biotic stress or pathogen infection. TF such as WRKY53, which participates in senescence, is antagonistically modulated by SA and JA, this suggests that SA and JA both plant hormone participates in senescence. SA enhance WRKY53 synthesis whereas WRKY53 interacts with JA- inducible protein ESR (epithiospecifying senescence regulator) inside nucleus, this association have negative effect on DNA-binding activity of WRKY53. Thus, it can be concluded that negative JA/SA crosstalk is mediated by the interaction between ESR and WRKY53 proteins during biotic stress, which is governed by the SA and JA equilibrium (Hu et al., 2017).

8 Interplay Between Salicylate and Jasmonate in Abiotic Stress Tolerance

SA and JA in association play a potent role in mitigating abiotic stress by up-regulating several defence signals, photosynthetic traits and antioxidant defence machinery and reducing ROS, MDA and electrolyte leakage (Fig. 8.2).

8.1 Salinity Stress

Salinity is one of the major threats to sustainable agriculture practices, that worldwide decreases plant production by hampering several morphological, physiological, biochemical, and molecular function. In particular, salinity reduces life sustaining processes including germination, growth, photosynthesis, transpiration, and stomatal conductance; it also reduces leaf water potential, turgor pressure, mineral uptake, imbalances ion homeostasis and increases ROS content; thus generates osmotic, ionic and oxidative stress respectively (Arif et al., 2020b). Application of SA and JA together plays crucial role in combating salt stress and augment soybean stress tolerance. SA and JA increases plant height, biomass, chlorophyll, photosynthesis and relative water content. Furthermore, they also induces the activity of H^+-ATPase, ATP and cation (potassium (K^+), magnesium (Mg^{+2}), iron (Fe^{2+})) content which was decreases due to salt. Thus, JA and SA synergistically play remarkable role in decreasing toxic effect of salinity (Ghassemi-Golezani & Farhangi-Abriz, 2018a). In strawberry, SA and JA together reduced salt toxicity by reducing ROS, MDA, electrolyte leakage and Na^+/K^+ ratio; and further enhanced plant height, fresh and dry weight, net photosynthetic rate and stomatal conductance. In particular, SOD, APX and POX activity was increased which help in depleting detrimental effects of salinity and inducing plant immunity (Faghih et al., 2017). Salinity stress reduced the amino acid content and reduced yield traits in *Glycine max*. Nonetheless, SA and JA application induced content of amino acids such as lysine, isoleucine, methionine, serine, leucine, alanine, valine, glycine, aspartic acid, glutamic acid, phenylalanine and tyrosine in seeds which was reduced by salt and increased economic value of seeds by inducing protein content. Furthermore, they also increased fatty acid and oil content which was declined due to salt (Farhangi-Abriz & Ghassemi-Golezani, 2016, 2018b). Salinity stress reduced the photosynthetic related traits, variable fluorescence, PSII efficiency, water –splitting efficiency and total performance index. However, treatment of SA and JA on safflower induced the chlorophyll content, photosynthesis, PSII efficiency, variable fluorescence, water-splitting efficiency and total performance index. Furthermore, these two hormones induced physio-biochemical traits and yield traits and combated salt induced ionic, osmotic and oxidative stress (Ghassemi-Golezani et al., 2020). Nasab et al. (2018) reported that application of salicylates and jasmonates increased plant immunity against salt stress by increasing proline, antioxidant enzyme and soluble sugar content, which increase growth, photosynthesis and yield traits.

8.2 Drought Stress

Drought stress impairs plants growth, developmental processes and productivity globally. It hampers morpho-physiological, cellular and biochemical traits which ultimately causes plant death. However, application of SA and JA in combination plays remarkable role in mitigating drought stress in wheat; they both improve germination traits, growth, biomass, leaf area, relative water content, membrane stability, water potential and osmotic potential. Furthermore, proline, soluble sugar and amino acid content were also modulated; thus SA and JA have synergistic interaction in conferring drought stress tolerance in wheat (Ilyas et al., 2017). SA and JA application increase maize plant tolerance to drought. SA and JA increased plant growth, biomass, CAT, POX and SOD activity and decreased ROS, lipid peroxidation and lipoxygenase activity. Furthermore, both hormones increased synthesis of osmolyte such as proline, total soluble sugar and total carbohydrate content which modulated plant turgor and water status (Tayyab et al., 2020). In *Triticum aestivum*, SA and JA application mitigated negative effect of drought. In particular, SA and JA application increased growth, biomass, relative water content, stomatal conductance, flag leaf area, photosynthetic traits, water use efficiency, ear length, grain number per ear, biological yield and grain yield; whereas both hormones reduced ROS, MDA and electrolyte leakage (Vahabi et al., 2017). Salicylates and jasmonates mutually enhance drought tolerance in chamomile plant, they detoxify free radicals, MDA and reduce electrolute leakage by modulating plant osmoticum, antioxidant system, proline, soluble sugar and amino acid content (Nazarli et al., 2014). Salicylates and jasmonates conferred PEG induced drought-tolerance in *Verbascum sinuatum*, both hormone synergistically modulated growth, biomass and photosynthetic traits. Furthermore, SA and JA also promoted antioxidant enzymatic machinery and polyphenol oxidase which scavenges ROS, MDA and reduce electrolyte leakage (Karamian et al., 2020).

8.3 Heavy Metal Stress

Heavy metal (HMs) pollution is caused by human activities, industrial waste, mining, and weather processes, inside soil and water. HMs in higher amount is highly toxic as it hampers plants growth and developmental processes; it declines plant physio-biochemical and yield traits (Sofy et al., 2020). SA and JA together induce nickel (Ni) resistance in *Alyssum inflatum*, they positively regulate growth, biomass and yield traits. Furthermore, they increased plant turgor, relative water content, photosynthesis, chlorophyll content, stomatal conductance and mineral uptake. Moreover, it promotes antioxidant enzymatic machinery which scavenges ROS and reduces MDA and electrolyte leakage (Karimi & Ghasempour, 2019). In maize, SA and JA synergistically attenuated toxic effect of lead (Pb) by increasing physio-biochemical traits such as growth, biomass, photosynthetic pigment and mineral nutrient content such as N, P and K. Furthermore, SA and JA together also increased CAT, POX, SOD and glutathione activity; also enhanced proline, total soluble sugar production, ascorbic acid and phenol content. They also reduced electrolyte leakage, MDA and ROS content; thus help in reducing Pb stress (Sofy et al., 2020). SA and JA play an important role in mitigating deleterious effect of chromium (Cr) HMs. Both hormones synergistically increased modulated growth, biomass, photosynthetic and yield component; they also increased activity of antioxidant enzyme which quenches ROS and oxylipins (Scalabrin et al., 2016).

8.4 Temperature Stress

High and low temperature can negatively impair plant growth, developmental, cellular and metabolic processes. Furthermore, several life sustaining processes such as photosynthesis and respiration also declines (Siboza et al., 2017; Wang et al., 2017). SA and JA synergistically activate several genes and modulate several physio- biochemical processes and provide *Gracilariopsis lemaneiformis* tolerance against heat stress. Moreover, both hormones interacted with each other and sustain membrane stability and integrity (Wang et al., 2017). Hou et al. (2018) reported that SA and JA synergistically provide tolerance against heat stress by up-regulating lipoxygenase (LOX) genes such as *lox1* and *lox2*, this induce plant growth and development and defence response against toxic effect of high temperature stress. Salicylates and jasmonates application induce chilling tolerance in lemon fruits; both hormone synergistically induce growth, biomass and biochemical traits; promoted antioxidant defence system (CAT, APX, GR) which detoxify ROS and reduces chilling injury (Siboza et al., 2017). SA and JA synergistically play crucial role in increasing production of heat shock proteins (HSPs) which confer chilling tolerance. HSPs serve as the molecular chaperones which prevent aggregation and accumulation of denatured proteins and also help in translocation of proteins to cellular compartments. Additionally, HSPs plays crucial role in protecting cells, tissues, cellular compartment and membrane against chilling injury and maintain plasma membrane stability and integrity; HSPs also induce protein refolding and maintain cellular homeostasis which enhance chilling tolerance (Siboza et al., 2017). Mangosteen experiences pericarp hardening due to diversion of phenolics and lignin during chilling injury. However, application of both salicylates and jasmonates are highly beneficial as they modulated several biochemical pathway; they up-regulated total soluble phenolics content, promoted antioxidant activity and polyphenol oxidase (PPO) activity. Thus, SA and JA together confer chilling injury tolerance (Mustafa et al., 2018). Table 8.1 illustrates the interplay of SA and JA in ameliorating biotic such as pathogen, insect, pest and herbivore and abiotic stress such as salinity, drought, heavy metals, heat and cold.

9 Conclusion and Future Prospective

Biotic and abiotic stresses are recognized as a potential agricultural threat for the sustainable agriculture practices. The research efforts of scientists are to provide mechanisms that could help in the survival of plants under different stresses. Thus, plant hormones play significant roles in modulation of growth, developmental and

Table 8.1 Interplay of salicylates and jasmonates in amelioration of stress in plants

Biotic / Abiotic stress	SA and JA associated induced effect on plants stress tolerance	Plant species	References
Pathogen (fungi, bacteria, nematode)	SA and JA together up-regulates expression of defense related genes such as serine/threonine-protein kinase, LRR receptor-like serine/threonine-protein kinase, enzymatic antioxidant which induce plant immunity against pathogen	*Solanum peruvianum*	Du et al. (2020)
	SA and JA interplay serves as potent elicitors that increases growth, development, biochemistry, production of camphor and phenolic compound	*Achillea gypsicola*	Açikgöz et al. (2019)
	SA and JA together plays key role in activating plant self- protecting system; it induces growth, signalling pathway and TFs that mitigate pathogenic infection	*Populus*	Luo et al. (2019)
	SA and JA mutually up- regulates activity of enzyme such as CAT, PAL and PPO; they both also triggers activity of MAPK, WRKY, Lexyl2, and atpA and decreases ROS, MDA content	*Solanum lycopersicum*	Wang et al. (2019)
	SA and JA together activated defense signaling that function to constrain virulence in pathogen population and increases plant innate immunity; modulates expression of TFs and protein that triggers growth and plant development	*Arabidopsis thaliana*	Volz et al. (2019)
	SA and JA interconnected to each other and help in plant defense by up-regulating NtWRKY50-OE2	*Nicotiana tabacum*	Liu et al. (2017)
Pest, insect and herbivore	SA and JA treatment negatively affect growth of insects, pests and bug by activating defense signaling pathway; further improve plant growth and development and yield traits	*Oryza sativa*	Stella de Freitas et al. (2019)
	SA and JA activates expression of defense related genes which enhances plant tolerance against brown planthopper; it augments plant productivity	*Oryza sativa*	Pan et al. (2018)
Salinity stress	SA and JA upregulates chlorophyll content, photosynthesis, PSII efficiency, variable fluorescence, water-splitting efficiency, total performance index, maintain ion homeostasis, mineral uptake and plasma membrane integrity	*Carthamus tinctorius*	Ghassemi-Golezani et al. (2020)

(continued)

Table 8.1 (continued)

Biotic / Abiotic stress	SA and JA associated induced effect on plants stress tolerance	Plant species	References
	SA and JA mutually improves amino acid, fatty acid and oil content; maintains low level of ROS and increased CAT, POX, SOD, APX activity; induces the activity of H^+-ATPase, ATP and cation K^+, Mg^{+2}, Fe^{2+} content	*Glycine max*	Ghassemi-Golezani and Farhangi-Abriz (2018a,b)
	Both hormone triggers antioxidant defense system and increase compatible osmolyte content which reduces electrolyte leakage ROS, RNS and LOX level and boost up plants immunity for better growth and development	*Lavendula officinalis*	Nasab et al. (2018)
Drought stress	SA and JA regulates synthesis of osmolyte such as proline, total soluble sugar and total carbohydrate content which modulated plant turgor and water status; increases plant growth, biomass, antioxidant activity and decreased ROS, lipid peroxidation and lipoxygenase activity	*Zea mays*	Tayyab et al. (2020)
	SA and JA interplay enhances activity of antioxidant enzyme, polyphenol oxidase and increases content of osmolyte which help in combating osmotic and oxidative stress and reduces ROS and MDA	*Verbascum sinuatum*	Karamian et al. (2020)
	SA and JA provide plant tolerance against stress and triggers seed germination trait, plants growth, physio-biochemical and yield traits; also modulates relative water content, membrane stability, water potential and osmotic potential	*Triticum aestivum*	Ilyas et al. (2017)
Heavy metal stress	SA and JA together increases growth, fresh and dry mass; promotes antioxidant enzymatic defense machinery for ROS scavenging and facilitates chlorophyll content and photosynthetic traits; also enhances proline, total soluble sugar production, ascorbic acid and phenol content	*Zea mays*	Sofy et al. (2020)
	SA and JA crosstalk increases plant turgor, relative water content, photosynthesis, chlorophyll content, stomatal conductance and mineral uptake which helps in reducing ROS and improving photosynthetic traits	*Alyssum inflatum*	Karimi and Ghasempour (2019)

(continued)

Table 8.1 (continued)

Biotic / Abiotic stress	SA and JA associated induced effect on plants stress tolerance	Plant species	References
	Both hormones SA and JA synergistically modulates growth, biomass, photosynthetic and yield component; also increases activity of antioxidant enzyme which quenches ROS and oxylipins and further which removes toxic metals from plant cells	*Nicotiana langsdorffii*	Scalabrin et al. (2016)
Heat stress	SA and JA synergistically increases heat tolerance by up-regulating *lox1* and *lox2* and enhances activity of CAT, POX, SOD, APX, GPX; also reduces ROS and electrolyte leakage and increases photosynthetic attributes and membrane stability	*Gracilariopsis lemaneiformis*	Hou et al. (2018)
Cold stress	SA and JA triggers HSPs level which provide plant resistance against stress and triggers plants physio-biochemical and molecular responses	*Citrus limon*	Siboza et al. (2017)

cellular processes, and signaling networks in plants under different stressful environment. Plant hormone and potent elicitors, salicylate and jasmonate serve as beneficial tool in increasing tolerance of plants to several biotic and abiotic stresses (Fig. 8.3). SA is the phenolic signaling molecule whereas JA is the lipid- derived bioactive compound, both alone or in combination serves in regulation of several physio-biochemical and molecular processes under biotic and climatic stress (Fig. 8.3). Jasmonates and its derivative are effective against necrotrophic pathogen, insect and herbivore whereas salicylate mediates defense against biotrophic pathogen and other environmental stress. This chapter gives better understanding of SA and JA signaling interaction and cross communication at multiple regulatory points; JA negatively regulates SA- responsive gene expression.

Thus, SA and JA work antagonistic of each other. Apart from this, modulation in physio-biochemical traits and yield component during stress after SA and JA application suggests that SA and JA are overlapped to certain extent and have cross-talk between signaling and metabolic pathway activation. In this chapter it is well documented that there is strong interplay between SA and JA pathway in induction of growth, biomass, physiological and biochemical traits in conferring stress tolerance and boosting plant immunity against several biotic and abiotic stress. Furthermore, this chapter highlights SA and JA synergistic crosstalk in enhancing osmolyte (proline) content and promoting antioxidant defence system (such as CAT, POX, SOD, APX GR, glutathione, ascorbate, phenolic compound, flavonoid, α- tocopherol, soluble sugar and amino acid) for quenching ROS and reducing MDA and electrolyte leakage. SA and JA together also participate in inducing translation of HSPs during stress. Furthermore, still the mechanism of interaction between both

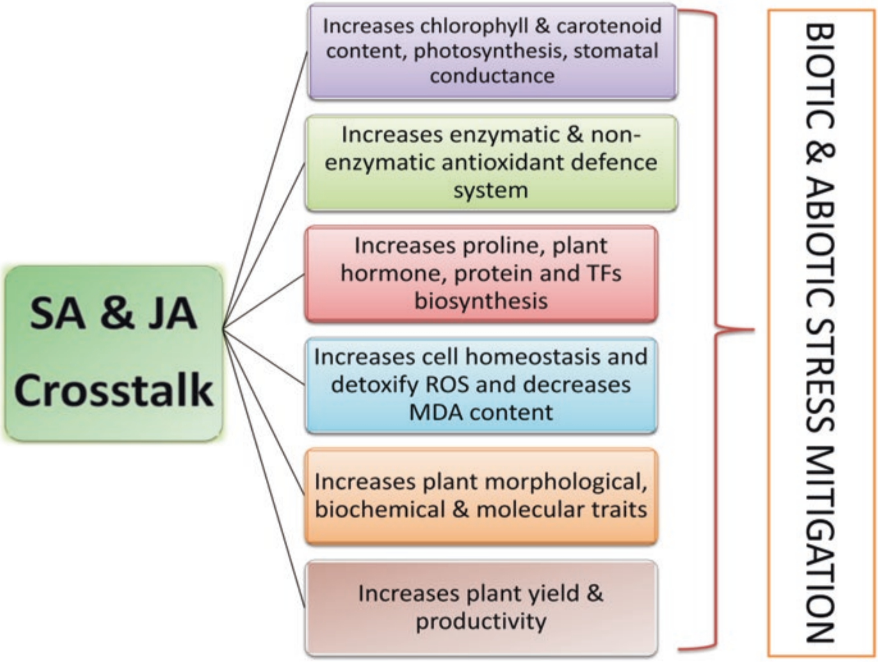

Fig. 8.3 Summary of interplay of SA and JA role during stress

hormones is unclear. Our hypothesis is that both salicyltae and jasmonate ae interlinked during stress and act tremendously beneficial in plant growth and metabolism. Additionally, unraveling the role of both SA nad JA in modulating several genes, proteins and signaling pathway in detal will be useful to develop a deeper understanding of mechanism involving in regulation of plant metabolism during stress. In current scenario, present knowledge on interplay of SA and JA biosynthesis, signaling, molecular mechanism and its action controlling plants metabolism during stress is too far from being complete and require further intensive research. Furthermore, more researches need to be conducted on interplay of salicylate and jasmonate in ameliorating biotic and abiotic stresses such as salinity, drought, HMs, temperature and UV rays to investigate physio- biochemical and molecular interaction associated with SA and JA. Undoubtedly, a diverge area for future investigation remains.

Acknowledgments Yamshi Arif acknowledges the receipt of Innovation in Science Pursuit for Inspired Research (INSPIRE) Fellowship (IF 180778) by the Department of Science & Technology, Government of India, New Delhi.

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Chapter 9
Increasing Evidences on the Relationship Between Salicylic and Polyamines in Plants

M. Pál, T. Janda, O. K. Gondor, and G. Szalai

Abstract Salicylic acid (SA) is generally present in plants in quantities of a few µg/g fresh mass or less, either in the free state or in the form of glycosylated, methylated, glucose-ester or amino acid conjugates. It directly or indirectly affects various physiological processes, including germination, vegetative growth, flower induction, thermogenesis, ion and nutrient uptake, nitrogen metabolism, transpiration, photosynthesis, and respiration. SA may also contribute to maintain cellular redox homeostasis, through the regulation of antioxidant enzymes. SA, as a signalling molecule, plays role in local and systemic acquired resistance against pathogens as well as in acclimation to abiotic stressors. Negative or positive interactions of SA with other hormones like abscisic acid, cytokinins, ethylene, gibberellins or jasmonic acid have an important role in the fine-tuning of the immune response during stress conditions. Natural polyamines (PAs) are water-soluble aliphatic amines present in all cells, but have not been given the status of plant hormones, because of their general relatively high, 10^{-9}–10^{-5} M concentration. They play an essential role in regulating plant development and life functions, such as plant growth, cell division and differentiation, transcription and translation. PAs are suggested to be considered not only as direct protective molecules, but also as versatile compounds that play key role in the regulation of stress tolerance, interacting directly with other metabolic pathways and hormonal crosstalk, and activate the expression of stress-responsive genes. In redox regulation, PAs have dual role, as they are both sources of reactive oxygen species and potential quenchers of them, so they also play a role in the regulation of plant redox homeostasis. In addition, despite of numerous reviews published on the interactions between SA and phytohormones, the synthesis of current information on the relationship between SA and PAs is scarce. This chapter will provide insight in the mode and levels of the connection between SA and PAs.

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S. Hayat et al. (eds.), *Salicylic Acid - A Versatile Plant Growth Regulator*,
https://doi.org/10.1007/978-3-030-79229-9_9

Keywords Biotic and abiotic stress · Polyamines · Putrescine · Salicylic acid · Spermidine · Spermine

1 Introduction

Plant hormones are low molecular weight organic compounds, which occur in low concentrations and produced by the plants as secondary metabolites to regulate their growth, development, reproductive processes, longevity, even death, and are responsible for the adaptation of plants to environmental stimuli. There are five general classes of hormones: auxins, cytokinins, gibberellins, ethylene, and abscisic acid. Later, this list has been expanded, so brassinosteroids, jasmonates, salicylic acid, and strigolactones are also accepted as plant hormones. However, the definition of a plant hormone has not been clearly established. Depending on which definition is considered, other plant growth regulators, such as polyamines (PAs) can also be classified as plant hormones (Munné-Bosch & Müller, 2013). PAs are much more abundant in plant cell than classical phytohormones; furthermore, their metabolism is in relation with the synthesis of other protective or signalling compounds and hormones, e.g. ethylene, proline, GABA, phytochelatins, H_2O_2 and NO (Moschou et al., 2008; Pál et al., 2015, 2017, 2018a, b, c), thus PAs do not appear to have just a strictly hormonal role. According to these, it might be better to classify them as phytohormonal-like regulator molecules.

Natural PAs are low molecular weight, positively charged aliphatic amines present in all plant cells, that are able to interact with negatively charged molecules, e.g. nucleic acids, acidic phospholipids and various types of proteins, protecting them under stress conditions. The involvement of PAs in the regulation of various cellular, physiological and biochemical processes (transcription, translation, cell division and elongation, photosynthesis, ion transport, antioxidant system and signalling) has general importance (Pál et al., 2015). Under stress conditions, plant species exhibit different responses in terms of PA levels, some accumulating PAs, while others have constant or even decreased endogenous PA content (Liu et al., 2007). In many cases, a correlation between PA content and plant stress responses has also been shown. However, even if PAs accumulate, this does not prove their direct role in protection and tolerance (Kovács et al., 2012; Pál et al., 2013, 2014). Changes in the levels of the most abundant PAs, putrescine (PUT), spermidine (SPD) and spermine (SPM), were characterised under biotic and abiotic stress effects in different cereals (Pál et al., 2006, 2013, 2014; Gardiner et al., 2010; Kovács et al., 2014a, b; Janda et al., 2016), demonstrating that PAs are involved in the acquisition of stress tolerance (Gill & Tuteja, 2010). The early activation of the PA biosynthesis has been reported in response to drought stress in rice (Yang et al., 2007), in response to both biotic and abiotic stresses in wheat (Asthir et al., 2010). Modulation of SPD and SPM levels was studied in maize seedlings under long-term salt stress (Jiménez-Bremont et al., 2007), in wheat under cold and drought stress (Kovács et al., 2010; Kocsy et al., 2011), and during UV-B radiation in different plant species (Mapelli et al., 2008). The protective effect of both PA treatment and priming also studied in several species (Farooq et al., 2007; Radyukina et al., 2011; Ghosh et al., 2012; Jafar et al., 2012; Szalai et al., 2017; Tajti et al., 2018; Doneva et al., 2020). The role of PAs during biotic stresses has been reviewed (Pál & Janda, 2017), and it was also reported that PAs can elicit plant defence responses against certain biotic stressors (Hussain et al., 2011).

Importance of PAs has been proved by the fact, that correlation was found between the grain filling rate and PA contents in rice plants (Wang et al., 2012), and similarly, exogenous applications of SPD or SPM increased the grain filling rate and weight of wheat grains (Liu et al., 2013, 2016). However, the accumulation of PAs does not prove their direct role in the tolerance (Kovács et al., 2012, Pál et al., 2013, 2014). It was also found that treatments with different PA compounds produce different changes in wheat, maize and rice plants, and depending on the actual metabolic changes and relations, the "more PA, the better" is not always true (Szalai et al., 2017; Pál et al., 2017).

PAs occur in free soluble form, non-covalently conjugated form, and covalently conjugated form, which can be divided into perchloric acid-soluble and perchloric acid-insoluble fractions. Earlier, only free PA content has been investigated. PAs can also be conjugated with small molecules, such as phenolic compounds. Phenolamides are conjugates of PAs with hydroxycinnamic acids are known as hydroxycinnamic acid amides (HCAAs) (Bassard et al., 2010). Growing evidences show that they are strongly involved in plant growth and development processes, as well as senescence, plant defence, cell division and plant response during biotic or abiotic stresses (Macoy et al., 2015). Besides these, PAs are capable to bind to pigment-protein complexes in the chloroplast, thus stabilize and influence their functions (Ioannidis & Kotzabasis, 2007; Sobieszczuk-Nowicka et al., 2007), directly bind and modify the activity of various ion channels (Pottosin 2015), antioxidant enzymes (Paul et al., 2018). Nevertheless, PAs can also bind to DNA or RNA, thus influence the gene expression and translation (Amarantos et al., 2002; Childs et al., 2003) (Fig. 9.1).

PUT is synthesized by the decarboxylation of ornithine through an enzymatic reaction catalysed by ornithine decarboxylase (ODC) or indirectly by the decarboxylation of arginine by arginine decarboxylase (ADC), via agmatine. Higher molecular weight PAs are produced by sequential addition of aminopropyl moieties to the PUT skeleton through enzymatic reactions catalysed by SPD and SPM synthases (SPDS and SPMS). The donor of aminopropyl groups is decarboxylated S-adenosylmethionine, which is synthesized from S-adenosyl-methionine (SAM) by SAM decarboxylase (SAMDC). The PA synthesis and gene expression is well studied in *A. thaliana*, and the use of synthesis mutant and transgenic *Arabidopsis* plants confirmed the importance of PAs in stress tolerance (Alcázar et al., 2012; Gill & Tuteja, 2010; Kusakabe et al., 2004). Besides the diamine oxidases (DAOs) and PA oxidase (PAOs), which catalyse the terminal catabolism of PAs in the apoplast, PAs also undergo rapid interconversion in the PA-cycle, where the partial and/or full back-conversion of SPM to SPD and of SPD to PUT are catalysed by special PAOs localised mainly in the peroxisome or cytoplasm. According to these, the amount of PAs changes extremely dynamically (Pál et al., 2015) (Fig. 9.1). In addition, the levels of free PAs depend not only on their biosynthesis, but also on their uptake, transport, conjugation and catabolism.

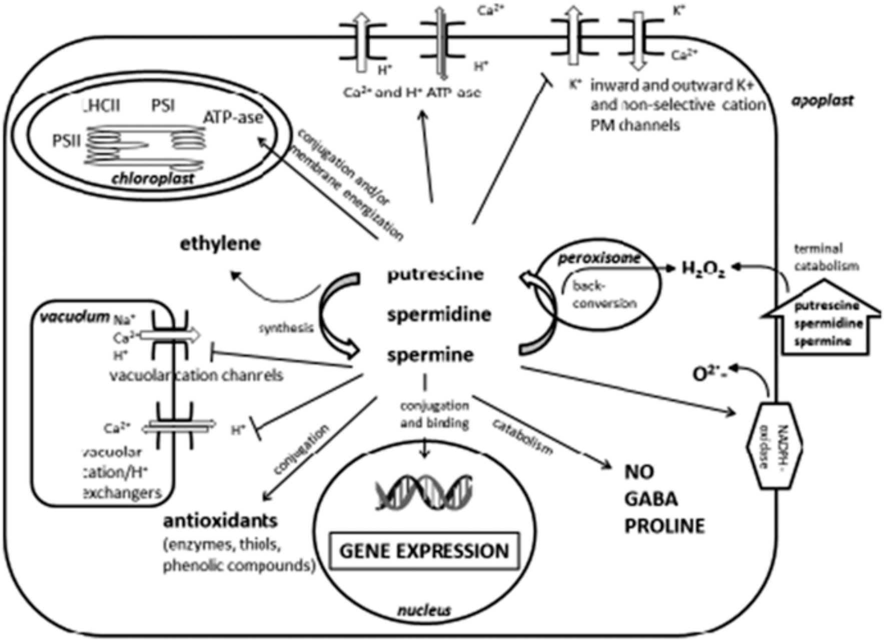

Fig. 9.1 Schematic diagram on the effects of polyamines on various plant processes resulted from their direct bind or conjugation, and from their metabolism

Mutant and transgenic *Arabidopsis* plants with altered PA synthesis pointed to involvement of PAs in different abiotic and biotic stresses responses and have elucidated their key functions in stress signalling networks in plants. As PAs play a critical role in plant stress adaptation, insight into the regulation of the metabolism of these compounds will provide new perspectives for crop protection against environmental changes. PAs should not be taken into consideration only as protective molecules, but rather like double-faced molecules that likely serve as a major area for further research efforts. Although recent results indicate that PA signalling is involved in direct interactions with other plant hormones, such as salicylic acid (SA), the precise mechanism by which PAs control plant responses in the hormonal crosstalk is largely unknown.

2 Correlation Between Salicylic Acid and Polyamine Contents

Salicylic acid (SA) has been known as a signal molecule in the induction of defence mechanisms in plants for a long time (Raskin, 1992), furthermore it also participates in the signalling of abiotic stresses (Khan et al., 2015). Earlier only in a few studies were described the parallel changes in SA and PA contents during plant growth or under stress conditions (Iqbal et al., 2006a, b; Majláth et al., 2011; Pál et al., 2014; Glaubitz et al., 2015). However, these studies did not payed any attention on the possible connection between these compounds, usually only the changes in their levels were discussed. Screening of high number of cereals has been performed under field condition determining the SA and PA contents to select genotypes with higher and lower initial SA and PA levels, separately with respect on biotic stress tolerance of the genotypes, but no correlation was found between the levels of SA or PAs and the degree of stress tolerance against powdery mildew or leaf rust (Kovács et al., 2012). While also on results on powdery mildew infection in wheat leaves revealed significant, positive correlations between the SPD and SPM contents (free and conjugated forms) or conjugated PUT, and the free SA level (Pál et al., 2013). Cadmium stress increased both the PUT and SA content in the leaves of wheat, which in turn lead to close, positive correlation between these compounds (Kovács et al., 2014a). Drought or cadmium stress alone or in combination with UV-B stress also induced pronounced changes in the levels of PAs and SA in wheat, and positive correlation was found in the leaves between SA and PUT, while in the roots between SA and cadaverin (Kovács et al., 2014b). Close positive correlation between SA and PUT or SPD was also found in Cd-treated *Scrophularia striata.* In addition, it was also revealed that Cd-induced changes in the PAs metabolism regulated the levels of PAs, abscisic acid and H_2O_2, which collection of metabolite changes in turn enhanced e.g. phenylalanine-ammonia-lyase (PAL) activity, and increased SA content (Beshamgan et al., 2019). However, correlation data did not reveal the causal relationships, only suggesting that relationship exists, without any information on the detailed background processes, despite of the fact that SA or PA treatments have similar beneficial effects under stress conditions, in addition the combined application of SA and PA was more positive under the same conditions than the SA or PA treatments alone (Darvizheh et al., 2018a, b; Koyuncu et al., 2019; Roumani et al., 2019; Neto et al., 2020).

3 Effects of Salicylic Acid Treatment on Polyamine Metabolism

One of the earliest works to report on the protective effect of SA against abiotic stress factors dealt with heavy metals. 0.1 or 0.2 mM SA was found to reduce the inhibitory effect of Pb^{2+} and Hg^{2+} on seed germination and seedling growth (Mishra & Choudhuri, 1997, 1999). This has been followed by a number of studies about SA-induced heavy or toxic metal tolerance in various plant species, which was accompanied with increased citrate efflux (Yang et al., 2003), accumulation of organic solutes leading to the protection of photosynthetic pigments and membrane integrity (El-Tayeb et al., 2006), and modified activity of antioxidant enzymes (Chen et al., 2007). Besides this, SA has been proved to be beneficial against drought stress (Hamada, 1998; Hamada & Al-Hakimi, 2001), mainly through enhancing the photosynthetic parameters, membrane stability, water potential and activities of nitrate reductase and carbonic anhydrase (Hayat et al., 2008). It was also reported that the exogenous application of SA induced the activity of antioxidant enzymes (Kadioglu et al., 2011), increased the accumulation of osmolytes (Tari et al., 2002, 2004, 2010; Szepesi, 2006, 2009; Gémes et al., 2008), ABA (Bandurska & Stroinski, 2005; Sakhabutdinova et al., 2004), and PA content in plants (Németh et al., 2002). It has been shown that SA could provide multiple stress tolerance at the same time, when applied in aqueous solution or as soil drenches or as seed soaking (Senaratna et al., 2000; Hamada & Al-Hakimi, 2001). These results suggested that exogenous SA could trigger acclimation processes involving the synthesis and accumulation of antioxidants and osmolytes, such as such as proline, PAs, sugars, polyphenols, sugars, polyalcohols, and amino acids. Despite the evidence that SA participates in abiotic stress responses, the stress tolerance imparted by SA appears to be dose-dependent, since SA deficiency or very high SA contents increase susceptibility (Yuan & Lin, 2008).

Enhanced levels of PAs have been reported to be in relation with increased abiotic stresses tolerance of plants, resulting from the overexpression of genes *ODC* in tobacco, *ADC* and *SAMDC* in rice, *SPDS* in tomato, and *ADC2* in Arabidopsis (Kumria & Rajam, 2002; Capell et al., 2004; Roy & Wu, 2002; Wen et al., 2010). In some cases, increased PUT, SPD, and SPM content showed close correlation between the PAs content and the degree of stress tolerance, but the relationship cannot be generalised (Pál et al., 2015). Nevertheless, 0.05 M SA treatment increased the PUT content in the leaves, and improved the salt acclimation in tomato (Szepesi, 2006). Application of SA at low concentrations may stimulate the PAs synthesis, in addition alterations in PA catabolism may also be involve in stress acclimation mechanisms in tomato plants (Szepesi et al., 2009, 2011). All the levels of the investigated PA compounds, namely PUT, SPD and SPM increased in the leaflets of SA-treated pea plants (El-Khawas, 2012). Németh et al. (2002) found in maize, that 0.5 mM SA treatment increased PUT, but decreased SPM content, but did not induced drought tolerance. Application of SA in oat under drought conditions reduced the level of PUT, while the content of SPM was increased (Canales et al., 2019). Exogenous SA changed the PA levels in yali pear and the different SA concentrations have different effects (Wang et al., 2012). Priming with 0.5 mM SA also led to an increase in the PUT content in the leaves of maize seedlings both under normal and low temperature conditions, and a slight decrease in the leaves was observed in SPD content under normal growth condition. It was also suggested that PAs may contribute to the stress tolerance of maize plants primed with SA (Szalai et al., 2016). Changes in PUT and SPM, but not SPD levels showed light dependent increase in SA-treated tomato. While the genes of PA biosynthesis (*ADC, ODC, SPDS* and *SPMS*) were up-regulated by 1 mM SA, the enzymes participating in PA catabolism (DAO and PAO) showed higher gene transcript level and enzyme activity at 0.1 mM SA concentration (Takács et al., 2016).

The effects of SA do not only depend on the plant species, but vary depending on the genotypes, as the salt sensitivity *Solanum lycopersicum* and the relative salt-resistant wild *Solanum chilense* responded differently to 0.01 mM SA treatment. In the resistant genotypes PUT, SPD and SPM contents increased both in the leaves and roots, while in the sensitive one only the root PUT level increased (Gharbi et al., 2016). The SA-mediated alleviation of drought stress might be also due to PAs in oat. 0.1 mM SA treatment reduced levels of PUT both in the resistant and sensitive genotypes under drought conditions, but not under the control conditions. SA could reduce the levels of SPD only in the resistant genotypes, while increased the SPM content in both genotypes either under control or drought conditions. The lower levels of PUT was due to the downregulation of *ADC* gene expression under drought stress, while increased SPM level was due to the upregulation the genes encoding enzymes involved in the synthesis of higher PAs, namely SPDS, SPMS and SAMDC (Canales et al., 2019). However, 0.1 and 0.5 mM SA treatments induced decrease in PA, especially SPD content in the root nodules of *Medicago sativa* during salt stress, due to the strongly induced synthesis of ethylene (ET), which manifested in the accumulation of 1- aminocyclopropane- l-carboxylic acid in nodules, indicating the prevalence of ET biosynthetic pathway over the synthesis of higher PAs (Palma et al., 2013). On the other hand, genes like SAM synthase and SAMDC are also up-regulated by SA, which was parallel with the repression of ET synthesis in tomato (Gharbi et al., 2016).

Methyl salicylate (MeSA) is a volatile organic compound that is synthesized from SA, and produced by plants in response to herbivore damage (Van Den Boom et al., 2004). MeSA treatment has been reported to increased *ADC* and *ODC* gene expression, which was accompanied by increased ADC activity, and increased PUT, SPD and SPM contents leading to decreased chilling injury in Me-SA-treated cherry tomato fruits (Zhang et al., 2011).

Different abiotic and biotic stresses lead to the accumulation of unfolded and misfolded proteins in the endoplasmic reticulum (ER), resulting in unfolded protein response (UPR), which can be regulated by SA. As SA regulates PA metabolism in plants in a concentration- and time-dependent manner, PA, such as SPM, could be significant candidates for the activation of UPR (Poór et al., 2019).

4 Effects of Polyamine Treatment on Salicylic Acid Synthesis

Although the roborating and anti-age effects of PAs are well-studied, in addition their protective role under stress conditions has been also demonstrated, the exact action mechanism is still not clear. PAs are multifaceted compounds, which have a key role in the regulation of plant development and stress responses, are involved in direct interactions with other metabolic routes and hormonal cross-talk, and also activate the expression of stress-responsive genes (Hussain et al., 2011; Pál et al., 2015; Chen et al., 2019; Takahashi 2020).

Microarray analysis of *ADC2* overexpression revealed both the up- and down-regulation of various stress-responsive, hormone- and signalling-related genes in Arabidopsis. These included genes encoding transcription factors belonging, genes involved in the biosynthesis of auxin (IAA), ET, abscisic acid (ABA), gibberellin (GA) and SA, genes for IAA transport, and genes coding for IAA-responsive proteins, ET- and ABA-responsive transcriptional factors, and also jasmonate (JA)-induced proteins. Arabidopsis plants with increased SPM levels caused by the overexpression of *SAMDC1* or *SPMS* also showed altered transcriptome profiles. Many of the genes responded similarly in these two genotypes, namely genes in the biosynthesis of JA and ABA, JA- and SA-responsive genes, receptor-like kinases, mitogen-activated protein kinases, genes with a role in calcium regulation, or the genes of transcriptional factors (Marco et al., 2011).

Interestingly, *Atpao4* plants (in which the lack of *PAO4* – responsible for the PA back-conversion – induced a decrease in SPD and an increase in SPM) several alterations in gene expression including the up-regulation of genes involved in flavonoid biosynthesis, such as PAL could be detected (Kamada-Nobusada et al., 2008).

Seed soaking with SPD and SPM enhanced SA content in wheat under salt stress (Iqbal et al., 2006a, b). It has also been demonstrated that SPM accumulation after TMV infection in tobacco plants stimulates the activity of two important mitogen-activated protein kinases (MAPK) involved in plant defence: wound-induced protein kinase (WIPK) and SA-induced protein kinase (SIPK) (Takahashi et al., 2003), furthermore NtMEK2–SIPK/WIPK cascade is involved in regulating PA synthesis in tobacco (Jang et al., 2009).

The chilling tolerance of maize was found to be improved PUT seed priming, and it was suggested PAs has role in SA-induced stress responses (Cao et al., 2008). A recent comparative study on PUT and SPD treatments applied as seed soaking or hydroponically revealed that the protective effect of the PA treatment under cadmium stress was in relation with decreased SA content in the leaves and roots of wheat plants, compared to that found after cadmium treatment alone (Tajti et al., 2018). Also in wheat, hydroponic PUT pre-treatment decreased the total SA content both in the leaves and roots, but increased it in case of maize roots. However, under PEG-induced osmotic stress, the PUT pre-treatment led to slightly lower total SA content in the wheat roots, but higher accumulation of SA in the roots compared to the PEG-treated ones. This difference in the PUT-induced changes in SA contents was responsible for more beneficial effect of PUT in wheat (Szalai et al., 2017). In contrast, SPM at 1 mM and SPD at 5 mM concentration sprayed on creeping bentgrass leaves did not induced significant changes in the SA content (Krishnan & Merewitz, 2017). Exogenous PAs, especially PUT also elicit MeSA emission – the herbivore-induced volatile – in lima bean leaves (Ozawa et al., 2009).

Effector triggered immunity and systemic acquired resistance in response to bacteria was dependent on the accumulation of hydrogen peroxide and SA, the expression of ENHANCED DISEASE SUSCEPTIBILITY (EDS1) and NONEXPRESSOR of PR GENES1 (NPR1) in Arabidopsis. PUT treatment elicits the local accumulation of SA, and in turn local and systemic transcriptional reprogramming, while SAR was compromised in loss-of-function *adc2–3* and *adc2–4* and n *cuao1–3* (CuAO: cooper containing amine oxidase) mutants. qRT-PCR analyses has also confirmed that SAR and PUT treatment induced similar up-regulation pattern, including gene such as *PATHOGENESIS RELATED1* (*PR1*), the SA biosynthesis gene *ISOCHORISMATE SYNTHASE 1* (*ICS1*), SAR-related *FLAVIN-DEPENDENT MONOOXYGENASE 1* (*FMO1*) and *L-LYSINE ALPHA-AMINOTRANSFERASE*

ALD1 (*AGD2-LIKE DEFENSE RESPONSE PROTEIN 1*). Based on these results it was concluded that, PUT induced the ROS-dependent SA pathway in the activation of plant defences (Liu et al., 2020). In order to study the contribution of SA and SA pathway on PUT signalling, the gene expression analysis was also performed after PUT treatment in *eds1–2*, *npr1–1* and *sid2–1* mutants, the latter one is deficient in *ICS1* gene. Significant part of the PUT-induced upregulation was diminished. It was also concluded that PUT elicits SA accumulation, but SA has no obvious influence on PA levels (Liu et al., 2020).

Also in Arabidopsis, investigation on *eds5–1*, which is a SA transport mutant and *sid2–2*, which has mutation in the SA biosynthesis ICS enzyme, revealed that mutations did not influence the PA contents, among the genes involved in PUT, SPD and SPM synthesis, back-conversion or terminal catabolism, only the expression levels of SPMS showed higher transcript level in *sid2–2* compared to the *eds5–1* and the wild type. The two SA biosynthetic pathways (PAL and ICS) showed different regulations. PA treatments in Col-0 wild type activated both pathways. In the *eds5–1* mutant, both pathways were active after PUT treatment, while the higher PAs (SPD and SPM) further stimulating the PAL pathway, but inhibited the gene expression levels of the *ICS*. Due to the mutation of *ICS* in *sid2–1*, the PAL pathway was activated by PA treatments and was responsible for induced SA biosynthesis (Tajti et al., 2019).

5 The Aspect of Salicylic Acid and Polyamines in Plant Hormone Interactions

As the positive feed-back loop between SA and PAs has been demonstrated in the subsections above, not surprisingly, the combined treatments of them may also have beneficial effects in plants. SA and SPM together enhanced the germination of *Echinacea purpurea* under normal and drought conditions by enhancing antioxidant enzyme activity, proline, chlorophyll, and carotenoid content, and dry shoot biomass (Darvizheh et al., 2018a, b). SA and SPD combined application in tomato helped to overcome the loss caused by salt stress, which manifested in increased growth, gas exchange activity, proline content and antioxidant enzyme activity (Fariduddin et al., 2018).

In generally, plant hormones can be divided in the two groups depending on their role in control of senescence. Ethylene (ET), jasmonic acid (JA), SA and abscisic acid (ABA), and to a minor extent strigolactons (SL) have been characterised as hormones that promote senescence, while cytokinins (CK), auxins (IAA), gibberellins (GA) and brassinosteroids (BR) are regarded as hormones that delay senescence (Kazan & Lyons, 2014; Ohri et al., 2015; Schippers et al., 2015; Shigenaga & Argueso, 2016; Verma et al., 2016). However, these hormones do not exert their effects alone, but in a very complex network, with several antagonistic and synergist connections between each other, which due to this fine tuning lead to the actual plant responses both under normal and stress conditions. Although PAs are not

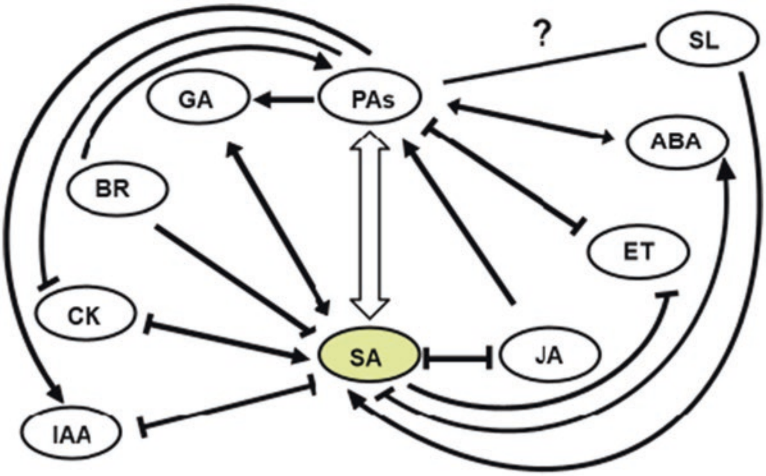

Fig. 9.2 Interaction between salicylic acid (SA) and polyamines (PAs) in the aspect of their relationship with other plant hormones. (*ABA* abscisic acid, *BR* brassinosteroids, *CK* cytokinins, *ET* ethylene, *GA* gibberellins, *IAA* auxins, *JA* jasmonic acid, *SL* strigolactones)

"classical" plant hormones, changes in PA levels have also been reported to be associated with aging, as their levels decline continuously with age (Minois et al., 2011). In addition, their relationship with plant hormones has been also well-documented (Pál et al., 2015). Figure. 9.2 shows the most dominant relations between PA/SA and other plant hormones under different growth conditions, in order to present their position in plant hormone signalling (Legocka & Żarnowska, 1999; Walters et al., 2002; Cui et al., 2010; Argueso et al., 2012; Shivani et al., 2015; Krishnan & Merewitz, 2017; Zhao et al., 2017; de Assis-Gomes et al., 2018; Hasanuzzaman et al., 2019; Omoarelojie et al., 2019).

6 Conclusion and Further Advances

Although numerous studies have been published investigating the effects of biostimulants aiming at their practical use in agriculture, in addition the benefits of their combined treatments have also been recognized, little is known about their interactions, and not enough attention has been payed to the fine-tuning of the combined treatments. In the present chapter, we focused on the interaction between SA and PAs at different levels during plant growth and development, and under stress conditions. The summarize of these recent findings allows us to highlight that the safe and practical use of SA as a plant growth regulator and/or stress protector can be considered after not only the determination of the effective concentration, mode of application and time duration for each specific plants species/varieties, but the crosstalk of SA with different plant growth regulators, such as PAs should be also taken into consideration. Based on the literature, positive feed-back relation of SA and PAs begins to emerge, thus the combined, coordinated use of these protective compounds shows new opportunities in sustainable agricultural production.

Acknowledgments Orsolya Kinga Gondor is a post-doctoral grantee of the Hungarian Academy of Sciences (PREMIUM-2019-462). This work was supported by the Hungarian National Scientific Research Foundation (NKFIH K134395).

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Chapter 10
Biosynthetic Convergence of Salicylic Acid and Melatonin, and their Role in Plant Stress Tolerance

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Abstract Phytohormones are small organic molecules that are found naturally in plants and regulate various physiological processes at a very low concentration. Salicylic acid (SA) and melatonin (MEL) are phytohormones discovered in plants during nineteenth and twentieth century, respectively. Since, SA and MEL share a common precursor during their biosynthesis many similarities exist between the two. SA and MEL regulate growth and physiology of plants specifically, under the stress (abiotic) conditions. The present chapter deals with SA and MEL coinciding biosynthetic pathway, response and interaction with each other in plants.

Keywords Antioxidants · Nitric oxide · Physiology · Phytohormone · Reactive oxygen species · Stress

1 Introduction

Salicylic acid (SA) chemically known as ortho-hydroxybenzoic acid is a phenolic compound and an active ingredient of willow (Salix sp.) bark. Use of aspirin (trade name for acetylsalicylic acid), replaced the use of SA as it produced similar medicinal properties but generated less gastrointestinal irritation (Hayat et al., 2013b). Most of the plant phenolic compounds are derived via shikimic acid pathway. In plants, SA synthesis occurs via phenylalanine pathway however, it may also take place by the isochorismic acid pathway. Phenylalanine by the action of enzyme phenylalanine ammonia lyase synthesizes trans-cinnamic acid which forms benzoic

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S. Hayat et al. (eds.), *Salicylic Acid - A Versatile Plant Growth Regulator*,
https://doi.org/10.1007/978-3-030-79229-9_10

acid via-oxidation of fatty acids or by the hydroxylation of trans-cinnamic acid to form ortho-coumaric acid followed by oxidation of the side chain which is a non-oxidative pathway. Benzoic acid 2-hydroxylase catalyzes the hydroxylation of benzoic acid to synthesize SA (Horváth et al., 2007; Vlot et al., 2009; Mustafa et al., 2009; An & Mou, 2011).

Melatonin (N-acetyl-5-methoxytryptamine; MEL) is found in both plants and animals, and well known for its various cellular and physiological functions. Lerner et al. (1958, 1959) discovered it in cow. Later, its presence in higher plants was also confirmed by Hattori et al. (1995) and Dubbels et al. (1995), and is often referred as phytomelatonin (Blask et al., 2004; Arnao & Hernández-Ruiz, 2018). Melatonin is an indoleamine derivative of tryptophan, and biosynthesis MEL from tryptophan has been investigated in plants and animals. In plants, tryptophan is primarily transformed into tryptamine which gets converted into serotonin (5-hydroxytryptamine), and further into N-acetylserotonin or 5-methoxytryptamine and finally into MEL. Various enzymes associated with MEL biosynthesis have been extensively studied and characterized in many species both animals and plants (Back et al., 2016).

2 Biosynthetic Convergence

MEL and SA biosynthetic pathways converge at a point i.e., chorismic acid. Chorismate is a condensation product of phosphoenol pyruvate obtained from glycolysis and erythrose 4- phosphate via pentose phosphate pathway generated from shikimic acid (Fig. 10.1). Chorismic acid acts as a precursor for the synthesis of three aromatic amino acids—tyrosine, phenylalanine and tryptophan. MEL is derived from tryptophan via anthranilate/indole pathway whereas SA from phenylalanine, besides the direct isochorismic acid pathway (Fig. 10.1).

Regarding catabolism, hydroxylation at different positions of the indole ring occurs in MEL, where 2-hydroxymelatonin is the major catabolite (Byeon et al., 2015). However, for SA, many conjugates for instance methyl SA, salicyloyl-L-aspartic acid, SA 2-O-β-glucoside and salicyloyl-glucose ester have been found.

3 Physiological Roles of SA and MEL in Plants

3.1 Role of Salicylic Acid in Plants

Salicylic acid and its derived products are phenolic compounds synthesized naturally by plants. It is primarily synthesized in cytoplasmic cell in plants. SA was initially discovered in Salix spp., which contains the salicin compound. In plants it occurs as free phenolic acid form or associated to different amino compounds in cells. In plants, SA is concerned in numerous processes like seed germination, root

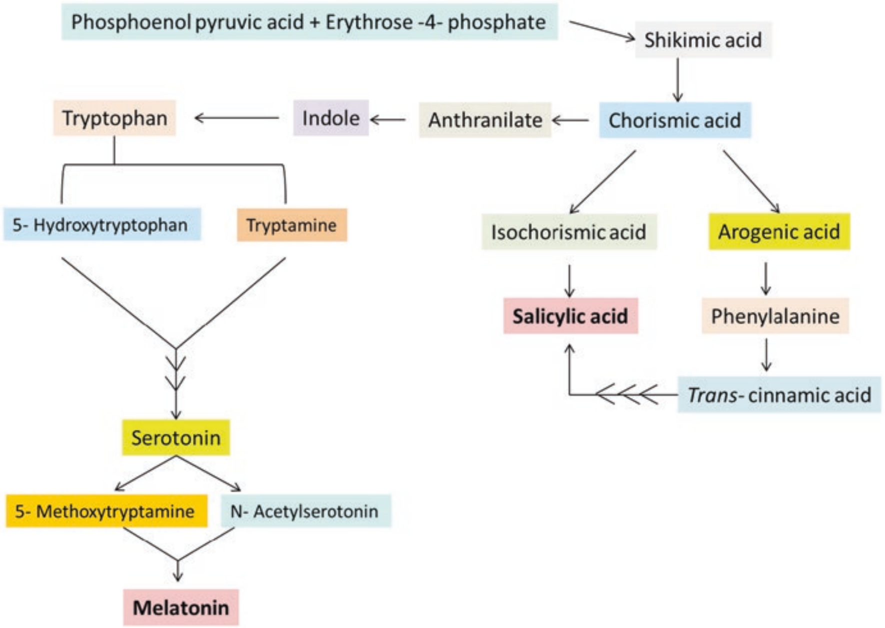

Fig. 10.1 Biosynthetic convergence of melatonin and salicylic acid

development, stomatal closure, stimulation of floral responses, and reductive response against biotic and abiotic stresses (Klessig et al., 2016).

3.1.1 Germination

Seed germination is the basic process that influences plant growth and development. Germination is generally regulated by plant growth regulators such as gibberellic acid and abscisic acid (Yamauchi et al., 2004). It is well recognized that exogenous treatment of SA promoted seed germination, and growth in various plant species such as in soybean, wheat, gloxinia, and Brassica juncea (Hayat et al., 2005). The impact of SA on seed germination process relies on plant genotype and applied experimental circumstances (Lee et al., 2010). Alamri et al. (2018) revealed that treatment of SA enhanced germination percentage, germination vigor and mean germination time in wheat. The reason behind this may be the role of SA to enhance the activity of α-amylase as well as improvement in nutrient uptake and oxygen levels in seeds. The exogenous application of SA enhanced germination in Broad bean as reported by Anaya et al. (2018). Comparable findings were also observed in wheat (Hayat et al., 2005), marigold (Carvalho et al., 2007) and barley (Pancheva et al., 1996). Espanany et al. (2015) while working on *Anethum graveolens* described the promoting impact of salicylic acid on germination, radical extension, plant height, dry weight, and vigor index. Also, the application of SA improved the GA content in *Limonium bicolor* seeds whereas the decline in ABA content was reported (Liu et al., 2019). Numerous studies also summarized the positive influence of SA on germination in different plants species like wheat, (Fardus et al., 2018), Echinacea spp. (Darvizheh et al., 2019), soybean (Aalam et al., 2019), and *Dracocephalum moldavica* (Shaikh-Abol-hasani & Roshandel, 2019).

3.1.2 Plant Growth and Development

Salicylic acid effects growth and development in dose dependent manner. At optimum concentration SA showed positive relation with growth and development in plants; however, Supra-optimal levels inhibit growth. The growth response to SA in plants depends on the plants species under consideration, stage of growth and concentration used. Kováčik et al. (2009) revealed that exogenous application of SA at lower concentration proved effective to improve root growth and leaf rosettes in chamomile plants however higher levels inhibits the same. In another study, higher levels of SA impart inhibitory effect on trichome development, trichome number, and density of trichomes in *Arabidopsis thaliana* (Traw & Bergelson, 2003). SA concentrations (35 and 70 mg), significantly improved the vegetative attributes like leaf area, number of branches and plant height, in *Brassica juncea* L. Moreover, yield traits were also enhanced on application of all SA Concentrations over the control. In *Rosmarinus officinalis* L., application of SA concentrations (450, 300, and 150) improved growth and photosynthetic characters. The best results were observed by the plants supplied with 300 Mg as compared to control (Najafian et al., 2009a). Similar results were also reported in *Thymus vulgaris* L (Najafian et al., 2009b). In *Datura stramonium* L. SA application improved plant height, fresh and dry weight of leaves, yield as well as nitrogen and potassium content, as compared to control (Al-Mohammadi & Al-Rawi, 2016). Fariduddin et al. (2003) reported the enhancement in dry matter accumulation in *Brassica juncea* at lower SA doses. In contrary to this, higher concentrations showed inhibitory effect and reduced the biomass accumulation in treated plants. Also, Hayat et al. (2005), reported the increase in leaf number, fresh and dry mass of *T. aestivum* plants cultivated from the seed grains soaked (10^{-5} M) concentration of SA. From the above literature, we conclude that SA regulates growth and development in dose dependent fashion. Lower doses of Exogenous SA improved all the growth processes while as higher concentrations downgraded the same. In support of this, various researches also reported the similar property of SA in plants (Manzoor et al., 2015; Saleem et al., 2020).

3.1.3 Photosynthesis

SA plays a crucial role in regulating photosynthesis in normal and stressful condition. Exogenous application of SA increased photosynthesis through activation of photosynthetic enzyme, carbohydrate metabolism and regulate the photosynthetic ability in wheat plant (Arfan et al., 2007). Mateo et al. (2006) reported that in *A thaliana*, SA aided photosynthesis and promotes plant growth, which ultimately aides in the adjustment of plants exposed to bright light. In addition, the treatment of SA to *Vigna radiata* plant, improved photosynthesis and several other physiological processes (Nazar et al., 2011). In *Brassica juncea*, exogenous application of SA improved nitrate reductase activity, rubisco activity, and photosynthesis, under normal environmental conditions (Nazar et al., 2015). Furthermore, SA treatment increased growth, photosynthetic rate, stomatal conductance and yield, and as reported in the case of barley (Habibi, 2012). In another study, SA Treatment (0.18 mM) on cucumber enhanced leaf area as well as enhancement chlorophyll pigments, which is directly linked to increased rate of photosynthesis (Sultan et al., 2016). Comparable findings were also observed in Artemisia (Aftab et al., 2010), grapevine (Wang et al., 2010), *Tectona grandis* (Li et al., 2014). In pistachio, different doses of SA, improved the chlorophyll content, stomatal conductance and photosynthesis in normal as well as under myriad conditions (Bastam et al., 2013). Poór et al. (2011) reported that SA improved the efficiency of photosynthetic quantum yield and carbon fixation by increasing RUBISCO activity in tomato. Hayat et al. (2012) concluded that SA acts as an effective plant growth regulator that increased the photosynthetic activity in chickpea. Additionally, SA supplementation enhanced chlorophyll levels, gaseous exchange, stomatal conductance, and CO_2 fixation in fenugreek plant (Babar et al., 2014).

3.1.4 Antioxidant Enzymes

The antioxidant system is crucial to protect cells from oxidative stress and prevents them from oxidation. As oxidation reaction produces various reactive oxygen species (ROS), which generates a web of oxidation reactions, thus causing cell injury or may lead to cell death. To overcome the ROS caused detrimental effects, plants had adopted a responsive system against these toxic reactive species recognized as the antioxidant system, which reduces the production and amelioration of these reactive oxygen species and their associated reactions. The positive role of SA to reduce these reactive radicals has been demonstrated in various studies. SA regulates various crucial enzymes of Asada and Halliwell pathway like GSH, GPX, GR, DHAR, and MDHAR, which demonstrates the beneficial impact of SA under myriad stress conditions in plants (Mustafa et al., 2018; Yan et al., 2018). SA improved the antioxidant enzyme activity such as SOD, CAT, and POX. For example, SA enhances the antioxidant enzyme levels in *Vigna radiate* exposed to toxic levels of (Al) (Ali, 2017), in soybean exposed to arsenic stress (Chandrakar et al., 2016), and in *Zea mays* grown in high chromium soils (Islam et al., 2016). Additionally, SA application improved the activity of APX, CAT, SOD, and GR, in bean cultivated under high Cd levels (Wael et al., 2015). SA also assists to sustain membrane permeability and cell homeostasis (Csiszár et al., 2018). El-Esawi et al. (2017) while working on *Rosmarinus officinalis* reported that, SA application upregulated the gene expression of SOD assessed by real time PCR. In another study, Tahjib-Ul-Arif et al. (2018) also concluded that SA alleviate stress by improving and regulating several antioxidant enzymes like CAT and APX activity in *Zea mays*, thus maintaining membrane integrity and protect the cells from oxidative damage. In another study, Tari et al. (2015) summarized that SA supplementation modulates the expression of antioxidant enzyme in *lycoperiscon esculentum* exposed to unfavourable stress conditions. APX level was enhanced in young leaves whereas elevated GR and APX were reported in roots. Similar enhancement in the levels of CAT and SOD was also found in roots and leaves. SA enhanced the activity of SOD, CAT, and POX in leaves and roots of *Vicia faba* grown under high salinity soil (Ahmad et al., 2018). Also the improvement in the level of SOD, GP and GPx, was observed in SA treated Arabidopsis under salt stress (Horváth et al., 2015). Moreover, SA Supplementation improved the level of total sugars, phenols and also the activity of antioxidant enzymes (CAT, APX and POD; Radwan et al., 2019). From the above literature, it is quite clear, that SA had a great influence on antioxidant enzyme activity by which it controls the oxidative stress and maintains plant growth and development.

3.2 Role of MEL in Plants

Melatonin is a pleiotropic molecule with many diverse functions in plants. It was initially discovered in the bovine pineal gland in 1958, however, in plants, it was primiraly detected in 1995 (Dubbels et al., 1995; Hattori et al., 1995). MEL primarily acts as an antioxidant with decisive actions against multiple oxidative stress responses like (ROS and RNS) and other toxic free radicals. Furthermore, it is involved in numerous physiological and biochemical processes, including seed germination, rooting, photosynthesis, growth, and protection against biotic and abiotic stressors (Mir et al., 2020a, b).

3.2.1 Seed Germination

Successful germination and seedling establishment are the two significant steps for establishing plant populations. MEL has been found to be very effective in promoting seed germination in plants. It exerts a positive effect on seed germination in a dose-dependent manner. MEL in higher concentrations restrains or has no impact on seed germination, while it promotes the seed germination at lower concentrations (Wei et al., 2015). Exogenous supplementation of MEL to seeds increased the germination percentage as well as germination rate as reported in *Phaseolus vulgaris* (Aguilera et al., 2015), *Glycine max* (Wei et al., 2015), and *Cucumis sativus* (Zhang et al., 2017). MEL concentration rises rapidly during the initial days of germination and achieves a maximum peak after 14 hours. However, it's level decline and attains a steady state as the germination process progresses to seedling establishment. Similar results were also described in *Cucumis sativus* primed with

1 μM of MEL. The initial rise of MEL in the cytoplasm may be a factor to the enhanced metabolic rate and nutrient mobilization to germinating seeds (Aguilera et al., 2015). Additionally, MEL remodels seed germination by diminishing the abscisic acid (ABA) levels, by down-regulating the genes related to ABA biosynthesis. On the other hand, gibberellin (GA) biosynthesis-related genes were upregulated leading to a sudden increase in GA levels.

3.2.2 Growth and Development

Various studies confirmed the beneficial role of MEL on plant growth and development. MEL modulates plant growth and development in a dose-dependent manner. At optimum level MEL enhanced the root development, rooting percentage and length of roots in sweet cherry; however, at higher concentrations, MEL inhibited the growth of the roots (Sarropoulou et al., 2012). In another study, Aguilera et al. (2015) reported that MEL significantly improved the root growth at the lower concentration of 20 μM in kidney bean and lentil sprouts. The results are consistent with the finding as reported in sweet cherry (Sarropoulou et al., 2012), Stevia seedlings (Simlat et al., 2018) and *A. thaliana* (Bajwa et al., 2014). The impact of MEL, not only relies on its concentration, but also depends on the growth stage ae well as on the species under consideration (Chen et al., 2009). MEL also induces growth in the aerial parts of the plant and roots, e.g. *Hordeum, Triticum, Helianthus, Brassica, Oryza, Zea mays* and *Lycoperiscon esculentum* plants (Arnao & Hernández-Ruiz, 2017). At optimal concentration it increases the overall growth characters viz., plant height, leaf area, shoot fresh mass and dry mass as reported in *Brassica juncea*; however at higher concentration it displayed negative effect on all growth parameters (Mir et al., 2020a, b). From the above literature, we conclude that MEL acts in a dose-dependent manner and improves numerous plant characteristics which include seed germination, plant growth (Kładna et al., 2003), and yield (Byeon & Back, 2014a), at lower concentrations. Several studies also confirmed the functional role of MEL in plant growth, plant development and its property to act as a growth promoter (Zhang & Zhang, 2014).

3.2.3 Photosynthesis

The primary molecule responsible for photosynthesis is chlorophyll (chl), and because of the fragility of this molecule, it is easily injured by ROS during the process of photosynthesis (Sharma et al., 2012). MEL has proven effective in protecting the degradation of chlorophyll and hence enhances the photosynthetic efficacy. Additionally, MEL enhances stomatal conductance and assists to monitor stomatal opening and closure, therefore, improving the higher rate of photosynthesis and CO_2 assimilation (Mir et al., 2020a, b). MEL enhances quantum yield in *Lycopersicon esculentum* by extenuating the photo-oxidative damage and assists the repair of damaged D1 protein (Zhou et al., 2016). Furthermore, it enhances the expression of genes related to photosynthesis, carbon assimilation, and ascorbate biosynthesis (Arnao & Hernández-Ruiz, 2015). MEL significantly improved the expression of photosynthetic proteins PsaK and PsaG, oxygen-evolving enhancer proteins PsbP and PsbO, the ferredoxin gene PetF, and the VTC4 gene involved in ascorbate biosynthesis (Wei et al., 2015). Similar improvement in photosynthetic was also reported *Triticum aestivum* (Turk et al., 2014), *A. thaliana* (Bajwa et al., 2014), and *Cynodon dactylon* (Hu et al., 2016). From the above literature, it may be concluded that MEL improves growth by improving photosynthesis via the upregulation of photosynthesis-associated genes (Hardeland, 2015).

3.2.4 Antioxidant

Melatonin plays a central, critical role in plants and maintains integrity against various biotic and abiotic stressors (Arnao & Hernández-Ruiz, 2019). It primarily acts as an antioxidant and monitors the expression of different redox mechanisms (Maldonado et al., 2007). MEL is a broad-spectrum antioxidant and possesses the ability to easily pass through cell membrane than other molecules or vitamins. Moreover, MEL controls the activity of many redox enzymes such as CAT, POX, SOD, ASX, GR, and the metabolites GSH, and ASC, therefore mitigate the oxidative stress in plants (Arano & Hernández-Ruiz, 2019). Additionally, its role in photoprotection has also been reported. Tan et al. (2007) while working on *Eichhornia crassipes* (Mart.) noted that MEL and AFMK protect the plant against free radical damage induced by destructive reactive oxygen and reactive nitrogen species. MEL also displayed its photoprotective ability against UV exposure in algae and higher plants (Tan et al., 2007).

The amphiphilicity of MEL supports its accumulation particularly in oily seeds, probably by contributing its antioxidative protection in a dormant seed and more or less dry system, in which enzymes are less effective (Hardeland et al., 2016). Guo et al. (2017) witnessed a rise in MEL level as a justification for the enhancement of 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity in germinating *Helianthus annuus* L. seeds. Posmyk et al. (2009) noticed a protective function of MEL during hydropriming in sprouting *Brassica oleracea rubrum* L. seeds and emerging seedlings against toxic levels of copper. MEL diminishes the oxidative burst of many biomolecules, such as lipids, proteins, and nucleic acids. In summary, the MEL protects plants via: (i) it free radicle scavenger activity, (ii) upregulates the expression of antioxidant enzymes, (iii) modulates the redox properties of the cell by regulating the synthesis and oxidation of glutathione, (iv) assures protection against oxidative impairment through antioxidant enzymes, (v) declines free radicle generation and electron leakage by enhancing the efficiency of mitochondrial electron transport chain. These properties make MEL a vigorous and powerful regulator by checking or nullifying MEL oxidative stress (Wang et al., 2012; Mir et al., 2020a, b).

3.2.5 Senescence

In the recent decade, the role of MEL in delaying senescence has been illustrated. MEL significantly delayed senescence by inducing the expression of CAB genes, reduced chlorophyll degradation, and facilitate the accretion of proteins and soluble sugars, in kiwifruit (Liang et al., 2018a). Additionally, MEL induced the enrichment of flavonoids and antioxidant enzymes like ascorbic acid, glutathione, in treated plants. MEL application decreased the MDA levels, which illustrates its ability in protecting the cell membrane and confirming its characteristic feature in dawdling leaf senescence. Moreover, MEL upregulated the expression many genes required in the biosynthesis of ABA, jasmonic acid (JA), salicylic acid (SA) and ethylene in *A. thaliana.* The majority of the ABA, JA, SA, and ethylene-responsive genes regulated by MEL are also upregulated during stress conditions (Weeda et al., 2014). Additionally, MEL down-regulated the ageing-related genes, genes encoding polygalacturonase, senescence-associated protein 29(SAG29), and JA-induced protein in *Oryza sativa* seedlings (Byeon & Back, 2014b). The decline in the expression of the senescence-associated gene 12(SAG12), as well as dropping of H_2O_2 levels, decrease in GSH levels, the rise of ascorbate content, and enhanced enzyme activity of APX, GSH,CAT, and POD were also reported in MEL treated plants (Byeon & Back, 2014b). MEL also governs the activity of senescence-associated genes, such as PAO, SAG12, ATGs and HXK1. These characteristic features emphasize the senescence-delaying role of MEL in plants (Hardeland, 2016). From the above literature, it is clear that MEL delays senescence, either by upregulating the senescence-delaying genes or by down-regulating the senescence-promoting genes.

4 Effect of Exogenous Salicylic Acid and MEL on Plants Exposed to Abiotic Stress

4.1 Salinity

Salinity is one of the major stresses that limits growth and productivity of plants (Zhu, 2001). It disturbs ionic equilibrium which is a fundamental criterion for the physiological activities in plants. Several general processes i.e. photosynthesis, energy and lipid metabolism and protein synthesis are enormously affected by salinity (Allakhverdiev et al., 2000). The damaging effects of salinity were ameliorated by the exogenous application of SA and MEL in several plants (Dehnavi et al., 2019; Yin et al., 2019). SA was shown to have ameliorating effect on growth, physiology and antioxidant activity of carrot under salinity (Eraslan et al., 2007). Pretreatment of cucumber seedlings with SA induced salt tolerance grown under moderate nitrogen conditions by controlling endogenous SA levels (Kim et al., 2017). In *Vigna angularis*, application of SA considerably reversed the damaging effects of salt stress by declining ROS generation and improving uptake of mineral ions (Ahanger et al., 2020). Foliar application of SA mitigated the salinity-induced damaging effect on growth, biomass and photosynthesis of fenugreek (Babar et al., 2014). In case of *Limonium bicolor,* 0.8 mM SA improved germination under salt stress by triggering hormonal balance between GA and ABA (Liu et al., 2019). In seedlings of *Oryza sativa*, seed priming with SA increased salt tolerance by promoting the activities of enzymatic and non-enzymatic antioxidant (Sheteiwy et al., 2019). SA improved the growth of sorghum plants by limiting the uptake Na and Cl and improvement of uptake and translocation of essential nutrients under salinity stress (Dehnavi et al., 2019). In *Mentha pulegium,* foliar spray of 1 or 1.5 mM SA ameliorated salt toxicity via improvement of antioxidant activity under different concentrations of NaCl (Farhadi & Ghassemi-Golezani, 2020). Exogenous application of 1 mM SA improved salt tolerance in *Brassica nigra* by diminishing Na content, proline and lipid peroxidation, and by up-regulating antioxidant activity, secondary metabolites and sugars under different levels of salinity (Ghassemi-Golezani et al., 2020). Foliar spray of varied concentration of SA (0.10, 0.50 or 1 mM) up-regulated photosynthesis and protected pistachio seedlings subjected to various doses of salt stress (Bastam et al., 2013). In *Nitraria tangutorum* seedlings, SA treatment (0.5–1.5 mM) ameliorated the adverse effect of salt stress by the up-regulation of osmolytes and antioxidant enzyme activities (Liu et al., 2016). In three barley cultivars, SA treatment modulated endogenous phytohormones, ROS and antioxidant enzyme activities under salinized conditions (Torun et al., 2020). Foliar spray of 0.5 mM SA relieved salinity-induced toxicity via enhanced photosynthesis, proline and antioxidant enzyme activites in soyabean (Ardebili et al., 2014). In *Torreya grandis* seedlings, SA induced salt tolerance by up-regulating photosynthesis and inducing antioxidative metabolism (Li et al., 2014).

In maize, application of 100 μM MEL (MEL) to the salt-treated seedlings improved photosynthetic capacity and antioxidant activity (Chen et al., 2018). Seed treatment and plant watering with 10 or 50 μM L^{-1} MEL improved germination and growth in *Cucumis melo* seedlings by stabilizing membrane integrity, ion status and root viability under salt stress (Castanares & Bouzo, 2019). Exogenous application of 50–150 μM MEL significantly improved salt tolerance by scavenging of ROS or up-regulating antioxidant metabolism in cucumber seedlings (Wang et al., 2016). Exogenous application of 20 μM MEL effectively improved seed germination in cotton seedlings by enhancing osmolytes and ion homeostasis under salt stress (Chen et al., 2020). In pistachio seedlings, 100 μM L^{-1} MEL mitigated salinity-induced inhibition in growth by up-regulating nutrient uptake, osmolyte balance, antioxidant enzymes and polyamine biosynthesis (Kamiab, 2020). Pre-treatment of *Solanum lycopersicum* with 150 μM L^{-1} MEL improved salt tolerance by stimulating the activity of the enzymes involved in ascorbate-glutathione (AsA-GSH) cycle (Yin et al., 2019). Treatment of watermelon seedlings with different doses of MEL (50, 100 or 150 μM) mitigated salt stress by improving redox homeostasis coupled with enhanced antioxidant metabolism (Li et al., 2017). In *Malus hupehensis*, pretreatment with 0.1 μM MEL improved salinity resistance by improving ion homeostasis (Li et al., 2012). Exogenously applied MEL improved salinity-mediated inhibition in growth and germination in alfalfa plants by scavenging of ROS and up-regulating antioxidant defense system (Chen et al., 2020).

4.2 Drought

Crops productivity is severely affected by drought stress as it significantly reduces photosynthetic pigments, photosynthesis and plant growth (Hayat et al., 2008; Lee et al., 2009). The positive effects of SA and MEL has been observed in several plant species under drought stress (Hosain et al., 2020; Abdelaal et al., 2020; Qiao et al., 2020; Bidabadi et al., 2020). In maize seedlings, SA pretreatment prevented drought-induced oxidative stress by modifying osmolytes, antioxidative defense system and endogenous ABA (Tayyab et al., 2020). Exogenous SA treatment mitigated drought-induced oxidative damage by up-regulating antioxidant enzymes in rice seedlings (Sohag et al., 2020). In *Hordeum vulgare*, 500 μM SA improved drought tolerance by modulating antioxidant defense system and photosynthesis (Habibi, 2012). In *Ctenanthe setosa,* application of 1 μM of SA induced antioxidative defense system in the plants that showed the symptoms of leaf rolling suffering from long-term drought stress (Kadioglu et al., 2011). In *Triticum aestivum*, SA induced drought tolerance towards by modulating membrane characteristics and antioxidative metabolism (Aldesuquy & Ghanem, 2015). Exogenous application of 0.05 mM SA to hydroponic solutions mitigated polyethylene glycol (PEG)-induced drought in wheat seedlings (Marcińska et al., 2013). Treatment with SA improved the yield in sunflower subjected to drought stress at various stages of growth (Hussain et al., 2008). SA sprays improved drought tolerance in wheat plants by up-regulating drought response gene *[dehydration responsive element binding gene (DREBs) or C-repeat binding factor gene (CBFs)]* (Kareem et al., 2019). Exogenous application of 0–3 mM SA to invitro grown *Impatiens wallerianato* induced physiological and molecular responses under PEG-induced drought stress (Antonić et al., 2016). Foliar application of 0.5 mM SA improved morpho-physiological, biochemical and yield characteristics in drought-stressed barley plants (Abdelaal et al., 2020). Exogenous application of different concentrations of SA improved growth and productivity of rice under drought stress (Hosain et al., 2020). SA supplementation to safflower improved drought tolerance by stimulating vegetative growth (Chavoushi et al., 2019). In peppermint plants, SA application enhanced drought tolerance by improving growth, photosynthesis and osmolytes (Elhakem, 2019). In corn seedlings, SA application alleviated drought stress by improving ion homeostasis, electrolyte leakage, pigment content and root anatomy structure (Bijanzadeh et al., 2019).

In *Malus domestica*, long term exposure to 100 μM MEL mitigated moderate drought stress by improving nutrient uptake (Liang et al., 2018b). Treatment of tomato seedlings with 0.1 mM MEL significantly ameliorated the negative outcomes of drought by maintaining photosynthetic capacity (Liu et al., 2015). In *Brassica napus* seedlings, application of 0.05 mM L^{-1} MEL alleviated the damage from drought stress by stimulating osmolyte and antioxidant activity (Li et al., 2018). Exogenous treatment with different concentration of MEL by leaf-spraying and root irrigation method improved drought tolerance in maize by improving photosynthetic activities (Huang et al., 2019). Maize seedlings when treated with 100 μM MEL as foliar spray and 50 μM MEL as soil drench method effectively mitigated drought stress by improving photosynthetic characteristics and the activity of antioxidative enzymes (Ahmad et al., 2019). Exogenous application of 100 μM MEL improved drought tolerance in maize by up-regulating photosynthesis and AsA-GSH cycle (Guo et al., 2020). Pre-treatments with 100 μM MEL mitigated drought stress in tea plants by detoxifying ROS and up-regulating antioxidative enzymes (Li et al., 2019). Exogenous application of 100 μM MEL improved tolerance to water deficit in tomato plants by promoting cuticle formation (Ding et al., 2018). In maize seedlings, application of 1 μM MEL enhanced tolerance to PEG-induced water deficiency by increasing hydraulic conductance (Qiao et al., 2020). Exogenous application of 100 μM L^{-1} MEL alleviated drought stress in *Glycine max* by up-regulating photosynthesis (Zhang et al., 2019). Foliar application of different concentration of MEL (50, 100, 150 or 200 μM) MEL improved drought tolerance in two species of salvia by improving essential oil production (Bidabadi et al., 2020).

4.3 Heavy Metal Stress

In our environment, heavy metal (HM) contamination is a huge problem that limits plant growth and development (Yadav et al., 2016). Exogenous application of SA and MEL improved HM tolerance to various extent in various plants (Liu et al., 2017; Gu et al., 2018; Kholodova et al., 2018; Xu et al., 2020). Exogenous SA pre-treatment improved plant resistance to cadmium (Cd) toxicity by stabilizing membrane integrity (Belkhadi et al., 2010). Exogenous SA treatment mitigated the deleterious effects of Cd by stabilizing the metabolism of antioxidants, phytochelatin (PC) and osmolytes in *Nymphaea tetragona* (Gu et al., 2018). In zinc-stressed safflower, SA application proved highly effective in mitigating the deleterious effects by the induction of antioxidant defense mechanism and PC biosynthesis (Namdjoyan et al., 2018). Pretreatment of rice seedlings with 100 μM SA greatly ameliorated the Cd-induced growth inhibition (Cao et al., 2013). Exogenously applied SA protected parsley plants from the deleterious effects of lead (Pb) toxicity by declining MDA content along with the increase in photosynthesis, anthocyanin and phenols in the leaves (Alamer & Fayez, 2020). Treatment of ryegrass seedlings with 0.2 mM SA mitigated the toxic effects of Cd by reducing root-shoot translocation of Cd and increasing antioxidative enzyme activities (Wang et al., 2013). Exogenous supplementation of 0.1 mM SA to Cd-stressed peanut seedlings improved growth by inducing photosynthesis, mineral nutrition and pigment content (Xu et al., 2015). In *Cucumis sativus*, SA application up-regulated antioxidants and the absorption of essential ions i.e. calcium, magnesium and zinc against manganese-induced toxicity (Shi & Zhu, 2008). Exogenously sourced SA inhibited aluminum (Al)-oxidative stress in *Cassia tora* roots by preserving membrane integrity and antioxidant metabolism (Wang et al., 2004). The exposure of 10 μM SA to the roots of soyabean mitigated Al-toxicity by modulating the metabolism of reactive oxygen species (Liu et al., 2017).

In strawberry seedlings, application of 100 μM MEL improved growth under Cd-induced toxicity by antioxidant activity, pigment content and ROS homeostasis (Wu et al., 2020). MEL treatment to *Solanum lycopersicum* mitigated Cd phytotoxicity enhancing phytochelatin biosynthesis and antioxidant potential (Hasan et al., 2015). Pretreatment of canola plants with varied concentrations of MEL (0.1–100 μM) mitigated copper toxicity by decreasing oxidative stress and proline accumulation (Kholodova et al., 2018). Foliar sprays of different concentration of MEL (10, 25, 50, 100, and 200 μmol L^{-1}) improved Cd tolerance ROS scavenging and detoxification (Xu et al., 2020).

4.4 Temperature Stress

Temperature is the major environmental factor affecting growth, metabolism, physiology and productivity of plants (Quint et al., 2016). Exogenous application of both SA and MEL positively alleviated temperature stress (Jahan et al., 2019; Li et al., 2019; Wassie et al., 2020). In ornamental pepper, application of 0.01 mM or 0.1 M SA alleviated high-temperature (HT) stress by modulating photosynthesis, pigment content, root activity and electrical conductivity (Wassie et al., 2020). Exogenous supplementation of 0.1 mM SA continuously for 7 days enhanced cold tolerance by modulating the metabolism of ROS and antioxidative enzymes in both tolerant and sensitive cultivars of barley (Mutlu et al., 2016). In tomato, exogenous SA (1 mM) enhanced heat tolerance by enhancing photosynthetic efficiency and up-regulating antioxidative defense system via ROS scavenging (Jahan et al., 2019). Exogenous SA treatment improved photosynthetic activity and antioxidants in chilling-stressed wheat plants (Yordanova & Popova, 2007). In *Digitalis trojana Ivanina,* SA application improved thermotolerance by inducing the synthesis of antioxidants and cardenolides (Cingoz & Gurel, 2016). SA enhanced tolerance of mango fruit against chilling stress by maintaining the balance between ROS, ascorbate and glutathione (Ding et al., 2007).

Exogenous application of 100 μM MEL improved cold tolerance in tomato plants by improving photosynthetic carbon fixation and antioxidant potential, accumulating proline, polyamine and sucrose and by inducing the expression of cold-responsive genes (Ding et al., 2017). In cucumber seedlings, MEL alleviated HT stress by maintaining active oxygen metabolism and integrity of lipid membranes (Xiang-dong et al., 2010). Pretreatment of tomato seedlings with 100 μM MEL alleviated heat-induced damage by improving antioxidant metabolism, inducing AsA-GSH cycle and by modulating polyamine and nitric oxide biosynthesis (Jahan et al., 2019). In *Solanum lycopersicum* seedlings, application of MEL ameliorated HT-induced oxidative stress by up-regulating efficient antioxidant defense system and ROS homeostasis (Ahammed et al., 2019). Exogenous application of 100 μM MEL improved cold tolerance by positively up-regulating the genes responsible for antioxidant enzyme biosynthesis (Li et al., 2019). In carrot suspension cells, pretreatment with 43–86 nM MEL significantly attenuated cold-induced apoptosis by ROS scavenging and its effect on polyamine levels (Lei et al., 2004).

MEL also plays a vital role in plant exposed to stressful conditions. Exogenous application or over expression of MEL biosynthetic genes also enhanced the resistance against various stresses, through enhancement of antioxidant enzymes as well as by its intermediates. MEL regulated stress tolerance involves the stimulation and biosynthesis antioxidants, and its related enzymes or through its ROS scavenging activity (Zhang & Zhang, 2014; Bajwa et al., 2014). During stress conditions, the growth-promoting effect of MEL has been clearly demonstrated in cold stressed *Arabidopsis* (Bajwa et al., 2014). MEL enhances growth upto three to fourfold over the control in *Triticum, Hordeum, Phalaris, Lupinus and Cucumis.* However, less pronounced activities were reported in other species (Arnao & Hernandez-Ruiz, 2017).

5 MEL, SA and Stress Signalling

Nitric oxide (NO) is proposed to have a relevant role in MEL responses in plants, primarily auxin-like and immune responses. There are many signalling factors that increase under abiotic/biotic stress such as NO, reactive oxygen species (ROS) and radical nitrogen species (RNS; Hayat et al., 2013a; Astier et al., 2016). Usually, ROS and RNS signalling is known to be highly coordinated. It is the levels of NO that regulate the ROS network via NO-dependent, post-translational modifications (León et al., 2016). It modifies various functions through modification of protein by nitration, S-nitrosylation and the ligation of NO to transition metals, but also via lipid (nitro-fatty acids) and DNA (8-nitroguanine) modifications (Pucciariello & Perata, 2017; Del Rio & López-Huertas, 2016; Saxena et al., 2016; Damiani et al., 2016; Molassiotis et al., 2016). Furthermore, NO triggers a set of responses to mitigate stress induced toxicity and damage at cellular level, which includes metabolic reprogramming of primary and secondary metabolic processes (León et al., 2016). Endogenous levels of MEL increases under stressful conditions via upregulation of MEL biosynthetic genes (Wei et al., 2018; Wen et al., 2016). Moreover, MEL increases the level of NO via upregulation of nitrate reductase (the enzyme responsible for catalysing the reduction of nitrate to nitrite, but could also reduce nitrite to NO using NADPH as a cofactor). During iron deficiency, MEL induced the NO synthase-like pathway in rice (Sun et al., 2017). It act as NO and ROS scavenger, and reciprocally, NO induces biosynthesis of MEL (Wen et al., 2016). Though the data suggests that MEL might act as an upstream signal of NO however, the intricate interaction between MEL–NO makes it difficult to interpret whether MEL is upstream or downstream of NO or not (Arnao & Hernández-Ruiz, 2018).

Phytohormones such as auxins (IAA), cytokinins and abscisic acid (ABA) stimulate NO production (Arnao & Hernández-Ruiz, 2018; Verma et al., 2016). MEL possess role in conferring tolerance against drought stress in maize plants (Fleta-Soriano et al., 2017). This study suggested that the endogenous level of MEL is directly proportional to stress-related phytohormones, especially SA, however exogenously applied MEL had no effects on the contents of any phytohormone.

6 Crosstalk Between SA and MEL in Plants Defense Response

SA is a hormone which is known for its defence responses and innate immunity in plants (Arnao & Hernández-Ruiz, 2018). It enhances the transcript levels of pathogenesis-related 1 (*PR1*) by a receptor nonexpressor of PR1 (*NPR1*). MEL when present in high level promotes the biosynthesis of SA by inducing the transcription of isochorismate synthase 1 (*ICS1*), via stimulation of mitogen-activated protein kinase (MAPK) cascade (MAPKKK3/OXI1-MAPKK4/5/7/9-MAPK3/6) (Lee et al., 2014; Yool, 2016). Apart from MAPKs, NO also influences the innate immunity in plant by regulating the expression levels of SA synthesis genes (*AtEDS1* and *AtPAD4*) as well as downstream SA resistant genes (*AtPR1*, *AtPR2*, and *AtPR5*) (Shi et al., 2012, 2015). Interestingly, MEL induces the transcription of *CBF*/*DREB1s*, resulting in the elevation of NO level by enhancing sugar accumulation (Shi et al., 2015).

7 Conclusion

SA and MEL share a common precursor chorismate and also present similarity in regulating various physiological processes in plants. SA and MEL when supplied exogenously (separately) confers tolerance against abiotic stress. Only limited evidence of interaction between SA and MEL is available so far, and therefore recommending a stable action model will require intense research of molecular mechanisms underlying such interactions.

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Chapter 11
A Dynamic Crosstalk Between GSH and Salicylic Acid to Mitigate Environmental Stress

Asma Sultana and Sharmila Chattopadhyay

Abstract Plants are exposed to challenging environmental conditions throughout the life cycle which greatly affect their potential growth, development, and productivity. To survive in these unfavourable situations, they have developed efficient and effective mechanisms to endure environmental catastrophe. Glutathione, the major source of plant non-protein thiols has been known widely for its functions in redox homeostasis, antioxidant biochemistry, detoxification and stress tolerance. It has been found that stress often resulted in the alteration of glutathione status which leads to changes in several signaling cascades and hence potential responses take place. An in-depth understanding at the molecular level can help to discern various stress response mechanisms in plants. Till date, the crosstalk between GSH and various phytohormones viz. ethylene (ET), salicylic acid (SA), jasmonic acid (JA), abscisic acid (ABA), etc. along with nitric oxide (NO) signaling has been studied. However, the molecular mechanism is yet to be explored in the context of plant defense response. Interestingly, a vibrant interaction of GSH, the reduced form of glutathione, with SA has been observed in model as well as in target plants. Here, we will discuss the crosstalk of GSH and SA at genomics and proteomics levels which eventually will open up further avenues in the understanding of stress mitigation *in planta.*

Keywords Glutathione · Salicylic acid · Cross-talk · Stress · Signaling

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S. Hayat et al. (eds.), *Salicylic Acid - A Versatile Plant Growth Regulator*,
https://doi.org/10.1007/978-3-030-79229-9_11

1 Introduction

1.1 Phytohormonal Signaling to Mitigate Environmental Stress in Plants

The perception and response of environmental stress in plants is mediated by several gene-to-gene interactions which are manifested downstream in the form of interactions and networking among the proteins along with several phytohormones. Phytohormones like SA, JA, ET and ABA (Dong, 1998; Thomma et al., 1998; Glazebrook, 2005; Howe & Jander, 2008; Zhu, 2016; Kuromori et al., 2018) along with brassinosteroids (Kagale et al., 2007; Planas-Riverola et al., 2019) has been long known to alleviate stress through a intermingle networking among themselves (Pieterse et al., 2009).

SA, a phenolic phytohormone is found to be widely distributed in plants. While initially it was widely known for its medicinal applications, however it quickly attracted the attention of the plant scientists as it got categorized as one of the ubiquitous phytohormonal molecule. SA level in model plant like *Arabidopsis* is below 1 μg g^{-1} fresh weight and the highest level of SA was found in thermogenic plants and in plants infected with necrotizing pathogens (Raskin, 1992). While the role of SA is well known for plant growth and development (Rivas-San Vicente & Plasencia, 2011), its role in defense is still getting unfolded. SA is known to be synthesized in plants in response to phytopathogenic challenges and is essential to the establishment of both local and systemic acquired resistance (SAR) (Loake & Grant, 2007). SA application induces accumulation of pathogenesis-related (PR) proteins which is also induced by the functional SA analogues benzoic acid (BA), 2,6-dichloroisonicotinic acid (INA) and benzo (1,2,3) thiadiazole-7-carbothioic acid S-methyl ester (BTH) (White, 1979; Lawton et al., 1995; Vernooij et al., 1995; Friedrich et al., 1996). Several potential components of the SA signaling pathway were identified, including (a) the hydrogen peroxide (H_2O_2)-scavenging enzymes ascorbate peroxidase (APX) and catalase, (b) a high affinity SA-binding protein (SABP2), (c) a SA-inducible protein kinase (SIPK), (d) an ankyrin repeat-containing protein that exhibits limited homology to IκBα and is required for SA signaling, non-expresser of PR genes-1 (NPR1), and (e) members of the TGA/OBF family of bZIP transcription factors. These bZIP factors interact physically with NPR1 and bind the SA-responsive element in promoters of defense genes, like *PR-1* (Klessig et al., 2000). DELLA protein is also known to fine tune the activity of SA (Grant & Jones, 2009), besides mitochondrial succinate dehydrogense (Belt et al., 2017). The interaction of SA with other phytohormones (Spoel et al., 2003; Wang et al., 2007; Navarro et al., 2008; Ghanta et al., 2014; Ding et al., 2016) and several other molecules (Klessig et al., 2000; Baxter et al., 2014) has been elaborated several times. However, the area concerning the crosstalk between GSH and SA is still emerging.

1.2 GSH and Plant Stress Signaling

The tripeptide thiol, glutathione has been known for its unique structural properties, broad redox potential and for its wide distribution among the living kingdom. Glutathione is known to be found in several forms. Its reduced form (2–5 mM) predominates over the oxidized form (GSSG) (10–200 µM) (Noctor et al., 2002; Ball et al., 2004) under physiological condition. Nitrosoglutathione (GSNO) represents another important form. GSH is known to contribute in cell cycle, signaling and epigenetic events (Foyer & Noctor, 2005; Diaz Vivancos et al., 2010). Along with GSH, its associated enzymes: glutathione reductase (GR), glutathione S-transferase (GST) and glutathione peroxidase (GPX) forms an efficient system to offer plant stress tolerance (Foyer et al., 1997; Dixon et al., 1998). The involvement of GSH in plant stress response has been documented by researchers worldwide several times. Although negative effect of GSH has been reported (Creissen et al., 1999), yet significantly huge amount of information has accumulated till date referring to the positive roles that GSH attribute in both abiotic and biotic stress management in plants (Dron et al., 1988; Wingate et al., 1988; May et al., 1998; Parisy et al., 2007; Ghanta & Chattopadhyay, 2011; Gill et al., 2013; Piślewska-Bednarek et al., 2018; Borgohain et al., 2019; Zechmann, 2020). GSH however participates in plant stress modulation by interacting with the phytohormonal signaling cascades as well as with several other molecules (Srivastava & Dwivedi, 1998; Dı́az et al., 2003; Mhamdi et al., 2013; Nazar et al., 2014; Cheng et al., 2015; Kaur & Bhatla, 2016; Sultana & Chattopadhyay, 2020). Beside being known to have its regulatory effect on GSH homeostasis (Kusumi et al., 2006), SA is popular for being one of the versatile phytohormone having wide range of interaction with GSH and its related family of enzymes (Sappl et al., 2004; Mhamdi et al., 2010). This investigation has been aimed to add to and fulfil the gaps left in understanding molecular interaction of SA with GSH in plants under stress condition. The present discussion has included a broad range of stresses that plants face under the natural condition, and also has been an effort to trace on the specific nature of SA-GSH interaction during those stress conditions, among the complex web of plant stress networking. Furthermore, the future prospect of the understanding the SA-GSH crosstalk on the perspective of plant stress tolerance/mitigation has also been discussed.

2 Role of SA and GSH in Conferring Stress Tolerance in Plants

The interaction between GSH and SA was observed while studying transgenic tobacco (*Nicotiana tabacum* cv. Xanthi) plants (*NtGB9* and *NtGB19*) overexpressing the glutamate cysteine ligase (GCL) or gammaglutamylcysteine synthetase (γ-ECS), the rate limiting enzyme, catalyzing the first step of GSH biosynthesis (Ghanta et al., 2011). *γ-ECS* was cloned from tomato, *Lycopersicon esculentum,* (*LeECS*) into the binary vector *pBI121* under the control of *CaMV35S* promoter and the resultant chimera was later used to transform tobacco leaves to develop transgenic tobacco lines. Though morphologically indistinguishable, HPLC analysis revealed that the overexpressed lines, *NtGB9* and *NtGB19* accumulated 2.6- and 2.07-fold higher GSH respectively compared to the wild type tobacco plants. The *NtGB* lines were also found to accumulate almost 4 times more SA as compared to the wild type plants. Gene expression profiling of GSH overexpressed *NtGB* lines showed higher expression of several genes viz. *NtNPR1* and *NPR1* dependent pathogenesis-related gene, *NtPR1*, the marker gene for SA mediated plant defense, etc. Interestingly, the expression level of *NtPR4*, the marker gene for ET signaling and 1-aminocyclopropane-1-carboxylate (ACC) oxidase (*NtACO*), the ET biosynthetic pathway gene in *NtGB* lines was increased in transgenic plants as compared to the wild type plants. The expression of allene oxide synthase (*NtAOS*) which synthesizes an enzyme of JA biosynthetic pathway however remained unchanged in *NtGB* lines. Furthermore, to estimate the stress tolerance potential, wild type tobacco while infected was found to be more susceptible to the biotrophic pathogen, *Pseudomonas syringae* pv. *tabaci* as compared to the *NtGB* lines. To gain a deeper insight into how GSH is interacting with various proteins species to confer biotic stress tolerance in plants, comparative 2 dimensional (DE)- gel electrophoresis were performed and the spots were identified using matrix assisted laser desorption/ionization (MALDI)-time of flight (TOF-TOF)-tandem mass spectroscopy (MS/MS) approach. Functional categorization using KEGG further classified the proteins into carbon metabolism, signaling, energy production, stress and defense, nucleic acid production and several hypothetical proteins. Among the differentially expressed proteins, 38% belonged to the stress and defense category. It was found that among the expressed proteins NtPR10, carbonic anhydrase (CA), calmodulin binding protein (CBP), arginine decarboxylase (ADC), and calcineurin, along with lectin proteins and NBS/LRR resistance protein like protein were found to be accumulated in *NtGB* lines. All these proteins or their related proteins are reported to be accumulated through the involvement of SA-mediated pathway (Ziadi et al., 2001; Slaymaker et al., 2002; Wang et al., 2004; van Loon et al., 2006; Blanco et al., 2009; Du et al. 2009; Jang et al., 2009; Zhao et al., 2018). The expression levels of the corresponding SA-related genes *NtPR10*, *CA*, *CBP*, *ADC* when validated by quantitative real time polymerase chain reaction (qRT-PCR), were found upregulated. Overall, the above study comprehends the fact that GSH plays a pivotal role in biotic stress tolerance against *P. syringae* pv. *tabaci* through NPR1 dependent SA-mediated pathway. Although GSH is known to act through NPR1 dependent SA mediated pathway in mitigating stress, but there is evidence where GSH seems to induce SA signaling via increased intracellular H_2O_2, in NPR1 independent manner as well (Han et al., 2013).

In another study with chloroplast-targeted γ-ECS overexpressed transgenic tobacco (*NtGp1* and *11*) (Ghanta et al., 2014), the indispensable role of SA in interacting with GSH and conferring stress tolerance, was once again evaluated. Significantly higher levels of GSH, SA and ACC were found in *NtGP* lines compared to the wild type. When a forward subtracted cDNA (SSH) library was constructed, using *NtGp* line as a tester, in addition to *ACO*, a key enzyme of ET biosynthesis, SA-related transcripts like *PR1a* and *SAR8.2m/2l,* was identified in the SSH library, which show similar nature of expressions on validation with qRT-PCR. Along with these, transcription factors like WRKY transcription factor 3 (WRKY3), WRKY1 and ethylene responsive factor 4 (ERF4), associated with SA and ET respectively, were also identified. To understand the association further, comparative 2 DE-proteomic profiling was performed with the chloroplast enriched fraction of the wild type and *NtGp11* line. ACC synthase (ACS), the enzyme involved in ET biosynthesis was found as newly detected protein in *NtGp11*. Proteins belonging to stress and defense like chaperone proteins viz. heat shock 70 protein (HSP70), GR, myb family transcription factor which is SA responsive (Yanhui et al., 2006), were also identified to be up accumulated in transgenic plants compared to the wild type. Thus, GSH was found to be interacting with SA as well as with ET in preparing the plants for their stress tolerability.

NtGp11 line when exposed to abiotic stress like drought stress mimicked by treating the plants with 200 mM of mannitol for 72 h, were found to perform better compared to wild type plants (Kumar et al., 2014). Comparative 2 DE-proteomic profiling of stress treated wild type and *NtGp11* line revealed several stress and defense related proteins like GPX, thioredoxin peroxidase, chalcone synthase along with the EIG-J7 which is known to be expressed under variety of stress conditions, including wounding, drought stress, viral infection and also under SA treatment (Takemoto et al., 2001; Chaves et al., 2009).

Additional studies with the model plant *Arabidopsis* also provided evidence on interaction between SA and GSH in imparting stress tolerance. When wild type plants of Columbia (Col-0) ecotype were treated with GSH they were found to be resistant to both abiotic (combined drought and cold treatments) as well as biotic stress (*P. syringae* infection), compared to the control Col-0, as observed by several transcript analysis (Sinha et al., 2015). Microarray analysis showed that among several genes, certain genes like *UGT85A1*, *SUS1*, *EDS16* and *EDS5* were upregulated in GSH-fed Col-0. These genes were either responsible for SA mediated signaling pathways or SA synthesis (Wildermuth et al., 2001; Nawrath et al., 2002).

Studies involving *Arabidopsis* mutant, like *phytoalexin deficient2-1* (*pad2.1*) helped further to shed light on the networking between SA and GSH. *pad2.1* with 22% of GSH content of wild type, has its mutation localized in *GCL* gene. Besides that, lack of pathogen triggered expression of *isochorismate synthase1* gene, coding for the SA-biosynthetic enzyme isochorismate synthase, results in lower accumulation of SA in the mutant (Dubreuil-Maurizi et al., 2011). Microarray analysis supported by comparative proteomics study of combined osmotic and cold treated *pad2.1* and Col-0 (Kumar et al., 2015) revealed that the expression of GST, SA responsive NBS-LRR (Wang et al., 2004; Zhao et al., 2018) were downregulated. Additionally, the transcriptomic profile also displayed that genes like *myb59*, *PTR3*, *LHY*, *CCA1*, were downregulated as well in combined stress treated *pad2*.1 compared to the stress treated Col-0. These genes are known to be regulated by the hormone SA (Yanhui et al., 2006; Karim et al., 2007; Zhou et al., 2015; Chen et al., 2018). Similar result was found when *P. syringae* treated Col-0 and *pad2.1* plants were subjected to comparative proteomics and when corresponding transcript validation of some differentially expressed proteins were done, using qRT-PCR (Datta & Chattopadhyay, 2015). It was found that GST and SA responsive NBS-LRR were downregulated in infected *pad2.1* compared to infected Col-0 plants. The control wild type plants also showed higher basal level of expression of genes, compared to control mutants.

Endogenous increment in phytohormonal levels is often found to have enhanced in response to stress in plants and can modulate plant stress tolerance. Also exogenously applied phytohormones can mimic plants under environmental stress conditions, that might lead to endogenous increment in the respective hormone levels, similar to conditions in plants under stress. Following this strategy Col-0, with optimum GSH level; transgenic plants overexpressing *LeECS* and having 2 times higher GSH content compared to Col-0 (*AtECS1*) (raised by Datta et al., 2015); and *pad2-1* mutant, were all treated with 1 mM of exogenous SA. The transcript level of *NPR1* and *PR*-genes like *PR1* and *PR2* were all found to be enhanced in treated Col-0, *AtECS1* and *pad2-1* compared to the untreated controls, yet *AtECS1* showed the highest expressions of all the genes compared to Col-0, and *pad2.1* having the lowest expressions. The basal levels of expressions of all the above-mentioned genes were highest in *AtECS1*. In the previous studies (Ghanta et al., 2011, 2014), it was found that enhanced GSH caused increment of SA content in plants. This corroborates with enhanced expression of *NPR1* and *PR*- genes in untreated control *AtECS1* and also with maximum increase of all the gene expressions in SA treated *AtECS1* plants compared to Col-0 and lowest expression in *pad2.1* (Sultana & Chattopadhyay, 2020). Thus, GSH might be acting as a positive modulator to control SA regulated signaling and mediating the expressions of several defense related genes in a beneficial way for the plants.

The role of GSH in biotic stress tolerance through SA pathway was re-established when AAL toxin were treated in *Arabidopsis* with varied GSH content (Sultana et al., 2020). The proteomic profile obtained after nano LC–MS/MS analysis, post-AAL toxin treatment of Col-0 and *AtECS1*, lead to the identification of handful of proteins belonging to stress and defense category, like, superoxide dismutases, GST, APX, catalases, peroxiredoxins, HSPs, myrosinases, PR4, etc. Among them the expressions of some viz., PR2, 5, CA, GST 8, glutamine synthetase, cytochrome b6-f complex iron-sulfur subunit, etc. has been known to get effected by SA (Uknes et al., 1992; Cao et al., 1994; Slaymaker et al., 2002; Blanco et al., 2009; Zhang et al., 2016). Later it was found that some of the corresponding transcripts like *CA*, *PR5* along with SA responsive *PR1* got upregulated in AAL treated *AtECS1* plants, compared to AAL treated Col0. However, the expressions of *PR4* and also *ACO1* got suppressed in AAL treated *AtECS1* compared to AAL treated Col-0. The basal levels of expression of all genes though, are higher in *AtECS1* under non-treated control condition. The western blot data also corroborated with the above findings when checked with the expressions of PR5, PR4 and ACO1. HPLC analysis showed that there was an increase in the SA level in AAL treated *AtECS1* compared to AAL treated Col-0, whereas, the ACC level diminished in *AtECS1* compared to Col-0 under treated condition. Thus, GSH while interacting with other molecules can be helping in alleviating the AAL induced stress. Nonetheless the role of GSH in this case in SA mediated suppression of toxic level of ET in AAL induced stress is worthy to be mentioned.

The above reports encompassing the expression analysis of several transcripts and proteins, substantiates the fact that GSH and SA might regulate each other either indirectly or directly, but in a positive way to diminish the effect of stress in plants.

In addition to the above studies, there are several other reports in different plant species, which confirmed about the involvement of GSH with SA signaling pathway to improve a wide range of stress tolerance in plants.

Pre-treatment of maize plants hydroponically with SA results in increased tolerance to chilling stress due to enhanced activities of antioxidants like peroxidase and GR (Janda et al., 1999). It was reported that ABA and SA when applied in small amount proved effective in relieving wheat plants against water stress and help in improving the antioxidative machinery of plants comprised of APX, GR, etc. (Agarwal et al., 2005). SA has been known to play an important role in alleviating high light induced oxidative stress in plants by increasing the glutathione pool in rice (Kusumi et al., 2006). In young grape plants it has been found that exogenous SA treatment causes protection of mesophyll cells against cold or heat stress in leaves, with changes in Ca^{2+} homeostasis, enzymes of ascorbate (Asc)-GSH cycle, as well as Asc and GSH contents (Wang & Li, 2006). Yoshida et al. (2009) reported that increased *de novo* biosynthesis of GSH induced by SA along with ET, protect plants against ozone-induced leaf injury. SA may increase drought tolerance of *Ctenanthe* by promoting the Asc-GSH cycle, by increasing activities of GR and dehydroascorbate reductase, hence elevating GSH content (Kadioglu et al., 2011). In wheat, SA pre-treatment is known to cause enhanced activity of the antioxidant system viz., APX, GR, as was evidenced from proteomics study (Kang et al., 2012). SA was also found to ameliorate UV-B and dimethoate (D_2) induced toxicity by relieving the inhibitions posed by the UV-B and the systemic insecticide by inducing catalase, GR and other antioxidant machineries, in mung beans (Singh et al., 2014). SA priming was found to be effective against salt stress in *Arabidopsis*. SA regulated H_2O_2 levels in plants and reduce peroxides by increased guaiacol peroxidase, GST and glutathione-dependent peroxidase activity (Horváth et al., 2015). Leaves of tomato plants hardened with SA results in enhanced activity of GR and increased GSH content which in addition to other anti-stress effectors were found to be effective against salt stress (Tari et al., 2015). It was noted that tobacco plants overexpressing *LcSABP* had enhanced photosynthetic activities, chlorophyll content. SABP2 helped in promoting stress tolerance induced by the triclosan, the most common organic pollutants in the sewage sludge, by increasing the SA content which resulted in increment of GSH content due to enhanced GR activity (Guan et al., 2019).

GSH mediated Nickel (Ni) tolerance has been found in *Thlaspi* Ni hyperaccumulators, in presence of constitutively overexpressed SA signaling (Freeman et al., 2005). Exogenous SA treatment on maize exerted beneficial effects on the growth and development by positively regulating the chloroplastic antioxidant system, involving enzymes of Asc-GSH system viz. GR and also the non-enzymatic components like Asc and GSH thus alleviating Ni toxicity (Wang et al., 2009). Exogenous SA might relieve the plants from mercury induced toxicity as well, as observed in *Medicago*. It was found that application of SA in the roots of *Medicago* enhances the level of GSH alongside Asc and proline (Zhou et al., 2009). In maize it has been found that SA along with sodium hydrosulfide pre-treatment decreased lead induced toxicity by increasing protein content, nitrate reductase activity and GSH content (Zanganeh et al., 2019). Maize was also found to show cadmium (Cd) tolerance under the effect of SA and silicon as was evidenced by increase in Asc and GSH contents as well by the stimulated activities of APX, GR, etc. (Singh et al., 2019). Application of SA as well as NO has been reported to enhance arsenic toxicity tolerance by increasing the antioxidant system that comprises the Asc-GSH cycle (Kaya et al., 2020).

Both SA and GSH are required for local and systematic acclimation responses observed in *Mesembryanthemum crystallinum* against non-host necrotrophic pathogen *Botrytis cinerea* (Kuźniak et al., 2013). Künstler et al. (2019) demonstrated that enhanced GSH can compensate for SA deficiency in tobacco to maintain resistance to tobacco mosaic virus. *Arabidopsis* plants overexpressing OXIDATION RESISTANCE 2 (AtOXR2), a mitochondrial protein, displayed enhanced protection against hemibiotrophic bacterial pathogen *P. syringae* and the overexpressed plants not only have activated SA signaling or enhanced free SA accumulation, but also have increased level of total glutathione (Mencia et al., 2020).

Despite the synergistic crosstalk of SA and GSH in plant stress tolerance, several reports also pointed out different outcomes and stress management strategies when both of them are involved. *Arabidopsis* plants expressing a salicylate hydroxylase (*NahG*) gene was found to perform better with reduced oxidative damage under NaCl and osmotic stress, compared to the wild type plants. NaCl treatment decreased the GSH/GSSG ratio by approximately 91% in wild type, whereas in *NahG* seedlings the same ratio decreased only about 71% after exposure to NaCl (Borsani et al., 2001). Endogenous SA was found to potentiate Cd mediated stress in *Arabidopsis*. The total glutathione content increased in both wild type and SA deficient Cd treated plants, due to increased GSSG in former and for GSH in case of later (Zawoznik et al., 2007). Transgenic *NahG Arabidopsis* plants were also able to adapt better to moderate salt stress compared to their wild type member (Cao et al., 2009). It has been found that SA deficient plants are also capable of adapting better to RNA virus infecting them and it was found that higher level of GSH and Asc has been responsible in alleviating the symptoms in those plants (Wang et al., 2011).

3 Conclusion and Prospects

The multifaceted role of SA signaling in attenuating plant stress is broadly known and GSH reputed as the master regulator, along with its arsenal of enzyme system is one of the key nodes where the signaling is implemented to crosstalk. Although a great advance had been made to understand the complex crosstalk between SA and GSH, yet certain crevices are yet to be studied in-depth. Although GSH and SA both are found to stimulate each other, however both can be stimulated separately by multitude of stresses and both can interact with several other phytoprotectants. It is yet to understand specifically that what causes the two to stimulate and crosstalk while not with others under a particular stress condition. Combined applications of the cutting-edge resources of genomics, proteomics, molecular biology and bioinformatics will be helpful in understanding the intermediate components of the signaling pathway connecting the two and in unravelling the sequential events happening in a particular cell during a whole defense response procedure. Moreover, the concentration of phytohormone is an important factor for acting as a stress reliever or as the stress enhancer. Thus, it is important to understand and optimize the specific concentration of SA required for a specific species leading to better management of stress the plants might be facing.

Acknowledgement The authors acknowledge the support of Council of Scientific & Industrial Research (CSIR) and Science Engineering Research Board (SERB), New Delhi. They are also thankful to Directors, CSIR-Indian Institute of Chemical Biology (CSIR-IICB), Kolkata for providing the necessary facilities. Dr. SC would like to acknowledge CSIR, New Delhi for Emeritus scientist fellowship. We would like to thank the editor for his kind invitation.

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Chapter 12
Regulation of SA-Mediated Signal Transduction in Plant Immune System

Hideo Nakashtia, Miyuki Kusajima, Hisaharu Kato, and Moeka Fujita

Abstract Salicylic acid (SA) is one of the major signaling molecule functioning in the plant immunity system. Systemic acquired resistance (SAR), activated though SA by attacks of certain kinds of pathogens, is an important defense mechanism to protect the whole plant body from the future attacks by pathogens. SAR has been practically used for stable crop production by developing SAR-inducing chemicals, plant activators. In addition to pathogenic infection, many evidences have been provided other regulation mechanism of SA-mediated resistance. This chapter covers the recent progress in our understanding of mechanism of SA-mediated disease resistance, especially about SA biosynthesis, which includes (1) mechanism of SA biosynthesis; (2) regulation of SA-mediated signaling by other signaling molecules; (3) up-regulation of SA biosynthesis by triggers other than pathogens; (4) the related metabolism accompanied by SA biosynthesis during development of disease resistance.

Keywords Disease resistance · Heat shock · Tocopherol

1 Introduction

1.1 Biosynthesis and Systemic Signaling

Salicylic acid is a phytohormone with a relatively small size and simple structure, which positively regulates plant immune system (Durner et al., 1997; Yang et al., 1997). The biosynthesis of SA is enhanced by pathogenic infection, which occurs in both infected and systemic tissues. In the infected tissues SA takes part to some extent in basal resistance against pathogens and the systemically enhanced SA

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S. Hayat et al. (eds.), *Salicylic Acid - A Versatile Plant Growth Regulator*,
https://doi.org/10.1007/978-3-030-79229-9_12

biosynthesis and accumulation function in induction of systemic acquired resistance (SAR) (Chester et al., 1933; Durner et al., 1997). Formerly SA was considered to be a mobile signal molecule for SAR induction by analyses using SA-deficient mutants such as *sid1*, *sid2* and *eds5* (see below) and NahG transgenic plant (Gaffney et al., 1993; Delaney et al., 1994), but recently other small molecules have been identified as critical components for SAR induction. In addition, recent studies have been providing various evidences for the biosynthetic mechanisms of SA.

In plants, SA is synthesized by two pathways, mediated by ICS1 (isochorismate synthase 1) and PAL (phenylalanine ammonia-lyase) activities (Zhang & Li, 2019). In *Arabidopsis*, a key enzyme for SA biosynthesis upon pathogenic infection is ICS1 (Wildermuth et al., 2001) that converts chorismic acid to isochorismic acid in plastid (Fig. 12.1). Isochorismic acid is transferred to cytosol by EDS5 (Enhanced disease susceptibility 5), the multidrug and toxin extrusion (MATE) transporter family protein (Nawrath et al., 2002), followed by the conversion to isochorismate-9-glutamate by PBS3 (avrPphB Susceptible 3) (Nobuta et al., 2007; Torrens-Spence et al., 2019). Degradation of Isochorismate-9-glutamate to SA can occur spontaneously, where EPS1 (Enhanced pseudomonas susceptibility 1) accelerates the degradation to SA (Torrens-Spence et al., 2019). Plants have another metabolic pathway mediated by PAL (Yalpani et al., 1993). Phenylalanine, produced thorough chorismic acid from shikimic acid, is converted to trans-cinnamic acid, and then converted to benzoic acid by AIM1 (Abnormal Inflorescence Meristem 1) (Richmond & Bleecker, 1999). The reaction from benzoic acid to SA is thought to be catalyzed by a benzoic acid-2-hydroxylase (BA2H), although the genetic evidence has not been demonstrated (Leon et al., 1995; Sawada et al., 2006). The importance of PAL pathway has been reported in tobacco and rice (Leon et al., 1995; Duan et al., 2014), however, reduction of disease resistance in quadruple *pal1/2/3/4* mutant demonstrated the contribution of PAL pathway to SA production also in *Arabidopsis* (Huang et al., 2010). On the other hand, rice also has OsICS1 that is up-regulated by OsWRKY6, a positive regulator for disease resistance (Choi et al., 2015), however, its contribution to SA production is thought to be low because of the weak enzymatic activity comparing with that of *Arabidopsis* (Yokoo et al., 2018). Together, the contribution of these two pathways for SA production in growth and/or stress responses would varies among plant species.

As a mobile signaling for systemic disease resistance, N-hydroxypipecolic acid (NHP) has been identified (Hartmann et al., 2018), however, which does not exclude completely the contribution of SA for signal transmission. Biosynthesis of NHP is induced by pathogenic infection and application of NHP induces locally and systemically expression of *PR* genes, SA biosynthesis, and disease resistance (Chen et al., 2018, Hartmann et al., 2018, Tian & Zhang, 2019). The NHP biosynthesis initiates from conversion of lysine to piperideine-2-carboxylic acid by ALD1 (aminotransferase1), and which is converted to pipecolic acid by SARD4 (SAR-deficient 4) (Ding et al., 2016). After transferred to cytosol by EDS5, pipecolic acid is converted to NHP by FMO1 (Flavin-dependent monooxygenase 1). Recently, glycosyltransferase UGT76B1 has been identified to inactivate NHP by converting into glycosylated NHP (Holmes et al., 2021; Mohnike et al., 2021).

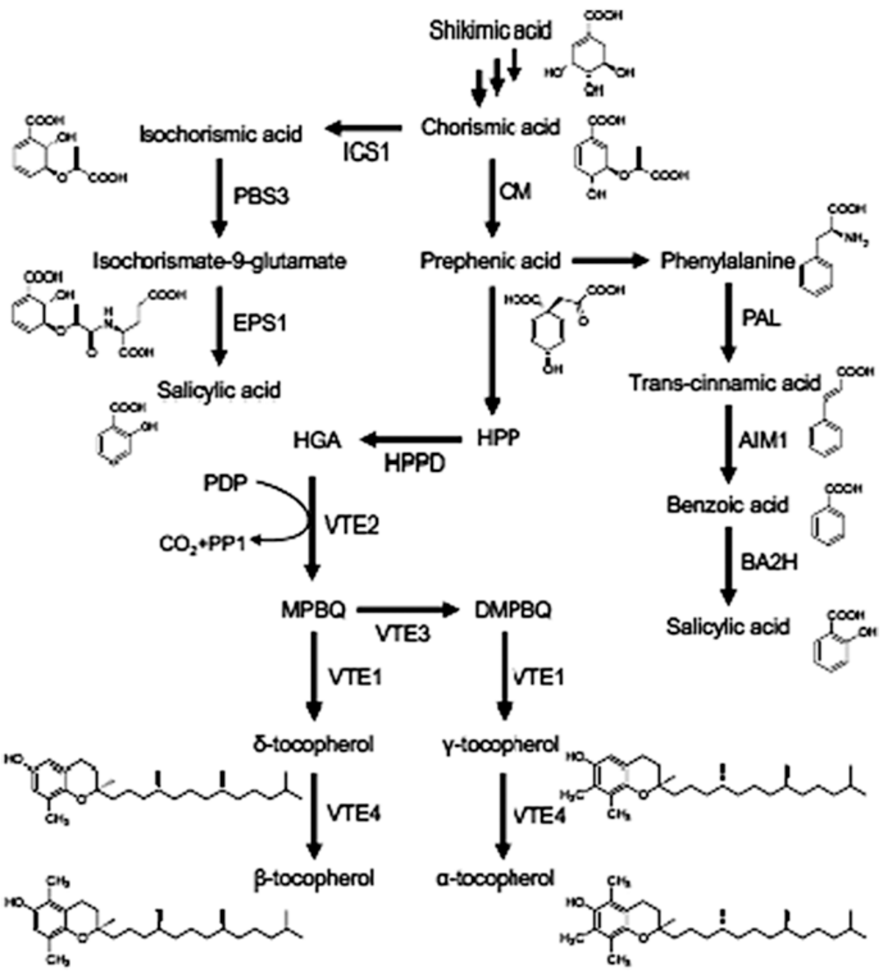

Fig. 12.1 Biosynthetic pathway for SA and tocopherols
HPP *p*-Hydroxyphenylpyruvic acid, *HGA* homogentisic acid, *PDP* phytyl diphosphate, *MPBQ* 2-methyl-6-phytylbenzoquinol, *DMPBQ* 2,3-dimethyl-5-phytylbenzoquinonol, *HPPD* *p*-hydroxyphenylpyruvate hydroxylase, *VTE2* homogentisate phytyltransferase, *VTE3* MPBQ/MSBQ methyltransferase, *VTE1* tocopherol cyclase, *VTE4* γ-tocopherol methyl transferase

2 Up-Regulation of SA Biosynthesis by Heat Shock Treatment

Greenhouse farming has many advantages to cultivate crops easier without influences from the outside weather. Controlling the inside temperature is important for the optimal plant growth, which is also useful to control insects and diseases. Temperature in a greenhouse can be controlled by using an air conditioning system or some kinds of heaters, but the easiest way is to maintain the temperature by opening and closing windows. In general, plants are more tolerant to higher temperature rather than insects and microbes. Sublethal or lethal temperature for insects is usually 35–40 °C (Ali et al., 2019). The optimal temperature for growth of plant pathogenic bacteria and fungi is about 25–30 °C. Although the lethal temperature varies depending on microbes, in general higher temperature such as 45 °C significantly reduces their growth (Castejón-Muñoz & Bollen, 1993) and in actual agriculture the seed sterilization by heat treatment with hot water (50–65 °C) is widely used. In contrast, plants are rather tolerant to higher temperature because plants can cool their aerial parts, such as leaves and stems, by transpiration using water taken up from soil. In the practical cultivation of tomato and cucumber in greenhouses, high temperature treatment has been developed to protect plants from insects and diseases, which was accomplished by raising the temperature to around 45 °C by closing the windows of greenhouse around noon for 1–2 h (Sato & Kubo, 2002; Sato et al., 2004). Interestingly, diseases can be controlled effectively by continuing the treatment at weekly intervals, because the effect lasts for about a week.

Phytopathological and plant physiological studies on its mechanism revealed that the protective effects are due not only to the direct action of high temperature on insects and microbes but also to the enhancement of plant disease resistance. Heat shock (HS) treatment induced the accumulation of SA and expression of defense-related genes in leaves of tomato and cucumber (Sato et al., 2003). A short-duration HS treatment, by dipping melon seedlings in hot water at 50 °C for 20 s can induce the expression of defense related genes in 12 h after the treatment (Widiastuti et al., 2011). The treated melon plant exhibits resistance against *Botrytis cinerea* only when inoculation is performed within 1 day of HS treatment (Widiastuti et al., 2011). These have suggested that some physiological system of plants involving SA-mediated signaling would function in the effect of high temperature treatment, however, the mechanism was not clarified in these plants.

Investigation using *Arabidopsis* has revealed the molecular mechanism of protective effect of high temperature treatment against diseases (Kusajima et al., 2012). Treating *Arabidopsis* wild-type plants (Col-0) at 45 °C for 1–3 h in a growth chamber induces expression of defense related genes in 3 days after the treatment. Pathogen inoculation assay with a virulent bacterial pathogen P*seudomonas syringae* pv. *tomato* DC3000 (*Pst*) at 3 days after the treatment has exhibited the reduced growth of bacteria in leaf tissues compared to the non-treated control plant, indicating that high temperature-treated *Arabidopsis* acquires enhanced resistance against *Pst.* Construction of a hot water dipping method has enabled further analyses to determine the molecular mechanism of this resistance. The effectiveness of high temperature treatment is dependent on the temperature and exposure time. Dipping the wild-type plant in hot water over 45 °C for 2.5 min induces the high-level accumulation of *PR-1* mRNA in leaves 2 days after treatment, whereas only the slight induction is observed by using the lower temperatures. Treating with higher temperature at 47.5° heavily affects the plant growth. Searching the optimal exposure time for 45 °C-hot water by the induction of *PR-1* gene expression has indicated that treatment for 2, 2.5 or 3 min give the maximum effect without wilting. Dipping at 45° for 2.5 min and culturing for 3 days can induce the resistance in the wild-type plants against *Pst*. Thus, during analyses of induced resistance, dipping at 45° for 2.5 min has been used as an optimal experimental condition for heat-shock (HS) treatment in *Arabidopsis*.

Induction of disease resistance and *PR* genes suggested that HS treatment activates SAR in *Arabidopsis*. This idea was confirmed by analyses using the SA biosynthesis-deficient mutant *sid2* (Wildermuth et al., 2001) and NahG transgenic plant defective in SA accumulation (Gaffney et al., 1993; Delaney et al., 1994); HS treatment did not induce disease resistance and *PR-1* expression in these plants. In nature, SAR is induced after the pathogenic infection and lasts for several weeks. Although the resistance in *Arabidopsis* wild-type plant is detected when inoculation is done 3 days after HS treatment, the resistance was not detected when inoculation was done 1 or 5 days after HS treatment, which is similar to the case of melon (Widiastuti et al., 2011). To clarify the reason why the disease resistance by HS treatment is activated only for a short period of time, time course analyses after HS treatment has been performed. The expression of *PR-1* initiates and reaches the highest level 2 days after the treatment, gradually reduces from the next day, and returns to the normal level by 7 days after the treatment (Fig. 12.2). The expression of *ICS1* is transiently observed only at 2 days after the treatment, which leads to the accumulation of both free SA and total SA (free SA ad SA glucoside) 2 days after treatment. Accumulation of free SA and total SA reaches the maximum levels 3 days after treatment and decreases rapidly from the next day. The accumulation of SA and *PR-1* expression at 1 day after the treatment are the normal levels and those of 5 days are also similar levels. These indicate that HS treatment in *Arabidopsis* induces the transient activation of SA biosynthesis and the SA-mediated signaling pathway resulting in enhanced disease resistance, which would explain the effects observed in tomato, cucumber and melon.

During SAR induction by pathogenic induction or plant activators, accumulation of SA and expression of *PR* genes gradually increase and last for more than a week or more (Lawton et al., 1996; Yoshioka et al., 2001; Yasuda et al., 2004). Thus, the transient activation is a different point between SAR by HS treatment and them. One of the plausible mechanisms for reduction of SAR is the SA-ABA crosstalk (Yasuda et al., 2008), because ABA-mediated signaling pathway is activated by high temperature treatment (Kotak et al., 2007). The enhanced expression of RAB18, the ABA-responsible gene, by HS treatment by dipping method and the suppressive effect of pretreatment with ABA on HS treatment supports the idea that the SA-ABA crosstalk would function to some extent in reducing SAR mechanism (Kusajima et al., 2012). As another mechanism for decrease of SA levels, strong induction of benzoic acid/SA carboxyl methyltransferase 1 (BSMT1) (Zheng et al., 2012), encoding an enzyme metabolizing SA to methyl salicylate, is detected 1 to 3 days after HS treatment (Kusajima et al., 2012). Although a trigger to induce SA biosynthesis after HS treatment is still unknown, production of reactive oxygen species (ROS), observed 6 h after HS treatment both in the wild-type and *sid2* mutant, possibly activates the SAR signaling pathway (Kusajima et al., 2012).

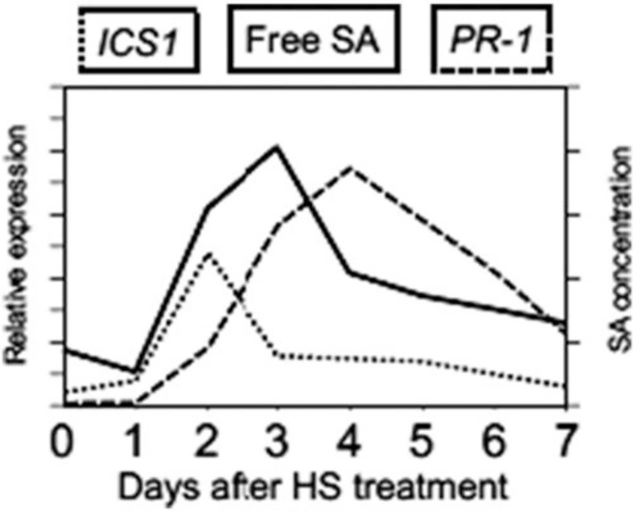

Fig. 12.2 Activation of SA biosynthesis by HS treatment
Relative gene expression and SA accumulation after HS treatment are indicated schematically. Gene expression levels were shown as relative value to UBQ in the same samples. The peaks of *ICS1* and *PR-1* are about 12-fold and 800-fold higher than controls, respectively. SA concentration of the peak in this graph is about 800 ng/g fresh weight

Antagonistic interaction between SA and ABA signaling can be seen in many physiological aspects, such as resistance against the ABA-producing pathogen *Botrytis cinerea* (Audenaert et al., 2002) and suppression of SAR induction by environmental stresses like drought and higher salt (Yasuda et al., 2008; Kusajima et al., 2010, 2017). Moderate low temperature activates ABA-mediated signaling and suppresses SA-mediated signaling for disease resistance, in which both the biosynthesis and the downstream signaling of SA are suppressed by ABA-mediated signaling (Yasuda et al., 2008). But very low temperature as 4 °C activates the SA biosynthesis (Wu et al., 2019). Taken together with HS treatment by dipping and in greenhouses, the biosynthesis of SA is up- and down-regulated by temperature changes.

3 Orchestrated Regulation of SA and Tocopherol Accumulation

During the activation of disease resistance by pathogenic infection, SA biosynthesis is one of the critical physiological changes in plant cells. In *Arabidopsis*, ICS1 play an important role in the response to pathogenic infection to produce SA (Wildermuth et al., 2001). In some other plant like tobacco, the PAL pathway may play important role in SA biosynthesis, which is not yet elucidated (Leon et al., 1995; Duan et al., 2014). In either case the substrates are supplied originally from shikimic acid pathway. The substrate for ICS1, chorismic acid, is produced thorough 3-step reactions from shikimic acid. The substrate for PAL, phenylalanine, is produced through 2-step reactions from chorismic acid. The metabolites produced from shikimic acid are used form various secondary metabolisms that produce many important compounds, including aromatic amino acids, phenylpropanoids, flavonoids, vitamin E and vitamin K, in addition to SA. Because some of these metabolites have antimicrobial activities, the metabolism for those could be activated at the same time as the activation of SA biosynthesis, which is reasonable mechanism to use the same substrate supply from shikimic acid pathway.

Among them, production of tocopherols, vitamin E, in relation to plant immunity system has been recently reported (Stahl et al., 2019; Kusajima et al., 2021). Vitamin E is a term used for similar molecules with antioxidant activity, which are 4 isomers (α, β, γ, δ) of tocopherol and 4 isomers (α, β, γ, δ) of tocotrienol. Because they are exclusively synthesized by photosynthetic organisms, they are important food ingredients for animals. In *Arabidopsis* tissues, α- and γ-tocopherols are major tocopherols, however, the endogenous levels of tocopherols vary between tissues (Lushchak & Semchuk, 2012). In green leaves α-tocopherol exists in chloroplasts, which prevent the lipid peroxidation of thylakoid membranes caused by photosynthesis-derived reactive oxygen species (ROS) (Munné-Bosch et al., 2005). In contrast, in seeds the γ-type is the main tocopherol (Falk & Munné-Bosch, 2010). *Arabidopsis* leaves during senescence also contain a higher amount of γ -tocopherol (Mishina et al., 2007).

Tocopherol has been shown to be involved in development of tolerance to abiotic stresses such as drought, salt, and osmotic stresses (Collakova & DellaPenna, 2003; Kanwischer et al., 2005; Abbasi et al., 2007; Liu et al., 2008; Ellouzi et al., 2013). As the responses in biotic stress, expression of the biosynthetic genes and accumulation of tocopherols in *Arabidopsis* leaves are induced by the infection with a virulent bacterial pathogen *Pseudomonas syringae* pv. *maculicola* (*Psm*) (Stahl et al., 2019). The *Psm* infection enhanced also accumulation of SA in leaves. The increasing ratio after/before inoculation is much higher in γ-tocopherol rather than α-tocopherol. The increased accumulation of γ-tocopherol by the *Psm* infection is SA signaling-independent and also independent of ethylene-, jasmonic acid-, ABA-, and NHP -related signaling pathways. EDS1 (Enhanced disease susceptibility 1) and PAD4 (Phytoalexin-deficient 4), important component for resistance development and positively regulate both SA and NHP, take part in the increased accumulation of γ-tocopherol.

The tocopherol biosynthesis-deficient mutant *vte2*, defective in homogentisate phytyltransferase, lacks basal resistance against *Psm*, whereas the biosynthesis of SA, pipecolic acid, NHP, and a phytoalexin camalexin are enhanced by *Psm* infection. Enhancement of the SA level by *Psm* infection in the *vte2* mutant is less than that in the wild-type plant. Thus, tocopherol production is necessary for basal resistance against bacterial pathogen and also contribute to SA accumulation to some extent.

Activation of tocopherol biosynthesis accompanied by SA biosynthesis occurs also by high temperature treatment. HS treatment by dipping in a hot water at 45 °C for 2 min induce tocopherol biosynthesis in *Arabidopsis*. The effect on γ-tocopherol level is more than on α-tocopherol, similarly to the case of pathogenic infection. Enhanced expression of some tocopherol biosynthetic genes, such as HPPD, VTE3, VTE1, and VTE4, is transient; they are rapidly induced in 6 h after HS treatment and returned to normal levels in 24 h (VTE3, VTE1, VTE4) and 72 h (HPPD). On the other hand, the induction of VTE2 initiates from 72 h. Tocopherols accumulate in leaves also transiently according to the expression of biosynthetic genes. Accumulation of γ-tocopherol initiates from 12 h after the treatment, reaches a peak at 48 h, and returns to the normal level at 120 h. Accumulation of α-tocopherol, produced from γ-tocopherol by VTE4, initiates from 48 h, reaches a peak at 72–96 h, and returns to the normal level at 120 h. These are also occurred in the *sid2* mutant, indicating that up-regulation of tocopherol biosynthesis by HS treatment is independent of the SA-mediated signaling pathway.

Both pathogenic infection and HS treatment activates the tocopherol biosynthesis in a similar way, although time course regulation has not been determined in the case of pathogenic infection. Their common characteristics are that tocopherol biosynthesis is activated with the same timing of activation of SA biosynthesis, but in a SA-independent manner, and that the increasing ratio by stimuli is more in the γ-tocopherol level rather than in α-tocopherol. Concerning the former feature, physiological property of tocopherols in plant cell would be important. Because both stimuli induce ROS generation, regardless of SA production, tocopherols would function to protect plant cell from damages by ROS, especially the peroxidation of lipids. Induced tocopherol biosynthesis by pathogenic infection and HS treatment indicates that both SA and tocopherol are up-regulated independently in the naturally occurring physiological change in the stressed cells. SA plays very important roles in local resistance and induction of SAR, which is exploited by artificial plant activators in practical fields, mainly to control diseases in rice (Lawton et al., 1996; Yoshioka et al., 2001; Yasuda et al., 2004). However, trade-off relationship between SAR induction and plant growth is an obstruction to utilized SAR in various crops. Although the detailed mechanism remains to be clarified, it is obvious that activation of SA signaling affect the plant growth, which is also supported by the dwarf phenotype of the SA-overaccumulation mutants *cpr1* and *cpr5* (Mateo et al., 2006). Extensive studies have reported that ROS function as an important component in SA-mediated signaling pathway (Herrera-Vásquez et al., 2015). Thus, tocopherols would be produced to avoid the damages by ROS that is produced during signal transduction for disease resistance such as SAR. Concerning the latter of common characteristics, some unknown physiological function of γ-tocopherol in cells may be implicated. Although antioxidant activity of γ-tocopherol is much less than α-tocopherol, the γ-type is the major tocopherol in leaf during senescence and seed, and furthermore it accumulates preferentially in stressed leaves. One possibility is that γ-tocopherol is stored in order for quick production of α-tocopherol and another is that γ-tocopherol has its own unique activity, which will be clarified in near future.

4 SA Biosynthesis in Age-Related Resistance

Generally, most plants acquire resistance according to their growth, which is called as age-related resistance (ARR) (Develey-Rivière & Galiana, 2007). It is not only due to physical barriers, such as hardness and thickness of organ surface, but also to some molecular immunity system. Extensive studies using *Arabidopsis* mostly with *Pst* or *Psm* have been revealing the mechanism of ARR. ARR occurs in *pad3–1*, a phytoalexin camalexin deficient mutant, and *eds7–1*, exhibiting enhanced disease susceptibility to *Pst* an *Psm* (Kus et al., 2002). Development of ARR in the wild-type is SA-dependent and not observed in SA-deficient *sid1*, *sid2* and NahG plant, although it is induced in the *npr1* mutant defective in SA signaling (Cameron & Zaton, 2004, Kus et al., 2002). These indicate that SA-mediated signaling is not required for ARR but that SA would function in ARR development or resistance directly to pathogens. Infiltration of 0.1 or 0.01 mM SA into intercellular space of *sid2* mutant restored ARR to *Pst* inoculated 3 or 72 h after SA application, but which is not observed in NahG plant. Even in the wild-type and *npr1* mutant, enhancement of ARR can be observed by inoculation assay 5 h after 0.1 mM SA application. In addition, infiltration of salicylate hydroxylase protein that converts SA to catechol, into intercellular space of the wild-type plant reduces the resistance against *Pst*. These support the idea that SA directly act as defensive compound on pathogens. Actually, SA has antimicrobial activity and reduce the growth rate of *Pst* (Cameron & Zaton, 2004), although the effective SA concentrations are different among pathogens (Amborabe et al., 2002; Brown et al., 2007).

More detailed analyses have revealed that the SA accumulation is also regulated by the interaction between plant and microbes (Wilson et al., 2014). Intercellular SA levels are different between younger plant and mature plant. Furthermore, when virulent bacteria *Pst* attacks young *Arabidopsis* wild-type plant, plant immune signaling network is hijacked by pathogen and reduces intercellular SA accumulation, resulting in successful infection. Coronatine produced by *Pst* act as an analogue of jasmonoyl-isoleucine, an active form of jasmonic acid (JA), to bind to the receptor protein COI1 and co-receptor protein JAZ (Katsir et al., 2008). This leads to degradation of JAZ, release of MYC2 transcription factor captured by JAZ, and then up-regulation of three NAC transcription factors (NAC019, NAC055, NAC072) (Zheng et al., 2012). NACs act suppressively on SA biosynthesis and positively on SA metabolism by BSMT1. Eventually, the virulent bacteria can proceed infection by inhibiting up-regulation of SA level and basal resistance. On the other hand, in mature plant that contains already a higher level of intercellular SA, the existence of some component to suppress the action of coronatine are speculated (Wilson et al., 2014). In the interaction between avirulent pathogen where hypersensitive response including cell death occurs, the SA biosynthesis is also enhanced (Nawrath & Metraux, 1999). Even in young plant, infection with *Pst(AvrRpt2)* induces accumulation of both intra- and intercellular SA, suggesting that accumulation of SA in intercellular space may take part in ETI (Wilson et al., 2014). Exploration of function and importance of intercellular SA is in progress, which will disclose some unexpected disease resistance mechanisms occurring outside of plant cells.

5 Conclusion

Plant utilizes SA as a signal compound for disease resistance, especially the resistance induction, provoked locally and systemically by pathogenic infection. SA is a very small molecule comparing with other secondary metabolites and other phytohormones, except for ethylene. Because the structure of SA is not so complicated as a metabolite, the biosynthesis of SA does not need so many steps of reactions from shikimic acid, which is convenient in plant self-defense system to respond quickly to pathogen infection. This quick response seems to play important roles in the quick and transient response to high temperature stress. In the relatively simple biosynthetic procedure form shikimic acid, the regulation of SA biosynthesis involves the regulation of the nearest key enzyme ICS1 and the shikimic pathway as the substrate supply mechanism. This regulatory mechanism resulted in the concerted up-regulation of salicylic acid biosynthesis and tocopherol biosynthesis, both are synthesized from same metabolite chorismic acid, in responses to heat-shock and pathogenic infection. These branched metabolic pathways would coordinate between the production of and protection from ROS during induction of resistance. This should be important to induce resistance effectively and safely, and furthermore which also contribute to relieving the trade-off effect between stress response and growth. In addition to as a signaling molecule, SA in intercellular space seems to function as an attacking weapon to inhibit bacterial growth, which is an important component of ARR. Further investigation concerning SA in defense system will reveal a part of fundamental mechanisms for homeostatic regulation of cells under outside stresses.

Acknowledgement The authors are grateful to grants from the Project of the NARO Bio-oriented Technology Research Advancement Institution (Research program on development of innovative technology 02016B) for financial support.

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Chapter 13
A Brief Insight on the Role of Various Phytohormones in Potato (*Solanum tuberosum L*) Tuber Development

Abhishek Pathak and Chandrama Prakash Upadhyaya

Abstract The potato tuber induction and subsequent developmental process are complex phenomenon involving several stages which includes the stolons formation, initiation of tuber by sub-apical swelling, longitudinal expanding of tissues and tuber growth. Potato tubers are known to serve as food storage organ and vegetative propagation for next generation. The potato tuberization process has been reported to be regulated via various extrinsic and extrinsic factors including light, temperature, photoperiod, phytohormones and balanced nutrition. The phytohormones including auxins, cytokinins, gibberellins, abscisic acid, ethylene, brassinosteroids, jasmonic acid, salicylic acid and strigolactones have been reported to play an important role in potato tuberization and development. Studies on endogenous hormone content and their synergestic relationship are of special interest in potato tuber formation. The gibberellins stimulate the tuberizationin potato tactfully via normalizing the shoot growth, while the cytokinins, auxins and abscisic acid evidently regulate the sink activity of the tubers via controlling cell division and cell expansion. The salicylic acid and associated compounds have also been reported to influence the tuber inducing activities and tolerance to biotic and abiotic stresses in potato, however, the tuber inducing activities of salicylic acid is unexplored. Molecular and proteomic studies have greatly extended the knowledge on the mechanism of phytohormone regulating the tuberization in potato. This chapter provides biochemical, genetic and molecular mechanism regulating the potato tuberization and further development mediated by different phytohormones with special emphasis on role of salicylic acid in tuber development.

Keywords Abscisic acid · Auxins · Jasmonic acid · Phytohormones · Salicylic acid Tuberization

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S. Hayat et al. (eds.), *Salicylic Acid - A Versatile Plant Growth Regulator*,
https://doi.org/10.1007/978-3-030-79229-9_13

1 Introduction

Potato being the third most important food crop after wheat and rice are valuable for the diets and livelihoods of millions of people worldwide (FOASTAT, 2017). The potato tubers have been considered as a good source of dietary energy, micronutrients, vitamins, and protein in comparison with other roots and tubers (http://www.fao.org/potato-2008/en/potato/index.html). Potatoes are an important source of income for the farmers playing a major role in food security and nutrition with thrust on social welfare of the people. The potato is vegetatively propagated, herbaceous, dicotyledonous and annual plant, however, can also grow as a perennial in select environments (Sarkar, 2008). In addition to the known difficulties associated with pests and diseases, growers continuously face various abiotic stresses hindering the overall potato yield. The increasing frequency of extreme weather events is generally responsible for decline of this important cash crop. According to an estimate, the world's population will approach 10 billion by 2050, and in order to meet the adequate food requirement, the agricultural production should increase by over 60%a as of the present. The International Potato Centre as estimated that 1 °C increase in temperature causes approximately 5% decline in the agricultural productivity; therefore, the global potato yields are estimated to decrease to 32% by 2060 (https://cipotato.org/annualreport2019/stories/next-generation-breeding/). The new verity of potato developed through the breeding improvement program has been considered to withstand the climate change and global warming. A potato plant tuberizes under short days and cool nights. The potato tuberization takes under short days and cool nights, well synchronized morpho-physiological process occurring on the underground stolons under the control of both extrinsic and intrinsic factors. The tuberization in potato has been considered as most sensitive stage of various developmental phases which limits its climate-associated geographical distribution as well as overall potato yield. Therefore, understanding the potato tuberization process becomes even more importantin view of the changing global climate which shall augment in development of new varieties. Development of potato tuber is the morphological transformation of underground stem into stolon which leads to the formation of tubers with an axillary dormant buds or an eye which help in the vegetative propagation under the favourable conditions. Moreover, the nutritional value of potato is conserved because of its vegetative mode of propagation (Navarre et al., 2016). The inherent plasticity of each tuber bud of vegetative organ to form a complete potato plants is certainly a biological impediment towards considering the precise in-planta mechanism regulating the tuberization process (Strunik et al., 1999). The most prominent environmental factor includes the lower temperature and day-length which are reported to be perceived by the above-ground parts of the plants. The hormones are major signalling pathways which operate between areal and below-ground parts playing major role in the regulation of tuber formation which are extensively reviewed in several literature (Vreugdenhil & Struik, 1989; Ewing & Struik, 1992; Ewing, 1995; Aksenova et al., 2012; Dutt et al., 2017).

Phytochromes are major photoreceptor of photoperiodic signalling resulting in changes in various morphological phenomenons including the tuberizationin potato. Currently, several forms of phytochrome are known with apoproteins encoded by different genes. Phytochrome B has been recognized to play a vital role controlling the photoperiod mediated regulation of tuberization. This is also reported to be involved in the inhibition of tuber initiation by long day (LD) photoperiodic. One of the ways for LD action on potato tuberization is the control of hormone content by a photoperiod (Chailakhyan, 1984). Several phytohormones, gibberellins (GAs) and cytokinins (CKs) primarily, affect substantially tuber formation (Ewing, 1995). It has been reported that the molecular mechanisms of phytochrome B action are tightly related to both GA and CK activities (Jackson et al., 2000; Fankhauser, 2002). Role of the phytohormone, the GA has also been recognized in different dimension of potato tuberization which includes stolon formation, elongation and branching (Vreugdenhil & Struik, 1989).

Several studies have also reported that higher level of GA content in stolon under non-inductive LDs (long days), whereas GA activity decreases when leaves are exposed to inductive SD (short days) condition (Machackova et al., 1998; Xu et al., 1998a). An inhibitory effect of the GA under the *In-vitro* conditions is also reported; however, the degradation of active GAs at the stolon tip during tuber formation have been recognized as an important condition for tuberization to advance in general (Ewing & Struik, 1992; Carrera et al., 2000; Kloosterman et al., 2007). The involvement of CKs in the conception of metabolic sinks gave an indication of its major role in the potato tuber initiation and development. Studies reported that the exogenous application of CK under the *In-vitro* conditions increased the total tuber yield. Similarly, the overexpression of the gene encoding regulatory enzyme of the CK biosynthesis pathway, the isopentenyltransferase (*IPT*) also induces tuber induction and development and overall tuber yield (Mauk & Langille, 1978. Romanov et al., 2000). The phytohormone, abscisic acid (ABA) has also been shown promoting effect on tuberization when applied exogenously and acting antagonistically to GA (Xu et al., 1998a). The salicylic acid (SA) and its related compounds have also been reported to influence the tuber inducing activities in potato. The current studies showed the role of auxins phytohormone in tuberization with an appropriate ratio of CK (Sergeeva et al., 2000). Expression analysis of the auxin biosynthetic pathway gene have also shown an association of auxin response factor 6 (ARF6) protein in tuberization and tuber dormancy release (Faivre-Rampant et al., 2004).

2 Hormonal Cascade Regulating Potato Tuberization

The tuberization is an important survival mechanism for the potato plant which has been considered as an intricate plant developmental phenomenon involving several (van den Berg et al., 1996). The potato tuber formation starts with the swelling of the modified stem referred as stolons which further elongates longitudinally via storage of carbohydrates subsequently initiating the tuber formation. During this development, the tissues of the stolons undergoes a series of cell division and cell enlargement followed by an adjustment in the orientation of cell growth at the subapical region of the tip of the stolon (Ewing & Struik, 2010; Vreugdenhil et al., 1999; Xu et al., 1998b). The principal biochemical process in potato tuberization involves synthesis and accumulation of storage carbohydrates and proteins which has been reported to be synchronized by precise expression patterns of different genes (Taylor et al., 1998; Bachem et al., 2000; Tauberger et al., 2000; Verhees et al., 2002). Studies on the cDNA microarray analysis, quantitative trait loci (QTLs) have revealed differential gene expression pattern and role of various endogenous phytohormones involving potato tuberization (Ewing et al., 2004; Kloosterman et al., 2005). Role of various phytohormones in tuberization has been studies extensively with an established fact that the coordinated levels of hormones regulate the potato tuberization under the natural conditions. Role of various hormones affecting the steps of the potato tuberization is represented in Table 13.1.

3 Role of Gibberelic Acid (GA) in Tuberization

The GA has been reported to play a key protagonist in the potato tuber development, hence studied extensively in literature. The biochemical characterizations have identified over 120 GAs in the plants species; however, only GA_1 and GA_4 are reported to be naturally vigorous participating in the tuber development (Jackson & Prat, 1996; Vreugdenhil & Struik, 1989). The GA has been reported as a prevailing regulator for potato development, and its endogenous level are controlled by the cellular content of the sucrose and abscisic acid (ABA). The GA also induces stolon elongation and branching through longitudinal cell extension and transverse direction of microtubules and microfibrils to the cell axis (Vreugdenhil & Struik, 1989). Moreover, the GA also participates in the photoperiodic control of tuberization (Xu et al., 1998a). GA play an important role in the stolons formation and analysis of diverse GA biosynthetic gene revealed enhanced expression of the gene during early stages of tuber induction; however a swift decline in the gene expression of biosynthetic gene confirmed decrees in the active GA during stolon tip swelling and initiation of the tubers. Analysis of the endogenous GAs content also exhibited lowering of active GAs content in the swelling stolon tips upon tuber induction (Koda & Okazawa, 1983). It was reported that the expression of the potato GA reducing gene ($StGA_2ox$), induced prior to stolon swelling immediately after the initiation of the stolons formation rendering their importance in the stolon formation (Kloosterman et al., 2007). Thereafter, Carrera et al. (2000) showed that the overexpression of an active GAs biosynthetic gene ($StGA_{20}ox1$ gene) in potato delayed the tuberization while or antisense expression of the gene advanced the time of tuberization. The antisense transgenic lines showed an increase in the active GA content, inferior stolon growth and consequent tuberization. In another experiment, the overexpression of $StGA_2ox1$ gene overdue the tuberization and change the tuber morphology (Kloosterman et al., 2007). Furthermore, another gene referred as $StGA_3ox2$

Table 13.1 Physiological effect of phytohormones in Potato tuberization

Hormones	Physiological effect	Tuber stages	Reference
Gibberellins	Stimulate stolons formation, elongation and branching but inhibit tuber initiation and growth in higher concentration.	• Favour stolon growth • Inhibit tuber initiation • Inhibit tuber growth	Koda and Okazawa (1983), and Carrera et al. (2000)
Auxins	Play important role in stolon tip swelling and tuber growth, its effect on tuber development possibly by regulating tuber growth through mediating meristem identity and re-orientation of the plane of cell division.	• Maintain stolon apical Dormance at low level • Stimulate tuber initiation • Favourtuber growth	Xu et al. (1998b), Roumeliotis et al. (2012), and Kolachevskaya et al. (2015)
Aba	Retards stolon growth and initiate tuber formation in high ABA:GA ratio.	• Inhibit stolon elongation and growth • Stimulate tuber initiation • Favour tuber growth	Wareing and Jennings (1979), Menzel (1980), Stallknecht and Farnsworth (1982), Ewing (1995), and Dutt et al. (2017)
Cytokinins	Activate starch biosynthesis-related geneslike ADP-glucose pyrophosphorylase and starch synthase during tuber initiation. Help in starch accumulation and enhance sink capacity of developing tubers.	• Role in stolon growth is not well known • Stimulate tuber initiation • Stimulate tuber growth	Ronzhina and Mokronosov (1994), Gális et al. (1995), Kefi et al. (2000), Romanov et al. (2000), Aksenova et al. (2012), and Hartmann et al. (2011).
Jasmonates	Induce tuber formation and tuber growth. JA and its derivatives TA and TGA have opposite role of GAs in tuber initiation and growth.	• Effect of JA on stolon formation is not well known • Induce tuber initiation • Stimulate tuber growth	Koda (1997), Pelacho and Mingo-Castel (1991), Matsuki et al. (1992), and Abdala et al. (2002)
Salicylic acid	Plays role in tuber induction and tuber growth when it is applied exogenously.	Role of SA on various stages of tuber development is not well studied.	Malik and Madhi (2017)

has been used for tissue specific or constitutive overexpression in the plants. The overexpression of the gene driven by the constitutive 35S promoter resulted in early tuberization and overall tuber yield; however, the tuber specific expression slightly delayed tuberization (Bou-Torrent et al., 2011). Further biochemical analysis revealed that the overexpression of gene in the leaves dynamically converted GA_{20} to GA_1 in the leaves, thereby; a lower GA_{20} transported to the stolon tips causing a

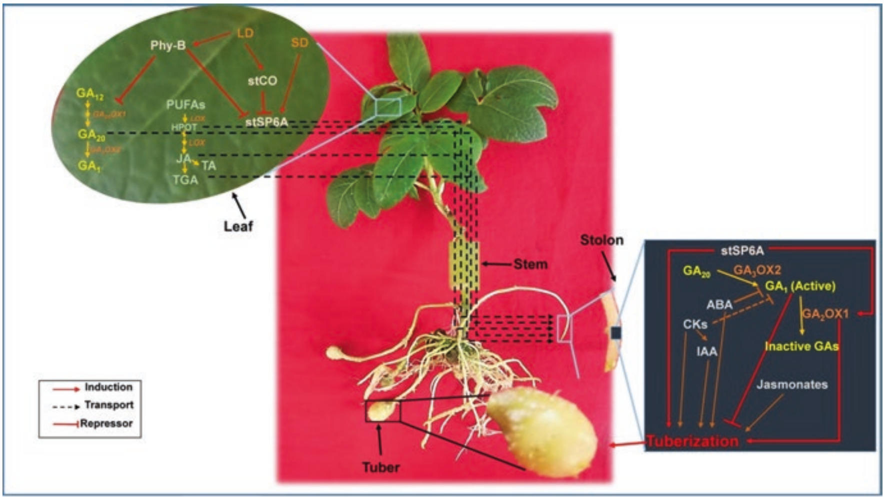

Fig. 13.1 Schematic representation of regulation of tuberization via various hormonal cascades
StSP6A and GAs act as mobile signals that are transported through phloem to stolon. Translocation of mobiles signals are regulated by a complex regulatory network. Phy-B and StCO repress tuberization in response to LDs. GAs seem to act as repressors, whereas StSP6A (GA biosynthetic regulator) help to induce tuber induction under SD conditions (Dutt et al., 2017). Under LDs, Phy-B represses the expression of *StSP6A* and *StGA20ox1*, which encodes an enzyme that catalyzes the synthesis of GA_{20}. Under LDs, *StCO* represses *StSP6A*. StGA3ox2 catalyzes the conversion of GA_{20} to GA_1, an active GA. Other phytohormones like Auxins, CKs, ABA and JA act as a inducer of tuber formation in stolon
Photograph of plant captured from Plant Molecular Biology Lab, Dept. of Biotechnology, Dr. Harisingh Gour Vishwavidyalaya, Sagar, M.P., India.
Abbreviation: *GAs* Gibberellins, *$GA_{20}OX1$* GA 20-oxidases-1, *GA_3OX2* GA 3-oxidases-2, *GA_2OX1* GA 20-oxidases-1, *LD* Long Day photoperiod, *SD* Short day photoperiod, *Phy-B* Phytochrome-B, *StCO Constans*, *StSP6A* Solanum tuberosum SELF PRUNING 6A, *PUFAs* Poly unsaturated fatty acids, *HPOT* hydroperoxides, *JA* Jasmonic acid, *TA* Tuberonic acid, *TAG* Tuberonic acid glucoside, *LOX* Lypoxigenase, *ABA* Abscisic acid, *CKs* Cytokinins, *IAA* Indole 3-acitic acid

lower accessibility of GA_{20} in the stolon tips resulting in reduced GA_1 content leading to quicker tuber formation. A line diagram representing the role of GA and other phytohormone regulating various stages of the potato tuberization is represented in Fig. 13.1.

4 Role of Cytokinin in Tuberization

Several other plant hormones have also been reported to play an important role in the tuber induction and development in potato. Plant phytohormone, the Cytokinins (CKs) have also been recognized to function as a common mechanism of storage-organ formation in the plants (Roitsch & Ehneß, 2000; Abelenda & Prat, 2013). Several reports existed in literature on the role of CKs in potato tuberization and development (Krauss & Marschner, 1976; Galis et al., 1995; Banfalvi et al., 1997; Miyazawa et al., 1999; Romanov et al., 2000; Guivarch et al., 2002). The CKs is known to promote potato tuber initiation and advance the commencement of tuber formation (Gális et al., 1995; Kefi et al., 2000; Romanov et al., 2000). Application of CKs in *in-vitro* tuberization medium could explicitly advance the yield of microtubers, tuber number and biomass in the potato (Levy et al., 1993; Romanov et al., 2000). Field application of CK prior to tuber formation has also been reported to induce the overall tuber yield in potato (Pavlista, 2001). The initial stage of tuber formation require the presence of CK in combination of GA which stimulate cell proliferation, cellular division, radial cell growth and starch accumulation via source–sink movement directing the development of initial tubers (Aksenova et al., 2009; Abelenda & Prat, 2013). Metabolic pathway analysis also showed that CK also activates the ADP-glucose pyrophosphorylase and starch synthase enzyme activity resulting in starch accumulation and enhancing the sink capacity of developing potato tubers (Ronzhina & Mokronosov, 1994; Aksenova et al., 2012). In non-tuberizing plants, an increase of active CK levels can stimulate morphogenesis, resembling tuber formation (Guivarc'h et al., 2002; Eviatar-Ribak et al., 2013). Further studies on the development of transgenic potato altering the CK biosynthetic pathways also showed importance of this plant hormone in the tuber development. The transformation of potato with the isopentenyl transferase gene (IPT) which catalyzes the rate-limiting step of CK biosynthesis, exerted a stimulating consequence in the tuber initiation process and thereby an increased tuber yield, fresh tuber weight per plant (Gális et al., 1995; Hirose et al., 2008). Hartmann et al. (2011) overexpressed *Arabidopsis* cytokinin oxidase/dehydrogenase1 gene (*CKX1)* in potato under the constitutive promoter. Biochemical analysis revealed a lowered endogenous CK levels which resulted in deformed tuber with reduced tuber yield.

5 Role of Auxin in Tuberization

Another important plant hormone, the auxin has also been shown to play an important role in several aspects of plant architecture including embryogenesis, lateral root formation and flower development (Marchant et al., 2002; Luo et al., 2011; Krizek, 2011). The potato tuberization include three stages which include tuber induction, tuber initiation, and tuber growth; however, during the initial stage of tuber initiation the longitudinal cell division at the stolon and horizontal cell elongation provides the space for the cell expansion resulting in development of younger tuber through swelling (Xu et al., 1998b). Therefore, changes in the orientation of cell division that result in swelling of the stolon tip in tuber development are due to the activity of auxin. The plain of cell division remains transversal during this phase. Auxin content remains relatively low and the role of auxin is to maintain stolon apical dominance (Roumeliotis et al., 2012). The *In-vitro* studies revealed the importance of an important auxin, the indoleacetic acid (IAA) in tuber initiation. Experiments showed that the tuber initiation in the presence of IAA depends upon the content of sucrose in the culture medium. Presence of IAA in the *in-vitro* medium with lower sucrose content enhanced tuber number, whereas higher sucrose content (up to 5–8%) in the medium, the IAA exerted negative impact on the number of tubers (Aksenova et al., 2000). In addition, removal of stolon tip (auxin source) from explants taken for *In-vitro* tuberization experiments also showed increased tuber formation from axillary buds (Roumeliotis et al., 2012).

Differential gene expression analysis showed expression of several auxin-responsive genes during early tuberization including auxin biosynthesis, auxin transport (PIN gene family) gene, auxin response (ARF) factors (Faivre-Rampant et al., 2004; Kloosterman et al., 2007). A few transgenic studies overexpressing the auxin pathway gene also prompted the auxin response in the potato tuberization. Kolachevskaya et al. (2015) reported development of transgenic potato generated potato by overexpressing *Agrobacterium* auxin biosynthetic genes tms-1. The gene was cloned in binary vector, pBinB33-tms1 fused to tuber-specific promoter of the class I patatin gene (B33-promoter) of potato. Molecular analysis revealed tms1 gene expression in the tuber tissues with enhanced auxin content in the tuber tissues and plants with increased auxin levels were predominantly in tubers which probably accelerated and intensified the process of tuber formation. Overall, progressive relationships were witnessed among tms1 expression, the IAA content in tubers and stimulation of tuber formation.

6 Role of Abscisic Acid (ABA) in Tuberization

Abscisic acid (ABA) is regarded as a general regulator that reduces GA-promoted processes in plant development. It has been reported to retard the plant growth, however, exerts a positive impact on the induction of tuberization in combination with the GA. Several studies suggested that ABA content increases under inductive conditions which indicate that ABA is a factor counteracting the GA effect on tuberization (El-Antably et al., 1967; Krauss & Marschner, 1976; Ewing, 1995). The exogenous applications of ABA to potato as foliar spray land to cultured plant sections (aqueous nutrient media) have been inconsistent in stimulating the tuberization. However, in contrast, no effect of ABA has been observed on sprout or stolon sections cultured *In-vitro* (Wareing & Jennings, 1979; Menzel, 1980; Stallknecht & Farnsworth, 1982). Another experiment with *droopy* mutant of *S. phueja* potato deficient in ABA synthesis showed that despite the blockage of ABA synthesis, this mutant transits to tuber formation normally under SD conditions, which indicated that ABA does not play a key role in tuber induction and that its stimulatory effect is due to its antagonism to GA signalling (Quarrie, 1982; Chailakhyan, 1984; Xu et al., 1998a).

7 Phytochrome Mediated Hormonal Regulation of Potato Tuberization

The phytochrome A (Phy-A) and B (Phy-B) as photoreceptors present at the aerial parts have been recognized to sense the photoperiod of which Phy-B arbitrate the photoperiodic regulation of tuberization in potato. Jackson et al. (1996) for the first time down regulated the Phy-B protein in potato via transgenic expression of the biosynthetic pathway gene which finally revealed that the plant lost the capability to react to an LD (Long day) photoperiod which directly reaffirmed their role as photoreceptor. Jackson et al. (1998) also showed that when the wild type potato was grafted to the Phy-B antisense plants, its regulated the grafting experiments with Phy-B antisense plants and wild-type potato plants showed that Phy-B regulates the transmission of Phy-B signal thus affecting the tuber induction in the under the LD photoperiod. The Phy-B has also been reported to controls the synthesis of an inhibitory signal that has a role in GA signal transduction. In the GA signalling cascade, a photoperiod responsive-1 (*PHOR1*) gene encoding a novel arm-repeat protein is a vital constituent of the GA-signalling pathway. The transgenic overexpression of this gene in potato resulted in the induced tuberization with overall tuber yield, however, the down regulation of the gene showed reduction in the PHOR1 protein with reduced stem length, earlier tuberization under SDs and enhanced insensitivity to applied Gas under the *In-vitro* conditions. Martinez-Garcia et al. (2002) reported the overexpression of the *Arabidopsi*s CONSTANS (CO) transcription factor (TF) in potato under the control of the constitutive promoter which resulted in delayed tuberization in potato. This also suggested that the *AtCO* (a flowering response gene of *Arabidopsis*) also had a role in the tuberization, therefore, the reconciliation of some unknown pathway regulating the flowering and the tuberization. Thereafter, González-Schain et al. (2003) reported the isolation and transcriptomic expression of the *AtCO* orthologue exists in potato regulating the photoperiodic control of tuberization.

8 Role of Jasmonic Acid, Salicylic Acid on Tuberization

Role of plant hormone, the Jasmonic acid (JA) is also recognized to modulate the plant development pathways. Another orthologue of JA regulating the potato tuberization is recognized as tuberonic acid (TA) and its aglycone the tuberonic acid glucoside (TAG) (Koda et al., 1988; Yoshihara et al., 1989; Koda et al., 1991; Matsuura et al., 1993). Several studies have already identified jasmonate-related compounds as potent tuber-inducing factors in potato (Koda et al., 1991). The lipoxygenase (LOX), a dioxygenases enzyme activity has been recognized as key enzyme regulating the JA biosynthesis. The LOX oxygenates the two polyunsaturated fatty acids, the linoleic and linolenic acid resulting in the synthesis of 9(S)-hydroperoxylinolenic acid (9(S)-HPOT) and 13(S)-hydroperoxylinolenic acid (13(S)-HPOT). The JA is then synthesized from 13(S)-HPOT and then catabolozed to tuberonic acid (TA) and tuberonic acid glucoside (TAG) which has been recognized to induce tuberization in case of the potato (Koda, 1997). The cellular activation of LOX requires lower temperature, which is also an important factor for the tuber induction. The activation of LOX also induces the increase in HPOT, JA, TA and TAG content in the aerial parts of a potato plant and then transported to the underground stolons (Yoshihara et al., 1996; Nam et al., 2008). The gene expression studies have also revealed that analogous to outcome of ABA, the LOX originated metabolites might support tuberization by antagonizing the effects of GA, (Jackson & Willmitzer, 1994; Jackson, 1999; Xu et al., 1998a). Various molecular analysis also exhibited that accumulation of Lox1 class mRNA occurs at the tips of stolons, and LOX activity was highest at nascent vigorously growing tubers, thereby, controlling the advance of tubers in potato (Kolomiets et al., 2001). Sarkar et al. (2006) showed for the first time that the derivatives of 13(S)-HPOT, the JA and methyl jasmonate, are mainly occupied in tuber development fairly than in the initial step of the tuber formation recognized as tuber initiation.

Studies have shown that application of JA and its derivatives induce tuber initiation in potato stolons (Pelacho & Mingo-Castel, 1991). While seeking inhibitors of JA biosynthesis, found that salicylic acid (SA) and acetylsalicylic acid (ASA) induce tuberization in stolons under the *in-vitro*. During the early steps of tuber initiation, the level of JA in stolons increased (Abdala et al., 2002). *In-vitro* studies have shown that application of the inhibitor of GA biosynthesis agent changes cell division in the stolon as opposite to that of the natural GA and the analogous changes in the cortical microtubule orientation were observed through the application of the JA and TA (Matsuki et al., 1992; Abdala et al., 2002). Treatment with JA induced complete reversion of GA3 inhibitory action on tuber formation by *In-vitro* cultivated potato explants (Castro et al., 2000). Involvement of JA and enzymes of their biosynthesis in processes of cell division and growth at the tuber initiation have been recognized in several studies. The apparent inductive effect of JA on tuberization might be due to the fact that a sub- optimal concentration of sucrose, one of the most important tuber-inducing signal molecules, was used in the *in-vitro* assay medium (Pelacho & Mingo-Castel, 1991). Although SA and related compounds affects a range of developmental and disease-response phenomena in plants, there is still little information on their tuber-inducing effects (Raskin, 1992; Pierpoint, 1994).

9 Conclusions

The regulation of potato tuberization and role of various extrinsic and intrinsic factors involved in tuberization have been studied extensively. Biochemical and molecular mechanism controlling in-plants tuberization and elucidation of specific role of phytohormones in tuberization still remains elusive. The photoperiod and temperature have been considered as most crucial environmental factor influencing the tuberization in potato. Evidence on the role the involvement of hormonal signals, numerous enzymes, transcription factors and genes in these processes have been reported, however, mechanisms of interaction between various phytohormones and their signalling pathways have been refined. Major hormones positively modulating the tuber induction are the GA, CKs, ABA, jasmonates and auxins. Studies suggest that SA also influences tuberization process positively, however, the salicylates have been recognized an important role in biotic and abiotic stress tolerance. Investigations on the composite hormonal, biosynthesis, metabolism genome modification might develop an opportunity to describe the influence of hormonal signalling in this important phenomenon.

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Chapter 14
Potato Virus Elimination as Short and Long-Term Effect of Salicylic Acid Is Mediated by Oxidative Stress and Induction of Tolerance to Thermotherapy or Cryotherapy

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Abstract Potato seed production requires virus-free plants. Potato virus X (PVX) is normally cleaned by thermotherapy, however, potato virus S (PVS) is one of the most difficult viruses to clean by thermotherapy. Cryotherapy is an optional technique to eliminate difficult viruses by traditional techniques. Both therapies induce oxidative damage leading to low survival. In the present investigation, the effect of SA to protect *Solanum tuberosum* plants infected with PVX or PVS from damage due to thermotherapy or cryotherapy was studied. Previously to therapies, nodal explants from plants PVX or PVS positive were sub-cultured for 30 d on MS medium containing SA 10^{-5} or 10^{-6} M. Thermotherapy: Plants PVX positive were subcultured for a supplementary 30 d period on MS SA-free, followed by thermotherapy (32–42 °C) for 35 d. Results showed SA induced thermotolerance during thermotherapy. PVX-free plants obtained in SA were significantly higher than in the control. SA increased CAT activity and reduced the H_2O_2 content. Cryotherapy: Plants PVS positive, following cryotherapy, plant development and virus testing were carried out. Between 66.6% and 100% of the treated plants were virus-free compared to control plants which exhibited 0% survival. Thus, SA treatment in combination with thermotherapy or cryotherapy described, enhanced plant survival and virus cleaning in potato plants.

Keywords Hight temperature tolerance · Reactive oxygen species · Salicylate treatment · Ultra low temperature tolerance · Virosis cleaning

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S. Hayat et al. (eds.), *Salicylic Acid - A Versatile Plant Growth Regulator*,
https://doi.org/10.1007/978-3-030-79229-9_14

1 Introduction

In potato, viral diseases are very common and they are transmitted via infected tubers, insect vectors, or by many ways from one generation to the next, or from one area to another in the same country (Kushnarenko et al., 2017). Crops infected with virus, especially S (PVS), a strong loss in crop production usually occur. Production of potato seed tubers, of basic and certified potato categories, requires virus-free plants by *in vitro* multiplication (Faccioli & Colombarini, 1996), phytosanitary norms of each country requires pathogen-free plants in order to obtain basic seed, followed by production and distribution (Naik & Khurana, 2003).

Thermotherapy is the most known technique for virus eradication, as virus concentration can be reduced or eliminated when plants are heat-treated. Thermotherapy used in temperature and the time of application depend on the virus present and genotype sensitivity to heat (Rosenberg, 2000). During the last 10 years, cryotherapy has appeared as an alternative technique for removing pathogens in various crops (Ding et al., 2008; Wang & Valkonen, 2008, 2009). The efficacy of using ultra-low temperatures (−196 °C) for virus cleaning, is based on physio-anatomical differences among meristematic cells (Wang & Valkonen, 2009). Nitrogen is likely to remove infected cells in the meristems, whereas healthy cells have more potential for regeneration (Wang et al., 2008). Virus-infected cells are more susceptible to damage or death during cryogenic methods (Wang et al., 2008).

Cryotherapy has been reported to eliminate PVY and Potato Leaf Roll Virus (PLRV), (Wang et al., 2006), and also in combination with chimiotherapy as reported by Kushnarenko et al. (2017) for elimination of PVS with a ribavirin pretreatment method.

Temperature as a stress factor in plants, in both high and low temperatures produces oxidative damage (Horvath et al., 2007; Khan et al., 2015). Plants under stressful conditions, due to biotic or abiotic conditions suffer oxidative stress, which increases the production of reactive oxygen species (ROS), including singlet oxygen ($^1O_2$), superoxide anion (O_2^-), hydroxyl radical (OH^-), and hydrogen peroxide (H_2O_2) (Larkindale & Huang, 2004; Shi et al., 2006; Ahmad et al., 2008; Hayat et al., 2010).

ROS are toxic depending on the concentrations, at adequate levels, ROS can function as signal molecules, particularly $H_2O_{2,}$ which triggers tolerance against various stress factors (Zhou et al., 2012). Due to its poor reactivity with most organic molecules, H_2O_2 is the most stable ROS. ROS can cause severe oxidative damage to both biomolecules and cellular structures (Smirnoff, 1996; Tatone et al., 2010). In plants 2 antioxidant systems are present as protection against ROS; non-enzymatic and enzymatic antioxidant systems (Larkindale & Huang, 2004). Enzymatic antioxidants, such as superoxide dismutase (SOD), peroxidases (POX), and catalases (CAT) protect by directly scavenging ROS radicals, converting these radicals into less reactive species (Suzuki & Mittler, 2006; Mhamdi et al., 2010).

Catalase enzyme permits the reduction of H_2O_2 to H_2O and O_2 without participation of a reducing agent (Willekens et al., 1995; Mhamdi et al., 2010). Salicylic acid

(SA) inhibits this enzyme (Kuźniak & Urbanek, 2000). SA is a phenolic compound with a hydroxyl group (An & Mou, 2011; Vlot et al., 2009). Hayat et al. (2010) reported that SA is able to mediate the efficiency of antioxidant systems in plants. SA can regulate enzymes such as catalase (CAT), peroxidase (POX), and superoxide dismutase (SOD) (Knörzer et al., 1999; Taşgın et al., 2006). We previously demonstrated SA's capability to induce thermotolerance during thermotherapy and subsequent PVX eradication in infected *S. tuberosum* (potato) microplants (López-Delgado et al., 2004). SA application induced protection against several stress factors, such as long-term drought (Kadioglu et al., 2011), heavy metals (Krantev et al., 2008), and heat (Lopez-Delgado et al., 1998; Dat et al. 1998a, b; He et al., 2005; Shi et al., 2006), Induction of heat shock and chilling tolerance by SA and H_2O_2 (Lopez-Delgado & Scott, 1997; Lopez-Delgado et al., 1998; Mora-Herrera et al., 2005), as well as microtuberization (López-Delgado et al., 2012) was previously reported. It has been demonstrated that SA can increase the survival and elimination of viruses by thermotherapy (Aguilar-Camacho et al., 2016; Lopez-Delgado et al., 1998, 2004), in addition to cryopreservation methods (Ruiz-Sáenz et al., 2019; Ayala-Hernández et al., 2019; Pathirana et al., 2016; Wang et al. 2009).

It has been suggested that SA induce changes in cellular redox homeostasis inhibiting CAT in peroxisomes (Serrano et al., 2015) leading to an increase in H_2O_2 accumulation, so activating pathogen defense pathways (Dat et al., 2000; López-Delgado et al., 2004; Tada-Oikawa et al., 2008). Information regarding the long-term effects of SA in thermotolerance and virus cleaning is scarce. Therefore, our objectives were as follows: (a) evaluate the short and long term effects of SA in thermotolerance induction and virus-free microplants obtained; (b) analyze the relationship between CAT activity and H_2O_2 content and (c) to evaluate the effect of SA on the induction of tolerance to oxidative damage due to cryotherapy applied for the elimination of PVS in *Solanum tuberosum* plants.

2 Materials and Methods

2.1 Thermotherapy

2.1.1 Plant Material and Culture Conditions

Solanum tuberosum L. microplants clone 040138 positive to PVX were obtained from the National Potato Program of the National Institute for Agriculture and Livestock Research (INIFAP), in Toluca, Mexico and used in all experiments. PVX virus presence was confirmed by ELISA technique. Microplants were micropropagated following the methods of Espinoza et al. (1986). Every 30 d, axillary buds were subcultured in jars on MS propagation medium (Murashige & Skoog, 1962) at 18 ± 1 °C with a 16 h photoperiod at low radiance (fluorescent lights, 35µmol m^{-2} s^{-1}, 400–700 nm).

2.1.2 Short Term Effects (STE)

SA Treatments: Axillary nodes were cultivated for 30 d on MS propagation medium ± SA (0, 10^{-5}, 10^{-6} M). These concentrations were selected based on previous reports, where SA was effective in enhancing thermotolerance and elimination of potato virus (López-Delgado et al., 2004).

2.1.3 Long-Term Effects (LTE)

The same SA concentration used in STE were applied in LTE experiments.

SA Treatments: Same methods as the STE, but microplants were grown an additional 30 d on MS without SA for a total of 30 d on SA followed of 30 d without SA before thermotherapy.

Thermotherapy: Prior to thermotherapy, 30 single nodes per treatment (SA) were subcultured in tubes for 5 days with MS propagation medium. Following subculture, thermotherapy was carried out in a Biotronette Mark III (Lab Line) environmental chamber under fluorescent lights (35μmol m^{-2} s^{-1}, 400–700 nm). A temperature regime was performed every day for 35 d. Temperature applications alternated between 32 ± 1 °C for 23 h and 42 ± 1 °C for 1 h. Survival was evaluated 1 day following thermotherapy, and microplants were subcultured to MS propagation medium. Following 30 days, survival and ELISA were evaluated in order to test for PVX.

2.1.4 CAT and H_2O_2 Measurements

CAT activity or H_2O_2 content were evaluated in shoots obtained immediately after the short and long term SA treatment. All samples were kept in liquid nitrogen until valuation.

Catalase Activity (CAT)

CAT activity was according to Aebi (1984). Soluble protein from tissue samples (0.1 g) was extracted by homogenizing the tissue powder in 0.4 mL extraction buffer (50 mM potassium phosphate buffer pH 7.2, 5 mM dithiothreitol (DTT), 1 mM EDTA, and 1% (*w*/*v*) PVP). Total reaction mixture (3 mL) included 50 mM potassium, sodium phosphate pH 7.0, and 20μL of enzyme extract. The addition of 30 mM H_2O_2 initiated the reaction. Decomposition was followed directly by decrease of absorbance at 240 nm every 20 s for 3 min at 26 °C. Protein content was determined using a spectrophotometer nanodrop 1000 (Thermo Scientific).

H_2O_2 Measurement

Hydrogen peroxide was measured in microplants without roots. Tissue samples (~ 0.2 g) were extracted in 1.2 mL ice-cold 5% (*w*/*v*) trichloroacetic acid (TCA). Following centrifugation (10 min, 10,000 g), 0.5 mL of the supernatant fraction was passed through Dowex-1 resin (0.5 g, Fluka) followed by 3.5 mL 5% TCA. H_2O_2 was measured in the eluates using luminol- dependent chemiluminescence (Warm & Laties, 1982): 0.5 mL of eluate was added to 0.5 mL 0.5 mM luminol (Sigma); this volume was composed of 4.5 mL 0.2 M NH_4OH (pH 9); a 0.452 mL mixture was analyzed in a polystyrene tube (12 × 75 mm, Fisher) using an Optocomp P luminometer (MGM Instruments, USA). Chemiluminescence was initiated by injecting 50μL of 0.5 mM potassium ferricyanide in 0.2 M NH_4OH and emitted photons were counted over 5 s. A parallel sample of each initial extract was processed after addition of a known H_2O_2 concentration to provide a recovery correlation factor.

2.2 Cryotherapy

2.2.1 Plant Material and Culture Conditions

Potato clones of *Solanum tuberosum* L. microplants positive only or PVS (04-2-4-6, 06-27, 976119, 981818, 990611, 940201-C, LP2S6, T01-7-70, T97-1-172, and Tollocan H) from the *in vitro* germplasm bank of the Laboratory of Biotechnology and Plant Physiology of the National Potato Program at the National Institute of Forestry, Agriculture, and Livestock Research (INIFAP) in Metepec, Mexico were used for experiments. These clones were subjected to a cryogenic protocol (Yamamoto et al., 2015, modified by Arizaga et al., 2017) and their growth was evaluated 15 days later.

Microplants clones, 981,818 and T01-7-70, showed the lowest rates of survival following cryotherapy and were selected for further study. Presence of virus in these clones was confirmed in double antibody sandwich (DAS)-enzyme-linked immunosorbent assays (ELISA) (Clark & Adams, 1977). The microplants were subsequently propagated according to the methods of Espinoza et al. (1986). Every 30 d, axillary buds were subcultured in jars containing Murashige and Skoog propagation medium (MS) (Murashige & Skoog, 1962) at 18 ± 1 °C with exposure to 16 h of fluorescent light (35μmolm^{-2} s^{-1}, 400–700 nm).

2.2.2 SA Treatment

Microplants clones, 981,818 and T01-7-70, were incubated in MS propagation medium (Murashige & Skoog, 1962), in the presence of SA (0 M, 10^{-5} M, or 10^{-6} M) for 28 d prior to receiving cryotherapy. These concentrations were elected based on previous reports of SA concentrations for inducing freezing tolerance and elimination of potato virus (Mora-Herrera et al., 2005; Aguilar-Camacho et al., 2016).

2.2.3 Evaluation of SA Treatments

Twenty axillary buds per treatment of each clone, 981,818 and T01-7-70, were dissected (1–2.5 mm in length) and subcultured in SA-free MS medium. Data about survival ($n = 20$), shoot fresh weight ($n = 5–20$/treatment) for the plants were obtained 15 days later.

2.2.4 Cryotherapy Protocol with Subculturing

Axillary buds from treated microplants were dissected (1–2 mm length) and precultured in SA-free MS medium containing 0.3 M sucrose at 21 °C. Then they subcultured for 3 days, cryotherapy was performed in aluminium $7 \times 37 \times 0.5$ mm^3 D-cryoplates containing 10 oval wells as previously described (Yamamoto et al., 2015 modified by Arizaga et al., 2017). Briefly:

1. Droplets of Na-alginate solution (2% *w*/*v* sodium alginate/ and 0.4 M sucrose in MS basal solution) were added to each cryoplate well.
2. Precultured axillary buds were transferred individually to single wells and then covered with Na-alginate solution. The cryoplate was covered with a disinfected sheet of BEMCOT paper (7×30 mm^2), followed by calcium chloride solution (0.1 M calcium chloride/0.4 M sucrose in MS basal solution) until it was covered completely. Na-alginate polymerization was completed after 15 min at room temperature Extra calcium chloride solution was removed.
3. Cryoplates containing buds and covered with BEMCOT paper were then transferred into a loading solution (LS; 2.0 M glycerol/1.0 M sucrose in MS basal solution).
4. After 45 min, cryoplates were separated from the LS and were dehydrated in a petri dish containing 35 g silica gel. Desiccation was achieved for 90 min at 24 °C.
5. Then cryoplate was transferred into cryotubes (2 mL) held on a cryocane and filled with liquid nitrogen (LN).
6. After 60 min, cryotubes were recovered from the LN and were immersed and rewarmed in cryotubes containing 2 ml of 1 M sucrose solution with MS basal medium.
7. After 15 min at RT, the paper-attached buds were detached from the cryoplates and plated in a petri dish containing 20 ml solid MS medium. The buds were then isolated from the alginate gel and plated onto fresh solid MS medium.

2.2.5 Survival Measurements

Survival was evaluated 15 days after cryotherapy ($n = 20$/ treatment) by counting the number of plants that resumed growth. Root length data was also obtained 15 days after cryotherapy ($n = 5$–20/treatment).

2.2.6 Das-Elisa

The presence of PVX and PVS was confirmed with the Enzyme-Linked Immunosorbent Assay DAS-ELISA (Clark & Adams, 1977) performed in the ICAMEX phytopathology laboratory. DAS-ELISA was performed with samples collected from the meristem, stem, and leaf tissues (0.3 g each) before SA treatment and 30 d after thermotherapy and cryotherapy. Data was recorded with a Multiskcan FC spectrophotometer (Thermo Scientific).

2.2.7 Statistical Analysis

Analysis of variance (ANOVA) and post hoc Duncan's Multiple Range Test (Duncan, 1955) were performed with the program, Statgraphics Centurion XVI. The a priori significance level was established as $P < 0.05$. All experiments were performed three times.

3 Results and Discussion

3.1 Thermotherapy

3.1.1 Short-Term Effects (STE)

SA strongly induced thermotolerance during thermotherapy. Both SA concentrations (10^{-5}, 10^{-6} M) significantly increased PVX-infected microplant survival to thermotherapy in contrast to the control (Fig. 14.1).

Treated microplant CAT activity and H_2O_2 content were assessed prior to thermotherapy. SA 10^{-6} M significantly increased CAT activity compared with the control (Fig. 14.2a). In contrast, SA (10^{-6} M) resulted in a significant reduction in H_2O_2 concentration (Fig. 14.2b).

SA significantly enhanced the percentage of PVX-free microplants contrasted with the control, SA treatments significantly raised PVX-free microplants by 88 and 53% for 10^{-6} and 10^{-5} M, respectively to the control (Fig. 14.3). Results reinforced that SA induced both, thermotolerance and a higher PVX-free microplants after thermotherapy.

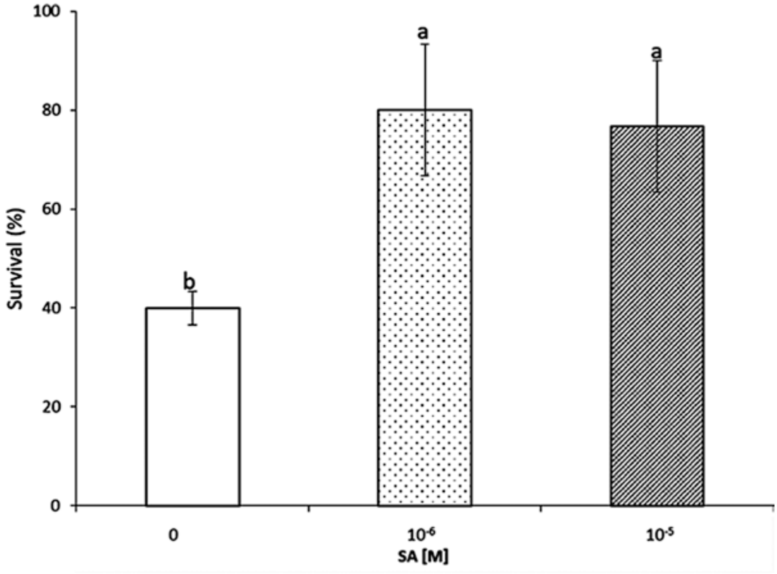

Fig. 14.1 Short term effect of SA on tolerance to thermotherapy (32–42 °C) of potato microplants clone 040138. Survival of microplants was evaluated 1 day after thermotherapy ($n = 30$). Data are means of 3 experiments ± S.E. Bars identified with same letter were not significantly different by ANOVA and Duncan test ($\alpha = 0.05$)

3.1.2 Long-Term Effects (LTE)

Similar to STE, SA LTE significantly augmented survival post thermotherapy treatment in PVX-infected *in vitro* plants (Fig. 14.4). Our original hypothesis was confirmed, since results reinforced that SA stimulated thermotolerance, even though SA was not present in the medium during thermotherapy. Both SA concentrations (10^{-5}, 10^{-6} M) improved survival up to 50%. Prior to thermotherapy, CAT activity and H_2O_2 content were assesed. SA did not induce significant effects on CAT activity (data not shown).

It is worth to mention that SA significantly reduced H_2O_2 concentration relative to the control (Fig. 14.5); 10^{-6} M reduced H_2O_2 concentration in 35% and 10^{-5} M in 38%.

Alike to STE, SA significantly augmented the percentage of virus-free microplants during LTE. PVX-free microplants augmented by 98% under SA 10^{-6} M treatment related with the control, while in SA 10^{-5} M, PVX-free microplants augmented by 69% (Fig. 14.6).

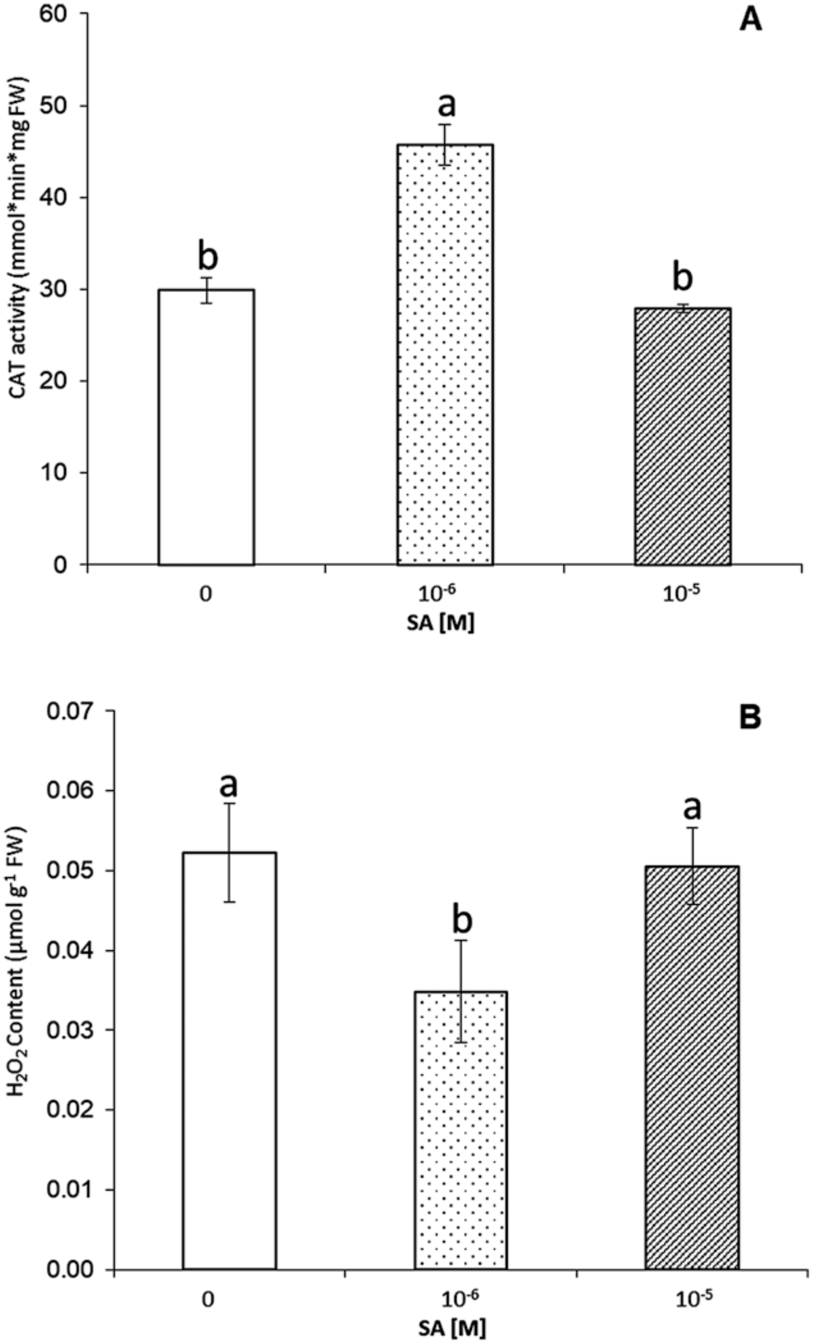

Fig. 14.2 Short term effect of SA on catalase activity (**a**) and hydrogen peroxide content (**b**) of potato microplants clone 040138. Data are means ± S.E. bars of three experiments (n = 3), each one with three samples, measured in triplicate. Bars identified with same letter were not significantly different by ANOVA and Duncan test ($\alpha = 0.05$)

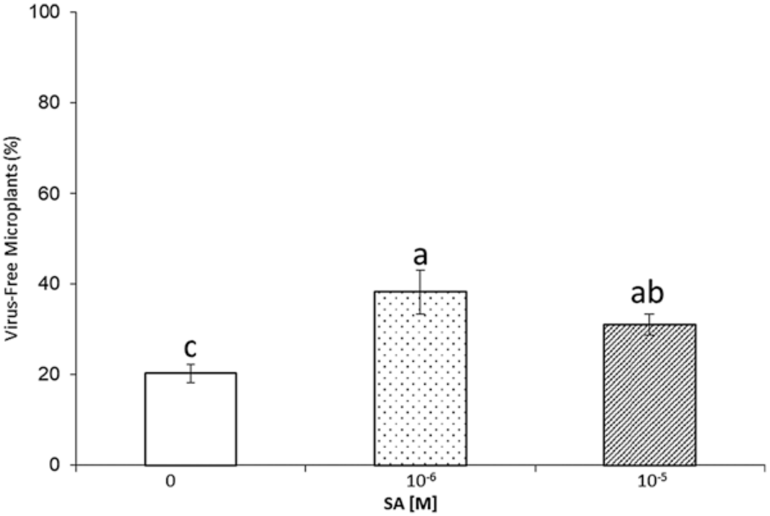

Fig. 14.3 Short term effect of SA and on the percentage of PVX-free microplants of potato clone 040138 subcultured to MS medium from *in vitro* thermotherapy-treated plants. Data are means of 3 experiments (n = 8–27). Bars identified with same letter were not significantly different by ANOVA and Duncan test (α = 0.05)

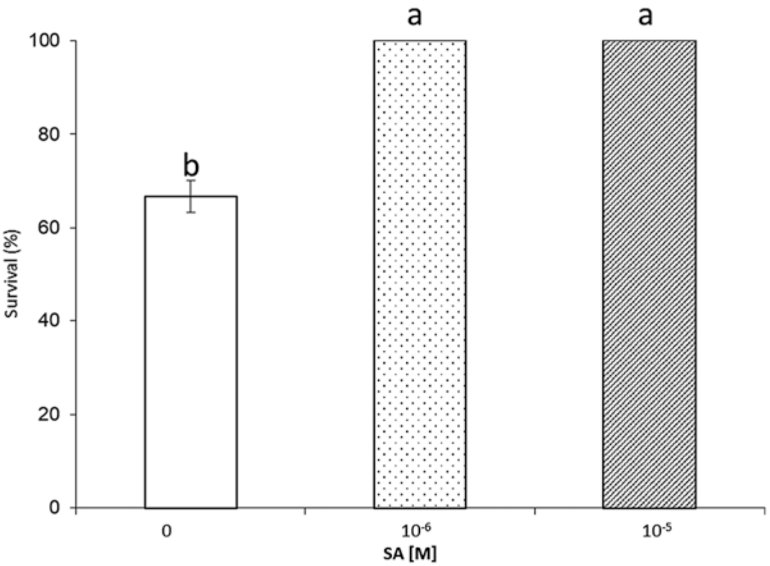

Fig. 14.4 Long term effect of SA on resistance to thermotherapy (32–42 °C) of potato microplants clone 040138. Survival of microplants was measured 1 day after thermotherapy (n = 30). Data are means of 3 experiments ± S.E. Bars identified with same letter were not significantly different by ANOVA and Duncan test (α = 0.05)

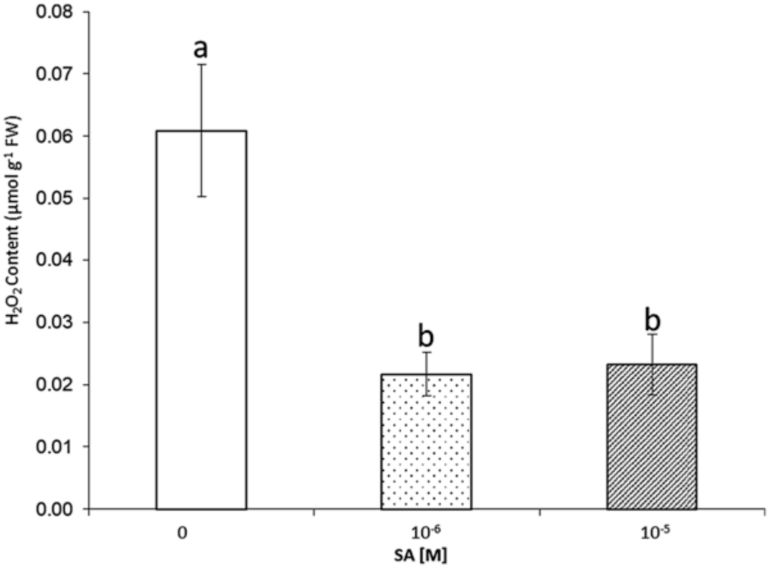

Fig. 14.5 Long term effect of SA on hydrogen peroxide content of potato microplants clone 040138. Data are means ± S.E. bars of three experiments (n = 3), each one with three samples measured in triplicate. Bars identified with same letter were not significantly different by ANOVA and Duncan test ($\alpha = 0.05$)

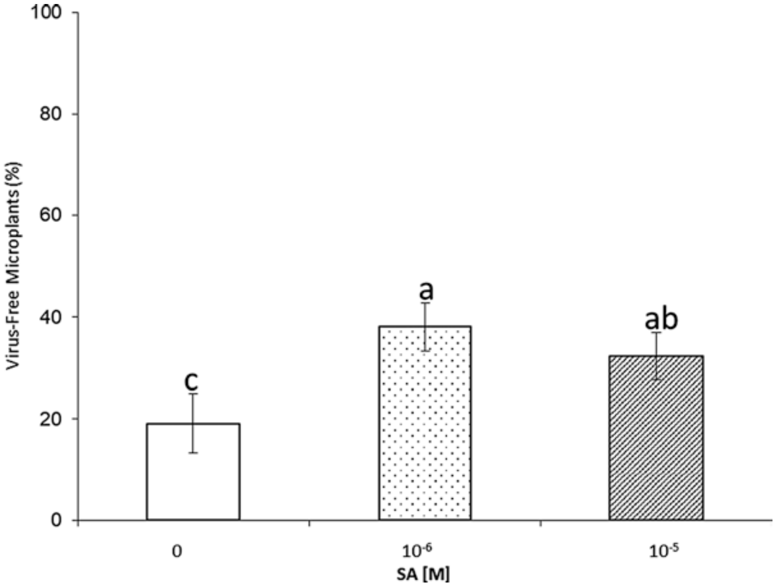

Fig. 14.6 Long term effect of SA on the percentage of PVX-free microplants of potato clone 040138 subcultured to MS medium from *in vitro* thermotherapy- treated plants. Data are means of 3 experiments (n = 8–24). Bars identified with same letter were not significantly different by ANOVA and Duncan test ($\alpha = 0.05$)

3.2 *Cryotherapy*

3.2.1 Selection of Plant Material

In order to study the effects of SA on induction of tolerance to cryogeny, clones T01-7-70 and 981,818 were chosen for carry on experiments, since they showed high susceptibility to cryogenic process; both genotypes showed null survival after cryogenic protocol (Fig. 14.7).

The treatment of 10^{-6} M SA induced the greatest increase for shoot fresh weight, since an augmentation of 12.18 fold was observed in clone T01-7-70 (Fig. 14.8) and of 1.52 fold for clone 981,818 (Fig. 14.8) in contrast to the control. Microplants treated with 10^{-5} M SA induced an increase in shoot fresh weight of 2.08 fold for clone T01-7-70 and for 1.20 fold for clone 981,818 of (Fig. 14.8) comparing to control.

3.2.2 Survival After SA-Cryotherapy Treatment

In vitro plants pre-incubated in SA and exposed to cryotherapy, presented survival percentages of 10–70%, whereas 100% of control plants of both clones died (Fig. 14.9). *In vitro* plants pretreated with 10^{-6} M SA, presented 70% and 28.33% of survival for clones T01-7-70 and 981,818 respectively after cryotherapy (Fig. 14.9). *In vitro* plants of clone 981,818 pretreated with 10^{-5} M SA showed 10% of survival after cryotherapy, though the same SA concentration induced a lethal response in clone T01-7-70 (Fig. 14.9).

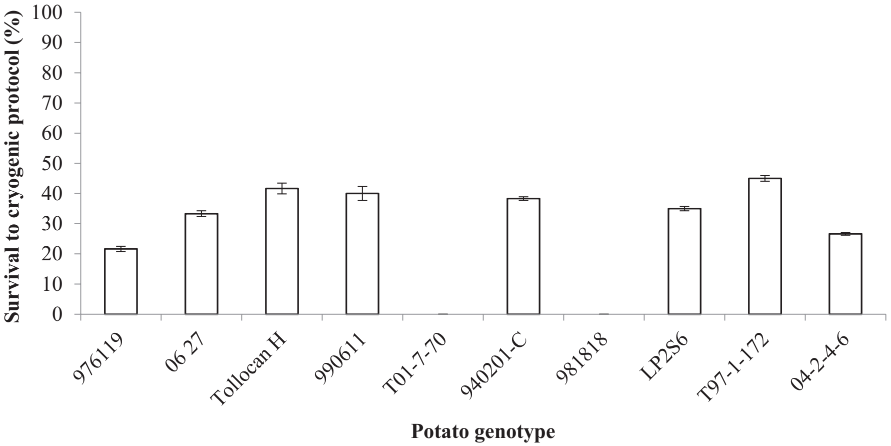

Fig. 14.7 Effect of cryogenic protocol on survival of 10 clones positives to PVS. Estimation was accomplished 15 d after subculture. Data are means ± S.E. bars of three experiments (n = 20). ANOVA and Duncan test (α = 0.05)

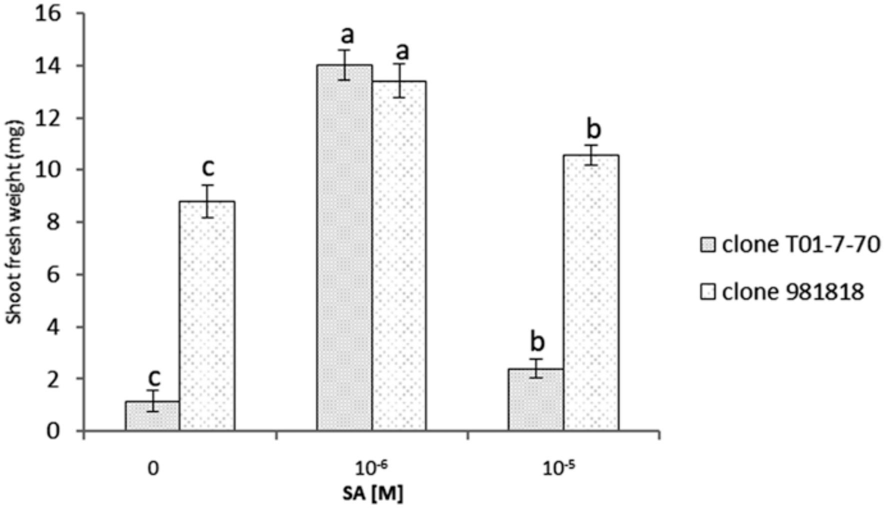

Fig. 14.8 Effect of SA on shoot fresh weight of clones T01-7-70 and 981,818 positives to PVS before cryotherapy. Variables were assessed 15 d after subculture. Data are mean of 3 experiments (n = 5–20 per experiment) ± SE. Values significantly different from controls according to the Duncan test ($\alpha = 0.05$)

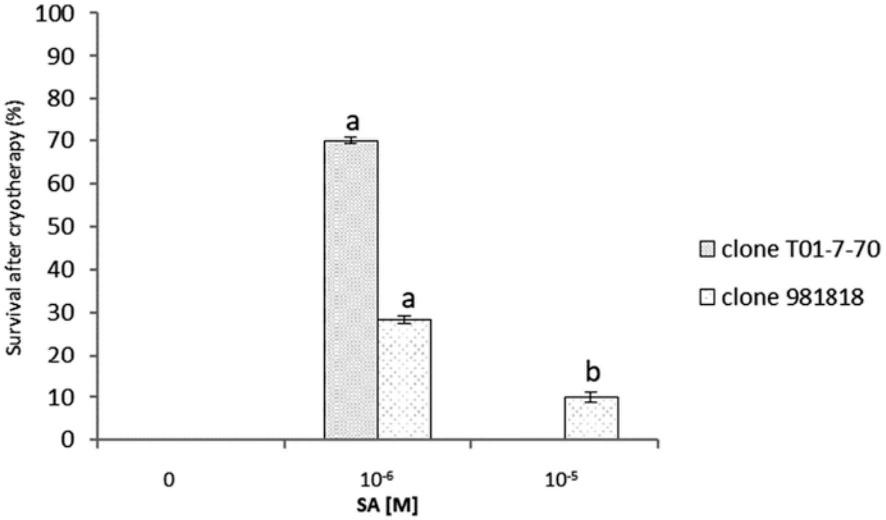

Fig. 14.9 Effect of incubation of potato microplants on SA for 28 days before cryotherapy on survival and assessed after cryotherapy. The data are mean of 3 experiments (n = 20 per experiment) ± SE. Values significantly different from controls according to the Duncan test ($\alpha = 0.05$)

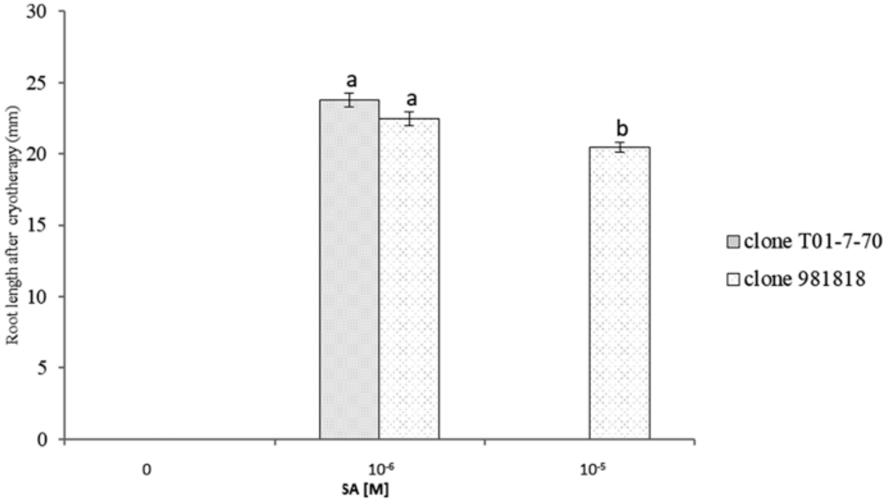

Fig. 14.10 Effect of incubation on SA for 28 days before cryotherapy on root length after cryotherapy. The data are mean of 3 experiments (n = 5–20 per experiment) ± SE. Values significantly different from 10^{-5} M treatment according to the Duncan test ($\alpha = 0.05$)

Root length was significantly different among microplants in both SA concentrations in clone 981,818 (Fig. 14.10). Microplants pre-incubated in 10^{-6} M SA presented and increase of 1.09 fold respect to those pre-incubated in 10^{-5} M SA (Fig. 14.10).

3.2.3 Percentage of Virus Free Plants

Microplants regenerated after cryotherapy, were subjected to the ELISA serological test to determine the presence or elimination of the PVS virus. *In vitro* plants pretreated in 10^{-6} M SA of clone T01-7-70, showed 96.66% of virus-free plants (Fig. 14.11). However, *in vitro* plants previously incubated in 10^{-6} M SA of clone 981,818, presented 100% of virus-free plants. No significant differences were obtained among the *in vitro* plants of both clones pre-incubated in 10^{-6} M SA (Fig. 14.11). Microplants from 10^{-5} M SA for clone 981,818 presented a 66.6% of virus-free plants (Fig. 14.11).

4 Discussion

4.1 Thermotherapy

The results confirmed that SA induced effects on thermotolerance to thermotherapy and supported the generation of PVX-free plants in the short and long terms. Previous studies reported that SA induced heat tolerance in potato, so the present

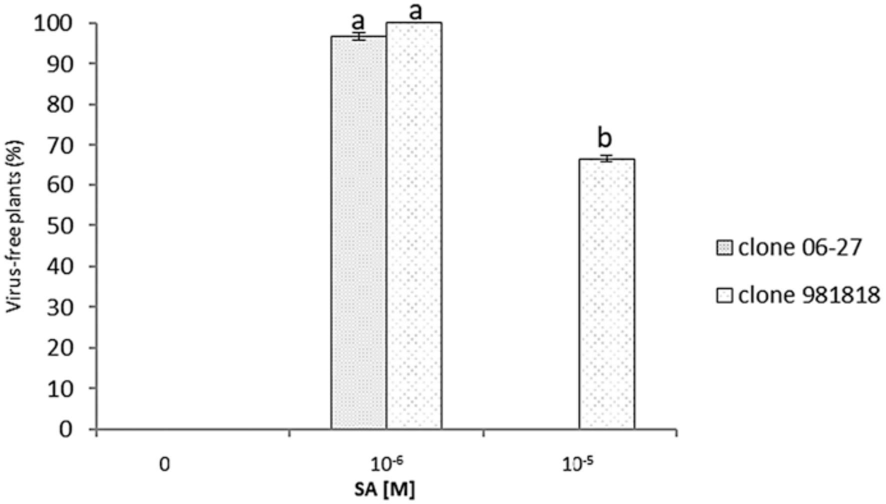

Fig. 14.11 Percentage of virus S free plants after cryotherapy previously incubated in SA. The data are the mean of 3 experiments (n = 5–20 per experiment) ± SE. Values significantly different from 10^{-5} M treatment according to the Duncan test ($\alpha = 0.05$)

experiments investigated the short and long-term effects of SA signaling in potato during thermotherapy. We previously demonstrated the short term effect of SA treatment on the induction of thermotolerance in thermotherapy and enhanced the percentage of PVX-free microplants (López-Delgado et al., 2004).

Combined long-term effects of SA on virus cleaning and thermotolerance had not been reported. Heat tolerance induced by SA has been claimed in many species, (Dat et al. 1998a, b; Senaratna et al., 2000; Larkindale & Knight, 2002; Larkindale & Huang, 2004; Chakraborty & Tongden, 2005; Wang & Li, 2006; Pan et al., 2006; He et al., 2005; Shi et al., 2006).

It was demonstrated that SA induced thermotolerance in short and long terms, which increased survival percentages after thermotherapy (López-Delgado et al., 2004; Aguilar-Camacho et al., 2016). SA is potentially useful for thermotherapy, as potato genotypes do not often survive long periods of heat in thermotherapy, on the other hand, heat tolerance is not automatically linked with virus eradication. The short and long term effects of SA significantly augmented the percentage of PVX-free plants comparing to the control. No significant differences were obtained in virus-free plants between both SA concentrations (Figs. 14.3 and 14.6). Improved CAT activity in SA 10^{-6} M during STE (Fig. 14.2a) was obtained, which was related with a greater percentage of PVX-free plants (Fig. 14.3). Clarke et al. (2002) claimed that SA in bean plants induced elevated CAT activity, inhibiting virus replication, this is compatible with our results. Moreover, SA treatments could increase the capacity of plants to scavenge ROS and hinder virus replication (Clarke et al., 2002).

Remarkably, SA 10^{-6} M treatment increased CAT activity in STE (Fig. 14.2a) and decreased microplant H_2O_2 internal concentration (Fig. 14.2b). These results differ with previous reports in potato, where SA decreased CAT activity leading to a internal H_2O_2 accumulation in heat shock (Lopez-Delgado et al., 1998), thermotherapy (López-Delgado et al., 2004), induction of freezing tolerance (Mora-Herrera et al., 2005), and protection to stress due to phytoplasma (Sánchez-Rojo et al., 2011).

The relationship between SA and H_2O_2 show different patterns of response, SA induction by H_2O_2 and catalase inactivation by SA function cooperatively in a cyclic way to amplify the SA/H_2O_2 signal (Chamnongpol et al., 1996; Leon et al., 1995). In this work, *in vitro* plants showed augmented thermotherapy survival and raised percentage of PVX-free microplants compared with control, especially at SA 10^{-6} M (Figs. 14.3 and 14.6). Oxidative stress physiology in thermotherapy is a very specific research topic, and an interesting area for research is the relationship between SA and H_2O_2 signaling responses, when two combined stress factors are present, high temperature and viral infection.

4.2 Cryotherapy

Genotypes of the same species can show different tolerance to cryogeny and between species, for example, very low survival to cryogeny has been reported in grapevine (*Vitis vinifera L.; Vitaceae*) (Wang et al., 2003) and potato (Benson et al., 1996; Grout & Henshaw, 1978; Arizaga et al., 2017), these results agree with the null survival observed in the controls of clones T01-7-70 and 981,818 in the present study (Fig. 14.7). In contrast, SA 10^{-6} M treated microplants of clone T01-7-70 and clone 981,818 infected with PVS prior to cryotherapy showed a greater survival, with survival percentages of 70% and 28.33% respectively. However, different response was observed in microplants pretreated in 10^{-5} M SA prior to cryotherapy, microplants from clone T01-7-70 exhibited 0% survival and 10% in clone 981,818 (Fig. 14.9). The results suggest that SA affects potato plant survival, and the response depends on the interactions between the dose-response of SA and the genotype (Hara et al., 2012; Miura & Tada, 2014; Mora-Herrera & López-Delgado, 2006).

SA regulates the vegetative growth via different signaling pathways, including via indole acetic acid and naphthaleneacetic acid production (Salisbury & Ross, 1994). In the present study, SA stimulated shoot fresh weight (Fig. 14.8). These results agree with results observed in other species, for example, in *Capsicum annuum L.* (Elwan & El-Hamahmy, 2009; Sánchez-Chávez et al., 2011), *Catharanthus roseus* (Echevarría-Machado et al., 2007), chamomile (Kováčik et al., 2009), *chrysanthemum* (Villanueva-Couoh et al., 2009), corn (El Tayeb & Ahmed, 2010; Tucuch-Haas et al., 2016), *Lycopersicum esculentum* Mill. (Larqué-Saavedra et al., 2010), *Pinus patula* (San-Miguel et al., 2003), *Solanum tuberosum* (López-Delgado et al., 2004), soybean (Gutiérrez-Coronado et al., 1998), and wheat (Hayat et al., 2005). Dowling and Simmons (2009), suggested that freezing and unfreezing enhance the production of ROS. Consequently, Lynch et al. (2011) suggested that it is possible to reduce oxidative damage induced by cryogenic processes.

It has been found that tolerance to abiotic stress induced by SA in potatoes was associated with reduced CAT activity, thus increasing H_2O_2 internal levels and triggering stress tolerance signaling pathways (Aguilar-Camacho et al., 2016; Romero-Romero & López-Delgado, 2009; Sánchez-Rojo et al., 2011). In the present study, regeneration after cryotherapy was observed only in SA 10^{-6} M and also presented root development (Fig. 14.10). This response is consistent with the effect of SA on root development as reported in other studies (Echevarría-Machado et al., 2007; Gutiérrez-Coronado et al., 1998). The potential of SA to induce regeneration of meristems via plant growth and cell division was confirmed, it has been associated with signaling and regulation of other hormones such as auxins, cytokinins, gibberellins, and abscisic acid (Zarghami Moghaddam et al., 2014). Cytokines have also been linked in the regeneration of shoots following a cryogenic process (Lambardi et al., 2000; Pennycooke & Towill, 2000; Wang et al., 2003).

Exposure of meristems to ultra-low temperatures (e.g., −196 °C) helps to the elimination of viruses, because many viruses are not able to infect meristematic cells (Martin & Postman, 1999). However, Kushnarenko et al. (2017) showed that cryotherapy alone did not eliminate Potato Virus M and PVS from potato. In contrast, PVX was eradicated with SA 10^{-6} M pretreatment in combination with thermotherapy (Aguilar-Camacho et al., 2016). In the present study, the same SA concentration in combination with cryotherapy, eliminated PVS in the clone 981,818 and in 96.66% in microplants of the clone T01-7-70 (Fig. 14.11).

Therefore, these results demonstrate the potential of SA pretreatment followed by cryotherapy to obtain high percentage of PVS-free microplants, particularly for that genotypes that are susceptible to cryotherapy. These effects are strong justification for continued research of the interactions between SA concentrations and a larger number of potato genotypes with null survival to cryogenic protocol.

5 Conclusion

The evaluation of ROS changes in response to thermotherapy or cryotherapy virus-infection-SA pretreatment, will help to further investigation and better understanding of the oxidative stress physiology involved in thermotherapy and ultimately get more efficient virus eradication protocols.

Acknowledgments We knowledge to M.C. Guadalupe Ríos Dominguez and Ing. Rocio Sara Valentina Hernández Sánchez, from the pathology laboratory of ICAMEX for performing DAS-ELISA tests.

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Chapter 15
Role of Salicylic Acid in Mitigation of Biotic Stress

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Abstract Plants are exposed to various types of stresses during their life cycle. Biotic stresses (fungi, virus, bacteria, nematodes and insects) to plants leads to tremendous economic loss and have damaging effects on agricultural production and yield. The increasing threat of these stresses poses a sincere challenge for plant researchers to discover out some effective alternatives. Various stress alleviating compounds play significant role to overcome the biotic stress conditions. Salicylic acid (SA) is a well-known promising phytohormone, which plays major role in neutralizing the biotic stress responses. It belongs to the diverse group of plant phenolics and ubiquitously distributed in plants. SA plays magnificent roles in the regulation of plant growth and development, seed germination, flowering, photosynthesis, thermogenesis, nitrogen metabolism, proline metabolism and antioxidative defense system under stress conditions. Beside this, it also acts as vital signaling molecule which is involved in maintenance and activation of plant defense via the regulation of many metabolic and molecular processes. The aim of this chapter is to summarize the role of the SA in response to biotic stress and extend our understanding in context to its role in overcoming the deleterious effects of biotic stresses and develop resistance among plants.

Keywords Biotic stress · Growth · Metabolism · Molecular aspects · Salicylic acid

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S. Hayat et al. (eds.), *Salicylic Acid - A Versatile Plant Growth Regulator*,
https://doi.org/10.1007/978-3-030-79229-9_15

1 Introduction

Phytoprotectant of phenolic nature i.e. Salicylic acid (SA; O-hydroxybenzoic acid) along with its derivatives or simply the salicylates as they are commonly called, were initially known to exist as secondary metabolites which served the profile of chemical messengers in various biological processes. It was only in the early 1990s that SA got its due recognition as the sixth chief plant growth hormone (Raskin, 1992). This particular compound like rest of other plant hormones regulates multiple aspects of plant growth and development via having a say in vital processes such as photosynthesis, stomatal conductance, transpiration, respiration, mineral uptake and transport, flowering and fruit yield. Furthermore, it has now been found to confer the plants with endogenous local and systemic resistance against a wide array of extraneous stress factors of biotic or abiotic nature which otherwise affect plant growth, yield, reproductive potential and survival quite adversely (Nazar et al., 2011; Khan et al., 2013; Miura & Tada, 2014; Sharma et al., 2020).

Plants subjected to stressful conditions produce SA which in turn activates the genes of defence system together with exhibiting a striking coordination among different signal transduction pathways in a direct or indirect manner leading to the formation of allochemicals and immunity strengthening proteins (Li et al., 2002). Stress is also found to be mitigated if SA is supplemented exogenously by means of pre-soaking of seeds, foliar spray or via simply adding it in nutrient solution (Horváth et al., 2007; Palma et al., 2013). Heat shock proteins (the molecular chaperones), antioxidants, different secondary metabolites like [sinapyl alcohol dehydrogenase (SAD), cinnamyl alcohol dehydrogenase (CAD), and cytochrome P450] and other resistance genes are induced by SA for battling out the stress generated issues (Jumali et al., 2011).

For mitigating biotic stresses alone, Salicylic acid and plant immunity have been lately reported to be intricately linked so much so that SA is best termed as a defense-related hormone (Durrant & Dong, 2004; An & Mou, 2011). SA is evidenced to manage regulation and expression of mitogen activated protein kinase (MAPK) and *non-expressor of pathogenesis-related genes 1 (NPR1)* (Chai et al., 2014). Its involvement in orchestrating effective face off of plants with respect to the nature of biotic invaders by interceding major resistance signaling pathways with that of Jasmonic acid (JA) and Ethylene (ET) has been proven and these pathways show SA to be engaged in mutualistic crosstalks with ethylene and jasmonic acid bothways, positively (Synergistic manner) as well as negatively (antagonistic manner). Moreover, this SA-JA/ETcrosstalk in resistance signaling act as a principle component of biotic stress induced defensive measures whereas growth regulators as the likes of Auxins, Cytokinins, Giberellins, Abscisic acid and Brassinosteroids etc. mitigate this problem in an indirect manner via plainly acting as modulators of SA-ET/JA signaling pathways.

2 Salicylic Acid: Excerpts from Historical Archives

Hippocrates, Galen and Pliny the Elder were reported to be well versed with the pain and fever mollifying properties of Salicylic acid and it is chronicled to be used as a folk remedy since aeons ago by the inhabitants of Egypt, Assyria, Indian, America and China (Norn et al., 2009). Though the active constituent 'Salicin' from the bark of the plant White Willow (*Salix alba*) was isolated and termed so, by a German chemist, Johann Andreas Buchner in 1828; it was only in the year 1838 that the Italian chemist, Raffaele Piria, succeeded in converting Salicin into another compound which gets transformed into Salicylic acid upon oxidation. The very first successful extraction of Salicylic acid in 1839 was performed by German researchers from the herbaceous plant Meadowsheet (*Filipendula ulmaria*) (Jeffreys, 2005). Eventually, different derivatives like methyl salicylate (MeSA), saligenin (alcohol of SA), and their glycosides also got extracted from willow and other plants. Germany is accredited for being first in the production of Salicylic acid for the purpose of trading in 1874.

Acetyl salicylic acid famously known as Aspirin was made known to the world in 1898 by Germany based pharma company Bayer which subsequently turned into a trusted brand as an analgesic and preferred over salicylic acid for being comparatively less baleful as a gastrointestinal irritant. Up to now, a number of salicylates have been employed medicinally incepting from the common colds to heart attacks with considerable efforts being still carried out to comprehend their mode of medicinal action. As far as plants are concerned, stressful conditions enhance the endogenous production of salicylic acid quite readily but the external supplementation of SA in right dosage to such stressed plants, taking into account the plant's age, species, particular organ and duration of treatment, has also been reported to alleviate stress (Kováčik et al., 2009; Miura & Tada, 2014).

Further, Raymond F. White (1979) was the first person to observe role of SA in fighting biotic stress imposed by tobacco mosaic virus (TMV) in virus-susceptible tobacco cultivar (*Nicotiana tabacum* cv. Xanthi-nc) upon the application of aspirin (acetyl-SA). The specific tobacoo cultivars possessing the genes resisting this virus have been observed to exhibit promoted production of SA upon viral attack and consequently piling up of pathogenesis-related (PR) proteins occurred; thus hinting at probable role of SA in conferring immunity against pathogens.

In 1990s, enhancement in the endogenous levels of SA was elicited preceding the development of local and/or systemic disease resistance in tobacco and cucumber plants, which substantiated role of SA as an intrinsic signal for disease resistance (Malamy et al., 1990; Métraux et al., 1990). Subsequent investigations on tobacco and *Arabidopsis* plants which are inefficient in accumulating SA owing to either mutations or expression of SA-degrading enzymes firmly established that SA is essential for plant innate immunity and systemic acquired resistance (SAR) (Vlot et al., 2009).

3 SA Biosynthesis

To completely ascertain the nature of plant- pathogen interaction during biotic stress conditions, endogenous synthesis of Salicylic acid ($C_7H_6O_3$) has to be discerned first. Biosynthesis of this important signalling phytoprotectant in plants, takes place via two major pathways: (i) Isochorismate (IC) pathway and (ii) Phenylalanine amino-lyase (PAL) pathway. Chorismic acid, synthesized via a seven step enzyme catalysed Shikimate pathway in plastids, acts as the common initial point for both of these biosynthetic processes; though both the pathways differ in plant species on account of their significance. Plants mostly prefer PAL pathway for synthesizing SA, nevertheless IC pathway is also utilized for the same at times (Mustafa et al., 2009). SA synthesis in plants under normal as well as stressed conditions being itself a multi- step enzyme catalysed process shows that both these pathways are indeed interlinked to each other instead of taking place independently as depicted in Fig. 15.1. In PAL pathway, trans- Cinnamic acid gives rise to benzoic acid indirectly from either β-oxidation of fatty acids or pathways without the involvement of oxidation. The benzoic acid upon oxidation by benzoic acid-2-hydroxylase enzyme result in the production of SA. Secondly, phenylalanine produces trans-Cinnamic acid when acted upon by phenylalanine amino lyase (PAL) enzyme which is then hydroxylated to produce o-Coumaric acid in the presence of Cinnamate 4-hydroxylase (C4H) enzyme. Subsequent oxidation of a side chain and hydroxylation of Coumaric acid synthesize SA (Horváth et al., 2007; An & Mou, 2011). IC

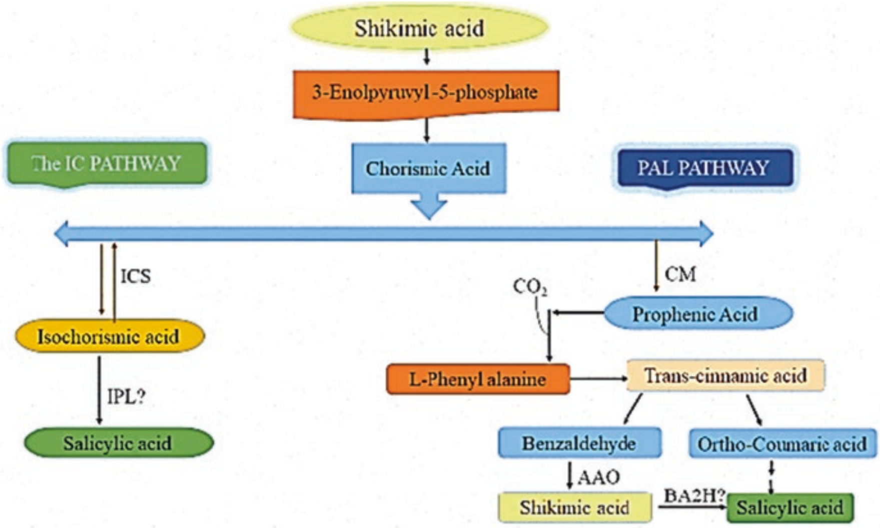

Fig. 15.1 Salicylic acid biosynthesis in plants (Abbreviations: (i) CM: Chorismate mutase (ii) ICS: Isochorismate synthase (iii) IPL: Isochorismate pyruvate lyase (iv) PAL: Phenylalanine ammonia-lyase (v) AAO: Aldehyde oxidase (vi) BA2H: benzoic acid 2-hydroxylase. Names of the enzymes with question mark specify the absence of their definite identification so far

pathway involves synthesis of Salicylic acid from Chorismic acid through the synthesis of Isochorismic acid first followed by its disintegration with the help of Isochorismate pyruvate lyase enzyme.

4 SA Accumulation Under Biotic Stress

All the major enzymes of SA biosynthesis undergo alteration upon their encounter with biotic stress factors, especially the peculiar upsurge observed in activity of Isochorismate synthase (ICS) and Phenyl alanine amino lyase (PAL) which consequently form SA in excess and guard the plant against the tension imposed by stress. For instance, *Arabidopsi*s and *Nicotiana tabacum* show enhancement in action of ICS and BA2H respectively for the biosynthesis of SA while displaying defensive response against pathogens (Biotic) stress in addition to abiotic ones (Wildermuth et al., 2001; Ogawa et al., 2005). A blend of positive and negative regulation mechanisms determines the effectiveness of SA for either inciting or subsiding immune responses as per the requirement. Mutant screening has helped in identifying these regulators of both positive and negative types operative in SA accumulation. Mutants of positive regulators promote disease vulnerability of plants via lowering down levels of SA whereas those of negative regulators act the other way round.

(a) **Positive regulation:** It shows the involvement of some major regulators as the likes of (i) EDS1 (a lipase-like protein), a major junctional node operating upstream of SA in Pathogen associated molecular pattern (PAMP)-triggered immunity (PTI) upon getting attacked by virus, bacteria, and fungi together with in Effector-triggered immunity (ETI) when stimulated by a subset of R genes (Chandra-Shekara et al., 2004; Xiao et al., 2005), (ii) putative lipases such as PAD4 and SAG101 which either transduce EDS1 signaling or coordinate their working with EDS1 via forming protein complexes in different subcellular components (Feys et al., 2001, 2005), (iii) NDR1 which is independent of EDS1, (iv) EDS5/SID1 (Nawrath et al., 2002), (v) ALD1, (vi) PBS3/WIN3/GDG1 (Nobuta et al., 2007; Lee et al., 2007; Jagadeeswaran et al., 2007), (vii) EPS1 (Zheng et al., 2009), and (viii) MOS (Monaghan et al., 2010) etc. In eds1 and pad4 mutants, exogenous supplementation of SA is involved in initiating defense gene induction whereas in wild type plants, it promotes expression of EDS1 and PAD4 hinting that SA modulated both these regulators through a feedback loop in a positive manner (Feys et al., 2001). On the similar basis, ndr1 mutants having SAR-deficient phenotype show reduced PTI and ETI while their overexpression decreases the bacterial growth and they are normalised by BTH (benzothiadiazole) application as reported by Shapiro and Zhang in 2001 and Coppinger et al. in 2004. Furthermore, the interactive mechanisms functional among positive regulators of SA can be effectively worked out if conventional epistatic analytical studies are combined with genomics techniques like microarray and RNA-Seq technologies.

(b) **Negative regulation:** Negative feedback regulation of Salicylic acid biosynthesis is also there but its analysis is tad bit difficult because the mutants exhibiting it are prone to adverse consequences like stunted growth or even cell death. Plants bearing such mutations are addressed as lesion mimic mutants (LMM), which are indirectly studied at genetic level by coupling them with mutants inoperative in positive SA regulation (Lorrain et al., 2003; Moeder & Yoshioka, 2008). This strategy serves the double purpose of divulging the information regarding the relationship SA shares with LMMs along with nature of interactive network among different SA regulators, this is substantiated by examining multiple LMMs such as lsd1, edr1, and vad1 (Rustérucci et al., 2001; Lorrain et al., 2004; Tang et al., 2005). Besides this, an accomplished tool in the form of a gain-of-function LMM, acd6-1, has helped extensively for evaluating the working relationship which SA regulators share among themselves by utilizing the antithetical correlation between SA regulators and dwarfism (Lu et al., 2003).

Furthermore, this refined attunement between positive and negatively regulated SA-mediated immune responses exhibited by plants under biotic stresses are actually modulated by a composite framework at genetic level which can be readily categorized into NPR1-dependent pathways, and NPR1-independent pathways.

4.1 NPR1-Dependent SA Signaling Pathway

Multitude of defense responses of stressed plants are chiefly controlled by NPR1 (NIM1/SAI1), a key regulator functioning downstream from SA (Durrant & Dong, 2004). Transcription factors belonging to TGA and WRKY family of proteins which bind to the activation sequence-1 (as-1) and W-boxes respectively present in the promoter region of NPR1 arbitrate the signaling process between NPR1 and SA (Weigel et al., 2001, 2005). Likewise, three structurally similar proteins of NIMIN type also help in the expression of NPR1. Upon getting inducted by invasion of pathogens, oligomeric NPR1 in the cytosol transforms into its monomeric form followed by its passage into the nucleus where it stimulates transcription of defense genes (Mou et al., 2003). The expression of these NPR1 genes is upregulated by SA supplementation following a pathogen attack.

4.2 NPR1-Independent SA Signaling Pathway

Existence of SA mediated but NPRI-independent resistance signaling pathways have been hinted at by the recent advances in the concerned areas (Kachroo et al., 2000; Murray et al., 2002). Confirmation regarding them came from the screening conducted in the absence of NPR1 on different mutants of *Arabidopsis* such as npr-1-5 mutants altered by Ethyl methylsufonate (EMS); ii) snc mutants like snc-1, snc2-1D and snc4-1D, which are able to transcribe PR genes consequently leading to heightened resistance against multiple pathogens with the help of amassing of SA (Li et al., 2001; Bi et al., 2010; Zhang et al., 2010). Transcription factors as the likes of WHY (WHIRLY) and MYB (e.g. AtMYB330) have been found to be involved in regulating SA signaling in NPR1-independent manner (Raffaele et al., 2006).

However, Shah et al. in 2001 had demonstrated the side by side functioning of both NPR1- dependent and independent pathways through his studies on ssi1 and ssi 2 mutants possessing wild type NPR1 allele vis a vis ssi1 npr1 and ssi 2 npr 2 double mutants where the former ones comparatively accumulate more SA than the latter after invasion of pathogen.

5 Interaction of SA with Other Phytohormones Under Biotic Stress

Whether the Salicylic acid is produced endogenously or applied exogenously, it is deeply gripped in cross talking with other elicitors or phytohormones like auxins, cytokinins, ethylene, jasmonic acid, abscisic acid etc. especially under biotically stressed conditions, carrying out intricate signal transduction pathways. In other words, this cross talking between all the phytohormones is fundamentally an essential feature of stress response in plants and diligent studies centred around them are required for developing pathogen resistant plant varieties (He et al., 2017). These phytohormonal interactions for the mitigation of biotic stress are discussed as follows:

5.1 Salicylic Acid – JA/ET Signaling Pathway Crosstalk

Nature of invading pathogen determine the type of signaling pathway which a plant is going to adopt for responding and defending itself from the potential harm. Immune responses arbitrated by SA play a central role in rendering local as well as systemic- acquired resistance (SAR) to oppose biotrophic pathogens like *Pseudomonas syringae*, which naturally tend to colonize between the cells and obtain nutrition without being lethal to the host. In contrast to this, ET/JA-mediated immune responses act quite effectively in opposition to the necrotrophic pathogens like *Botrytis cinerea* which are known for deriving nutrition only after killing their hosts. When these two separate SA and ET/JA- mediated signaling pathways come across each other, their crosstalks are revealed to be both positive (Synergistic) and negative (antagonistic) in nature. Further, Mur et al. in 2006 reported that SA and JA are engrossed in synergistic talks in low concentrations by allowing the expression of *PR1*(SA target gene) and *PDF1.2* (JA- marker gene) while antagonism between them is displayed if present in higher concentrations. However, it is the negative interactions between them which contribute majorly in disease resistance signaling in plants. This can be corroborated with the fact that the SA-mediated defense signaling which gets triggered by biotrophic pathogen *Pseudomonas syringae*, coerce the plants to be more responsive to the necrotrophic parasite *Alternaria brassicae* via subduing the ET/JA-mediated defence signaling (Spoel & Dong, 2008). Though definite nature of intermutual relations prevalent between SA and ET/JA signalling is still inadequate at molecular level, yet subjugation of genes of JA biosynthesis like *LOX2*, *AOS*, *AOC2 and OPR3* is inferred to be linked to SA. Moreover, exogenous application of SA is found to prevent JA-marker gene *PDF1.2* from being expressed in the wild as well as in aos mutant forms. Additionally, ERF1 or ORA59 induced expression of *PDF1.2* in JA-receptor coi I (coronotine insensitive 1) mutant has also been reported to be suppressed by SA (Leon-Reyes et al., 2010).

5.1.1 Transcriptional Regulation of This Crosstalk

The transcriptional control of this dual natured i.e. antagonistic and synergistic relationship prevalent among these phytohormones has been depicted in Fig. 15.2. The negative modulators invoked by SA defense signaling cause hindrance to the activities of ET/JA-regulated transcription factors belonging to the ERF branch. JA-induced marker gene *PDF1.2* is inhibited from getting expressed by the activation plus ectopic expression of *WRKY* transcription factor *WRKY70,* by SA (Li

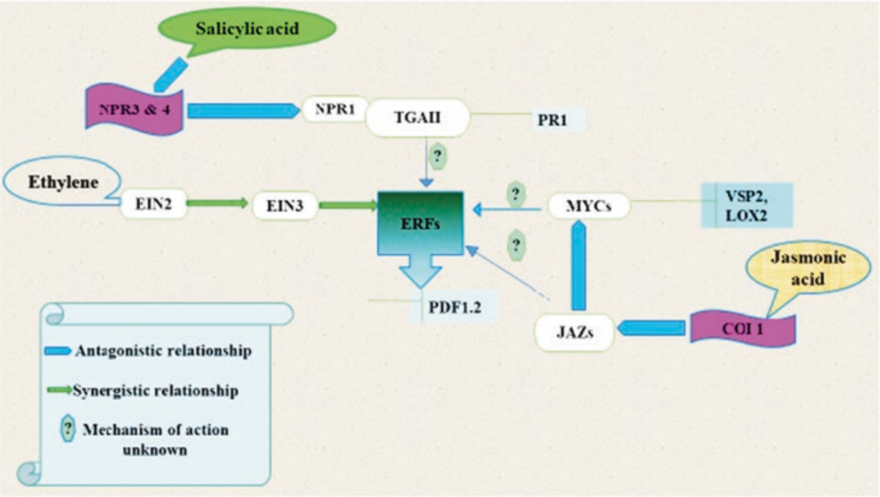

Fig. 15.2 Depicting the signaling nexus among SA and ET/JA under biotic stress, focusing mainly upon their interactive talks at transcriptional level. (Abbreviations: NPR-non expressor of pathogenesis related gene; TGA-TGACG binding factor; PR-Pathogenesis related gene; EIN2 and3-Ethylene insensitive transcriptional factor; ERF-Ethylene response factor; PDF-Pathogen defense factor; COI-coronatine insensitive factor; JAZ-Jasmonate ZIP domain; MYC-Jasmonate responsive signaling branch; LOX- 13-lipoxygenase; VSP-Vegetative storage protein)

et al., 2004, 2006). Moreover, the TGA transcriptional factors of SA signaling of clade II type are managed by SA to manipulate and control ET/JA-signaling pathway. This was further affirmed after the characterization of SA-activated plant-specific redox enzymes discovered in *Arabidopsis* like glutaredoxin (*GRX*) *ROXY19* or as *GRX480* or *GRXC9*), which repress induction of *ORA59* and *PDF1.2* quite immensely during ET/JA-signalling via expressing themselves ectopically and interacting with clade II TGA-factors (Ndamukong et al., 2007).

The negative regulation of expression of SA-induced transcription factors *WRKY70* and *ROXY19* by the master transcriptional regulator NPR1 plays a noteworthy role in SA-ET/JA crosstalk as it causes the suppression of ET/JA-signaling pathway. It has been substantiated in *npr1* mutant plants in which the repression activity of SA is quashed with by the non-functioning of NPR1 (Spoel et al., 2003). Expression of NPR1 merely in its chimeric form in the cytosol is the simple requirement for suppression of JA-induced *PDF* expression which is in turn brought about by the external application of SA. Furthermore, the activated ET/JA-pathway reciprocates and modulates the biosynthesis of SA negatively. Deletion of JA receptor COI1 (coronatine insensitive 1) and JA-responsive MYC branch (which handles insect attacks on plants) has been reported to escalate the endogenous levels of SA leading to amplification of resistance in opposition to the biotrophic pathogen *Pseudomonas syringae* (Spoel & Dong, 2008).

5.2 Salicylic Acid and Other Phytohormones' Crosstalk

Novel perceptions providing insights into the regulatory mechanisms involving SA and other phytoprotectants (except ET and JA) in conferring resistance against pathogens by getting engaged in complex crosstalks among themselves have also come to the fore lately, however, a lot more concerted efforts are still required to consummately decipher the modulation of their signal transduction pathways under biotic stress. Few precedents highlighting the intermutual relationship which SA and these plant growth regulators share to alleviate biotic stresses are summed up as follows:

5.2.1 Salicylic Acid – Auxin Crosstalk

Production of auxin by plant pathogens by themselves or the hindrance they cause to the endogenous biosynthesis of auxin inside plants for hampering normal plant growth and development have been confirmed which are otherwise maintained in optimal conditions by auxin (Chen et al., 2007). Therefore, plants employ the suppressive measures to inhibit signaling of auxin after pathogen attack, in which they are helped by SA. SA is evidenced to sustain auxin repressor proteins via suppressing auxin linked genes so that auxin is restricted from manifesting responses, thereby enhancing resistance in plants (Wang et al., 2007). In addition to this, auxins are found towards increasing the susceptibility of host plants along with frequency of diseases when augmented exogenously, indicating the nature of its interaction with SA to be negative (Navarro et al., 2006). Auxin responsive gene GH3.5 of GH3 family plays a twofold role in controlling the signaling of both SA and Auxin/IAA during invasion of *Arabidopsis* with *Pseudomonas syringae*, and adversely affect its disease resistance potential by positively regulating SA biosynthesis plus modulating auxin signaling simultaneously to promote disease susceptibility (Zhang et al., 2007). Though, the amplified SA biosynthesis, in turn, displays a synergistic feedback and intensifies the expression of GH3.5 genes resulting in more SA production so that it gets piled up in higher concentrations, whereas Auxin fails to get accumulated like SA, thus making the defense mechanism of plants more strong.

5.2.2 Salicylic Acid – Gibberellins Crosstalk

Navarro et al. (2008) reported that negative modulators of Gibberellins/GA signaling i.e. DELLA proteins bestow the plant with resistance to necrotrophic pathogens and susceptibility to biotrophic pathogens via dwindling the balance between the signaling pathways mediated by SA and ET/JA. But Gibberellins are found to be indulged in degrading these DELLA proteins by acting antagonistically, thus providing resistance against biotrophic pathogens and susceptibility to necrotrophic pathogens again via arbitrating in SA and ET/JA signal transduction pathways.

5.2.3 Salicylic Acid – Cytokinin Crosstalk

With the help of SA-JA mediation, Cytokinins are also reported to confer immunity to the plants. This was proven from the characterisation of the Cytokinin response factor (CRF) gene by Liang et al. in 2010. Escalated transcript levels of SA-activated genes of PR family in response to the attack of *Pseudomonas syringae* pv. *tomato* DC3000 (Pst DC3000) have been observed to upregulate the overexpression of this particular gene (Rashotte et al., 2006; Cutcliffe et al., 2011). The crosstalk between CK and SA has been exemplified in *Arabidopsis thaliana* as well, in which the resistance potential of plant against Pst DC3000 attack was enhanced if the plant is given prior treatment with CK (Choi et al., 2010).

5.2.4 Salicylic Acid – Abscisic Acid Crosstalk

ABA is associated with making of plants more prone to diseases, substantiating its negative role in disease regulation (Ton et al., 2009). Restrainment in disease resistance has been observed in *Arabidopsis* and other plants in response to *P. syringae* if ABA is supplemented externally because it is found to modify SA-arbitrated defense signaling followed by inhibition of SA accumulation (Mohr & Cahill, 2003;

Koga et al., 2004). This particular treatment of plants with ABA further substantiates the negative nature of interaction between ABA and SA by impairing SA-mediated systemic acquired resistance (SAR) (Yasuda et al., 2008). The mutants as the likes of myb96-1d with defective ABA biosynthesis, show significant enhancement in the measures of SA to provide forbearence against pathogens in *Arabidopsis* and *Solanum persicum* (de Torres Zabala et al., 2007; Asselbergh et al., 2008). This is reasoned out to be associated with the fact that the gene which expresses enzyme isochorismate synthase i.e. SA-INDUCTION DEFICIENT 2 (SID2) gets stimulated in response to infection (Wildermuth et al., 2001; Seo & Park, 2010). Furthermore, this antagonistic interaction of SA and ABA is linked to the ABA-ET/JA crosstalk in an indirect way (Adie et al., 2007).

5.2.5 Salicylic Acid – Brassinosteroids Crosstalk

To deal with biotic stresses Brassinosteroids are considered utmost important because of the broad spectrum resistance they provide against a plethora of pathogens ranging from viruses, bacteria to fungi. However, if their crosstalks with SA are evaluated, they turn out to be of antagonistic in nature. The tolerance provided by benzothiadiazole (BTH), a synthetic analogue of SA is reported to be dampened by Brassinolide, thereby making the host plant more responsive to manifestation of diseases. Brassinosteroids are also found to antagonise the basal resistance provided by SA in rice (*Oryza sativa*) plants when the fungal pathogen, *Pythium graminicola* attack these rice plants. When the resistance potential of two genotypes SA-deficient *NahG* transgenics and wild-type Nipponbare was tested upon getting attacked by *P. graminicola* after giving them both a treatment with brassinosteroid BRZ on regular basis. These experiments showed enhanced susceptibility of *NahG* plants after getting infested with fungal pathogen as compared to their wild type counterparts, indicating the importance of SA mediated defense signaling which is negated by BRZ (De Vleesschauwer et al., 2012).

6 Conclusion

Multitudes of biotic stress conditions are dealt with by the plants via entailing different immune responses for which the help of a number of phytohormones is sought after. The role of SA, one of such plant growth regulators, in the mitigation of biotic stress at individual level and by being engaged intricately in interactive talks with other plant growth regulators, has become unquestionable. Extensive research activities with this existing complex defensive network at molecular level as a focal point is a necessary requirement to thoroughly comprehend SA- mediated immunity mechanism of plants. This will certainly help in apprehending the role of member genes of NPR family in SA biosynthesis and its signaling mechanism in doing away the imposed stress. Investigatory efforts at proteomic, transcriptomic and genomic assisted with bioinformatics and computational biology will surely unravel the intricacies underlying SA-signaling and crosstalking mechanisms in biotically stressed plants which will further explain consequent rise or fall in endogenous SA levels. Detailed and comprehensive studies especially in the field of genetic engineering, regarding the usage of SA in right amount plus remodelling of SA/ET-JA crosstalk so that SA either surpasses or disconnects from the ET-JA signaling under biotic stress are quite fascinating and promising as this would enable the plants with the resistance potential of fighting off biotrophic and necrotrophic pathogens in one go. Keeping in consideration such outcomes which could be put to use by science of plant breeding for developing disease/pathogen resistant varieties leading to effective management of agricultural losses incurred otherwise due to different biotic stresses.

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S. Hayat et al. (eds.), *Salicylic Acid - A Versatile Plant Growth Regulator*,
https://doi.org/10.1007/978-3-030-79229-9