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STUDIES REGARDING THE EFFECTS OF SALICYLIC ACID ON MAIZE (*ZEA MAYS* L.) SEEDLING UNDER SALT STRESS

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ABSTRACT. Abiotic stresses such as heavy metals, salinity, drought, temperature, UV-radiation, ozone, cause drastic yield reduction in most crops. Plants have complex and dynamic systems of response to stress. Salt stress affects around 20% of the world's cultivated areas. Salicylic acid (SA) plays an important role in response to biotic and abiotic stress. Pre-treatment of maize seeds with SA may cause a low level of oxidative stress, improving the antioxidative capacity of the plants. Salicylic acid can increase the plant tolerance to salt stress induced in our experiment by 150 mM NaCl treatments. In our experiment we determined the effect of pretreatment of maize seeds with 0.5 mM concentration of salicylic acid (SA) solution on growth, peroxidase activity, assimilatory pigments and protein content of the 3thd leaves of maize seedlings under salt stress. The results obtained showed that exogenous application of SA induced an increase in growth parameters of maize seedlings and the peroxidase activity in the roots of maize seedlings was lower than in untreated plants. The treatment with SA ameliorates the total clorophyllian pigment content and the content of total soluble protein in maize seedling leaves under salt stress.

Keywords: maize, salicylic acid, growth, peroxidase activity, protein content, assimilatory pigments

INTRODUCTION

Salicylic acid play an important role in the plant response to adverse environmental conditions such as low temperature stress (Janda et al., 1999; Tasgin et al, 2003), salt and osmotic stress (Senaratna et al., 2000, Borsani et al., 2001). Abiotic stresses such as, heavy metals, drought, temperature, UV-radiation, ozone, cause drastic yield reduction in most plants crops (Nafees and Sarvajeet, 2007).

Salicylic acid belongs to a diverse group of plant phenolics, is a natural signaling molecule involved in the regulation of different physiological and biochemical processes, including membrane permeability (Barkosky and Einhellig, 1993), ion uptake, enzymes activities, photosynthesis (Hayat et al, 2005), growth and development of plants (Hayat and Ahmad, 2007), and may function as a plant growth regulator (Arberg, 1981). Quiroz-Figueroa et al., in 2001 suggested that it is possible as these phenolic compounds act as a signal, which induce the differentiation processes. An explanation is the possibility that, due to the chelating properties of these compounds, some inhibitors present in the embryogenic cultures are inactivated.

The application of salicylic acid, acetylsalicylic acid or other analogues of salicylic acid, to leaves of corn and soybean accelerated their leaf area and dry mass production, but plant height and root length remained unaffected. However the leaves of corn and soybean treated with acetylsalicylic acid or gentisic acid exhibited no change in their chlorophyll contents (Khan et al., 2003).

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 also the ratio of chlorophyll a/b, in wheat plantlets (Moharekar et al., 2003).

The soaking of wheat (*Triticum aestivum* L.) seeds in 0.05mM SA also reduced the damaging effects of salinity on seedlings growth and accelerated the growth processes (Shakirova et al., 2003).

Salicylic acid pre-treatment also provided protection against salinity in tomato plants, probably due to the increased activation of aldose reductase and APx enzymes and the accumulation of osmolytes, such as sugar, sugar alcohol or proline (Tari et al., 2002; Tari et al., 2004; Szepesi et al., 2005).

Deef, 2007, demonstrated that the application of exogenous SA enhanced the drought and salt stress resistance of plants. During the germination period a considerable increase was observed in proline levels (up to 185% in *T. aestivum* and about 128% in H. vulgare) in the seedlings subjected to saline stress.

Purcarea and Cachita (2008 a, b) studying the influence of SA and acetylsalycilic acid (ASA) on the growth of sunflower (Helianthus sp.), seedling roots, on their total absorbtion capacity and on content of assimilatory pigments, in their primary leaves, observed that on the 6-th day of germination the diluted ASA solutions, with concentrations of 0.01, 0.1 and 0.5 mM had greater effects on the growth and determined the highest increase of the total absorption capacity of sunflower root system, with 159.6% compared to the control group being recorded in case of the treatment with 0.1 mM ASA solution. The diluted ASA solution, with 0.01 mM the 0.1 mM concentration, determined an increase in the total chlorophyllian and carotenoid pigments content in the primary leaves of sunflower plantlets especially for 0.01 mM the 0.1 mM concentration. Higher

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concentrations than 0.5 mM decreased the same parameter, the greatest inhibitions being obtained for the SA or ASA solutions of a 5.0 mM concentration.

The aim of this study was to determine the effect of pretreatement of maize caryiopsis with 0.5 mM concentration SA solution on growth, peroxidase activity, assimilatory pigments and total soluble protein content of the 3thd leaves of maize seedlings stressed by NaCl solution.

For the future we will continue to study the role of SA and its derivatives, like ASA in the response of the plants to other abiotic stress factors or on other agricultural plant.

MATERIALS AND METHODS

Sample preparation

Surface sterilized maize seeds (*Zea mays* L) were soaked for 24 h in water or in 0.5 mM SA.

The germination was made in plastic recipients, on a filter paper, moistened with 20 ml treatment solution:

- Control group (C) – 24 h soaked in water and germination in water.
- Sample 1 (S_1) – 24 h soaked in water and germination in 150 mM NaCl solution;
- Sample 2 (S_2) – 24 h soaked in salicylic acid and germination in 150 mM NaCl solution.

Each recipient contained 20 seeds. The germination was made at room temperature. Every day, the quantity of solutions from the recipients was brought to the level of 20 ml. The germination temperature was around 20-23˚C.

Plant growth measurement

For the biometrical determination, in the 3, 5 and 7 days of germination we measured the length of the roots and shoots of 10 maize seedlings. In addition the plant growth was estimated by measuring of root and shoot weight, after drying the plants material at 70°C for 72 h.

Preparation of protein and enzyme extract

0.5g fresh sample (roots – for peroxidase and leaves for protein) were collected from each variant in the 6th day of germination, and were blended with 8 ml phosphate buffer solution, pH 7.0, diluted 1:9 with distilled water, cooled at 4˚C. The samples were centrifuged at 15,000 g, for 20 minutes at 4˚C, and supernatant was separated. The extract is kept in the refrigerator, for 2 hours for stabilizing and expressing enzyme activity.

Peroxidase activity determination

Peroxidase activity (POX) was determined at 483 nm wavelength, with Shimadzu-UV-mini-1240 spectrophotometer, using phosphate buffer of 7.0 pH, hydrogen peroxide as substrate and p-phenilendiamine as chromogen (Hans-Luck, 1970). The extension for each sample was noted after 30 seconds of reaction period.

Determination of total soluble protein

The protein concentration from the 3rd leaves of maize seedlings was determined as described by Bradford (1976), using bovine albumin serum (BSA) as standard. The extract obtained from fresh leaves were used for spectrophotometric determination of protein at λ=540 nm (Shimadzu UV-Visible mini-1240).

Assimilatory pigments

After 7 days of germination we planted the seedlings in sand, leaving them there for an additional 7 days, and sprayed their primary leaves each day with 1 ml of water.

On the 14th day we determined the content of chlorophyllian pigments of the sunflower plantlets primary leaves, using *N,N-dimethylformamide* 99.9%, (Moran and Porath, 1980) for the extraction. The extraction of assimilatory pigments in higher plant tissue using *N,N-dimethylformamide* (DMF), expedites the process and enables the determination of small samples with low pigment level (Moran, 1982). There is a vast array of solvents used for the extraction and determination of the chlorophyllian pigments, but most of them necessitate grinding and centrifuging of material with or without heating. The use of DMF renders the process simpler and faster, since the pigments can be extracted from intact tissue. For extraction, 50 mg fresh weight of primary leaves, were collected separately from each sample, and were blended with 5ml DMF and then cooled at 4˚C for 72 hours. The supernatant was separated and the content of the pigment was determined using a UV-visible mini–1240 Shimadzu spectrophotometer, at 664nm wave length for chlorophyll a, 647 nm for chlorophyll b and 480 nm for carotenoids.

The data obtained after the spectrophotometrycal determination, was mathematically processed using formulae proposed by Moran and Porath (1980).

$$\text{Chlorophyll a (mg/g sp)} = (11.65\ a_{664} - 2.69\ a_{647}) \cdot V/sp$$

$$\text{Chlorophyll b (mg/g sp)} = (20.81\ a_{647} - 4.53\ a_{664}) \cdot V/sp$$

$$\text{Carotenoids (mg/g sp)} = (1000\ A_{480} - 1.28\ \text{chloroph.a} - 56.7\ \text{chloroph.b})/\ 245 \cdot V/sp$$

The results obtained for all parameters are averages of 3 determinations and were statistically processed by the "t- test" using *Prisma 5 for Windows*. The values of the probabilities were determined from tables using the values of the "t" distribution and the freedom degrees based on which the variance of the empiric series was calculated.

RESULTS AND DISCUSSIONS

Plant growth

Studying the length of the maize seedlings obtained from the germination of the maize seeds under laboratory conditions, after 3; 5 and 7 days of

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germination, we observed that the salt treatments significantly reduces growth in length (between 15.2 and 17%, in comparison with the control group), and the dry weight content of the maize plantlets was very significantly reduced, between 17.3 and 22.3%, as compared with the control group). In case of the seeds pre-treated with 0.5 mM SA solution, the negative effect of salt stress was reduced therefore the growth in length was insignificantly reduced in comparison with the control group (1.82% and 3.6%, as compared with the control group). Dry weight was significantly reduced (between 2.35 and 5.21%) (Table 1 and figure 1). We can observe that treatment with 0.5 mM SA solution ameliorate growth parameters of maize seedlings.

Table 1

Effect of Salicylic acid pretreatment on the length (mm) and on dry weight (DW %) of maize (*Zea mays L.*) seedling (root and shoot)

Day of germination	Parameters	C (Control group)	S_1 (salt)	S_2 (SA + salt)
3	Seedling length (mm)	55.3± 1.3	46.3± 1.1 ***	53.3± 1.9 ns
	DW %	8.5± 0.1	6.6 ± 0.2 ***	8.3 ± 0.2 *
5	Seedling length (mm)	147.7 ± 2.2	123.2± 1.7 ***	144.2 ± 2.1 ns
	DW %	11.5 ± 0.2	9.4 ± 0.1 ***	12.1 ± 0.2 *
7	Seedling length (mm)	181.5 ± 2.1	153.9 ± 1.6 ***	178.2 ± 1.5 ns
	DW %	12.7 ± 0.1	10.5 ± 0.1 ***	13.1 ± 0.3 *

*p>0,05=non-significant; p<0,05 * significant; p<0,01=** distinctly significant; p<0,001=*** very significant in comparison with control group*

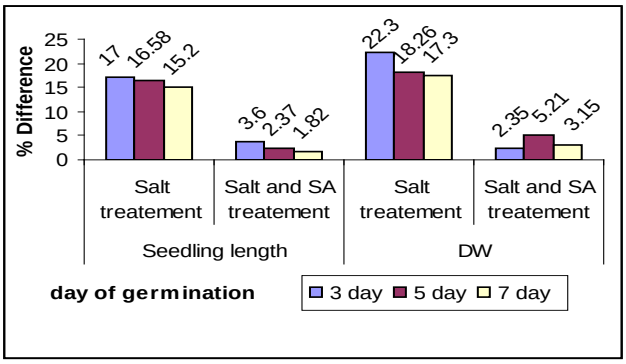

Fig. 1 Percentage differences which reflect the effect of SA pretreatment on the length (mm) and on dry weight (DW %) of maize (*Zea mays* L.) seedling (root and shoot) under salt stress condition, treated or untreated with SA as compared with the control group

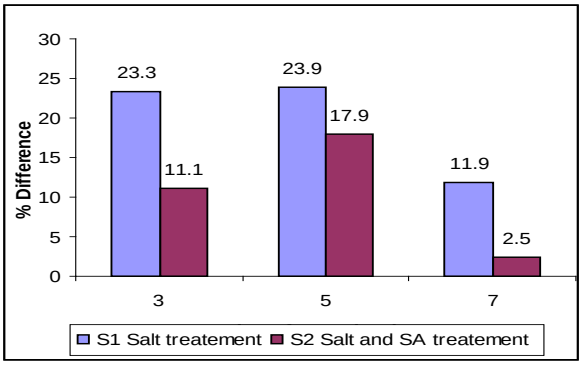

Fig. 2 Percentage differences which reflect the effect of SA pretreatment on the peroxidase activity in the roots of maize seedlings under salt stress condition, treated or untreated with SA, as compared with the control group

Peroxidase activity determination

In the case of samples treated with a 150 mM NaCl solution, there was a very significant increase of the peroxidase activity (between 11.95 and 23.91%, in comparison with the control group), in the roots of maize plantlets.

Pre-soaking the seeds for 24 h in a 0.5 mM SA solution significantly reduced the increase of the peroxidase activity in the 3rd, 5th and 7th days of germination (between 2.5 and 11.1%, in comparison with the control group) (Table 2, fig 2).

Salicylic acid pre-treatment ameliorate the peroxidase activity under salt stress.

Total soluble protein content

The standard curve of bovine albumin serum (BSA) stock solution (100mg/ml), is shown in fig. 3A.

In all the samples the content of soluble protein was increased. In case of salt treatment the increase of total soluble protein was non-significant. The highest value has obtained in the case of salt treatment after pre-treatment with salicylic acid (fig.2B). The treatment with SA solution improved the total amino acids profile (essential and non-essential) in corn leaves Hussein et al., (2007), and in that way increased the total soluble protein content also. Amino acids like glycin, betaine or proline have a protective role against stress condition.

Assimilatory pigments content

Studying the content of chlorophyllian pigment (chlorophyll a and b) and carotenoids on the 3rd leaves of the maize seedlings obtained from each experimental variant, we observed that the aplication of exogenous 0.5 mM SA solution ameliorate the effect of salt stress. The results obtained were presented in table 3 and fig. 4.

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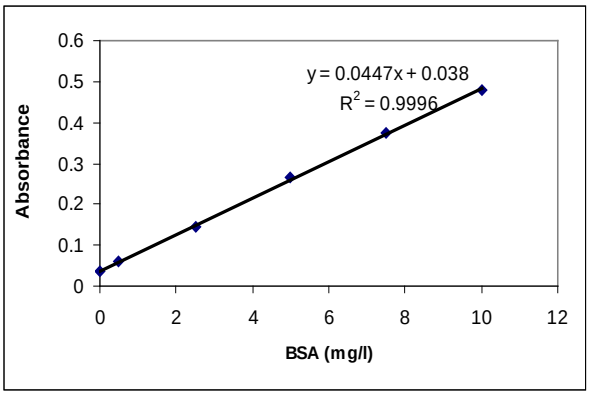
Table 2

Effect of Salicylic acid pretreatment on the peroxidase activity in maize (*Zea mays* L.) seedling roots

Day of germination	Extinction of enzymes extract		
	C (Control group)	S_1 (salt)	S_2 (SA + salt)
3	0.938 ± 0.04	1.157 ± 0.03 ***	1.042 ± 0.004 *
5	1.037 ± 0.02	1.285 ± 0.035 ***	1.106 ± 0.02 *
7	1.188 ± 0.02	1.321± 0.02 ***	1.218 ± 0.017 ns

*p>0,05= non-significant; p<0,05 * significant; p<0,01=** distinctly significant; p<0,001=*** very significant in comparison with control group*

A.



B.

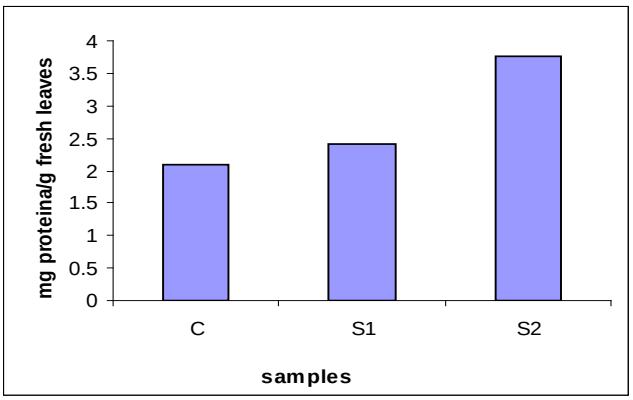


Fig. 3: A) Standard curve of BSA using Bradford assay; B) Variation of soluble protein contents of maize (*Zea mays* L.) seedlings under salt stress condition, treated or untreated with SA, as compared with the control group (C = control group; S1=salt treatment; S2=salt +SA treatment)

Table 3

The level of the assimilatory pigments content of maize (Zea mays L.) seedling leaves under salt stress condition, treated or untreated with Salicylic acid, as compared with the control group

Samples	Chlorophyllian pigments (mg/g)				Carotenoid pigments (mg/g)
	Clorophyll a	Clorophyll b	Total chlorophyllian pigments mg/g	Clorophyll a / b	
C	2.03	0.97	3.0	2.1	0.323
S1	1.42	1.28	2.7	1.1	0.193
S2	1.78	1.03	2.81	1.72	0.281

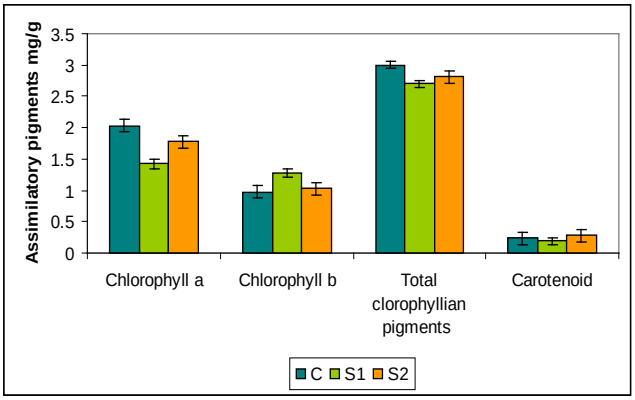


Fig. 4 Assimilatory pigments content estimative mean value and standard deviation under salt stress condition, treated or untreated with SA, as compared with the control group

For the samples treated with a 150 mM NaCl solution, we determined a significant decrease of the chlorophyll a pigments quantity (Table 3), while chlorophyll b registered an increase compared with the control sample. A decrease in the proportion of clorophyll a/b, indicates senescence, poor plant growth or stress condition (Ryang, 2009).

In the case of NaCl treatment, the total quantity of chlorophyllian pigments was 2.7 mg/g leaves, comparatively much less than in control sample or in case of pre-treatment with 0.5 mM SA. Similar values were recorded regarding carotenoid pigments. The recorded values were 0.323 mg/g in the control sample; 0.193mg/g in the case of NaCl treatment, respectively 0.281 in case of pre-treatment with 0.5 mM SA.

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CONCLUSIONS

The analysis of the results obtained in this study shows that salt induced stress inhibits the growth parameters in 3, 5 and 7 days of germination in maize (*Zea mays* L.) plantlets and determined an increase in peroxidase activity, in comparison with the control group.

Exogenous applications of 0.5 mM SA solution induced an increase in growth parameters, in comparison with the untreated samples. The peroxidase activity in the roots of maize seedling was significantly lower than in SA untreated plantlets.

The treatment with 0.5 mM SA significantly increased the total soluble protein.

According to obtained results, there is a clear correlation between the treatment applied and the content of clorophyllian pigments in the leaves. The content of chlorophyll a decreased in case of salt stress, while the chlorophyll b increases compared with the control sample. The SA treatment ameliorates the chlorophyllian pigment contents.

The content of carotenoid pigments also decreases because of exposure to salt stress; therefore the pre-sowing treatment in SA solution increased these pigment contents.

Salicylic acid application as a 24 hours pre-sowing seed treatment enhanced salt tolerance in the studied maize plants.

REFERENCES

Arberg B. – Plant growth regulators. Monosubstituted benzoic acid, ,Sweed. Agric.Res., 11, p. 93-105, 1981.

Barkosky RR., Einhellig FA., Effects of salicylic acid on plant water relationship, Journal of Chemical Ecology, 19, p 237-247, 1993.

Borsani O.,Valpuesta V., Botlleb M.A., Evidence for a role of salicylic acid in the oxidative damage generated by NaCl and osmotic stress in Arabidobsis seedlings. Plant Physiology 126, p.1024-1030, 2001.

Bradford M.M., -A rapid and sensitive method for the quantitation of microgram quantities of protein-dye binding, Anal. Biochem. 72, p.248-254, 1976.

Deef H.E., - Influence of Salicylic acid on stress tolerance during seed germination of Triticum aestivum and Hordeum vulgare, Advances in Biological Research 1 (1-2): p.40-48, 2007.

Hans-Luck, Methdos of Enzymatic Analysis Verlag Chemie Gmb H. (Ed).Weinheim Bergstr. Academic Press, New york and London.p.885-888, 1970.

Hayat S., Ahmad A., Salicylic acid: a plant hormone, Springer (ed) dortrecht, the Netherlands, 2007.

Hayat S., Fariduddin Q., Ali, B., and Ahmad A., Effects of salicylic acid on growth and enzymes activities of wheat caryopsislings, Acta.agron.hung., 53, p.433-437, 2005.

Hussein M.M., Balbaa L.K., and Gaballah M.S., Salicylic Acid and Salinity Effects on Growth of Maize Plants;
Research Journal of Agriculture and Biological Sciences, 3(4), p. 321-328, 2007.

Janda T., Szalai G., Tari I., Páldi E., (1999) - Hydroponic treatment with salicylic acid decreases the effect of chilling injury in maize (Zea mays) plants. Planta 208, p.175-180.

Khan W., Prithiviraj B, Smith DL, Photosynthetic responses of corn and soybean to foliar application of salicylates, Journal of plant physiology, 160, p.485-492, 2003.

Moharekar ST., Lokhande SD, Hara, T., Tanaka, R., Tanaka, A., and Chavan PD., Effect of salicylic acid on clorophyll and carotenoid contents of wheat and moong caryopsislings, Photosynthetica, 41,p. 315-317, 2003.

Moran R, Porath D, Clorophyll determination in intact tissue using N,N- dimethylformamide, Plant physiol, 65, p.478-479, 1980.

Moran R., Formulae for determination of chlorophyllous pigments extracted with N,N-dimethylformamide, Plant physiol. 69 (6), p.1376-1381, 1982.

Nafees K. A.; S., Sarvajeet, 2007, Abiotic Stress and Plant Responses, New Delhi, India: Narosa Publishing House.

Purcărea C., Cachiţă-Cosma D., - Comparative studies about the influence of salicylic and acetylsalicilic acid on content of assimilatory pigments in the primary leaves of sunflower (Helianthus sp.) plantlets. Arad, May 2008, "Studia Universitatis Vasile Goldis, Arad", Seria Stiinţele vieţii, vol.18, ISSN: 1584-2363, pp. p. 51-54, 2008. (a)

Purcărea C., Cachiţă-Cosma D., - The influence of salicylic acid and acetylsalicylic acid on the growth of sunflower (Helianthus sp.) seedling roots and on their total absorption capacity. – Arad, May 2008, "Studia Universitatis Vasile Goldis, Arad", Seria Stiinţele vieţii, vol.18. ISSN: 1584-2363, p. 55-60, 2008 (b).

Quiroz-Figueroa F., Mendez-Zeel M., Larque-Saavedra. Loyola-Vargas, VM., Picomolar salicylate levels enhance cell growth and embryogenesis, Plant cell reports 20, p 679-684, 2001.

Ryang S.Z., Woo S.Y., Kwon S.Y., Kim S.H., Lee S.H., Kim K.N., Lee D.K., Changes of net photosynthesis, antioxidant enzyme activities, and antioxidant contents of Liriodendron tulipifera under elevated ozone. Photosynthetica. 47, p.19-25.2009.

Shakirova F.M., Sakhabudinova A.R., Bezrukova M.V., Fakhutdinova R. A., Fakhutdinova D.R.– Changes in the hormonal status of wheat seedlings induced by salicylic acid and salinity. Plant. Sci. 164, p.317-322, 2003.

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Senaratna, T., D. Tuochell, T. Bunn and K. Dixon. Acetylsalicylic acid (aspirin) and Salicylic acid induce multiple
stress tolerance in bean and tomato plants. Plant Growth Regul., 30, p.157-161. 2000.

Szepesi, Á., J. Csiszár, S. Bajkán, K. Gémes and F. Horvath. Role of Salicylic acid pre-treatment on the acclimation of tomato plants to salt- and osmotic stress. Acta Biologica Szegediensis, 49 p.123-125, 2005

Tari, I., J. Csiszár, G. Szalai, F. Horváth, A. Pécsváradi, G. Kiss, Á. Szepesi, M. Szabó and L. Erdei. Acclimation of tomato plants to salinity stress after a Salicylic acid pre-treatement, Acta Biol. Szeged, 46, p.55-56, 2002.

Tari, I., Simon L.M., Deer K.J., . Csiszár, J., Bajkan Sz., Kis Gy., and Szepesi, Á. Influence of salicylic acid on salt stress acclimation of tomato plants: oxidative stress response and osmotic adaptation. Acta Physiol. Plant, p.268:237, 2004.

Tasgin E., Atici O., Nalbantoglu B. – Effects of salicylic acid and cold on freezing tolerance in winter wheat leaves. Plant Growth Regul. 41, p. 231-236, 2003.

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