

## BRIEF COMMUNICATION

**Salicylic acid influences net photosynthetic rate, carboxylation efficiency, nitrate reductase activity, and seed yield in *Brassica juncea***

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*Plant Physiology Section, Department of Botany, Aligarh Muslim University, Aligarh 202002, India***Abstract**

Aqueous solutions of salicylic acid (SA) were applied to the foliage of 30-d-old plants of mustard (*Brassica juncea* Czern & Coss cv. Varuna). The plants sprayed with the lowest used concentration ( $10^{-5}$  M) of SA were healthier than those sprayed with water only or with higher concentrations of SA ( $10^{-4}$  or  $10^{-3}$  M). 60-d-old plants possessed 8.4, 9.8, 9.3, 13.0 and 18.5 % larger dry mass, net photosynthetic rate, carboxylation efficiency, and activities of nitrate reductase and carbonic anhydrase over the control, respectively. Moreover, the number of pods and the seed yield increased by 13.7 and 8.4 % over the control.

*Additional key words:* carbonic anhydrase; chlorophyll; dry mass; pod and seed number.

Natural occurrence of salicylic acid (SA) has been detected in the leaves and reproductive organs of about 34 important species (Raskin *et al.* 1990) where it is recognised as the endogenous regulator of plant metabolism. However, its exogenous application to the plants generates diverse physiological effects, such as inhibition of dry mass accumulation (Schettel and Balke 1983), promotion of stomatal closure (Larque-Saavedra 1979), control of ion uptake and their transport (Harper and Balke 1981), and inhibition of ethylene synthesis (Leslie and Romani 1986). SA is involved in the initiation of an alternative respiratory pathway (Elthon *et al.* 1989) through the regulation of some specific nuclear gene, encoding the alternative oxidase protein in *Sauromatum guttatum* (Rhoads and McIntosh 1991). Moreover, SA checked the breakdown of ribulose-1,5-bisphosphate carboxylase/oxygenase, RuBPCO (Pancheva and Popova 1998). SA also affected Hill activity, kinetic of  $O_2$  evolution (Maslenkova and Toncheva 1998) in association with the changes in net photosynthetic rate ( $P_N$ ), and carotenoid and sugar contents (Chandra and Bhatt 1998). The growth of barley seedlings improved when the grains were pretreated with SA (Pancheva *et al.* 1996).

In certain aspects of plant responses, SA may be compared with other regulators, *e.g.* jasmonic acid and abscisic acid (Popova *et al.* 1987, 1988). Its involvement in the functioning of stomata, regulation of transpiration, respiratory pathway, and chlorophyll (Chl) contents (Khurana and Mahishwari 1980, Larque-Saavedra 1979, Elthon *et al.* 1989) tempted us to assume that SA should have regulatory role in other important physiological functions. With this goal in mind the mustard seedlings were assessed for  $P_N$  and the activities of carbonic anhydrase (CA) and nitrate reductase (NR) following SA treatment.

The seeds of *Brassica juncea* Czern & Coss cv. Varuna were purchased from *National Seed Corporation*, New Delhi, India. The healthy seeds were surface sterilised with 0.1 % mercuric chloride solution followed with repeated washings with double distilled water. The seeds were sown in earthen pots (25-cm diameter), lined with polythene sleeves and filled with acid washed sand (Hewitt 1966). Each pot was supplied with 200 cm<sup>3</sup> of full nutrient solution (Hoagland and Arnon 1950) on alternate days, up to day 30. Thereafter, the quantity of the nutrient solution was increased to 500 cm<sup>3</sup>. De-ionised water (250 cm<sup>3</sup>) was

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*Abbreviations:* CA – carbonic anhydrase; Chl – chlorophyll; CE – carboxylation efficiency; DAS – days after sowing; NR – nitrate reductase;  $P_N$  – net photosynthetic rate; SA – salicylic acid.

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also given daily, in the evening to each pot. 30-d after sowing (DAS), aqueous solutions of  $10^{-3}$ ,  $10^{-4}$ , and  $10^{-5}$  M SA were sprayed on the foliage. Control plants were sprayed with double distilled water. Each treatment was replicated five times and the experiment was conducted twice. Dry mass (DM) per plant (recorded after dehydration at  $80^{\circ}\text{C}$ ), leaf chlorophyll ( $a+b$ ) content (Chl), activities of CA and NR,  $P_N$ , and carboxylation efficiency (CE) were assessed in 60-d-old plants. The yield characteristics were recorded at harvest (140 DAS). Chl content was estimated following the method of Mackinney (1941). The procedures of Dwivedi and Randhawa (1974) and Jaworski (1971) were used for measuring the activities of CA and NR, respectively. CE was calculated by the formula given

by Tiwari *et al.* (1998).  $P_N$  in intact leaves was measured by using a LI-6200 portable photosynthetic system (LI-COR, Lincoln, NE, USA). The results of both the years were pooled and analysed statistically following the procedures described by Gomez and Gomez (1984).

The plants responded significantly to SA (Fig. 1, Table 1). The lowest concentration ( $10^{-5}$  M) of SA generated highest values for all the characteristics (DM, Chl, CA, NR,  $P_N$ , and CE) at the 60-d-stage, and the yield and its parameters at harvest. However, the values decreased as the concentration of SA was increased and reached below that of the control at maximum concentration ( $10^{-3}$  M).

Table 1. Effect of leaf-applied salicylic acid (SA) on dry mass (DM) per plant, chlorophyll (Chl) ( $a+b$ ) content, carboxylation efficiency (CE), and nitrate reductase (NR) activity in 60-d-old *Brassica juncea* plants, and on number of pods and seeds and seed yield at harvest. Different subscripts within column represent values significant at  $p = 0.05$ .

SA [M]	DM [g]	Chl [g kg <sup>-1</sup> ]	CE [mol m <sup>-2</sup> s <sup>-1</sup> ]	NR [nmol kg <sup>-1</sup> (leaf FM)]	Pod number	Seed number	Seed yield [g]
control	2.26 <sup>b</sup>	1.22 <sup>b</sup>	0.043 <sup>a</sup>	270.15 <sup>b</sup>	196.12 <sup>b</sup>	12.23 <sup>a</sup>	6.90 <sup>a</sup>
$10^{-5}$	2.45 <sup>a</sup>	1.46 <sup>a</sup>	0.047 <sup>a</sup>	305.35 <sup>a</sup>	223.15 <sup>a</sup>	12.25 <sup>a</sup>	7.48 <sup>a</sup>
$10^{-4}$	2.13 <sup>c</sup>	1.13 <sup>c</sup>	0.040 <sup>b</sup>	259.15 <sup>c</sup>	191.25 <sup>b</sup>	12.18 <sup>a</sup>	6.56 <sup>c</sup>
$10^{-3}$	1.98 <sup>c</sup>	1.02 <sup>d</sup>	0.038 <sup>bc</sup>	243.12 <sup>d</sup>	184.80 <sup>c</sup>	13.19 <sup>a</sup>	6.11 <sup>d</sup>

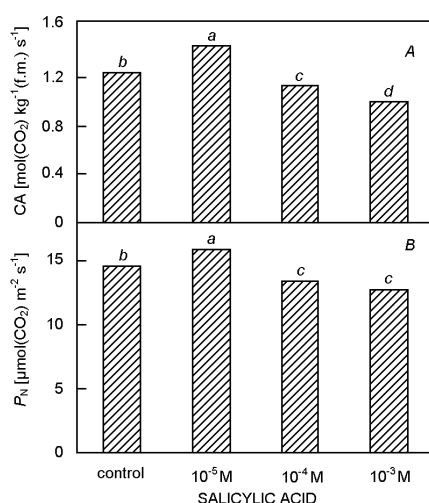


Fig. 1. Effect of leaf applied salicylic acid on carbonic anhydrase (CA) activity (A) and net photosynthetic rate ( $P_N$ ) (B) of 60-d-old *Brassica juncea* plants. Different subscript represents the difference significant at  $p = 0.05$ .

Phenolic compounds are characterised with chelating property (Clemetson and Anderson 1966), therefore, SA could have formed chelate(s) with some of the component(s) of the membrane of the cell/organelle facilitating the diffusion of certain substances. Alternatively, SA also prevents auxin oxidation (Schneider and Whitman 1974) whose elevated content increases  $P_N$  in leaf (Arteca 1997, Zhao *et al.* 1995).

The increased activity of NR by the dilute concentration ( $10^{-5}$  M) of SA could have either been an expression of the interaction of the acid with NR specific inhibitors whose presence is claimed by Srivastava (1980) and/or through the mediation of the other hormone(s). Auxins (IAA and Cl-IAA) are protected by phenols (Schneider and Whitman 1974) and elevate the activity of NR in pea and mustard (Ahmad 1988, 1994, Ahmad and Hayat 1999, Ahmad *et al.* 2001). Moreover, the content of any active protein (enzyme) represents a fine balance between its synthesis/activation and degradation/inactivation (Jain and Srivastava 1981). The concentration of SA might play an active role in such a regulation where the lower concentration favoured an increase in the NR protein and higher quantity of SA decreased it by affecting the above processes.

CA is the other most abundant soluble zinc containing protein (Lawlor 1987) in the chloroplasts of  $C_3$  plant, after RuBPCO (Okabe *et al.* 1984). CA facilitates the diffusion of  $\text{CO}_2$  across the chloroplast membrane by catalysing the hydration of dissolved  $\text{CO}_2$  as it enters the more alkaline environment of the stroma (Majeau and Coleman 1994) where CA also catalyses reversible hydration of  $\text{CO}_2$  and maintains a constant supply for RuBPCO. Otherwise, the rate of  $\text{CO}_2$  generation, from its reduced form  $\text{HCO}_3^-$ , will be relatively very slow that will naturally restrict the activity of RuBPCO at the ambient concentration of  $\text{CO}_2$ .

The content of the enzyme protein is under the regulation of transcription and/or translation (Okabe *et al.* 1980). However, either of these processes was favoured by the lower concentration of SA that increased the activity of CA (Fig. 1). Higher CA activity was significantly correlated (+0.994) with  $P_N$  that is also supported by Ohki (1978) and Hayat *et al.* (2000).

The plants treated with low concentration ( $10^{-5}$  M) of SA acquired higher  $P_N$ , which was naturally reflected in the form of healthy growth of the plants and

increased dry mass production. These healthy plants will naturally bear strong inflorescence axis with more pods per plant (Table 1 and Ghai *et al.* 2002). It may also be an expression of the involvement of phenols in the mobilisation of photosynthates to the sink (Nanda *et al.* 1976). The higher concentration of SA made a permanent change at the level of membrane organisation of the cells that proved injurious for plant metabolism and growth (Table 1 and Uzunova and Popova 2000).

## References

- Ahmad, A.: Nitrate accumulation and nitrate reductase activity during rooting of pea cuttings treated with auxins. – Indian J. exp. Biol. **26**: 470-472, 1988.
- Ahmad, A.: Shoot apex as a source of auxin for nitrate uptake and activity of nitrate reductase in pea cuttings. – Indian J. exp. Biol. **32**: 65-67, 1994.
- Ahmad, A., Hayat, S.: Response of nitrate reductase to substituted indole acetic acids in pea seedlings. – In: Srivastava, G.C., Singh, K., Pal, M. (ed.): Plant Physiology for Sustainable Agriculture. Pp. 252-259. Pointer Publishers, Jaipur 1999.
- Ahmad, A., Hayat, S., Fariduddin, Q., Ahmad, I.: Photosynthetic efficiency of plants of *Brassica juncea*, treated with chlorosubstituted auxins. – Photosynthetica **39**: 565-568, 2001.
- Arteca, R.N.: Plant Growth Substances: Principles and Applications. – CBS Publishers and Distributors, New Delhi 1997.
- Chandra, A., Bhatt, R.K.: Biochemical and physiological response to salicylic acid in relation to the systemic acquired resistance. – Photosynthetica **35**: 255-258, 1998.
- Clemetson, C.A.B., Anderson, L.: Plant polyphenols as antioxidants for ascorbic acid. – Ann. New York Acad. Sci. **136**: 339-378, 1966.
- Dwivedi, R.S., Randhawa, N.S.: Evaluation of a rapid test for the hidden hunger of zinc in plants. – Plant Soil **40**: 445-451, 1974.
- Elthon, T.E., Nickels, R.L., McIntosh, L.: Mitochondrial events during the development of thermogenesis in *Sauromatum guttatum*. – Planta **180**: 82-89, 1989.
- Ghai, N., Setia, R.C., Setia, N.: Effects of paclobutrazol and salicylic acid on chlorophyll content, Hill activity and yield components in *Brassica napus* L. – Phytomorphology **52**: 83-87, 2002.
- Gomez, K.A., Gomez, A.A.: Statistical Procedures for Agricultural Research. – John Wiley & Sons, New York 1984.
- Harper, J.R., Balke, N.E.: Characterization of the inhibition of  $K^+$  absorption in oats roots by salicylic acid. – Plant Physiol. **68**: 1349-1353, 1981.
- Hayat, S., Ahmad, A., Mobin, M., Hussain, A., Fariduddin, Q.: Photosynthetic rate, growth and yield of mustard plants sprayed with 28-homobrassinolide. – Photosynthetica **38**: 469-471, 2000.
- Hewitt, E.J.: Sand and water culture methods used in the study of plant nutrition. – Commonwealth Agricultural Bureaux, Farnham Royal 1966.
- Hoagland, D.R., Arnon, D.I.: The water culture method for growing plants without soil. – Univ. California Exp. Stat. Circular 347, 1950.
- Jain, A., Srivastava, H.S.: Effect of salicylic acid on nitrate reductase activity in maize seedlings. – Physiol. Plant. **51**: 339-342, 1981.
- Jaworski, E.G.: Nitrate reductase assay in intact plant tissue. – Biochem. biophys. Res. Commun. **43**: 1274-1279, 1971.
- Khurana, J.P., Maheshwari, S.C.: Some effects of salicylic acid on growth and flowering in *Spirodela polyrrhiza* SP<sub>20</sub>. – Plant Cell Physiol. **21**: 923-927, 1980.
- Larque-Saavedra, A.: Stomatal closure in response to acetyl-salicylic acid treatments. – Z. Pflanzenphysiol. **93**: 371-375, 1979.
- Lawlor, D.W.: The chemistry of photosynthesis. – In: Lawlor, D.W. (ed.): Photosynthesis: Metabolism, Control and Physiology. Pp. 127-157. Longman Publishers, Singapore 1987.
- Leslie, C., Romani, R.: Salicylic acid: a new inhibitor of ethylene biosynthesis. – Plant Cell Rep. **5**: 144-146, 1986.
- Mackinney, G.: Absorption of light by chlorophyll solutions. – J. biol. Chem. **140**: 315-322, 1941.
- Majeau, N., Coleman, J.R.: Correlation of carbonic anhydrase and ribulose-1,5-bisphosphate carboxylase/oxygenase expression in pea. – Plant Physiol. **104**: 1393-1399, 1994.
- Maslenkova, L., Toncheva, S.: Salicylic acid induced changes in photosystem II reduction in barley plants. – Proc. bulg. Acad. Sci. **51**: 101-104, 1998.
- Nanda, K.K., Kumar, S., Sood, V.: Effect of gibberellic acid and some phenols on flowering of *Impatiens balsamina*, a quantitative short day plant. – Physiol. Plant. **38**: 53-56, 1976.
- Ohki, K.: Zinc concentration in soybean as related to growth, photosynthesis and carbonic anhydrase activity. – Crop Sci. **18**: 79-82, 1978.
- Okabe, K., Lindlar, A., Tsuzuki, M., Miyachi, S.: Effects of carbonic anhydrase on ribulose 1,5-bisphosphate carboxylase and oxygenase. – FEBS Lett. **114**: 142-144, 1980.
- Okabe, K., Yang, S.Y., Tsuzuki, M., Miyachi, S.: Carbonic anhydrase: its content in spinach leaves and its taxonomic diversity studied with anti-spinach leaf carbonic anhydrase antibody. – Plant Sci. Lett. **33**: 145-153, 1984.
- Pancheva, T.V., Popova, L.P.: Effect of salicylic acid on the synthesis of ribulose 1,5-bisphosphate carboxylase/oxygenase in barley leaves. – Plant Physiol. **152**: 381-386, 1998.
- Pancheva, T.V., Popova, L.P., Uzunova, A.N.: Effects of

- salicylic acid on growth and photosynthesis in barley plants. – *J. Plant Physiol.* **149**: 57-63, 1996.
- Popova, L.P., Tsonev, T.D., Vaklinova, S.G.: A possible role for abscisic acid in regulation of photosynthetic and photorespiratory carbon metabolism in barley leaves. – *Plant Physiol.* **83**: 820-824, 1987.
- Popova, L.P., Tsonev, T.D., Vaklinova, S.G.: Changes in some photosynthetic and photorespiratory properties in barley leaves after treatment with jasmonic acid. – *J. Plant Physiol.* **132**: 257-261, 1988.
- Raskin, I., Skubatz, Z., Tang, W., Meeuse, B.J.D.: Salicylic acid levels in thermogenic and non-thermogenic plants. – *Ann. Bot.* **66**: 369-373, 1990.
- Rhoads, D.N., McIntosh, L.: Isolation and characterization of a cDNA clone encoding an alternative oxidase protein in *Sauromatum guttatum*. – *Proc. nat. Acad. Sci. USA* **88**: 2122-2126, 1991.
- Schettel, N.L., Balke, N.E.: Plant growth response to several allelopathic chemicals. – *Weed Sci.* **31**: 293-298, 1983.
- Schneider, E.A., Whitman, F.: Metabolism of auxin in higher plants. – *Annu. Rev. Plant Physiol.* **25**: 487-513, 1974.
- Srivastava, H.S.: Regulation of nitrate reductase activity in higher plants. – *Phytochemistry* **19**: 725-733, 1980.
- Tiwari, H.S., Agarwal, R.M., Bhatt, P.K.: Photosynthesis, stomatal resistance and related characteristics as influenced by potassium under normal water supply and water stress condition in rice. – *Indian J. Plant Physiol.* **3**: 314-316, 1998.
- Uzunova, A.N., Popova, L.P.: Effect of salicylic acid on leaf anatomy and chloroplast ultrastructure of barley plants. – *Photosynthetica* **38**: 243-250, 2000.
- Zhao, H., Lin, X., Shi, A., Chang, S.: The regulating effects of phenolic compounds on the physiological characteristics and yield of soybeans. – *Acta agron. sin.* **21**: 351-355, 1995.