Rahat Nazar · Noushina Iqbal
Nafees A. Khan *Editors*

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Preface

The rapid increase in industrialization and urbanization during the past few decades has posed various unpredicted disturbances in the environment resulting in stressful conditions. Plants are constantly exposed to changes in environmental conditions. When these changes are rapid and extreme, plants generally perceive them as stresses which are of two types, biotic and abiotic, depending on the source of stress. A broad range of abiotic stresses includes osmotic stress caused by salinity, drought, and low and high temperatures, as well as ionic, nutrient, or metal stresses. The responses to abiotic stresses are not the linear pathways but are the complicated integrated circuits involving the interaction of additional cofactors and/or signaling molecules to coordinate a specified response to a given stimulus.

Plant hormones regulate a number of signaling networks involving developmental processes and plant response to environmental stresses. Phytohormones, the chemical messengers, play a vital role in the resistance of plants to the changing environments by regulating physiological and molecular processes. Their signaling pathways are not isolated but rather interconnected with a complex regulatory network involving various defense signaling pathways. To understand how plants coordinate multiple hormonal components in response to various developmental and environmental cues is a major challenge for the future. The role of phytohormones under abiotic stress is critical in modulating physiological responses that will eventually lead to adaptation to an unfavorable environment. Among the recognized major classes of phytohormones, attention has largely been focused on salicylic acid (SA). In recent years, salicylic acid has been the focus of intense research due to its function as an endogenous signal mediating local and systemic plant defense responses against pathogens and also because it participates in the regulation of physiological processes and plant resistance to biotic and abiotic stress. Salicylic acid regulates photosynthetic events, nutrient metabolism, osmotic relations, and defense mechanisms in plants growing under optimal and changing environmental conditions.

This book primarily deals with the importance of SA in regulating plant growth and development under stress conditions along with its interaction with other hormones or molecules in controlling the process. The editors and contributing authors hope that this book will include a practical update on the current knowledge of abiotic stress tolerance and lead to new discussions and explore the mechanisms responsible for the perception and signal transduction of salicylic acid under control and stress conditions and the efforts to use the informative tools for the improvement of crop plants in the era of global climatic change.

The chapters of the book deal with the importance of salicylic acid and/or its structural analogs in response to some biotic and abiotic challenges in relation to their effect in the antioxidative metabolism in plants. It also emphasizes on the recent understanding to underpin the interaction of defense regulators, such as salicylates, jasmonates, hydrogen peroxide, nitric acid, and abscisic acid, with growth phytohormones, viz., auxins, cytokinins, gibberellic acid, and ethylene, in correlation with disease development in different plant-microbe interactions. The perception and transduction of a signal from salicylic acid induced defense response under biotic and abiotic stress conditions are also studied. It deals with the current knowledge of the role of SA on plant growth and development, and explores the identification of potential targets for the modulation of salicylic acid signal pathways in response to plant stress tolerance. Critical evaluation of and cross-talks in salicylic acid signaling pathways under optimal and stressful conditions is also discussed. It also gives an insight to the genetic and molecular aspects of plant resistance to stress through recent advancements and the role of salicylic acid in stress resistance. The mechanism to induce thermotolerance in plants by SA interaction is also studied. This book presents an overview of stresses on crop plants and effects of SA on different stresses on plant physiology and stress agronomy, as well as the synergies between types of stresses. In addition, an understanding on the mechanisms underlying between SA and nutrient signal transduction pathways in plants for abiotic stress tolerance is also covered.

The book covers interesting topics dealing with the role of SA and the mechanistic approaches for abiotic stress tolerance to pave the path for agricultural scientists and breeders to develop high-yielding sustainable transgenic crops.

We extend our gratitude to all those who have contributed in making this book possible. Also, we would like to apologize unreservedly for any mistake or failure to acknowledge fully.

New Delhi, India Rahat Nazar
Aligarh, India Noushina Iqbal
Nafees A. Khan

Acknowledgments

First and foremost, we would like to thank the Almighty without whose blessings the task would be impossible. We thankfully acknowledge the DS Kothari postdoctoral fellowship for providing the research grant. We also acknowledge the academic staff of Jamia Hamdard, New Delhi, and AMU, Aligarh, for their support.

We are also thankful to our families for their continuous support and inspiration. And lastly, we thank Springer for providing us the chance to widen the audience for our work.

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Dr. Rahat Nazar received her master's degree and PhD from Aligarh Muslim University, Aligarh. She did her post doc at Jamia Hamdard University, New Delhi, India. She worked as JRF and SRF in DST- and UGC-funded projects during her research. She has published many research papers in leading journals of the world with a high impact factor. She has also edited two books and authored one book. Dr. Nazar is a life member of the Indian Society for Plant Physiology (ISPP) and the National Environmental Science Academy (NESA), New Delhi, India. She has been awarded with Junior Scientist Award (2010) by NESA. Dr. Nazar's is actively engaged in studying the role of phytohormones and mineral nutrition with special emphasis on photosynthetic efficiency and nutrient-use efficiency under optimal and abiotic stress conditions.

Dr. Noushina Iqbal has done her Ph.D. at Aligarh and then postdoc at Jamia Hamdard, New Delhi, India. She has many good publications in journals of national and international repute with high impact and has written five chapters and presented four papers in conferences. She has edited two books and authored one book. She is an eminent researcher and has worked as a project fellow in a UPCST-funded research project and received UGC-BSR and CSIR-SRF fellowship during her research. She is a member of the Indian Society for Plant Physiology (ISPP). Dr. Iqbal works on the role of phytohormones, osmolytes and nutritional factors particularly nitrogen and sulphur in combating abiotic stress.

Nafees A. Khan, a professor of plant physiology, is affiliated with AMU, Aligarh (India). Prof. Khan obtained his bachelor's, master's and doctoral degrees at AMU, Aligarh, and also earned his D.Sc. at the same university. In addition to innovative teaching, he is fond of researching and publishing and has to his credit more than 170 significant peer-reviewed scientific papers and a dozen of edited volumes on important aspects including plant stress physiology, abiotic stress tolerance, phytohormones, S and N nutrition, nutrient-use efficiency and source-sink relations. A member of academic bodies of universities, Prof. Khan is also a life member of major professional societies including the Indian Society for Plant Physiology (ISPP) and National Environmental Science Academy (NESA), New Delhi, India, and the Indian Botanical Society. He also worked as subject expert for national and international bodies and was honoured with the position of editor-in-chief of the *Journal of Functional and Environmental Botany* (2011–2013). Since 2013, he is serving as an executive editor of the *Journal of Functional and Environmental Botany*. In recognition of his contributions, Prof. Khan was elected as a fellow of the 'Indian Society for Plant Physiology' and the 'Indian Botanical Society' and was also awarded with the UGC Research Award (2002); Distinguished Scientist Award (2015) by VIFRA International Foundation (India); Group Leader, DBT-BUILDER Programme (2013–2016), DBT, Government of India, New Delhi; and Scientist of the Year Award (2005) and Eminent Scientist of the Year Award (2006) by NESA, New Delhi, India.

Role of Salicylic Acid in the Control of General Plant Growth, Development, and Productivity

1

Cesar J. Tucuch-Haas, Jesica V. Pérez-Balam, Karen B. Díaz-Magaña, José Manuel Castillo-Chuc, María G. Dzib-Ek, Gabriel Alcántar-González, Silvia Vergara-Yoisura, and Alfonso Larqué-Saavedra

Abstract

Applications of low concentrations of salicylic acid (SA) to the shoots of seedlings of horticultural plants such as habanero pepper (*Capsicum chinense*) or to perennial trees such as the Ramon (*Brosimum alicastrum*) significantly increase their growth, development and productivity.

In chili pepper it was found that the positive effect of SA on root growth is correlated with an increased uptake of macro nutrients and micronutrients which are allocated in the plant tissues. Data have shown that plant tissues treated with SA had significantly higher levels of macronutrients. Accumulation of nitrogen, phosphorus and potassium 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%) with respect to the control plants. The levels of other nutrients, such as copper, zinc, manganese, boron, calcium, magnesium and iron, were also higher.

The application of 1 µM SA to shoots of trees, affected the root length. The control plants had 42 cm, and those of the treated plants 65.5 cm, equivalent to an increase of 55.7%. Fresh weight of the root was 158.3% higher in the treated plants and the dry weight increased by 160.7%. Increases were also observed in stem length (46%), stem diameter (25.9%), fresh weight (78.3%), and dry weight

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(89%), in comparison with the control. The number of leaves presented in treated plants averaged 12.6, whereas the control plants showed an average of 9 leaves with a lower leaf area.

Keywords
Salicylic acid • Root • Growth regulation

1.1 Introduction

As examples of the results obtained from the research carried out by our working group on the effect of applications of salicylic acid (SA) on plant growth, development, and productivity over a period of 40 years, three basic contributions can be highlighted. The first is the publication, in 1978, in which it was proposed that the application of aspirin to bean plantlets had an effect on plant water status, a fact that was confirmed in specific bioassays conducted with bean explants and with stomata, using the bioassay of *Commelina communis* epidermis strips (Larqué-Saavedra 1978, 1979). These results were widely commented on in the international press, and it was emphasized that aspirin could be used to save crops under conditions of stress from the impact of droughts (Times, UK 1978). This aspect, i.e., that salicylates had the potential to participate in the physiology of plants subjected to stress, was subsequently demonstrated by Shimakawa and collaborators in 2012.

The second observation was published in 1998 (Gutiérrez-Coronado et al. 1998) in which it was reported that the application of salicylates to intact soya plantlets favored growth in the radical system of the plants. This discovery was accompanied by a third contribution which referred to the fact that concentrations in the order of micromoles, nanomoles, and femtomoles were sufficient to stimulate root growth and differentiation of secondary roots (Echeverria et al. 2007; San Miguel et al. 2003; Larqué-Saavedra et al. 2010), a fact that was confirmed using the bioassay of transformed roots of *Catharanthus roseus*. This discovery can be considered of significant importance for science given that it demonstrated the high sensitivity and capacity of response of the plant tissues to the application of low concentrations of salicylates which were reported in bioassays with animal tissues.

Based on these three observations, a number of experiments have been carried out in these laboratories, in which we have been able to confirm that SA plays a role in the control of plant growth, development, and productivity (Larqué-Saavedra and Martin-Mex 2007; Martin-Mex et al. 2013).

Salicylic acid (SA) is a phenolic compound produced by plants and possesses growth-regulating functions as well as the capacity to mediate in responses to pathogens (Delaney 2010; Hayat et al. 2007; Hayat et al. 2013; Rivas-San Vicente and Plasencia 2011; Yusuf et al. 2012). Since 1979, endogenous levels have been associated with physiological processes which include the induction of flowering in *Lemna* (Cleland and Tanaka 1979, Shimakawa et al. 2012) and the induction of heat production in plants such as the lily (Raskin 1992).

Numerous physiological effects resulting from the applications of SA have been reported. One effect of particular interest refers to its capacity to stimulate rooting in explants of *Phaseolus*, *Acer*, *and Vigna* (Basu et al. 1969; Li and Li 1995) and growth of the radical system in species such as soya or habanero pepper (Gutiérrez-Coronado et al. 1998; Deef 2007) or to stimulate the length and differentiation of secondary roots of *Catharanthus roseus* (Echeverría et al. 2007).

There are also a number of reports dealing with its capacity to increase protection against damage caused by ions such as boron, arsenic, lead, and cadmium (Choudhury and Panda 2004; Chen et al. 2007; El-Feky et al. 2014), to favor the accumulation of ions such as magnesium, calcium, and potassium (El Tayeb and Ahmed 2010) and to reduce the adverse effects caused by salinity and high temperatures (Pirasteh-Anosheh et al. 2014), drought (Habibi 2012; Farzane et al. 2014), and cold (Mutlu et al. 2013). The effects estimated support the proposal that this molecule can be considered an elicitor, which is a biotic inductor that triggers measurable physiological and morphological processes (Zhao et al. 2005). It is possible that the receptors and mechanisms of action stimulated by the application of this growth regulator could be similar to those reported in 1989 (Larqué-Saavedra and Rodríguez 1989).

It has also been reported that the application of SA produces alterations in processes such as the stimulation of nitrate reductase activity (Fariduddin et al. 2003), the increase in photosynthetic activity, electron transport, and chlorophyll (Arfan et al. 2007; Sánchez-Chávez et al. 2011). It has been published that SA also increases seedling height and vigor (Anwar et al. 2013) and that it affects processes relating to the bioproductivity of cultivated plants (Hayat et al. 2007, 2012).

The effect of SA applications in woody perennial species, however, has been poorly studied; thus the present research study was carried out to measure the effect of SA on the development of a tree from the Mexican tropics denominated *Brosimum alicastrum* (common name, Ramon); this species has drawn much attention due its high potential as a food source and its role in mitigating climate change (National Academy of Science 1975; Pardo-Tejeda et al. 1976).

1.2 Studies of SA on Horticultural Plants: *Capsicum chinense*

1.2.1 Salicylic Acid on Root and Shoot Growth

It is already known that a larger, more vigorous root system will contribute to better crops and horticultural plants. Our results are consistent with previous publications which have reported that 1 μM or less is sufficient to favor root growth. In *Pinus patula*, for instance, concentrations of 1.0 and 0.01 μM increased root growth in 33% and 30%, respectively, (San Miguel et al. 2003), while in *Chrysanthemum* a concentration of 0.01 μM SA increased dry root weight significantly (Villanueva et al. 2009). Dry root weight was also favored by the application of 1.0 μM or less in tobacco and cotton (Gutiérrez-Coronado et al. 1998).

Table 1.1 Effect of spraying 1 μM of salicylic acid on habanero pepper plantlets (*Capsicum chinense*) in different morphological variables

Morphological variable	Control	Concentrations of salicylic acid 1 μM
Height (cm)	18.44 ± 0.43 c	24.51 ± 0.18 a
Stem diameter (cm)	0.28 ± 0.05 b	0.34 ± 0.05 a
Leaf area (cm^2)	150.4 ± 13.3 b	204.3 ± 7.68 a
Fresh weight of the aerial part (g)	2.85 ± 0.15 b	3.65 ± 0.14 a
Dry weight of the aerial part (g)	0.37 ± 0.03 b	0.45 ± 0.02 a
Fresh weight of the root (g)	0.49 ± 0.04 b	0.69 ± 0.04 a
Dry weight of the root (g)	0.05 ± 0.004 b	0.08 ± 0.005 a

The values are the means with their respective standard errors. Different letters in the same line represent different statistics (Tukey, $\alpha = 0.05$). $n = 25$ (Data adapted from preliminary research work to be published)

One observation that was particularly relevant in studies of salicylate applications to habanero pepper plants (*Capsicum chinense*) was the evidence indicating that the plantlets which were sprayed with this compound showed much greater vigor in comparison with the control plants. It is reported that applications of 1 μM salicylic acid (SA) to seedling shoots significantly increased growth and fresh and dry weight of roots, stems, leaves, and fruits of this plant species at harvest time (Table 1.1).

1.2.2 The Uptake of Nutrients

Experiments were conducted to determine if the proposed positive effect of SA on root growth could be correlated with an increase in the uptake of macronutrients and micronutrients; to achieve this, these elements were measured in the plant tissues of treated plants.

The results showed that the tissues of plants treated with SA had significantly higher levels of macronutrients. Accumulation of nitrogen, phosphorus, and potassium 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%), respectively, in comparison with the control plants. The levels of other nutrients, such as copper, zinc, manganese, boron, calcium, magnesium, and iron, were also higher in the majority of the plant tissues treated with SA (Table 1.2, Figs. 1.1 and 1.2).

1.2.3 Effect on Flowering

The effect of SA on flower induction was analyzed by means of an experiment in which plantlets of habanero pepper (*Capsicum chinense*) were sprayed with 1 μM of this growth regulator. The results demonstrated that 50% of the treated plants

Table 1.2 Effect of spray applications of 1 μM of salicylic acid to habanero pepper plantlets (*Capsicum chinense*) on the content of macronutrients in the different organs harvested 128 days after application

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

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)

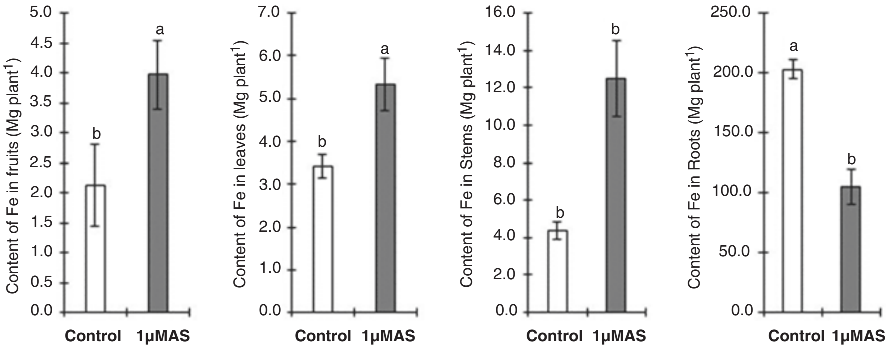

Fig. 1.1 Effect of spray application of 1 μM of salicylic acid on the iron content in the root and leaf. Stem and fruit of habanero pepper plants. Values with the same letter are not significant (Tukey $p = 0.05$). Each value is the mean of five individuals ± s.e. (Data adapted from preliminary research work to be published)

initiated flowering 25 days after spraying, while the control plants presented flowering after 45 days (Fig. 1.3). Moreover, at 55 days after spraying, flowering was observed in 97 of the treated plants and in 82 of the control plants. The number of flowers per plant observed at 80 days, in the plants sprayed with SA, was 112, while the control plants presented only 66 flowers per plant (Fig. 1.4).

1.2.4 Effect on Fruit Formation

Once the effect of SA on the flowering process was demonstrated, fructification of the plants treated with this growth regulator was evaluated. The results show that the plants sprayed with 1 μM SA formed 342 fruits, while the control plants formed

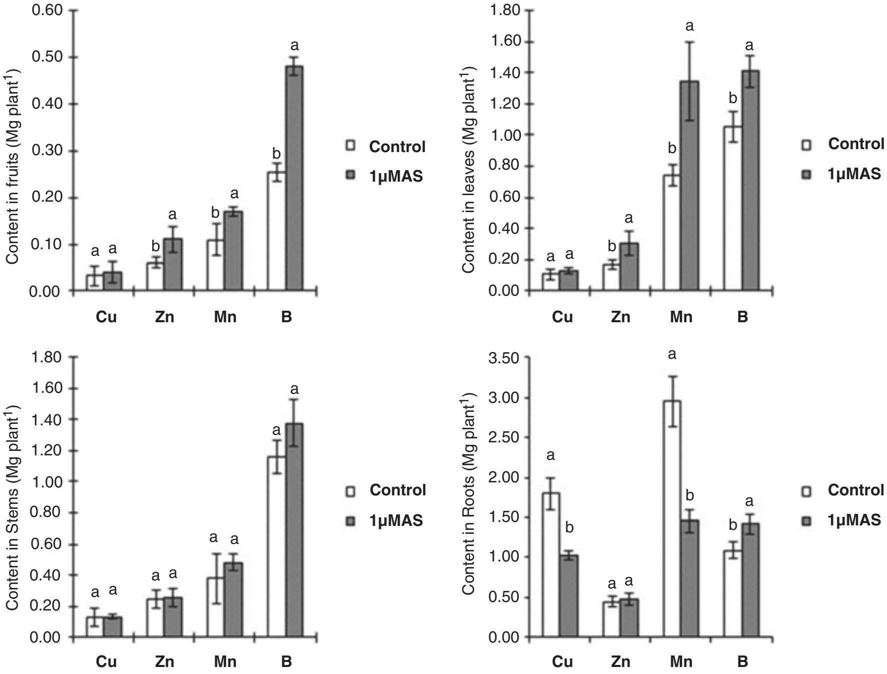

Fig. 1.2 Content of copper (Cu), zinc (Zn), manganese (Mn), and boron (B) in root, leaf, stem, and fruit of habanero pepper plants sprayed with 1 μM of salicylic acid. Values with the same letter are not significant (Tukey $p = 0.05$). Each value is the mean of five individuals (Data adapted from preliminary research work to be published)

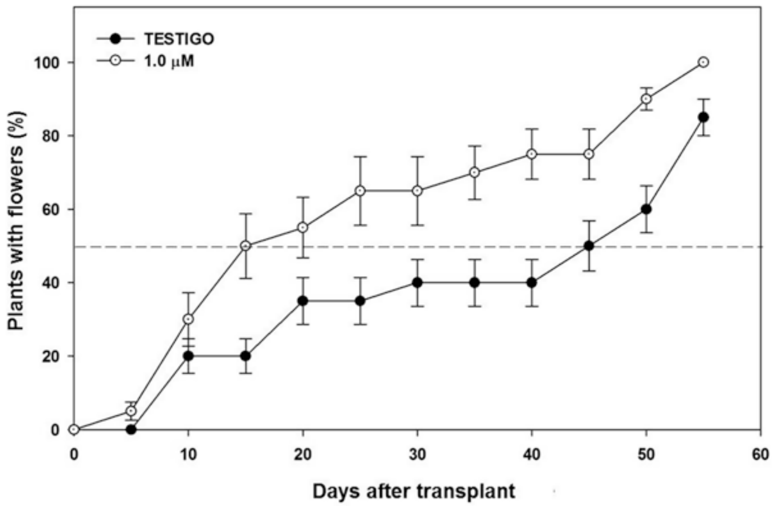

Fig. 1.3 Days to flowering of the habanero pepper plants treated with 1 μM of SA. The *dotted line* indicates 50% of the individuals with flowers. The values represent means with their standard error. An ANOVA ($\alpha = 0.05$) was performed; significant differences are identified with an *asterisk* $n = 20$ (Data adapted from preliminary research work to be published)

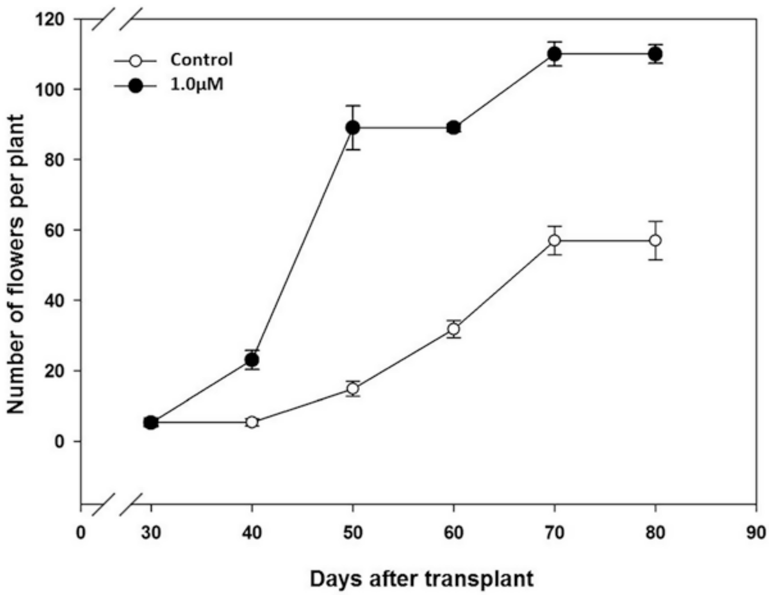

Fig. 1.4 Number of flowers in plants sprayed with 1 μM of SA. The values represent means with their standard error. ANOVAS (α = 0.05), significant differences are indicated with an *asterisk*. n = 20 (Data adapted from preliminary research work to be published)

only 244. In other words, the treated plants produced 40% more fruits in comparison with the control. Measurements of fruit form showed that the apical diameter and the medial region showed no significant differences (Table 1.3).

1.3 Studies on the Effect of SA on the Growth and Development of Woody Perennial Plants

The effect of SA applications in woody perennial plant species has been poorly studied; thus the present research study was carried out to measure the effect of SA application on the growth and development of *Brosimum alicastrum* (common name, Ramon), a tree of the Moraceae family from the Mexican tropics. This plant species has drawn much attention due to the environmental services it offers to mitigate climate change and its high potential as a food source (National Academy of Science 1975; Pardo-Tejeda et al. 1976).

First, a series of experiments were carried out to measure the effect of sprayed applications of this hormone on the root length. A specific bioassay was set up. Seedbeds consisting of PVC tubes 23 cm high and 4 cm in diameter were filled with perlite substrate. One seed was planted in each PVC tube, and these were placed in

Table 1.3 Effect of spray applications of 1 μM salicylic acid to habanero pepper plantlets (*Capsicum chinense*) on different estimators of the fruit. The mean of 20 repetitions ± s.e. is presented

Variable	Treatments	
	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, $\alpha = 0.05$ for NFP, PPF; Duncan, $\alpha = 0.05$ for PFP) (Data adapted from preliminary research work to be published)

a randomized experimental block designed with six replicates per treatment. The seedbeds were kept in a growth room at a temperature between 28 and 30 °C with daily watering and a photoperiod of 12 h of light and 12 h of darkness.

The SA solution was spray applied to the shoots of 13-day-old Ramon seedlings on ten occasions. When the seedling roots from the different treatments were seen to protrude from the base of the PVC tube, the seedlings were harvested in order to measure the root and stem lengths of the seedlings. For the measurement of the biomass weight, after the roots were harvested and measured, they were dried in an oven at 40 °C, for a 12 h period.

The results showed that SA treatment significantly increased the length of the roots of *Brosimum* (Fig. 1.5). This value was, on average, 4.3 cm longer than that of the control treatment. Fresh weight of the root also increased significantly in comparison with the control. Dry weight of the roots, however, was not significant, although the pattern does indicate that the treatment with SA showed a higher value.

SA treatment also favored stem height of *Brosimum* seedlings with 4.2 cm higher than that of the control (Fig. 1.6). From these results, we can infer that spraying SA on the leaves of *Brosimum* will stimulate root growth while increasing the capacity to absorb water and nutrients which will facilitate a more successful development, particularly in adverse conditions, such as drought.

With the results obtained, it was found that the sprayed application of 1 μM SA to the leaves of *Brosimum alicastrum* stimulates growth of the roots by a length of 4.3 cm, in comparison with the control. The treatment also increased fresh weight by 0.3 g with respect to the control, and stem height showed an increase of 4.2 cm over the control. Taking into consideration these results, one can infer that sprayed applications of salicylic acid to the leaves of *Brosimum alicastrum* seedlings stimulate growth of the roots and stems, which could possibly increase their capacity to absorb water.

Further work was carried out to estimate the effect of SA on plantlets of this perennial tropical tree. An experiment was conducted with potted plants, cultivated under shade netting in the open. Six weeks after planting, when the seedlings reached an average height of 13.8 cm and presented the first two complete leaves,

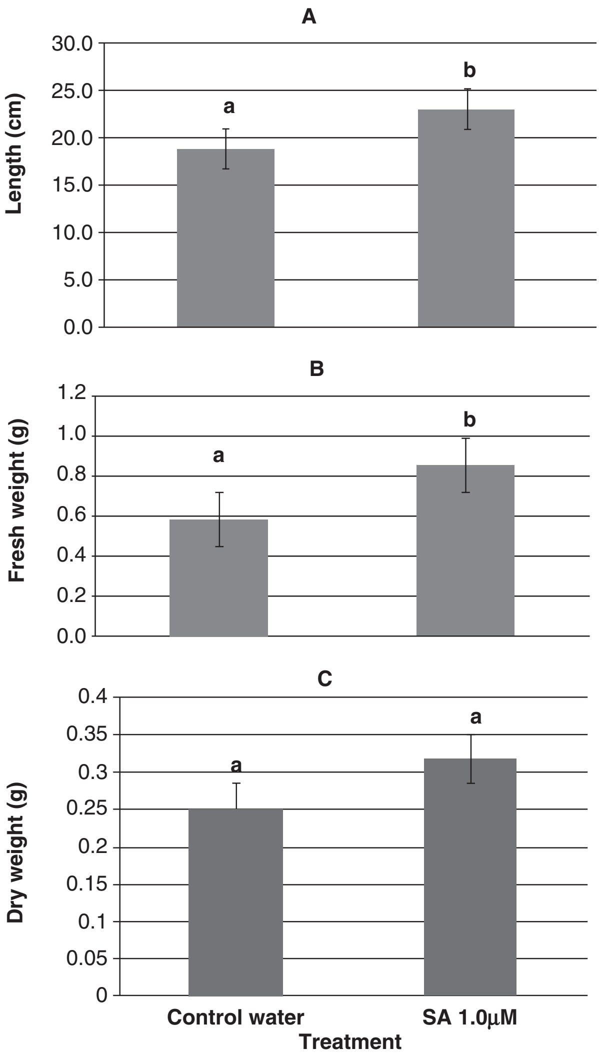

Fig. 1.5 Effect of spray applications of 1 µM of salicylic acid to *Brosimum alicastrum* seedlings on the length (**a**), fresh weight (**b**), and dry weight (**c**) of roots. Average of six repetitions ± standard error is shown. *Similar letters* indicate no significant difference (Fisher, $p = 0.05$) (Data adapted from preliminary research work to be published)

they were sprayed with 1 µM SA or water on the canopy, twice a week for a period of 3 weeks, after sunrise. The concentration of 1.0 µM of SA was evaluated as it was considered to be the optimal, based on the results obtained from previous experiments carried out in these laboratories (Larqué-Saavedra and Martin-Mex 2007; Martin-Mex et al. 2013).

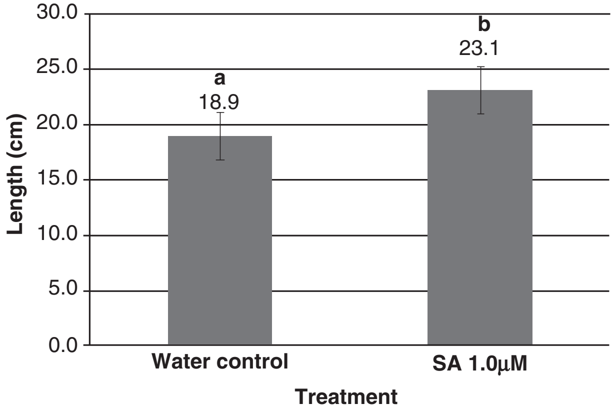

Fig. 1.6 Effect of spray applications of 1 µM of salicylic acid to *Brosimum alicastrum* seedlings on the length of the stems. Data are the mean value of six replicate samples ± s.e. *Similar letters* indicate no significant differences (Data adapted from preliminary research work to be published)

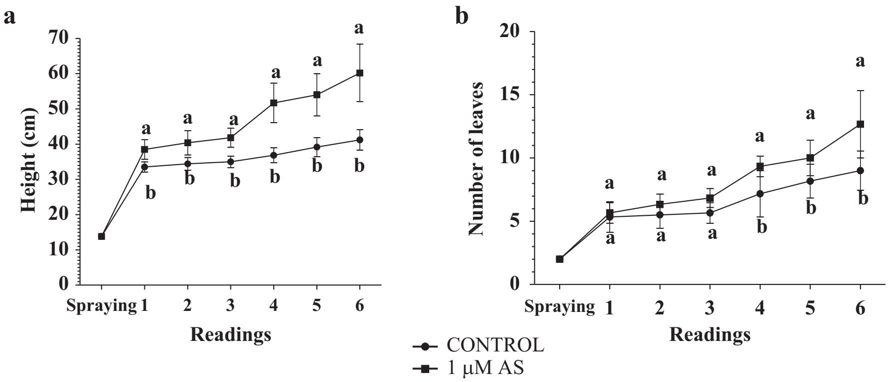

Fig. 1.7 Effect of spray application of 1 µM of SA on the pattern of height (**a**) and leaf exposure (**b**) in *Brosimum alicastrum* seedlings after initiation of treatment (AIT). Each point is the average of six plantlets ± standard error. Same *letters* indicate no significant difference (Tukey, $p \leq 0.05$) (Data adapted from preliminary research work to be published)

After 8 months, six plants were harvested to measure the length of root and canopy, as well as fresh and dry weight. The different parts of the plants were dried in an oven at 60 °C, until constant weight. Stem diameter was measured 5 cm above the vital node.

The results showed that the application of SA increases the growth pattern and rate of leaf exposure in comparison with the control. These differences were maintained and amplified, as can be appreciated in Fig. 1.7; thus, after 8 months, the increase observed, due to the effect of SA, was 46% in height and 40% more leaves.

Similarly, the spraying of 1 μM of SA on the canopy of Ramon plantlets stimulates root length significantly, in comparison with the control (Fig. 1.8). In fact, at the end of the test, root length of the control plants was 42.0 cm, and the treated plants registered 65.5 cm, equivalent to an increase of 55.7%, as a result of SA application. Fresh weight and dry weight of the root were also increased significantly, compared to those of the control. Fresh weight was 158.3% higher in the plants treated with SA, and dry weight increased by 160.1%.

The treatment with SA also favored stem length, with the treated plants presenting 60.2 cm, while the control was 41.2 cm, an increase equivalent to 46% in comparison with the control. Moreover, the effect of SA resulted in a stem diameter of 4.46 mm, and that of the control was 3.54 mm, equivalent to an increase of 25.9 %. The total fresh weight of the shoot was 78.3% greater in treated plants in comparison with the control, and the dry weight increased by 89.7% (Fig. 1.9).

The number of leaves was also affected by SA spraying. Treated plantlets increased the number of leaves by 40% over the control, presenting an average of 12.6 leaves with a foliar area of 387 cm^2, while the control had 9 with a foliar area of 231 cm^2. Fresh weight of leaves for the control was an average of 3.5 g, while that of the treated plants was 5.6 g, equivalent to an increase of 61.9%. Dry weight of control leaves was 1.37 g, and that of the treated plants was 2.22 g, equivalent to an increase of 62% (Fig. 1.10).

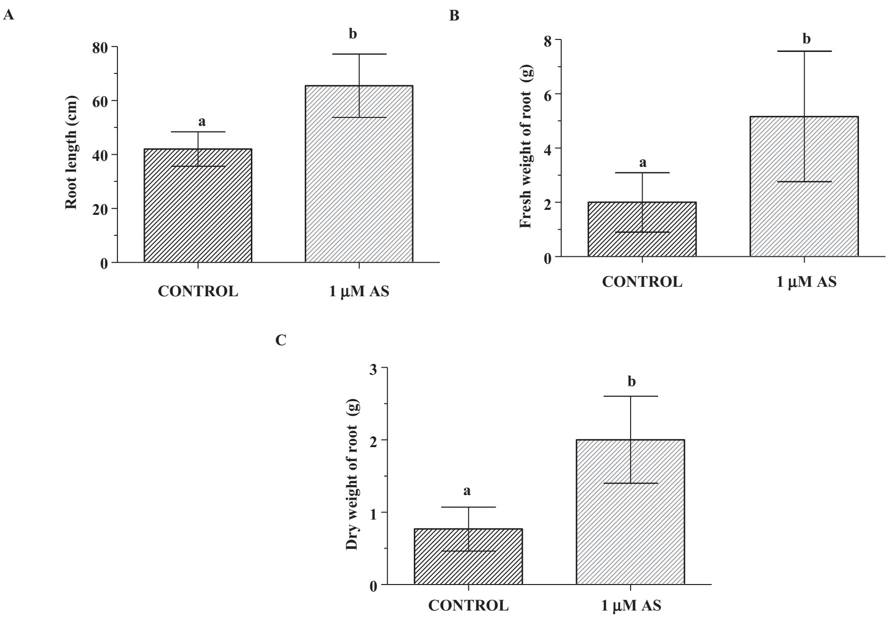

Fig. 1.8 Effect of spray application of 1 μM of SA to the shoots of seedlings on length (**a**), fresh weight (**b**), and dry weight (**c**) of the roots of *Brosimum alicastrum* plantlets, 8 months after the application of the growth regulator. Each block is the mean of six repetitions ± standard error. Same *letters* indicate no significant difference (Tukey, $p \leq 0.05$) (Data adapted from preliminary research work to be published)