

SALICYLIC ACID: A PLANT HORMONE

SALICYLIC ACID: A Plant Hormone

Edited by

S. Hayat

*Department of Botany
Aligarh Muslim University
Aligarh 202002, India*

and

A. Ahmad

*Department of Botany
Aligarh Muslim University
Aligarh 202002, India*

 **Springer**

A C.I.P. Catalogue record for this book is available from the Library of Congress.

ISBN-10 1-4020-5183-2 (HB)
ISBN-13 978-1-4020-5183-2 (HB)
ISBN-10 1-4020-5184-0 (e-book)
ISBN-13 978-1-4020-5184-0 (e-book)

Published by Springer,
P.O. Box 17, 3300 AA Dordrecht, The Netherlands.

www.springer.com

Cover photo: Female branch of *Salix tetrasperma* Roxb

Cover photo provided by kind permission of
Dr. Athar Ali Khan
Department of Botany
Aligarh Muslim University
Aligarh 202002
India

Printed on acid-free paper

All Rights Reserved

© 2007 Springer

No part of this work may be reproduced, stored in a retrieval system, or transmitted in any form or by any means, electronic, mechanical, photocopying, microfilming, recording or otherwise, without written permission from the Publisher, with the exception of any material supplied specifically for the purpose of being entered and executed on a computer system, for exclusive use by the purchaser of the work.

**Dedicated To The
Institution and Nation Where We Work**

Contents

Preface	ix
Contributors	xi
1. Salicylic acid: Biosynthesis, metabolism and physiological role in plants S. Hayat, B. Ali and A. Ahmad	1
2. Effects of salicylic acid on the bioproductivity of plants Alfonso Larqué-Saavedra and Rodolfo Martin-Mex	15
3. Effect of salicylic acid on solute transport in plants M. S. Krasavina	25
4. Role of hormonal system in the manifestation of growth promoting and antistress action of salicylic acid F. M. Shakirova	69
5. Role of salicylic acid in the induction of abiotic stress tolerance T. Janda, E. Horváth, G. Szalai and E. Páldi	91
6. The role of salicylates in <i>Rhizobium</i>-legume symbiosis and abiotic stresses in higher plants Fazli Mabood and Donald Smith	151
7. Stress and antistress effects of salicylic acid and acetyl salicylic acid on potato culture technology H.A. Lopez-Delgado, I.M. Scott and M.E. Mora-Herrera	163
8. Salicylic acid and reactive oxygen species in the activation of stress defense genes Loreto Holuigue, Paula Salinas, Francisca Blanco and Virginia Garretón	197
9. The interplay between salicylic acid and reactive oxygen species during cell death in plants J.F. Dat, N. Capelli, F. Van Breusegem	247
10. Salicylic acid as a defense-related plant hormone: Roles of oxidative and calcium signaling paths in salicylic acid biology Tomonori Kawano and Takuya Furuichi	277

11. Salicylic acid and local resistance to pathogens	323
Igor V. Maksimov and Lubov G. Yarullina	
12. Salicylic acid in plant disease resistance	335
Ratnesh Chaturvedi and Jyoti Shah	
13. Transcriptomic analysis of salicylic acid-responsive genes in tobacco BY-2 cells	371
I. Galis and K. Matsuoka	
Index	397

Preface

The organism that we recognise as plant is the result of integrated metabolic functions administered by a number of factors, including hormones. Out of these chemicals, five (Auxins, Gibberellins, Cytokinins, Abscissic acid and Ethylene) are well recognized for their functions but the more recent ones (Brassinosteroids, Salicylic acid, Polyamines and Jasmonates) can not be excluded from the list of hormones because of their involvement in important functions of plants.

This book is providing information related with Salicylic acid (SA) that was first noticed to be a major component in the extract from *Salix* (willow) bark and used as an anti-inflammatory drug. It belongs to phenolic group and is ubiquitous in plants. SA is involved in signal transduction, pondering over the plant resistance to stress and generates significant impact on photosynthesis, transpiration, uptake and transport of ions and growth and development. However, the observations related with this hormone are very much scattered it was, therefore, decided to compile all in the form of a book, based on 13 chapters written by various experts, working in this field. A total of 31 experts have explained their results based on the practical work carried over by them and of others on various selected aspects of plant growth and development. After going through these chapters it may be concluded that the hormone has a wide range of actions by involving genes and / or the membranes.

With great pleasure, we extend our sincere thanks to all the contributors for their timely preparation of excellent and up to dated contributions and also for their consistent support and cooperation.

Thanks are also due to Springer, The Netherlands for expeditious acceptance of our proposal and completion of the review process. Subsequent cooperation and understanding of their staff, especially, Malanie van Overbeek, publishing assistant, is also gratefully acknowledged.

We express our sincere thanks to the members of our family for all the support they provided and the neglect, what so ever, they suffered during the preparation of this book.

Finally we are thankful to the Almighty God.

S. Hayat
A. Ahmad

Contributors

Ahmad A.

Department of Botany,
Aligarh Muslim University,
Aligarh-202002, India

Alfonso Larqué-Saavedra

Centro de Investigación Científica de Yucatán,
A. C., Calle 43 No. 130,
Chuburná de Hidalgo, Mérida, Yucatán,
México CP 97200

Ali B.

Department of Botany,
Aligarh Muslim University,
Aligarh-202002, India

Capelli N.

Laboratoire de Biologie Environnementale (EA 3154 MRT, UC
INRA), Université de Franche- Comté,
UFR Sciences et Techniques,
Place Leclerc, F-25030 Besancon Cedex,
France

Dat J.F.

Laboratoire de Biologie Environnementale (EA 3154 MRT, UC
INRA), Université de Franche- Comté,
UFR Sciences et Techniques,
Place Leclerc, F-25030 Besancon Cedex,
France

Donald Smith

Plant Science Department,
Macdonald Campus of McGill University,
21,111 Lakeshore Road,
Sainte Anne de Bellevue, Qc,
Canada, H9X 3V9

Fazli Mabood

Plant Science Department,
Macdonald Campus of McGill University,
21,111 Lakeshore Road,
Sainte Anne de Bellevue, Qc,
Canada, H9X 3V9

Francisca Blanco
Facultad de Ciencias Biológicas,
Universidad Católica de Chile,
P.O.Box 114-D, Santiago,
Chile

Galis I.
RIKEN Plant Science Center,
1-7-22 Suehiro-cho,
Tsurumi-ku, Yokohama
Japan

Hayat S.
Department of Botany,
Aligarh Muslim University,
Aligarh-202002, India

Horváth E.
Agricultural Research Institute of the Hungarian Academy of
Sciences,
H-2462, Martonvásár, POB. 19.
Hungary

Igor V. Maksimov
Institute of Biochemistry and Genetics,
Ufa Research Centre,
Russian Academy of Science,
450054, Ufa, pr. Oktyabrya, 69,
Russia

Janda T.
Agricultural Research Institute of the Hungarian Academy of
Sciences,
H-2462, Martonvásár, POB. 19.
Hungary

Jyoti Shah
Division of Biology,
Kansas State University,
Manhattan, Kansas 66506
USA

Krasavina M.S.

Timiryazev Institute of Plant Physiology,
Russian Academy of Science,
Botanicheskaya ul. 35, Moscow,
Russia

Lopez-Delgado H.A.

Programa Nacional de Papa,
Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias
(INIFAP), Metepec, Méx. 52141, A. P. 1-4.
México

Loreto Holuigue

Facultad de Ciencias Biológicas,
Universidad Católica de Chile,
P.O.Box 114-D, Santiago,
Chile

Lubov G. Yarullina

Institute of Biochemistry and Genetics,
Ufa Research Centre,
Russian Academy of Science,
450054, Ufa, pr. Oktyabrya, 69,
Russia

Matsuoka K.

RIKEN Plant Science Center,
1-7-22 Suehiro-cho,
Tsurumi-ku, Yokohama
Japan

Mora-Herrera M.E.

Programa Nacional de Papa,
Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias
(INIFAP), Metepec, Méx. 52141, A. P. 1-4.
México

Páldi E.

Agricultural Research Institute of the Hungarian Academy of
Sciences,
H-2462, Martonvásár, POB. 19.
Hungary

Paula Salinas

Facultad de Ciencias Biológicas,

Universidad Católica de Chile,
P.O.Box 114-D, Santiago,
Chile

Ratnesh Chaturvedi
Division of Biology,
Kansas State University,
Manhattan, Kansas 66506
USA

Rodolfo Martin-Mex
Centro de Investigación Científica de Yucatán,
A.C., Calle 43 No. 130,
Chuburná de Hidalgo, Mérida, Yucatán,
México CP 97200

Scott I.M.
Institute of Biological Sciences,
University of Wales,
Aberystwyth, Ceredigion, SY23 3DA,
UK.

Shakirova F.M.
Institute of Biochemistry and Genetics,
Ufa Scientific Centre,
Russian Academy of Sciences,
69 pr. Octyabrya, 450054, Ufa,
Russia

Szalai G.
Agricultural Research Institute of the Hungarian Academy of
Sciences,
H-2462, Martonvásár, POB. 19.
Hungary

Takuya Furuichi
Graduate School of Medicine,
Nagoya University,
Showa-ku, Nagoya 466-8550,
Japan

Tomonori Kawano
Graduate School of Environmental Engineering,
The University of Kitakyushu,
Kitakyushu 808-0135,
Japan

Van Breusegem F.
Department of Plant Systems Biology,
Ghent University, VIB,
Technologie Park 927, B-9052 Gent,
Belgium

Virginia Garretón
Facultad de Ciencias Naturales,
Universidad Santo Tomás,
Ejército 41, Santiago.
Chile

Chapter 1

SALICYLIC ACID: BIOSYNTHESIS, METABOLISM AND PHYSIOLOGICAL ROLE IN PLANTS

S. HAYAT, B. ALI AND A. AHMAD

Department of Botany, Aligarh Muslim University, Aligarh, INDIA

Abstract: Salicylic acid (SA) is a phenolic derivative, distributed in a wide range of plant species. It is a natural product of phenylpropanoid metabolism. Decarboxylation of transcinnamic acid to benzoic acid and its subsequent 2-hydroxylation results to SA. It undergoes metabolism by conjugating with glucose to SA glucoside and an ester. SA has direct involvement in plant growth, thermogenesis, flower induction and uptake of ions. It affects ethylene biosynthesis, stomatal movement and also reverses the effects of ABA on leaf abscission. Enhancement of the level of chlorophyll and carotenoid pigments, photosynthetic rate and modifying the activity of some of the important enzymes are other roles assigned to SA. This chapter gives a comprehensive coverage to all the above aspects.

Key words: Biosynthesis, chlorophyll, metabolism, photosynthetic rate, salicylic acid

1. INTRODUCTION

Salicylic acid, at one stage of time, was the world's best selling drug synthesized in 1898 in Germany (Raskin, 1992b). However, it was John Buchner (1928) who isolated salicyl alcohol glucoside (Salicine) from willow bark at Munich that was later on named by Rafacle Piria (1938) as salicylic acid (SA). The word salicylic acid (SA) was derived from Latin word "Salix", meaning willow tree. It is ubiquitously distributed in the whole plant kingdom (Raskin *et al.*, 1990) and is classified under the group of plant hormones (Raskin, 1992a). SA is assigned diverse regulatory roles in the metabolism of plants (Popova *et al.*, 1997).

Chemically, SA belongs to an extremely diverse group of plant phenolics, that possess an aromatic ring with a hydroxyl group or its functional derivatives (Figure 1). Free salicylic acid is a crystalline powder that melts at 157-159°C. It is moderately soluble in water but highly soluble in polar organic solvents. The pH of its aqueous solution is 2.4; pKa is 2.98 and log K_{ow} is equal to 2.26 (Raskin, 1992b). Aspirine, an analogue of SA, undergoes spontaneous hydrolysis to SA, in aqueous solution (Mitchell *et al.*, 1967).

Using most modern analytical techniques, SA has been characterized in 36 plants, belonging to diverse groups (Raskin *et al.*, 1990). In plants, such as rice, crabgrass, barley and soybean the level of salicylic acid is approximately 1 mg g⁻¹ fresh mass. Floral parts of seven species and the leaves of twenty seven thermogenic species exhibited substantial variation in the level of SA (Raskin, 1990). Nonetheless, a maximum quantity of 37.19 mg g⁻¹ fresh mass was detected in the leaves of rice. However, SA could not be detected in the thermogenic flowers of water lily, *Victoria regia* Lindl. (Nymphaeaceae) and Palmae, *Bactris major* Jacq. (Raskin, *et al.*, 1990).

Presently there is no clear cut direct evidence that may be used to prove the transportability of SA. However, the physical properties of SA suggest that it could be transported, metabolized and/or conjugated in the plants. Moreover, the exogenously applied SA seems to be carried away from the sites of its initial application to different other tissues of the plants to generate response (Raskin, 1992b).

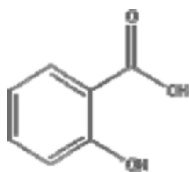


Figure 1. Structure of salicylic acid

2. BIOSYNTHESIS OF SALICYLIC ACID

It is largely believed that SA (ortho-hydrobenzoic acid) is a natural derivative of cinnamic acid, an intermediate in shikmic acid pathway, operative for the synthesis of phenolic compounds. However, two possible routes have been proposed in this direction (Figure 2).

(i) Decarboxylation of the side chain of cinnamic acid to generate benzoic acid, that undergoes hydroxylation, at C-2 position. Recently, this scheme, for the synthesis of SA has been reported in tobacco plants (Yalpani *et al.*, 1993) and also in rice seedlings (Silverman *et al.*, 1995). The enzyme that catalyzes β -oxidation of cinnamic acid to benzoic acid has been identified in *Quercus pedunculata* (Alibert and Ranjeva, 1971; Alibert *et al.*, 1972). However, the other enzyme that is responsible for the conversion of benzoic acid to salicylic acid has not been characterized, so far.

(ii) Hydroxylation of cinnamic acid to o-coumaric acid followed by its decarboxylation to salicylic acid. The conversion of cinnamic acid to o-coumaric acid is believed to be catalyzed by trans-cinnamate-4-hydroxylase (Alibert and Ranjeva, 1971; Alibert *et al.*, 1972) that was first detected in pea seedlings (Russel and Conn, 1967). Later on it was also identified in *Quercus pedunculata* (Alibert and Ranjeva, 1971; Alibert *et al.*, 1972), tubers of *Jerusalem artichoke* (Garbiac *et al.*, 1991) and *Melilotus alba* (Gestetner and Conn, 1974). However, the enzyme that activates the conversion of o-coumaric acid to SA has not yet been identified.

Moreover, incorporation of radioactive ^{14}C -benzoic acid or ^{14}C -cinnamic acid resulted in the formation of labeled SA in *Gaultheria procumbens* (Ellis and Amrchein, 1971). This observation strongly favours the belief that SA is synthesized from cinnamic acid, mediated by benzoic acid as an intermediate but El-Basyuni *et al.* (1964) believe that both the above systems are operative, in higher plants, in the synthesis of SA.

3. METABOLISM OF SALICYLIC ACID

Salicylic acid is known to form conjugates with a number of molecules (Ibrahim and Tower, 1959; Griffiths, 1959; Lee *et al.*, 1995) by glycosylation and less frequently by estrification (Popova *et al.*, 1997). The glucose esters of SA have been reported in suspension cultures of soybean, mung bean (Apte and Laloraya, 1982) and that of sunflower hypocotyls (Klamt, 1962) and also in other higher plants (Griffith, 1959; Ibrahim and Tower, 1959). Similarly, the conjugate (glucoside), β -glucoside-SA, has been identified in suspension culture of *Mallotus japonicus* (Tanaka *et al.*, 1990) and in the roots of *Avena sativa* seedlings (Balke and Schulz, 1987; Yalpani *et al.*, 1992a). Nonetheless, they also identified an enzyme, SA-glycosyltransferase (Gtase), that catalyzes the metabolism of SA to β -glucoside-SA. The presence of a conjugate with amino acids (salicyl aspartic acid) is also reported in wild grapes (Silverman *et al.*, 1995) and French bean (Bourne *et al.*, 1991).

Although, SA-2-O- β -D-glucoside is a predominant conjugate in plants, but other metabolites could be formed by estrification or additional hydroxylation of the aromatic ring. Out of them, 2,3-dihydroxybenzoic acid (O-pyrocatechuic acid) and 2,5-dihydrobenzoic acid (Gentisic acid) were identified in the leaves of *Astilbe sinensis* and *Lycopersicon esculentum*, fed with ^{14}C -cinnamic acid and ^{14}C -benzoic acid (Billek and Schmoock, 1967; Chadha and Brown, 1974).

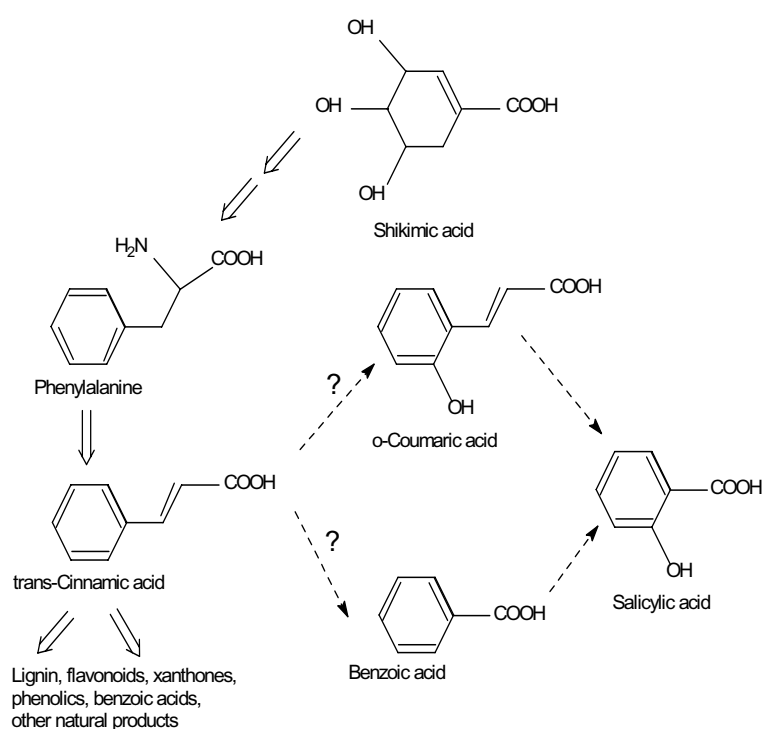


Figure 2. Proposed pathway for salicylic acid biosynthesis in plants.

4. PHYSIOLOGICAL ROLE OF SALICYLIC ACID

4.1 Effect of SA on plant growth

It is well documented that phenolic compounds exert their influence on physiological and biochemical processes including, photosynthesis, ion

uptake, membrane permeability, enzyme activities, flowering, heat production and growth and development of plants. One, such a natural compound is salicylic acid that may function as plant growth regulator (Arberg, 1981). The application of salicylic acid (SA), acetylsalicylic acid (ASA) gentisic acid (GTA) or other analogues of SA, to the leaves of corn and soybean accelerated their leaf area and dry mass production but plant height and root length remained unaffected (Khan *et al.*, 2003). Out of the various concentrations of SA used, Fariduddin *et al.* (2003) observed maximum increase in dry matter accumulation at a concentration of 10^{-5} M, supplemented to the leaves of the standing plants of *Brassica juncea* but the concentrations, above that proved inhibitory (Figure 3). Moreover, wheat seedlings, raised from the grains soaked in 10^{-5} M of SA possessed more number of leaves and higher fresh and dry mass (Figure 4), compared with water soaked, control (Hayat *et al.*, 2005). Likewise, Pancheva *et al.* (1996) treated 2-day old seedlings of barley with SA whose growth was significantly enhanced but leaf emergence was delayed. The blades expanded slowly, over a longer span of time and the mature blades were narrow and shorter. The stem cuttings of some ornamental plants, treated with SA, exhibited a rapid rate of root differentiation (Singh *et al.*, 1993). In contrary to the above, Pancheva *et al.* (1996) observed an inhibition in the growth of leaves and roots of barley seedlings, treated with salicylic acid and an increase in the concentration of SA enhanced it further. Similarly, a dose dependent, inhibition in bud formation in *Funaria hygromatica* was reported by Christianson and Duffy (2002).

4.2 Effect of SA on attributes, related to photosynthesis

The metabolic aspects of plants, supplied with SA or its derivatives, shifted to a varied degree, depending on the plant type and the mode of application of SA. The application of SA (20 mg ml^{-1}) to the foliage of the plants of *Brassica napus* improved the chlorophyll contents (Ghai *et al.*, 2002). Similarly, soaking the grains of wheat in 10^{-5} M of SA resulted in the plants with higher pigment contents which declined as the concentration of SA was increased above that (Hayat *et al.*, 2005). Moreover, 30 day old plants of *Brassica juncea* sprayed with 10^{-5} M of SA possessed chlorophyll 20% higher than those sprayed by water only, however the maximum concentration (10^{-3} M) decreased the chlorophyll contents and the values were below that of water sprayed control, at 60 day stage (Fariduddin *et al.*, 2003; Figure 3). However, the leaves of corn and soybean applied with acetyl salicylic acid (ASA) or gentisic acid (GTA), exhibited no change in their chlorophyll contents (Khan *et al.*, 2003). Nonetheless, soaking the seeds of *Vigna mungo* in aqueous solutions of SA (10-150 μm) lead a

decrease in the contents of chlorophyll and carotenoid in the leaves of subsequent plants, but supplementing SA through irrigant did not prove as severe as seed-treatment (Anandhi and Ramanujam, 1997). Similarly, the leaves of barley plants had less chlorophyll, if added with 100 μM to 1 mM of SA to the culture medium (Pancheva *et al.*, 1996). Salicylic acid activated the synthesis of carotenoids, xanthophylls and the rate of de-epoxidation but decreased the level of chlorophyll pigments, both in wheat and moong plants and also the ratio of chlorophyll a/b, in wheat seedlings (Moharekar *et al.*, 2003). Furthermore, *Spirodela* fed with salicylic acid, possessed less anthocyanin and chlorophyll pigments (Khurana and Maheshwari, 1980).

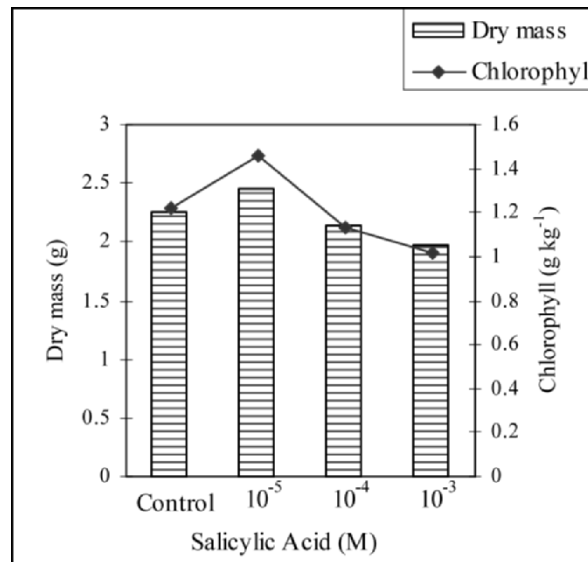


Figure 3. Effect of leaf applied SA on dry mass and chlorophyll content of 60 day old *Brassica juncea* plants

Leaf-applied SA induced stomatal closure in *Phaseolus vulgaris* (Larqué-Saavedra, 1979) and decreased transpiration rate in *Phaseolus vulgaris* and *Commelina communis* (Larqué-Saavedra, 1978, 1979). However, Khan *et al.* (2003) observed an increase in transpiration rate and stomatal conductance in response to the spray of SA, ASA and gentisic acid (GTA) on the foliage of corn and soybean. Moreover, the leaves of soybean exhibited increased water use efficiency, higher rate of transpiration and increased internal CO₂ concentration, on being supplemented with SA (Kumar *et al.* 2000). Similarly, barley plants, exposed to SA for a week, expressed an increase in the CO₂ compensation point and stomatal resistance (Pancheva *et al.*, 1996).

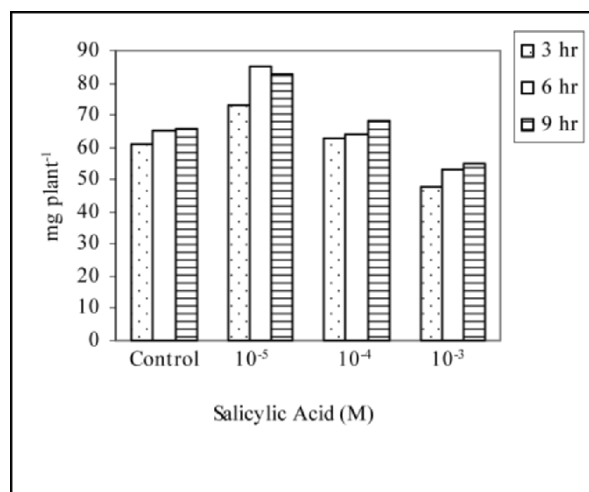


Figure 4. Effect of presowing seed treatment with salicylic acid for different durations on dry mass in wheat at 30 days after sowing

The response of the intact plants and/or their parts to SA-treatment involves a shift in the activity of the enzyme/s, of the process. The activity of carbonic anhydrase in the leaves of mustard, supplied with 10^{-5} M of SA to the foliage (Fariduddin *et al.*, 2003) or as pre-sowing seed treatment to wheat grains (Hayat *et al.*, 2005; Figure 5) was significantly enhanced. In both these studies, the authors observed a decline in the activity of carbonic anhydrase at the two subsequent higher concentrations (10^{-4} and 10^{-3} M) and 10^{-3} M proved to be supraoptimal. The activity of the other very important enzyme, ribulose-1,5-biphosphate carboxylase/oxygenase (RuBPCO) in barley plants, exposed to SA treatment for a week, decreased by about 50% and this loss was in proportion to the concentration (100 μ M to 1 mM) of SA (Pancheva and Popova, 1997). Moreover, they also noted an increase in PEPCase in barley and a decline in the photosynthetic rate. Contrary to the above observations, the activity of rubisco in stressed-maize plants (Khodary, 2004) and photosynthetic rate in mustard plants (Fariduddin *et al.*, 2003; Figure 3) increased under the influence of SA. Moreover, Fariduddin *et al.* (2003) also observed increased water use and carboxylation efficiencies in association with high photosynthetic rate in mustard plants, although the higher concentrations (10^{-4} and 10^{-3} M) of SA proved deleterious. Likewise, SA stimulated photosynthetic rate in soybean, barley and/or maize (Khodary, 2004, Pancheva *et al.*, 1996, Kumar *et al.*, 2000, Khan *et al.*, 2003).

4.3 Effect of SA on nitrate metabolism

A concentration (0.01-1.0 mM) of $\text{Ca}(\text{NO}_3)_2$ in association with SA activated the uptake of nitrogen and the activity of nitrate reductase (NR) both in the leaves and roots of maize plants, although higher concentration (5 mM) proved inhibitory (Jain and Srivastava, 1981). Similarly, SA increased the activity of NR in the presence of NO_3 and also favoured protection of the enzyme against protease, trypsin (Rane *et al.*, 1995). The plants resulting from the wheat grains, soaked in aqueous solution (10^{-5}M) of SA, exhibited high NR activity (Hayat *et al.*, 2005) and also in mustard leaves whose foliage was fed with SA (10^{-5}M) (Fariduddin *et al.*, 2003; Figure 7). In the former, 10^{-5}M of SA enhanced the activity of the enzyme by 36 % and by 13 % in the latter, as compared to their respective controls. Moreover, in both these cases the maximum concentration (10^{-3}M) of SA proves to be inhibitory, that decreased the activity of NR by 14 % in wheat seedlings and 10 % in mustard plants. Higher NR activity, under the influence of SA, in *Glycine max* was coupled with protein content (Kumar *et al.*, 1999) but SA decreased the level of soluble proteins in barley (Pancheva *et al.*, 1996). Nonetheless the level of sugars, starch and phenols exhibited a decrease in *Vigna mungo* cultivars in response to SA (10-50 μM) treatment (Anandhi and Ranjeva, 1997).

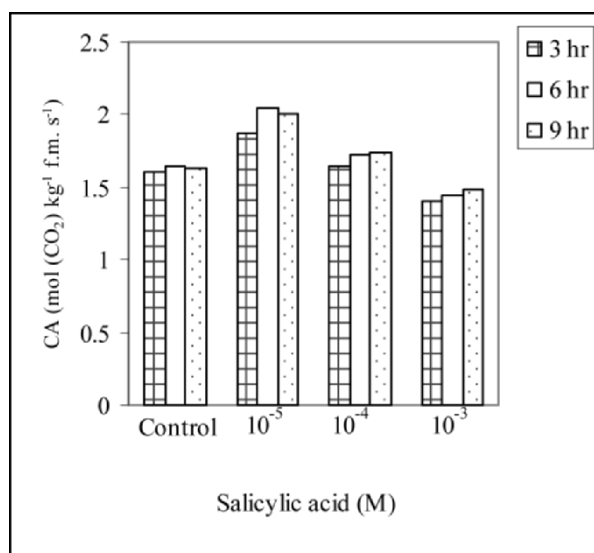


Figure 5. Effect of presowing seed treatment with salicylic acid for different durations on CA activity in wheat at 30 days after sowing

4.4 Effect of SA on ethylene production

Comparing the effect, generated by 22 related phenolic compounds, it was demonstrated that out of them SA and ASA inhibit ethylene production where the effect of SA was inversely dependent on the pH of culture medium and did not require continuous supply of salicylate. Moreover, the inhibitory actions of SA most closely resembled with that of dinitrophenol, a known inhibitor of ethylene forming enzyme (Leslie and Romani, 1980). In a similar study Romani *et al.* (1989) observed that SA and ASA inhibited ethylene production by about 90%, in apple discs, within three hours.

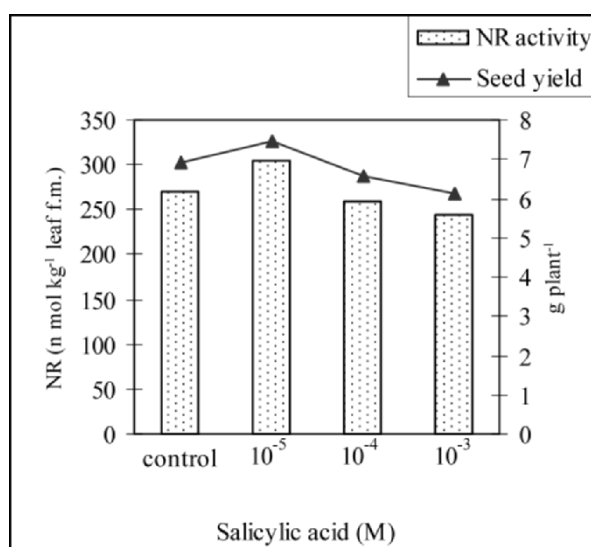


Figure 6. Effect of leaf applied SA on NR activity at 60 day stage and seed yield of *Brassica juncea* plants at harvest

4.5 Effect of SA on mineral nutrients

The plants also exhibit a shift in their nutrient status, under the impact of salicylic acid. The uptake of phosphate (Glass, 1973) and subsequently that of potassium (Glass, 1974) by barley roots was reduced by SA. However, the inhibition of the absorption of potassium by oat roots, under the impact of SA, was dependent on the pH and the concentration of the element in the medium. This inhibition was more prominent at lower pH, suggesting higher activity of protonated form of salicylic acid (Harper and Balke, 1981; Gordon *et al.* 2002). SA also caused the collapse of the transmembrane

electrochemical potential of mitochondria and the ATP dependent proton gradient of tonoplast enriched vesicles (Macri *et al.*, 1986).

4.6 Effect of SA on heat production

The involvement of SA in heat production in plants is well documented (Raskin, 1992b). The contents of SA, in five aroid species and in male cones of four thermogenic cycads, during heat production, increased by about 1 mg g⁻¹ (Raskin *et al.*, 1990). Besides this significant observation, the use of modern analytical techniques (Raskin *et al.*, 1989) proved, SA to be a clerigen (heat producer) and an integral endogenous regulator of heat production, in plants (Popova *et al.*, 1996). Therefore, the exogenous application of SA @ 0.13 mg g⁻¹ fresh mass to the sections of immature appendix of lilies led to an increase in temperature by 12°C (Raskin *et al.*, 1989). The mechanism of action of SA in thermogenic species, tobacco leaves is suggested to be through its action on respiration whose increased rate elevates the surface temperature (Van-Straten *et al.*, 1995).

4.7 Effect of SA on flowering

The very first physiological response, ever attributed to SA in plants, was its impact on flower induction in tobacco tissue culture, supplemented with kinetin and indole acetic acid (Lee and Skoog, 1965; Eberhard *et al.*, 1989). This impact of SA was later demonstrated in a number of plant species, belonging to different families. SA accelerated flower initiation in *Lemma*, although the effect on the rate of subsequent flower development was not so significant (Cleland and Ajami, 1974; Cleland and Tanaka, 1979). Similarly, SA stimulated flowering in *Xanthium strumarium* (Cleland and Ajami, 1974). Moreover, aspirine, an analogue of SA, hastened flowering under non-inductive photoperiod, in *Spirodela polyrrhiza* (Khurana and Maheshwari, 1980), *Spirodela punctata* (Scharfetter *et al.*, 1978) and *Wolffia microscopica* (Khurana and Maheshwari, 1987; Tamot *et al.*, 1987) and in combination with sucrose it enhanced flower opening in *Oncidium* (Hew, 1987). Similarly, flowering in *Pisita stratiotes*, a member of family Araceae, was also accelerated by incorporating the culture medium with SA (Piterse, 1982). The application of SA to the foliage of soybean also hastened flower bud and pod formation by 2-5 days (Kumar *et al.*, 1999). This compelled the scientists to conclude that salicylic acid somehow acts as an endogenous regulator of flowering (Cleland and Ajami, 1974).

Comparing the individual effect of GA, β-naphthol and that of SA on flowering in *Impatiens balsamina*, Nanda *et al.* (1976) and Sood and Nanda (1979) reported that SA generated an effect synergistic to that of GA.

Similar synergism on flowering has also been reported in *Arabidopsis thaliana* (Goto, 1981). In another study Kumar *et al.* (2000) compared the flower inducing effect of SA with that of GA, kinetin, NAA, ethrel and chloro choline chloride (CCC). They found that SA and/or GA were most effective than any other combination of the hormones. The specific flower inducing mechanism that involves salicylic acid is yet to be explored. However, Ooto (1975) hypothesized the belief that free o-hydroxyl group on Benzoic acid confers metal chelating characteristic that favours flower induction. This view gets the support from the observations where chelating agents induce flowering in Lamnaceae (Seth *et al.*, 1970; Ooto, 1972) and were comparable with that of the SA (Pitersen, 1977). Raskin (1992) on the basis of flower inducing (florigenic) activity of benzoic acid (Watanab and Takimoto, 1979; Fujioka *et al.*, 1983) and other non-chelating phenolic compounds (Watanab *et al.*, 1981), concluded that additional flower inducing mechanisms, other than the general belief, may be involved, that made the question wide open for further work.

5. REFERENCES

- Alibert, G., and Ranjeva, R., 1971. Recherches sur les enzymes catalysant la biosynthese des acides phenoliques chez *Quercus pedunculata* (Ehrn): I – Formation des series cinnamique et benzoique. *FEBS Lett.*, **19**: 11-14.
- Alibert, G., and Ranjeva, R., 1972. Recherches sur les enzymes catalysant la biosyntheses des acid phenoliques chez *Quercus pedunculata* (Ehrn): II- Localization intercellulaire de la phenylalanin mmonique-lyase, de la cinnamate 4-hydroxylase, et de la “benzoate synthase” *Biochem. Biophys. Acta*, **279**: 282-289.
- Anandhi, S., and Ramanujam, M. P., 1997. Effect of salicylic acid on black gram (*Vigna mungo*) cultivars. *Ind. J. Plant Physiol.* **2**: 138-141.
- Apte, P. V., and Laloraya, M. M., 1982. Inhibitory action of phenolic compounds on abscisic acid induced abscission. *J. Exp. Bot.*, **33**: 826-830.
- Arberg, B., 1981. Plant growth regulators. Monosubstituted benzoic acid. *Swed. Agric. Res.*, **11**: 93-105.
- Balke, N. E., and Schulz, M., 1987. Potential impact of enzymatic glucosidation of allelopathic phenolic compounds. In : Invited Lectures, Sec. 4: Industrial chemistry, 31st Int. Cong. Pure Appl. Chem. Pp. 17-29, Sophia, Bulgaria : Bulg. Acad. Sci.
- Billek, G., and Schmook, F. P., 1967. Zur biosynthese der gentisinaure. *Monatsch Chem.*, **98**: 1651-1664.
- Chadha, K. C., and Brown, S. A., 1974. Biosynthesis of phenolic acids in tomato plants infected with *Agrobacterium tumefaciens*. *Can. J. Bot.*, **52**: 2041-2046.
- Christianson, M. L., and Duffy, S. H., 2002. Dose-dependent effect of salicylates in a moss, *Funaria hygrometrica*. *J. Plant Growth Regul.*, **21**: 200-208.
- Cleland, C. F., and Ajami, A., 1974. Identification of a flower-inducing factor, isolated aphid honeydew as being salicylic acid. *Plant Physiol.*, **54**: 904-906.
- Cleland, C. F., and Tanaka, O., 1979. Effect of day length on the ability of salicylic acid to induce flowering in the long-day *Lema gibba* G₃ and the short-day plant *Lemma paucicostata* 6746. *Plant Physiol.*, **64**: 421-424.

- Eberhard, S., Doubrava, N., Marta, V., Mohnen, D., Southwick, A. *et al.* 1989, Pectic cell wall fragments regulate tobacco thin cell layer explant morphogenesis. *Plant Cell*, **1**: 747-755.
- El-Basyouni, S., Chen, D., Ibrahim, R., Neish, A.C., and Towers, G. H. N., 1964. The biosynthesis of hydroxybenzoic acids in higher plants. *Phytochem.* **3**: 485-492.
- Ellis, B. E., and Amichein, N., 1971. The "NH-shift" during aromatic orthohydroxylation in higher plants. *Phytochem.*, **10**: 3069-3072.
- Fariduddin, Q., Hayat, S., and Ahmad, A., 2003. Salicylic acid influences net photosynthetic rate, carboxylation efficiency, nitrate reductase activity and seed yield in *Brassica juncea*. *Photosynthetica*, **41**: 281-284.
- Fujioka, S., Yamaguchi, I., Murofushi, N., Takahashi, N., Kaihara, S., and Takimoto, A., 1983. The role of plant hormones and benzoic acid in flowering of *Lamium paucicostata* 151 and 381. *Plant Cell Physiol.*, **24**: 241-246.
- Gabriace, B., Werck-Reichhart, D., Teutsch, H., and Durst, F., 1991. Purification and immunocharacterization of a plant cytochrome P450: the cinnamic acid 4-hydroxylase. *Arch. Biochem. Biophys.*, **288**: 302-309.
- Gestetner, B., and Conn, E. E., 1974. The 2-hydroxylation of transcimannic acid by chloroplasts from *Melilotus alba* Desr. *Arch. Biochem. Biophys.* **163**: 617-624.
- Ghai, N., Setia, R.C., and Setia, N., 2002. Effects of paclobutrazol and salicylic acid on chlorophyll content, hill activity and yield components in *Brassica napus* L. (cv. GSL-1). *Phytomorphol.*, **52**: 83-87.
- Glass, A. D., 1973. Influence of phenolic acids on ion uptake. I. Inhibition of phosphate uptake. *Plant Physiol.*, **51**: 1037-1041.
- Glass, A. D., 1974. Influence of phenolic acids upon ion uptake. III. Inhibition of potassium absorption. *J. Exp. Bot.*, **25**: 1104-1113.
- Gordon, L. K., Minibayeva, F. V., Ogerodnikova, T. I., Rakhmatullina, D. F., Tzentzevitzky, A. N., Maksyntin, D. A., and Valitova, J. N., 2002. Salicylic acid induced dissipation of the proton gradient on the plant cell membrane. *Doklady Biol. Sci.*, **387**: 581-583.
- Goto, N., 1981. Enhancement of gibberellic acid by 5-bromodeoxyuridine, salicylic acid and benzoic acid on the flowering of *Arabidopsis thaliana*. *Arabidopsis Inf. Serv.*, **18**: 157-160.
- Griffiths, L. A., 1959. On the distribution of gentisic acid in green plant. *J. Exp. Bot.*, **10**: 437-442.
- Harper, J. P., and Balke, N. E., 1981. Characterization of the inhibition of K⁺ absorption in oat roots by salicylic acid. *Plant Physiol.*, **68**: 1349-1353.
- Hayat, S., Fariduddin, Q., Ali, B., and Ahmad, A., 2005. Effect of salicylic acid on growth and enzyme activities of wheat seedlings. *Acta Agron. Hung.*, **53**: 433-437.
- Ibrahim, R. K., and Towers, G. H. N., 1959. Conversion of salicylic acid to gentisic acid and o-pyrocatechuic acid, all labeled with carbon-14, in plants. *Nature*, **184**: 1803.
- Jain, A., and Srivastava, H. S., 1981. Effect of salicylic acid on nitrate reductase activity in maize seedlings. *Physiol. Plant.*, **51**: 339-342.
- Khan, W., Prithviraj, B., and Smith, D. L., 2003. Photosynthetic responses of corn and soybean to foliar application of salicylates. *J. Plant Physiol.* **160**: 485-492.
- Khodary, S. F. A., 2004. Effect of salicylic acid on the growth, photosynthesis and carbohydrate metabolism in salt stressed maize plants. *Int. J. Agric. Biol.*, **6**: 5-8.
- Khurana, J. P., and Maheshwari, S. C., 1980. Some effects of salicylic acid on growth and flowering in *Spirodela polyrrhiza* SP20. *Plant Cell Physiol.*, **21**: 923-927.
- Khurana, J. P., and Maheshwari, S. C., 1987. Floral induction in *Wolffia microscopica* by non-inductive long days. *Plant Cell Physiol.*, **24**: 907-912.

- Klambt, H. D., 1962. Conversion in plants of benzoic acid to salicylic acid and its β -glucoside. *Nature*, **196**: 491.
- Kumar, P., Dube, S. D., and Chauhan, V.S., 1999. Effect of salicylic acid on growth, development and some biochemical aspects of soybean (*Glycine max* L. Merrill). *Ind. J. Plant Physiol.*, **4**: 327-330.
- Kumar, P., Lakshmi, N.J., and Mani, V. P., 2000. Interactive effects of salicylic acid and phytohormones on photosynthesis and grain yield of soybean (*Glycine max* L. Merrill). *Physiol. Mol. Biol. Plants*. **6**: 179-186.
- Larqué-Saavedra, A., 1978. The anti-transpirant effect of acetylsalicylic acid on *Phaseolus vulgaris* L., *Physiol. Plant.*, **43**: 126-128.
- Larqué-Saavedra, A., 1979. Stomatal closure in response to acetylsalicylic acid treatment. *Z. Pflanzenphysiol.*, **93** (4): 371-375.
- Lee, T. T., and Skoog, F., 1965. Effect of substituted phenols on bud formation and growth of tobacco tissue culture. *Physiol. Plant.*, **18**: 386-402.
- Leslie, C. A., and Romani, R. J., 1988. Inhibition of ethylene biosynthesis by salicylic acid. *Plant Physiol.*, **88**: 833-837
- Macri, F., Kanello, A., and Pennazio, S., 1986. Salicylate-collapsed membrane potential in pea stem mitochondria. *Physiol. Plant.*, **67**: 136-140.
- Mitchell, A. G., and Broadhead, J. F., 1967. Hydrolysis of solubilized aspirine. *J. Pharm. Sci.*, **56**: 1261-1266.
- Moharekar, S. T., Lokhande, S. D., Hara, T., Tanaka, R., Tanaka, A., and Chavan, P.D., 2003. Effect of salicylic acid on chlorophyll and carotenoid contents of wheat and moong seedlings. *Photosynthetica*, **41**: 315-317.
- Nanda, K. K., Kumar, S., and Sood, V., 1976. Effect of gibberellic acid and some phenols on flowering of *Impatiens balsamina*, a qualitative short-day plant. *Physiol. Plant.*, **38**: 53-56.
- Oota, Y., 1972. The response of *Lemma gibba* G3 to a single long day in the presence of EDTA. *Plant Cell Physiol.*, **13**: 575-580.
- Oota, Y., 1975. Short-day flowering of *Lamma gibba* G3 induced by salicylic acid. *Plant Cell Physiol.*, **16**: 1131-1135.
- Pancheva, T. V., and Popova, L. P., 1998. Effect of salicylic acid on the synthesis of ribulose-1,5-biphosphate carboxylase/oxygenase in barley leaves. *J. Plant Physiol.*, **152**: 381-386.
- Pancheva, T. V., Popova, L. P., and Uzunova, A. M., 1996. Effect of salicylic acid on growth and photosynthesis in barley plants. *J. Plant Physiol.*, **149**: 57-63.
- Pitersen, A. H., 1982. A review of chemically induced flowering in *Lemma gibba* G3 and *Pistia stratiotes*. *Aquat. Bot.*, **13**: 21-28.
- Pitersen, A.H., and Muller, L. J., 1977. Induction of flowering in *Lemma gibba* G3 under short day conditions. *Plant Cell Physiol.*, **18**: 45-53.
- Popova, L., Pancheva, T., and Uzunova, A., 1997. Salicylic acid : Properties, biosynthesis and physiological role. *Bulg. J. Plant Physiol.*, **23**: 85-93.
- Rane, J., Lakshini, K. C., Kumar, P. A., and Abrol, Y. P., 1995. Salicylic acid protects nitrate reductase activity of wheat leaves. *Plant Physiol. Biochem.*, **22**: 119-121.
- Raskin, I., 1992a. Role of salicylic acid in plants. *Ann. Rev. Plant Physiol. Plant Mol. Biol.*, **43**: 439-463.
- Raskin, I., 1992b. Salicylate, a new plant hormone. *Plant Physiol.*, **99**: 799-803.
- Raskin, I., Skubatz, H., Tang, W., and Meeuse, B. J. D., 1990. Salicylic acid levels in thermogenic and nonthermogenic plants. *Ann. Bot.*, **66**: 376-383.
- Raskin, I., Turner, I. M., and Melander, W. R., 1989. Regulation of heat production in the inflorescences of an *Arum* lily by endogenous salicylic acid. *Proc. Natl. Acad. Sci. USA*, **86**: 2214-2218.

- Romani, R. J., Hess, V. M., and Leslie, C. A., 1989. Salicylic acid inhibition of ethylene production by apple discs and other plant tissues. *J. Plant Growth Regul.*, **8**: 62-69.
- Russell, D. W., and Conn, E. E., 1967. The cinnamic acid 4-hydroxylase of pea seedlings. *Arch. Biochem. Biophys.*, **122**: 256-258.
- Scharfettez, E., Rottenburg, T., and Kandeler, R., 1978. The effect of EDDHA and salicylic acid on flowering and vegetative development in *Spirodela punctata*. *Z. Pflanzenphysiol.* **87**: 445-454.
- Seth, P. N., Venkatarman, R., and Maheshwari, S. C., 1970. Studies on growth and flowering of a short-day plant, *Wolffia microscopica* III. Role of metal ions and chelates. *Planta*, **90**: 349-359.
- Silverman, P., Seskar, M., Kanter, D., Schweizer, P., Mettraux, J. P., and Raskin, I., 1995. Salicylic acid in rice, biosynthesis, conjugation and possible role. *Plant Physiol.*, **108**: 633-639.
- Singh, S. P., 1993. Effect of non-auxinic chemicals on root formation in some ornamental plant cuttings. *Adv. Hortic. For.*, **3**: 207-210.
- Sood, V., and Nanda, K., 1979. Effect of gibberellic acid and monophenols on the flowering of *Impatiens balsamina* in relation to the number of inductive and non-inductive photoperiodic cycles. *Physiol. Plant.*, **45**: 250-254.
- Tomot, B.K., Khurana, J.P., and Maheshwari, S.C., 1987. Obligate requirement of salicylic acid for short day induction of flowering in new duck weed, *Wolffia hyaline* 7378. *Plant Cell Physiol.*, **28**: 349-353.
- Van der Straeten, D., Chaerle, L., Sharkov, G., Lambers, H., and Van Montagere, M., 1995. Salicylic acid enhances the activity of the alternative pathway of respiration in tobacco leaves and induces thermogenesis. *Planta*, **196**: 421-419.
- Watanabe, K., Fujita, T., and Takimoto, A., 1981. Relationship between structure and flower inducing activity of benzoic acid derivatives in *Lemna paucicostata* 151. *Plant Cell Physiol.*, **20**: 847-850.
- Watanabe, K., and Takimoto, A., 1979. Flower inducing effect of benzoic acid and some related compounds in *Lemna paucicostata* 151. *Plant Cell Physiol.*, **22**: 1469-1479.
- Weissmann, G., 1991. Aspirine. *Sci. Am.*, **264**: 84-90.
- Yalpani, N., Leen, J., Lawthon, M. A., and Raskin, I., 1993. Pathway of salicylic acid biosynthesis in healthy and virus-inoculated tobacco. *Plant Physiol.*, **103**: 315-321.
- Yalpani, N., Schulz, M., Davies, M. P., and Balke, N. E., 1992. Partial purification of an inducible uridine-5'-diphosphate glucose : salicylic acid glucosyltransferase from oat roots. *Plant Physiol.*, **100**: 457-463.

Chapter 2

EFFECTS OF SALICYLIC ACID ON THE BIOPRODUCTIVITY OF PLANTS

ALFONSO LARQUÉ-SAAVEDRA AND RODOLFO MARTIN-MEX

Centro de Investigación Científica de Yucatán, A. C., Calle 43 No. 130, Chuburná de Hidalgo, Mérida, Yucatán, México CP 97200.

Abstract: Salicylic acid is a plant growth regulator that increases plant bioproductivity. Experiments carried out with ornamental or horticultural plants in greenhouse conditions or in the open have clearly demonstrated that they respond to this compound. Moreover, lower quantities of SA are needed to establish positive responses in the plants. The effect on ornamental plants is expressed as the increase in plant size, the number of flowers, leaf area and the early appearance of flowers. In horticultural species, the effect reported is the increase of yield without affecting the quality of the fruits. It is proposed that the increase in bioproductivity is mainly due to the positive effect of SA on root length and its density.

Key words: Salicylic acid, bioproductivity, root length, root density, earliness, yield, flowering.

1. INTRODUCTION

In the early seventies it was demonstrated that abscisic acid (ABA) was involved in controlling the water status of plants. Moreover, it was suggested that its presence could be linked to drought tolerance of plants (Larqué-Saavedra and Wain, 1974). Thereafter, in a series of experiments an attempt was made to demonstrate how the ABA production was inherited (Larqué-Saavedra and Wain, 1976). The induction to accumulate ABA by plant cells was then considered as a key question in this line of research, where it was proposed that receptors for drought may be localized in the membrane. A hypothetical model was set, in order to demonstrate this and compounds

such as prostaglandins and aspirin were tested. However, when the bioassays were conducted, it was detected that Aspirin affected the stomatal aperture of beans and *Commelina* plants (Larqué-Saavedra, 1978, 1979a, b). This unexpected effect was followed by a series of other assays that provided the fundamental basis to consider that salicylates affect the physiology of plants. Further studies were also carried out in these laboratories to study the salicylates and their relation to bioproductivity. In the literature, it was evident that the efforts were concentrated on learning the role of salicylic acid in systemic acquired resistance (SAR) to pathogens.

2. BIOPRODUCTIVITY

Bioproductivity of plants has been one of the main topics of agricultural sciences and different experts (geneticists, plant breeders, biotechnologists, plant nutritionists, plant physiologists, etc) have been trying to describe it. One of the main proposals suggests that the root system plays a key role in the productivity of any plant and salicylates were reported to favor the effect of indolacetic acid in rooting of mung beans (Basu *et al.*, 1969) and neem rootstocks (Mohinder *et al.*, 1992). In this respect in 1974, an observation was made in one of the bioassays where Aspirin favored the rooting of bean explants (Larqué-Saavedra *et al.*, 1975). Since then research work has focused on determining if salicylates could play a role in promoting better root systems in plants and if such an effect could give a better performance of the plant. However, it is important to mention that reproducible results were obtained by the applications of lower concentrations of salicylates.

2.1 Effect of salicylates in root system

Increasing the total soil volume explored by the root of the plant is one of the best ways to improve yield since more water and nutrients may be taken up by the plants. A second important aspect is to increase rooting density which occurs as a result of increased initiation of secondary roots. In experiments carried out with salicylic acid both aspects (root length and density) were found to be affected. Such studies did not include the resistance to water flow into the root, however, it is expected that a positive relationship may be observed.

Once it was observed that SA affected rooting, additional assays were conducted in order to determine the effect of SA on root growth and development. In a study carried out in 1996 it was reported that foliar application of SA to the shoots of soybean plants significantly affected the size of the root (Gutierrez-Coronado *et al.*, 1998; Figure 1). Further work

has reproduced similar findings with *Tagetes erecta*, using concentrations as low as 10^{-10} M solutions of SA (Sandoval-Yepiz, 2004; Figure 2).

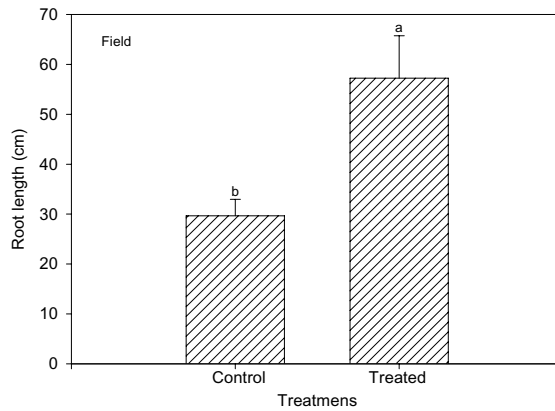


Figure 1. Effect of shoot applied salicylic acid (10^{-8} M) on root length of *Glycine max*, under field conditions, 7 days after the treatment.

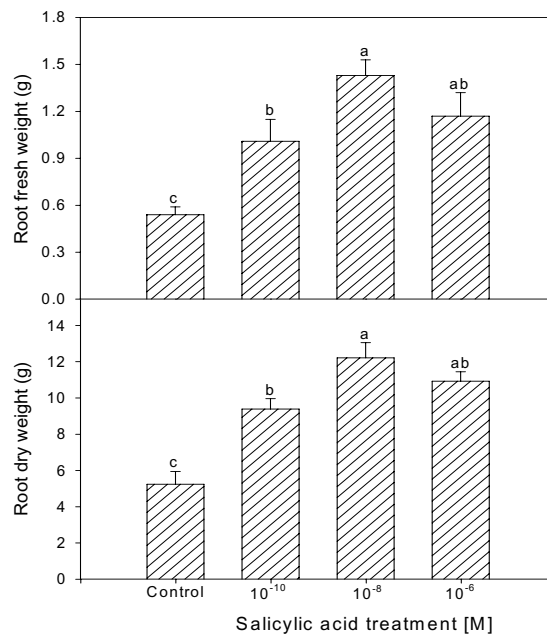


Figure 2. Effect of salicylic acid applications on the fresh and dry mass of *Tagetes erecta*.

2.2 Effect of salicylates on shoot growth

Application of salicylates to plants increased the shoot growth in different plant species such as *Clitoria* where the biomass production is important as forage for animal feed (Martin-Mex and Larqué-Saavedra, 2001). Similarly, when it was applied to ornamental plants the biomass always increased. Data showed that values for stem diameter, number of leaves, shoot fresh and dry mass, were higher, in a response to SA treatment.

In ornamental plants, such as gloxinia and violet, SA increased the number of leaves formed, and leaf area had values over 10% of that of the control. Similar values were recorded for the diameter of the rosette plant (Tables 1 and 2).

Table 1. Effect of salicylic acid on the growth of *Sinningia speciosa* 'Ultra' (gloxinia) Values are the mean of 25 replicate samples per treatment, \pm standard error.

Treatments	Number of leaves	Leaf area (cm ²)
Control	13 \pm 0.6a	456.4 \pm 3.0c
10 ⁻¹⁰ M AS	13 \pm 0.4a	496.2 \pm 2.3bc
10 ⁻⁸ M AS	13 \pm 0.6a	680.9 \pm 1.2a
10 ⁻⁶ M AS	14 \pm 0.4a	565.2 \pm 1.6b

Means with the same letter are not significantly different at $P \geq 0.05$, Tukey's

Table 2. Effect of salicylic acid on the growth of *Saintpaulia ionantha* (violet) Values are the mean of 20 replicate samples per treatment, \pm standard error.

Treatments	Number leaves	Rosette diameter (cm)
Control	16 \pm 1 b	139 \pm 16 b
10 ⁻¹⁰ M AS	19 \pm 2 a	177 \pm 12 a
10 ⁻⁸ M AS	18 \pm 3 ab	152 \pm 11ab
10 ⁻⁶ M AS	18 \pm 2 ab	156 \pm 10 a

Means with the same letter are not significantly different at $P \geq 0.05$, Tukey's

In a series of experiments run with *Tagetes erecta* cultivated in open conditions, it was found that beside the floral characters, the biomass of the shoot was significantly affected by the application of low concentration of SA (Sandoval-Yepiz, 2004; Figure 3).