SALICYLIC ACID AND SALT STRESS IN WHEAT (*TRITICUM AESTIVUM* CV CRISANA*)* PLANTS IN VEGETATIVE STAGE

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Abstract

Salicylic acid (SA) is considered to be a very important signal molecule involved in the plant development processes and mainly involved in some agricultural plants response to different abiotic stress factors and plays a major role in the physiology of stress in plants. Salinity is one of the major abiotic stresses. Many crops species are sensitive to salinity. Salt stress causes oxidative damage and alters the amounts and activities of the enzymes involved in scavenging oxygen radicals. In this paper we study the effect of pre-soaking seeds in 0.05 or 0.1 mM SA solutions. The experiments will be conducted under field conditions, growing in pots, on some physiological and biochemical parameters modification like: plant height, photosynthetic rate, stomatal conductance, assimilatory pigment contents, proline and other amino acid content in salt stressed wheat seedlings. Salt stress was simulated by irrigation of the wheat seedlings with 0.2M 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.

Key words: wheat, salt stress, salicylic acid, growth, photosynthesis, amino acids, proline.

INTRODUCTION

Global agriculture will be under significant pressure to meet the demands of rising populations using finite, often degraded, soil and water resources that are predicted to be further stressed by the impact of climate change. The impact of climate change on agriculture could result in water shortages and drought, new diseases, heat stress and we can expect to see flooding and drought becoming more frequent and more severe. Simultaneously, lack of irrigation water will cause the salinization of fertile lands. (Banati, 2010).

In developing countries 80% of the necessary production increase would come from increases in yields and cropping intensity and only 20% from expansion of arable land. In recent years, the growth rates of cereal yields have been falling. They dropped from 3.2% per year in 1960 to 1.5%. In 2000 Bogdan et al. (2010) emphasized, in their researches, that a sustainable economy of the future has to become a bio-economy, adapted to the rural area based on Agrifood Biodiversity.

There are many ways needed to be applied to save food and feed. One of them is *in vitro* conservation, an important method of germplasm conservation, as traditional conservation of crop for plants of agricultural interest (Petruş, 2011). To reduce the consumption of electric energy used in biotechnological vitroculture processes, in order to obtain cheaper seedling and keep the environment cleaner, Pop and Cachiţă, (2011) replace CFLs with ultrabright LEDs.

Salinity is one of the major abiotic stresses. Many crop species are sensitive to salinity. Salt stress causes oxidative damage (Borsani et al, 2001) and alters the amounts and activities of the enzymes involved in scavenging oxygen radicals (Hernandez et al, 1993). Soil salinity causes reduction in crop productivity because plants may suffer four types of stress: osmotic conductance, specific ion toxicity, ion imbalance, and oxidative stress with production of reactive oxygen species (Tester and Devenport, 2007).

Salinity decreased the contents of dry mass, chlorophyll, soluble proteins and enhanced the content of free amino acids on *Vicia faba* (Gadallah, 1999), like proline, a protective, free amino acid, one of the potential biochemical indicators of salinity tolerance in plants involved in plant protection (Ashraf and Harris, 2004).

Amino acids, the building blocks of all cell formation, are necessary components in many processes in the plant, among which the photosynthesis which produces carbohydrates necessary for plant growth. Stressful conditions reduce amino acid content with a corresponding decrease in crop quality and quantity.

The aim of this work was to study the influence of the exogenous SA solution on some physiological and biochemical parameters in wheat *(Triticum aestivum* cv. Crisana*)* seedlings, in pot experience under salt stress in comparison with the same parameters of the control lot which was treated with water.

MATERIAL AND METHOD

The experiments were performed in 2010-2011, at the Agrifood Biochemistry Laboratory in the Faculty of Environmental Protection, University of Oradea and at the Institute of Food Science University for Agricultural Sciences of Debrecen. For the study we used wheat (*Triticum aestivum* cv. Crisana), a cultivar created at the Agricultural Research and Development Station Oradea.

The experiments were conducted under field conditions, growing in pots. All experiments were performed in parallel on plants grown under normal and stress conditions in the treated groups compared with untreated ones. Growing vessel size differed depending on the studied species. Pots

had a diameter of 35 cm and depth of 50 cm and were filled with soil collected from the field, ground, sieved and homogenized (Kauffman and Gartner, 1978).

Sample preparation

Wheat seeds (*Triticum aestivum cv*. Crisana l) were soaked for 12 h in water for control lot or in 0.05 mM and 0.1mM SA solution in October and 25 wheat seeds were sown in every pot. Pots were placed in the ground to create similar conditions to those in field conditions.

Irrigation water or NaCl solution was applied through a vertical tube of 2.5 cm diameter, so watering was done as it was mentioned above. After 3 weeks the seed was first treated with SA (20 ml per pot), and after another 2 weeks the 2nd spray treatment with SA was done. The control groups were sprayed with tap water.

A number of physiological and biochemical analyses were performed during the vegetative stage, in March, before the straw formation.

Experimental variants were as follows

- Control lot (C) –12 h soaked in water, sown in pots and irrigated with water.
- Sample 1 (S_1) - 12 h soaked in water, sown in pots and irrigated with 0.2M NaCl solution
- Sample 2 (S_2) – 12 h soaked in 0.05 mM SA, sown in pots and irrigated with 0.2M NaCl;
- Sample 3 (S_3) – 12 h soaked in 0.1mM SA, sown in pots and irrigated with 0.2M NaCl;

A number of physiological and biochemical analyses were done during the vegetative stage, in March, before the straw formation: plant height, dry weight content of the wheat plantlets, photosynthesis rate (PR), stomatal conductance (SC), chlorophyllian pigments contents, proline and other amino acids content.

Physiological parameters

Biometrical determination

For the biometrical determination we measured the length of the roots and shoots of 10 wheat seedlings. The wheat seedling leaves area was measured with leaf area-meter. Three independent repetitions for each determination were made.

Photosynthetic rate and stomatal conductance

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). Three measurements / plot were undertaken.

Biochemical parameters

Assimilatory pigments

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 lengths 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).

Proline determination

Proline was determined following Bates et al.(1973). The absorbance was read at λ=520 nm using toluene for a blank and the proline concentration was determined from a standard curve and calculated on a fresh weight basis and expressed as μmoles proline/g of fresh weight material.

Amino acid analysis

The amino acid spectrum of different vegetative organs in treated lots in comparison with the ones not treated was determined by HPLC - amino acid analyzer**.**

Statistical analysis

The results represented the averages of 3 independent determinations and were statistically processed using the "t- test" - *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 DISSCUSIONS

Studying the height of the wheat seedlings obtained from the wheat seeds under *field experience* in *the vegetative stage in March*, we observed that the salt treatments significantly reduced growth for the entire plant (with 37.5% in comparison with the control lot). In case of the seeds pre-treated with 0.05 mM and 0.1 mM SA solution, the negative effect of salt stress was reduced; therefore, the growth in length was insignificantly reduced in comparison with the control lot, and very significantly increased in comparison with salt stress lot. (Table 1 and fig.1). We could observe that treatment with 0.1 mM SA solution determined an increase in the growth parameters of wheat seedlings.

The lowest leaf area was obtained again for salt stressed wheat seedling. The treatment with 0.05 mM SA solution and 0.1 mM SA solution significantly reduced the negative effect of salinity. Similar effect was

obtained by Gholinezhad et al, 2009 in case of water deficit stressed sunflower seedlings.

Photosynthetic rate (PR) and stomatal conductance (SC) were very significantly reduced with the addition of 0.2 M NaCl. (Table 1, fig.1). In 1990 Brugnolli and Lauteri studied the effects of salinity on stomatal conductance and photosynthetic capacity, of salt-tolerant (*Gossypium hirsutum* l.) and salt-sensitive (*Phaseolus vulgaris* l.) C3 non-halophytes and found that the assimilation rate and stomatal conductance always declined when cotton and bean plants were exposed to salinity.

Salicylic acid treatment 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 plantlets. Therefore, the highest value for the photosynthetic rate and stomatal conductance was obtained in the case of treatment with 0.1 mM SA solution (with 46.7% for PR and 75% for SC, higher in comparison with the salt stressed lot).

Table 1

Estimative mean values for plant characteristic of the salt stressed wheat (*Triticum aestivum* cv *C*rişana) seedling with or without treatment with different concentration salicylic acid solutions in comparison with the same parameters of the control lot. The measurements were taken in March (vegetative stage) before the straw formation.

Treatment	**Plant height (cm)**	**Leaf area mm^2/plant**	**PR ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$)**	**SC ($mol\ m^{-2}\ s^{-1}$)**
C	21±3	2148±136.5	12.09±0.95	0.19±0.002
S_1	13.33±0.57 *	722±3 ***	5.73±0.67 ***	0.08±0.001 ***
S_2	18.33±2.08 ns	1513±45 **	7.57±0.22 **	0.13±0.004 ***
S_3	20.61±1.52 ns	2004±69.8 ns	8.41±0.58 **	0.14±0.01 ***

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

Studying the *content of chlorophyll pigment (chlorophyll a and b) and carotenoids* on the 3rd leaves of the wheat seedlings obtained from each experimental variant, we observed that salt stress decrease the assimilatory pigments content (by 3.9% for chlorophyll a, 4.53% for chlorophyll b and by 7.6% for carotenoids in comparison with the control lot). Similar results were obtained by Kaydan et al. (2007), who observed that the photosynthetic pigments greatly decreased under the influence of salinity. El Tayeb, in 2005, found that chlorophyll a, b and carotenoids decreased significantly in NaCl treated plants in comparison to control samples of barley plants. Sinha et al. pointed out that chlorophyll and carotenoid contents of maize leaves were increased upon treatment with SA.

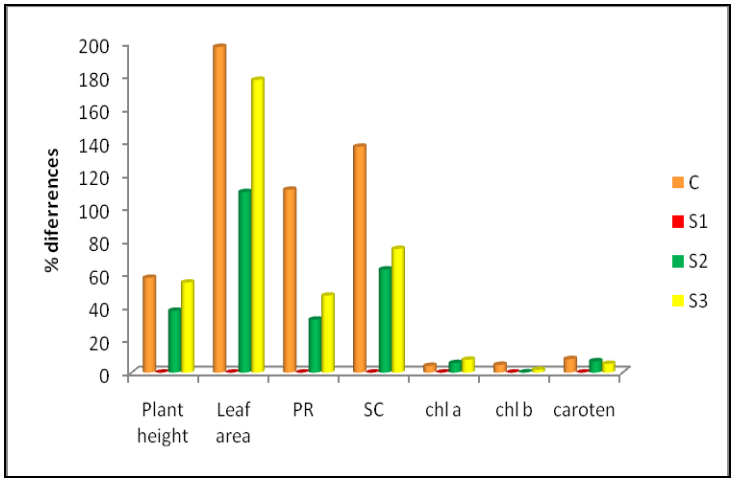

Fig 1. Percentage differences which reflect the effect of salicylic acid pretreatment on some physiological parameters of wheat (*Triticum aestivum* cv. Crisana*)* seedlings under salt stress condition, treated or untreated with SA as compared with the salt stressed lot marked with 0. The measurements were taken in March (vegetative stage) before the straw formation.

Table 2

Estimative mean values for assimilatory pigments content of the salt stressed wheat (*Triticum aestivum* cv. Crisana*)* seedling leaves with or without treatment with different concentration salicylic acid solutions in comparison with the same parameters of the control lot. The measurements were taken in March (vegetative stage) before the straw formation.

Parameters		**Treatment**			
		C	**S_1**	**S_2**	**S_3**
Assimilatory pigments mg/g FW	chl a	1.4±0.001	1.35±0.003 ***	1.43±0.012 ***	1.45±.0.004 ***
	chl b	0.66±0.002	0.63±0.008 ***	0.63±0.006 ***	0.64±0.002 ***
	Carot.	0.51±0.01	0.47±0.01 *	0.51±0.01 *	0.50±0.02 *
Proline µmoles proline/g FW	leaves	1.33±0.2	2.73±0.02 ***	1.83±0.01 *	1.91±0.02 **

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

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 2, fig.1). The treatment with 0.05 mM SA solution has a better effect in the case of carotenoid pigments content and the treatment with 0.1 mM SA solution increased more the chlorophyll a and b content in wheat seedling 3rd leaves.

Under stress conditions, free proline level increased in the leaves of wheat seedlings. Studying the value after spectrophotometric determination of proline content, we observed that under salt stress, with or without SA treatment, the proline content increased very significantly, but in the case of SA treated seedling leaves the increase of proline content was higher than in untreated leaves. For the salt stressed leaves the increase was 105.2% higher in comparison with the control lot. The treatment with 0.1mM SA alleviated the effect of salt stress and had a protective effect, in this condition the increase was of 37.5% and 43.6% in comparison with control lot.

Table 3

Estimative mean values for amino acids content (g/100g FW) of the salt stressed wheat seedling roots with or without treatment with different concentration SA solutions in comparison with the same parameters of the control lot

Amino acid %	**C**	**S_1**	**S_2**	**S_3**
ASP	1.16±0.02	1.06±0.01 ***	1.08±0.1 *	1.08±0.09 *
THR	0.58±0.04	0.55±0.03 ns	0.55±0.02 ns	0.59±0.02 Ns
SER	0.61±0.01	0.54±0.03 ***	0.57±0.04 *	0.59±0.04 *
GLU	2.07±0.03	1.88±0.02 ***	1.96±0.02 **	1.90±0.03 ***
GLY	0.51±0.05	0.51±0.07 ns	0.49±0.02 ns	0.48±0.05 Ns
ALA	0.60±0.08	0.60±0.1 ns	0.58±0.09 ns	0.57±0.08 Ns
CYS	0.14±0.008	0.15±0.005 ns	0.15±0.001 ns	0.15±0.004 Ns
VAL	0.80±0.04	0.77±0.04 ns	0.75±0.05 ns	0.75±0.04 Ns
MET	0.19±0.008	0.14±0.008 ***	0.15±0.009 ***	0.19±0.008 Ns
ILE	0.65±0.05	0.65±0.05 ns	0.58±0.07 ns	0.61±0.05 Ns
LEU	1.09±0.1	1.11±0.08 ns	1.06±0.07 ns	1.04±0.09 Ns
TYR	0.38±0.009	0.36±0.004 *	0.39±0.008 ns	0.36±0.005 Ns
PHE	0.73±0.08	0.70±0.07 ns	0.77±0.06 ns	0.72±0.05 Ns
HIS	0.65±0.07	0.59±0.08 ns	0.60±0.06 ns	0.61±0.06 Ns
LYS	1.10±0.01	1.03±0.1 **	1.05±0.08 *	1.09±0.1 Ns
Total amino acids	11.26±0.2	10.65±0.1 *	10.74±0.2 *	10.69±0.1 *

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 and treated with SA in comparison with salt stressed seedlings. Taken together, the results of the previous authors support our findings.

Similar results were obtained by Gauham and Singh in 2009. They registered an increased content of proline in maize seedlings subjected to stress induced by salinity, whereas seedlings subjected to salinity but pre-treated with 0.5 mM AS showed improvement in growth parameters. However, the free proline content decreased in comparison with the salt stressed lot, which means that the destructive effects of salinity on corn plants were significantly reduced by pre-treatment with AS.

In the case of determination of amino acids, salt stress significantly reduced the content in amino acids. Treatment with SA solution determined an enhancement of these values in comparison with the salt stressed lot, differences from the control lot becoming insignificant. Significant changes, in comparison with the salt stressed lot, were found for aspartic acid, serine, glutamic acid, methionine, tyrosine and lysine, and the total amino acids.

The highest enhancement value, in most cases, was registered in roots of salt stressed plantlets treated with 0.1mM SA solution (table3).

Hussein et al, 2007, have obtained similar results. Studying the effect of salinity and SA treatment on maize plantlets, they registered lower values for amino acid content except proline and glycine.

CONCLUSIONS

The analysis of the results obtained in this study shows that salt induced stress inhibits the growth parameters in wheat (*Triticum aestivum l.*) seedlings in comparison with the control lot.

Exogenous applications of 0.05 mM and 0.1 mM SA solution induced an increase in growth parameters in comparison with the untreated samples.

According to the obtained results, there is a clear correlation between the treatment applied and the physiological and biochemical parameters.

As a final conclusion of our studies - the results showed that exogenous SA solution administrated to the wheat seeds significantly ameliorated the negative effects of salt stress. Positive effects were more pronounced in the case of 0.1 mM SA solution.

The controlled addition of SA can create the possibility to use this substance as a growth regulator, a treatment that could result in increased crop, which could explain the possible involvement of this substance in future agro technical procedures.

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REFERENCES

1. Ashraf. M., Harris. P.J.C., 2004, Potential biochemical indicators of salinity tolerance in plants. *Plant Science.* 166: 3-16.
2. Banati D., 2010, Ethical considerations in the food chain - Analecta Technica Szegediensia vol. 2-3:18-22.
3. Bates. S., 1973, Rapid determination of free proline for water stress studies. *Plant and Soil*. 39: 205–207.
4. Bogdan A.T., Miresan V., Mironov A, Chelmu S, Boboc V, Surdu I., Burlacu R, Diaconecu D., Strateanu A, 2010, Prospects of Agrifood Green Power in 2050 and forecasting for 2100 with Sustenable Solutions Based on Ecobioeconomics new Paradigm. *Buletin USAMV Animal Science and Biotechnologies*. 67(1-2):1-18.
5. Borsani O., V. Valpuesta and M.A. Botella,2001, Evidence for a role of salicylic acid oxidative damage generated by NaCl and osmotic stress in Arabidopsis seedlings. *Plant Physiol*.. 126:1024-1030.
6. Brugnoli E.,Lauteri M., 2001, Effects of salinity on stomatal conductance. photosynthetic capacity. and carbon isotope discrimination of salt- tolerant (*Gossypium hirsutum* l.) And salt-sensitive (*Phaseolus vulgaris* l.) C3 non-halophytes. Plant physiology. 95:628-635.
7. Deef H.E., 2007, Influence of salicylic acid on stress tolerance during seed germination of *Triticum aestivum* and *Hordeum vulgare*. Advances in biological research 1 (1-2):40-48.
8. El-Tayeb MA., 2005, Response of barley grains to the interactive effect of salinity and salicylic acid. *Plant growth regul.* 45:215–224.
9. Gadallah M.A.A., 1999, Effects of proline and glycinebetaine on Vicia faba responses to salt stress. *Biologia Plantarum*. 42(2): 249-257.
10. Gauhan S.. P.K.Singh**,** 2009. Salicylic acid –induced salinity tolerance in corn grown under NaCl stress. Acta Physiol. Plant 31:1185-1190.
11. 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.
12. Hernandez J.A., E.J., Corpas. M.Gomez, L.A. Del Rio and F. Sevilla, 1993, Salt induced oxidative stress metiated by activated oxygen species in pea leaf mithocondria. *Physiol.Plant*. 89:103-110.
13. Hussein MM, Balbaa LK, Gaballah MS, 2007, Salicylic Acid and Salinity Effects on Growth of Maize Plants. Res. J. Agric. Biol. Sci.3(4): 321-328.
14. Kaufman M.D.. Gardner E.H., 1978, Segmental limming of soil and its effect on the growth of wheat. Agronomy J.. 70:331-336.
15. 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.

16. Moran R.,1982, Formulae for determination of chlorophyllous pigments extracted with N.N- dimethylformamide. Plant physiol. 69 (6). 1376-1381.
17. Moran. R.. D. Porath.,1980, Clorophyll determination in intact tissue using N.N-dimethylformamide. Plant Physiol. 65:478-479.
18. Petrus-Vancea A., 2011, New techniques in *Asparagus officinalis* L. micropropagation. Proceedings.UASVM Bucharest. Series A. LIV: 431-438.
19. Pop L.. Cachiţă D., 2011. contribution to *Solanum tuberosum* L. tubergenesis. vitrocultivated under ultrabright color L.E.D. Analele Universităţii din Oradea - Fascicula BiologieTom. XVIII. Issue: 2. 2011. pp. 124-127.
20. 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.
21. Tester M.. R.. Davenport, 2003, Na^+ tolerance and Na^+ transport in higher plants. *Annals of Botany*. 91: 503-507. ISSN 0305-7364.