EXOGENOUS SALICYLIC ACID INVOLVEMENT IN AMELIORATING THE NEGATIVE EFFECT OF 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 0.2mM 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 irigation water causing the salinisation 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, yield growth rates of cereal yields have been falling. It 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 themis *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 Cachiță, (2011) replace CFLs with ultrabright LEDs.

Salinity is one of the major abiotic stresses. Many crops 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: osmotical conductance, specific ion toxicity, ion imbalance, 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 them the photosynthesis which produces carbohydrates necessary for plant growth. Stressful conditions reduced 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 will be conducted under field conditions, growing in pots. All experiments will be performed in parallel on plants grown under normal and stress conditions in the treated groups compared with untreated. Growing vessel size differs depending on the studied species. Pots have a diameter of 35 cm and depth of 50 cm and will be 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 in every pot were sown 25 wheat seeds. Pots were be placed in the ground to create similar conditions to those in field conditions.

Irrigation water or NaCl solution is applied through a vertical tube with 2.5 cm diameter, so watering will be done based on the above. After 3 weeks the seed is first treated with sa (20 ml per pot), and after another 2 weeks will be realized the 2nd spray treatment with SA. The control groups will be sprayed with tap water.

A number of physiological and biochemical analysis will be done 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 analysis will be 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 were measured with leaf area-meter. Three independent repetitions for each determination was 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 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).

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 will be determined by HPLC - aminoacid 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 reduces growth for 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 can observed that treatement with 0.1 mM SA solution determine increasing of growth parameters of wheat seedlings.

The lowest leaf area were obtained again for salt stressed wheat seedling. The treatement 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 adition of 0.2 M NaCl. (tabel 1, fig.1). Brugnolli and Lauteri in 1990, 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 assimilation rate and stomatal conductance always declined when cotton and bean plants were exposed to salinity.

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 and stomatal conductance was obtained in case of treatement 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 measurment were taken in march (vegetative stage) before the straw formation.

Treatment	**Plant height (cm)**	**Leaf area mm^2/plant**	**PR (µmol 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 chlorophyllian 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 (with 3.9% for chlorophyll a, 4.53% for chlorophyll b and with 7.6% for carotenoids in comparison with the control lot). 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 chlorophyll a, b and carotenoids decreased significantly in NaCl treated plants in comparison to controls of barley plants. Sinha et al pointed out that chlorophyll and carotenoid contents of maize leaves were increased upon treatment with SA.

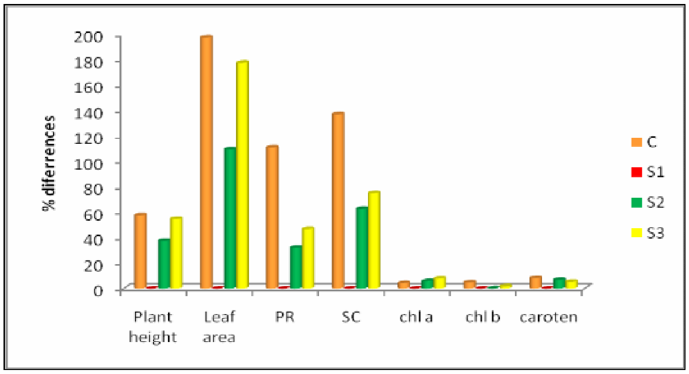

Fig. 1 Percentage differences which reflect the effect of salicylic acid pretreatment on some physiological parameters of wheat (*Triticum aestivum* cv. Crisana*)* seedlings under salt stress condition, treated or untreated with SA as compared with the salt stressed lot marked with 0. The measurment 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 measurment 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 treatement with 0.05 mM SA solution has a better effect in case of carotenoid pigments content and treatement 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 spectrophotometrycal determination of proline content, we observed that under salt stress, with or without SA treatment the proline content increased very significantly, but in 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 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

Aminoacid %	**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 aminoacids	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. Taking together, the results of the previous authors support our findings.

Similar results was obtained by Gauham and Singh, în 2009. They registered an increased proline content in maize seedlings subjected to stress induced by salinity, whereas seedlings subjected to salinity but pre-treated with 0.5 mM AS showed improvement from growth parameters, however the free proline content decreases in comparison with the salt stresed 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 getting to be insignificant. Signified 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 value of enhancement, 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 treatement 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 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 obtained results, there is a clear correlation between the treatement 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 agrotechnical procedures.

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