EXOGENOUS SALICYLIC ACID INVOLVEMENT ON SOME PHYSIOLOGICAL PARAMETERS AMELIORATION IN SALT STRESSED WHEAT (*TRITICUM AESTIVUM* cv.CRISANA) PLANTLETS

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Abstract
Abiotic stress causes drastic yield reductions in most crops. Salinity is one of the major abiotic stresses and researchers are trying to find the most suitable substance to enhance plant tolerance to stress factors. One of these substances is Salicylic Acid (SA). It has a significant impact on the various aspects of the plant life. In this paper we study the effect of presoaking seeds in 0.05 or 0.1 mM SA solutions in pot experience, on plant height, leaf relative water content (RWC), photosynthetic rate, stomatal conductance and assimilatory pigment contents of salt stressed wheat seedling. Salt stress was simulated by irrigation of the wheat seedlings with 200mM NaCl solution. The highest enhancements of the tolerance to salinity on Triticum aestivum cv. Crisana, plantlets were recorded in the case of treatments with 0.1 mM SA solution.

Keywords:. wheat, salt stress, salicylic acid, growth, photosynthesis.

INTRODUCTION

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, growth and development of plants (Hayat and Ahmad, 2007). One of these substances is salicylic acid (SA). Salicylic acid, a natural signaling molecule, could be raised to the status of the above phytohormones because it has significant impact on the various aspects of the plant life.

The application of salicylic acid, acetylsalicylic acid or other analogues of SA, 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 applied with acetylsalicylic acid (ASA) or gentisic acid (GTA) 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); SA also increased the chlorophyll and carotenoid content in maize plant (Khodary, 2004). Enhancing effect of SA on photosynthetic capacity can be attributed to its stimulatory effects on Rubisco activity and pigment contents.

Abiotic stresses cause drastic yield reduction in most crops, Therefore biodiversity conservation is one of the most important concerns of researchers. In plants the damaging effects of these abiotic stress factors take the shape of alterations in the plant physiology which leads to a reduction of growth and a decrease of their bioproductivity. Salicylic acid could ameliorate the damaging effects of heavy metals in rice (Mishra and Choudhuri, 1999), drought stress in wheat (Waseem et al, 2006) and salt stress in wheat (Arfan et al, 2007) and in sunflower plants (Noreen et al, 2009).

Salinity are limiting plant growth and development in most part of the world. Over 6% of the world's total land's are affected by salinity. Soil salinity causes reduction in crop productivity, because plants may suffer four types of stress: osmotically conductance, specific ion toxicity, ion imbalance, oxidative stress, i.e. production of reactive oxygen species (Tester and Davenport, 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.,2004; Szepesi et al., 2005)

Sinha et al (1993) pointed out that chlorophyll and carotenoid contents of maize leaves were increased upon treatement with SA by lead stress. The metabolic aspect of plants supplied with SA or its derivates shifted to a varied degree depending on the plant type and the mode of application of SA. The application of SA (20 mg/ml) to the foliage of the plants of *Brassica napus*, improved the chlorophyll contents (Ghai et al 2002).

Therefore the aim of this study was to evaluate the effect of Salicylic acid on growth and photosynthesis of wheat (*Triticum aestivum*, cv Crisana).

MATERIAL AND METHODS

This study was conducted in the Agrifood Biochemistry laboratory and in the Plant Physiology of the Faculty for Environmental Protection of the University of Oradea in 2010.

For study we used wheat (*Triticum aestivum* cv. Crisana), a cultivar created at Agricultural Research and Development Station Oradea.

To study the action of SA treatments under laboratory conditions, the wheat seeds were soaked for 12 hours in 0.1mM and 0.05 mM SA solutions and with tap water for the control lot.

Than the seeds were germinated in plastic recipients, for 7 days, on a filter paper, moistened with 20 ml treatment solution:

- control lot (**C**) – 12 h soaked in water and germinated in water.
- sample 1 (**S_1**) – 12 h soaked in water and germinated in 200 mM NaCl solution;
- sample 2 (**S_2**) – 12 h soaked in 0.1 mM SA solution and germinated in 200 mM NaCl solution.
- sample 3 (**S_3**) – 12 h soaked in 0.05mM SA solution and germinated in 200 mM NaCl solution;

Each recipient contained 50 seeds. The germination was made on filter paper moistened with tape water, at 20±3°C in a Sanyo MLR 351H phytotron, day/night, and relative humidity 65-85%, under natural photon flux density. Every day, the quantity of solutions from the recipients was brought to the level of 20 ml.

After 7 days of germination we planted the plantlets in pots containing equal amounts of clay and sand, leaving them there for an additional 14 days. The seedlings were irrigated with water or 0.2 M NaCl solution, and sprayed their primary leaves each day with water or SA solutions.

After 21 days we determined some physiological parameters: fresh and dry weight (FW and DW), relative water content (RWC), leaf area (LA), photoshynthesis rate (PR), stomatal conductance (SC) and chlorophyllian pigments contents.

For the biometrical determination we measured the length of the roots and shoots of 10 wheat seedlings, after 21 days of germination, and we made 3 independent repetitions for each determination.

Plant growth was estimated measuring accumulation of root and shoot weight, after drying the plants material at 60°C for 72 h.

Relative water content was also measured and express according to the following equation:

$$RWC\,(\%) = \frac{Wf - Wd}{Ws - Wd} \times 100$$

RWC = relative water content
Wf = Fresh leaf Weight
Wd = Dry leaf weight
Ws = Satured leaf weight

The wheat seedling leaves area were measured with Leaf area-meter.

Photosynthetic rate (µmol CO_2 m^{-2} s^{-1}) and stomatal conductance (mol m^{-2} s^{-1}) were measured with the LCi-pro- leaf chamber Analysis (ADC). 3 measurement/plot were undertaken.

The assimilatory pigments contents of the wheat seedling leaves were determined using N,N-dimethylformamide (DMF), 99.9%, for the extraction (Moran and Porath, 1980). The use of DMF renders the process simpler and faster, since the pigments can be extracted from intact tissue. The content of the pigment was determined using a UV-visible mini–1240 Shimadzu spectrophotometer, at 664 nm wave length for chlorophyll a, 647 nm for chlorophyll b and 480 nm for carotenoids. The data obtained from the spectrophotometric determinations, were mathematically processed using the formulas proposed by Moran and Porath 1982.

The results obtained after the content of chlorophyllian pigments determination are averages of 3 independent determinations and were statistically processed with 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 DISCUSSION

Plant growth

Studying the height (length of the roots plus shoots) and the dry weight of the wheat plantlets obtained from the germination of the wheat seeds under laboratory conditions, after 21 days of germination, we observed that the salt treatement very significantly reduced growth in height and dry weight leaf area and RWC of wheat plantlets. In case of the seeds pre-treated with SA solutions the negative effect of salt stress was reduced for both concentration of SA solution but the highest enhancements of the tolerance to salinity were recorded in the case of treatments with 0.1 mM SA solution (table 1).

The height measurement demonstrated that the wheat plantlets remained significantly shorter in case of salt stress (with 49.4%) and in case of treatement with 0.05mM SA (with 29.0%) in comparison with the control lot. Plant height decreased insignificantly, only with 2.8%.

The lowest dry weight was measured for salt stressed plantlets (with 42.4%) and for 0.05 mM SA treatement, with 41.3% lower that in comparison with the control lot. The dry weight obtained after treatement with 0.1 mM SA solution was very significantly higher, with 23.9% in comparison with the control lot, and also the values for stomatal conductance was higher for the same concentration of SA solution (with 75% higher in comparison with the salt stressed lot).

Table 1

Estimative mean values for plant characteristic of the salt stressed wheat seedling leaves with or without treatment with different concentration SA solutions in comparison with the same parameters of the control lot

Treatement	**Plant height (cm)**	**Leaf Fresh weight (g)**	**Leaf Dry weight (g)**	**Leaf saturated wieght (g)**	**Leaf area (cm^2)**	**RWC %**
Control (C)	46.5±0.79	0.0815±0.002	0.0092±0.0007	0.0974±0.005	7.14±0.06	81.9±0.85
Salt (S_1)	23.5±0.22 ***	0.0575±0.001 ***	0.0053±0.0004 ***	0.0821±0.002 *	5.23±0.04 ***	67.9±0.92 ***
Salt+ 0.05mM SA(S_2)	33±0.31 ***	0.0646±0.002 ***	0.0054±0.0003 ***	0.0844±0.003 *	6.25±0.04 ***	74.9±1.05 ***
Salt+ 0.1 mM SA (S_3)	45.2±0.7 ns	0.0839±0.002 ns	0.0114±0.0006 ***	0.0995±0.005 ns	6.98±0.06 *	82.9±0.89 ns

Data are presented as mean ± sd (standard deviation) (n=3). p>0.05= non-significant; p<0.05= * significant; p<0.01=** distinctly significant; p<0.001=*** very significant in comparison with the control lot.

The lowest leaf area and leaf relative water content were obtained again for salt stressed wheat plantlets and for samples treated with 0.05 mM SA solution followed by treatement with 0.1 mM SA solution. This treatement reduced significantly the negative effect of salinity. Similar effect was obtained by Gholinezhad et al, 2009 in case of water deficit stressed sunflower seedlings.

Photosynthetic rate* and *Stomatal conductance were very significantly reduced with adition of 0.2 M NaCl. Salicylic acid treatement can improve photosynthetic capacity in wheat under salt stress. SA treated plants had significantly higher photosynthetic rate and stomatal conductance in comparison with salt stressed plantles. Therefore, the highest value for the photosynthetic rate was obtained in case of treatement with 0.1 mM SA solution (with 68% higher in comparison with the salt stressed lot) and the

Table 2

Estimative mean values for physiological parameters of the salt stressed wheat seedling leaves with or without treatment with different concentration SA solutions in comparison with the same parameters of the control lot

Treatement	**Photosynthetic rate** (µmol CO_2 m^{-2} s^{-1})	**Stomatale conductance** (mol m^{-2} s^{-1})
Control (C)	2.33±0.02	0.08±0.004
Salt (S_1)	1.29±0.01 ***	0.04±0.003 ***
Salt+ 0.05 mM SA (S_2)	1.35±0.01 ***	0.06±0.002 ***
Salt+ 0.1 mM SA (S_3)	2.17±0.02 *	0.07±0.002 *

Data are presented as mean ± sd (standard deviation) p>0.05= non-significant; p<0.05=* significant; p<0.01=** distinctly significant; p<0.001=*** very significant in comparison with the control lot

Assimilatory pigments

Studying the *content of chlorophyllian pigment (chlorophyll a and b) and carotenoids* on the primary leaves of the wheat seedlings obtained from each experimental variant, we observed that salt stress decrease the assimilatory pigments content (with 20% for chlorophyll a, 11.8% for chlorophyll b and with 37.5% for carotenoids). Similar results were obtained by Kaydan et al 2007, they observed that under the influence of salinity the photosynthetic pigments greatly decreased. El Tayeb in 2005 found that chl a, b and carotenoids decreased significantly in NaCl treated plants in comparison to controls of barley plants.

Salicylic acid increased the content of assimilatory pigments in comparison with salt stressed samples.The influence of the exogenous SA solutions treatment was dependent on the concentration which was used. The results obtained were presented in (Table 3, fig.3).

The content of *chlorophyll a* increased nonsignificantly (with 3.4% from control lot considered 100%) after seeds presoaking in 0.05 mM SA solution. A very significant increase of chlorophyll a contents, with 35.6% from the control lot, was observed in the case of treatment with 0.1 mM SA solution. In the case of the *chlorophyll b* contents a nonsignificant increase could be observed, with 5.1% from control lot when using a 0.01 mM SA solution, and a very significant increase, with 47% from the control lot, in the case of treatment with 0.1 mM SA solution.

Table 3

Estimative mean values for assimilatory parameters of the salt stressed wheat seedling leaves with or without treatment with different concentration SA solutions in comparison with the same parameters of the control lot

Parameters		**Treatement**			
		Control (C)	**Salt (S_1)**	**Salt+ 0.05 mM SA (S_2)**	**Salt+ 0.1 mM SA (S_3)**
Assimilatory pigments mg/g FW	**chl a**	1.15±0.02	0.92±0.04 ***	1.19±0.03 ns	1.56±0.05 ***
	chl b	0.51±0.03	0.45±0.02 *	0.53±0.04 ns	0.75±0.03 ***
	carotenoids	0.40±0.01	0.25±0.006 ***	0.30±0.01 ***	0.36±0.004 **

p>0.05= non-significant; p<0.05=* significant; p<0.01=** distinctly significant; p<0.001=*** very significant in comparison with the control lot

Studying the *carotenoid* pigments content in the case of treatment with 0.05 mM concentrations SA solution, the results show that the accumulation of these pigments in the leaves of sunflower seedling on the 21th day of germination, increased very significantly, with 20%, in comparison with the same parameter determined from the salt stressed lot. The treatment with 0.1 mM SA solution significantly increased this pigment contents, with 44%, from salt stressed lot.

In soybean plants, treatment with salicylic acid, increased pigments content as well as the rate of photosynthesis (Zhao et al.,1995; Sinha et al.1993) pointed out that chlorophyll and carotenoid contents of maize leaves were increased upon treatment with SA.Taking together, the results of the previous authors support our findings.

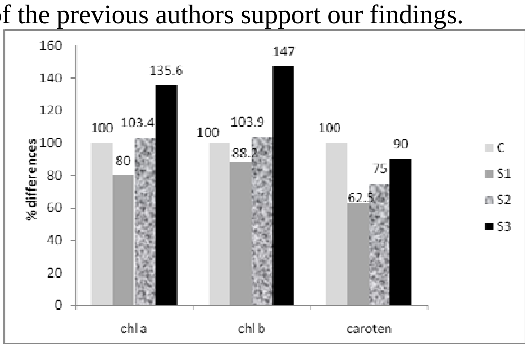

Fig.1: Percentage differences of assimilatory pigments content measured in primary leaves of wheat seedlings in stressed condition with or without SA treatment, in comparison with the same parameters measured in wheat seedlings from the control lot soaked in water. The value for the control lot was considered 100%.

CONCLUSION

- Salicylic acid treatement determined wheat plantlets growth and stimulate wheat salt tolerance by activating photoshyntethic process..
- Diluted SA solutions, with 0.05 mM and 0.1 mM concentration determined an increase in the chlorophyllian and carotenoid pigments content in the primary leaves of wheat seedlings in comparison with the salt stressed samples.
- The highest enhancements of the tolerance to salinity were recorded in the case of treatments with 0.1 mM SA solution

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REFERENCES

1. Arfan M., H.R. Athar and M Ashraf, (2007): Does exogenous application of salicylic acid through the rooting medium modulate growth and photosynthetic capacity in two differently adapted spring wheat cultivars under salt stress? J.Plant Physiol. 6(4): 685-694.
2. El-Tayeb MA. (2005) Response of barley grains to the interactive effect of salinity and salicylic acid. *Plant Growth Regul.* 45:215–224.
3. Ghai N.,Setia RC.,(2002): Effect of paclobutrazol and salicylic acid on chlorophyll content, hill activity and yield components in *Brassica napus* l (cv. gsl-1), phytomorphol. 52, 83-87.
4. Gholinezhad E., Aynaband A., Hassanzade Ghorthapeh A., Noormohamadi G., Bernousi I., (2009), Study of the effect of drought stress on yield, yield components and harvest Index of sunflower Hybrid Iroflor at different levels of nitrogen and Plant Population. Not.Bot.Hort.Agrobot. Cluj.37(2): 85-94.
5. Hayat S., A. Ahmad, (2007): Salicylic Acid: A Plant Hormone, SPRINGER (Ed) Dortrecht, The Netherlands
6. Kaydan, D., Yagmur, M., Okut, N., (2007): Effects of salicylic acid on the growth and some physiological characters in salt stressed wheat (*Triticum aestivum* L.). Tarim Bilimleri Dergisi, Ankara Universitesi Ziraat Fakultesi, 13(2): 114-119.
7. Khan W., Prithiviraj B, Smith DL, (2003) Photosynthetic responses of corn and soybean to foliar application of salicylates, Journal of plant physiology, 160:485-492.
8. Khodary S.E.A .(2004): Effect of SA on growth, photosynthesis and carbohydrate metabolism in salt stressed maize plants. Int. Journal Agri. Biol 6: 5-8.
9. Mishra A., M.A.Choudhuri (1999): Effect of salicilyc acid on heavy metal-induced membrane degradation mediated by lipoxigenase in rice. Biol.Plant, 42:409-415.
10. Moharekar ST., Lokhande SD, Hara, T., Tanaka, R., Tanaka, A., and Chavan PD. (2003), Effect of salicylic acid on clorophyll and carotenoid contents of wheat and moong caryopsis, Photosynthetica, 41, 315-317.
11. Moran R., (1982): Formulae for determination of chlorophyllous pigments extracted with N,N- dimethylformamide, Plant physiol. 69 (6), 1376-1381.
12. Moran, R., D. Porath, (1980), Clorophyll determination in intact tissue using N,N- dimethylformamide, Plant Physiol, 65:478-479.
13. Shakirova F.M., Sakhabudinova A.R., Bezrukova M.V., Fakhutdinova R. A., Fakhutdinova D.R. (2003)– Changes in the hormonal status of wheat seedlings induced by salicylic acid and salinity. Plant. Sci. 164, p.317-322.
14. Sinha S.K., SH Srivastava, Tripath RD, (1993): Influence of some growth regulators and cations on inhibition of chlorophill biosynthesis by lead in maize. Bul Env. Contamin Toxic; 51: 241-246
15. Szepesi, Á., J. Csiszár, S. Bajkán, K. Gémes and F. Horvath. (2005) Role of Salicylic acid pre-treatment on the acclimation of tomato plants to salt- and osmotic stress. Acta Biologica Szegediensis, 49:123-125.
16. Tari, I., Simon L.M., Deer K.J., Csiszár, J., Bajkan Sz., Kis Gy., and Szepesi, Á. (2004). Influence of salicylic acid on salt stress acclimation of tomato plants: oxidative stress response and osmotic adaptation. Acta Physiol. Plant, p.268:237.
17. Tester M., R., Davenport, (2003), Na^+ tolerance and Na^+ transport in higher plants. Ann.Bot. 91: 503-507.
18. Waseem M., H.U.R Athar and M. Ashraf, (2006): Effect of salicylic acid applied throught rooting medium on drought tolerance of wheat. Pak.J.Bot. 2006: 38(4), 1127-1136 p.
19. Zhao, H.J., X.W. Lin, H.Z. Shi, and S.M. Chang, (1995). The regulating effects of phenolic compounds on the physiological characteristics and yield of soybeans. *Acta Agron. Sin.,* 21: 351–355.