STUDIES REGARDING THE EFFECT OF SALICYLIC ACID ON WHEAT (*TRITICUM AESTIVUM* CV CRISANA*)* PLANTLETS RESPONSE TO SALINITY IN MILK STAGE

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Abstract

Salicylic acid (SA) is considered to be one of the new plant growth regulator. They have a significant impact on the various aspects of the plant life including different agricultural plants response to different abiotic stress factors. Salinity is one of the major abiotic stress. In this paper it was studied the influence of the exogenous applied SA solution on some physiological and biochemical parameters of plants, like plant height, leaf area, photosynthetic rate, stomatal conductance, antioxidative enzymes activity, assimilatory pigment contents and proline content in wheat (Triticum aestivum cv. Crisana) plantlets under salt stress, in pot experience in milk stage, in comparison with the same parameters of the control lots which were treated with water. The results showed that exogenous SA solution, administrated to the wheat seedlings ameliorated the negative effect of salt stress. Positive effects were more pronounced in the case of 0.1 mM SA solution. The experiments were performed between at the Agrifood Biochemistry Laboratory in the Faculty of Environmental Protection, University of Oradea and at the Agricultural Research and Development Station Oradea.

Key words: salicylic acid, field experience, wheat, generative stage, salinity

INTRODUCTION

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 (www.fao.org). 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.

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).

Salinity is one of the major abiotic stresses. Soil salinity causes reduction in crop productivity, because plants may suffer four types of stress: osmotical conductance, specific ion toxicity, ion imbalance, oxidative stress, production of reactive oxygen species (Tester and Devenport, 2007).

To overcome this inconvenient, plants need to possess mechanisms of avoidance and tolerance to stress.

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).

Salinity decreased the contents of dry mass, chlorophyll, soluble proteins and enhanced 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).
Salicylic acid is considered to be a very important signal molecule involved in the plant development processes and mainly involved in some agricultural plants' responses to different abiotic stress factors, and plays a major role in the physiology of stress in plants. This substance used in optimal concentrations can temporarily reduce the oxidative stress level in plants improving their antioxidative capacity and stimulating the synthesis of some protective components.

Salicylic acid activated the synthesis of carotenoids and xanthophylls, but decreased the level of chlorophyll pigments, both in wheat and moong plants and 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). The enhancing effect of SA on photosynthetic capacity can be attributed to its stimulatory effects on Rubisco activity and pigment contents.

The application of SA (20 mg/ml) to the foliage of the plants of *Brassica napus*, improved the chlorophyll contents (Ghai and Setia, 2002).
Sinha et al, 1993, pointed out that chlorophyll and carotenoid contents of maize leaves were increased upon treatment with SA by lead stress.
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 (Szepesi al, 2005; Tari et al 2004).

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.
Proline, a protective, free amino acid, is one of the potential biochemical indicators of stress tolerance in plants. Proline contributes to membrane stability and mitigates the effect of NaCl on cell membrane disruption (Ashraf, M., Harris, 2004).
The aim of this work was to study the influence of the exogenous SA solution on some physiological and biochemical parameters of plants, like plant height, leaf area, photosynthetic rate, stomatal conductance, antioxidative enzymes activity, assimilatory pigment contents and proline content in wheat *(Triticum aestivum* cv. Crisana*)* plantlets under salt stress, in growing vessels for simulate field conditions, in comparison with the same parameters of the control lots which were treated with water.

MATERIALS AND METHODS

The experiments were performed between 2010-2011 at the Agrifood Biochemistry Laboratory in the Faculty of Environmental Protection, University of Oradea and at the Agricultural Research and Development Station Oradea.
For the study we used wheat (*Triticum aestivum* cv. Crisana), a cultivar created at the Agricultural Research and Development Station Oradea. Crisana variety was classified in A_2 (B_1) valuable group, being appreciated like an ameliorative one (Bunta, 2009).
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. In every pot were sown 25 wheat seeds. Pots were be placed in the ground to create similar conditions to those in field conditions. The seedlings were thinned, leaving finally 10 plants.

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), after another 2 weeks will be realized the 2nd treatement with SA, and after the straw formation it was realised the last treatment with SA. The control groups will be sprayed with tap water.

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 in the milk stage of wheat: plant height, fresh and dry weight (FW and DW) of roots, leaf area (LA), photoshynthesis rate (PR), stomatal conductance (SC) and some biochemical parameters: assimilatory pigments contents, antioxidative enzymes activity and proline content.

1.Physiological parameters

Biometrical determination

For the biometrical determination we measured the length of the roots and shoots of 10 wheat plants, in the milk stage. It was made 3 independent repetitions for each determination. The wheat seedling leaves area were measured with leaf area-meter.

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

Photosynthetic rate and stomatal conductance

Photosynthetic rate (μmol $CO_2m^{-2}s^{-1}$) and stomatal conductance (mol $m^{-2}s^{-1}$) were measured with the LCi-pro-leaf chamber Analysis (ADC). Three measurements/plots were undertaken.

2. 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 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, 1982.

Antioxidative enzymes activity

Preparation of enzyme extract – 0.5g fresh sample (roots or leaves) were collected from each variant in the 21th day of experiment, and were blended with 8 ml phosphate buffer

solution, pH 7.0 cooled at 4˚C. The samples were centrifuged at 15000 x g, for 20 minutes at 4˚C, and the supernatant was separated. The extract is kept in the refrigerator, for 2 hours for stabilizing and expressing enzyme activity.

Peroxidase (POX) activity determination- activity was determined at 30ºC, with a Shimadzu–UV-mini–1240 spectrophotometer, following the formation of tetraguaiacol at 470 nm wavelength, $\varepsilon=26.6mM^{-1}cm^{-1}$, in a 3 ml reaction mixture containing 0.1 M phosphate bufer, pH=6.0; 15mM guaiacol; 3 mM H_2O_2, and 50µl of enzyme extract. One unit of peroxidase activity (U) represents the amount of enzyme catalyzing the oxidation of 1 µmole of guaiacol in 1 min, method cited by Kim and Yoo, 1996.

Catalase (CAT) activity determination - the colorimetric assay of CAT (Sinha, 1972) is based on the reduction of dichromate in presence of acetic acid to chromatic acetate when heated in the presence of H_2O_2. The absorbance was measured at 570 nm and expressed CAT activity as mmoles H_2O_2/min/g at 25°C.

Proline determination

Proline was determined following Bates et al (1973). For the proline determination 0.5g of leaves was homogenized with 10 ml of 3% aqueous sulfosalicylic acid and the homogenate filtered. The filtrate was treated with acid ninhydrin and glacial acetic acid in a test tube for 1 hour at 100ºC, and the reaction terminated in an ice bath. The reaction mixture was extracted with toluene. The cromophore containing toluene was aspired from the aqueous phase warmed to room temperature and the absorbance read at λ=520 nm using toluene for a blank. 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.

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 DISCUSSION

1.Physiological parameters

Biometrical determination

Studying the physiological parameters of the wheat plantlets obtained, we observed that the salt treatment very significantly reduced growth in height, leaf area, the roots dry and fresh weight. In case of the seeds pre-treated with SA solutions the negative effect of salt stress was reduced for both concentrations 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 (table1).

Erdal et al, 2011, studying the effects of foliar treatment with SA on wheat plantlets, observed that roots and shoots fresh and dry weight increased significantly in case of exposure to salt stress without SA treatment.

Table 1

Estimative mean values for wheat plantlets physiological parameters under salt stress, with or without treatment with different concentration SA solutions in comparison with the same parameters of the control lot

Treatment	Plant height (cm)	Leaf area (mm^2)	Dry weight roots (g)	Fresh weight Roots (g)
Control (C)	65.1±4.66	3301.5±164.5	0.83±0.08	1.75±0.113
Salt (S_1)	38±3.6 ***	951±18.24 ***	0.19±0.02 ***	1.23±0.02 ***
Salt+ 0.05mM SA(S_2)	53.1±3.6 */***	1568±57.9 ***/***	0.26±0.04 ***/***	1.6±0.02 ns/***
Salt+ 0.1 mM SA (S_3)	56.8±2.1 */***	1789±69.8 ***/***	0.68±0.03 */***	1.53±0.05 ns/***

(p>0.05= unsignificant; p<0.05=* significant; p<0.01=** distinctly significant). In comparison with the control lot / with the salt stressed lot.

Photosynthetic rate and stomatal conductance

Photosynthetic rate and Stomatal conductance were very significantly reduced with addition of 0.2 M NaCl. 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 case of treatment with 0.1mM SA solution (71.25% and 200% higher in comparison with the salt stressed lot).

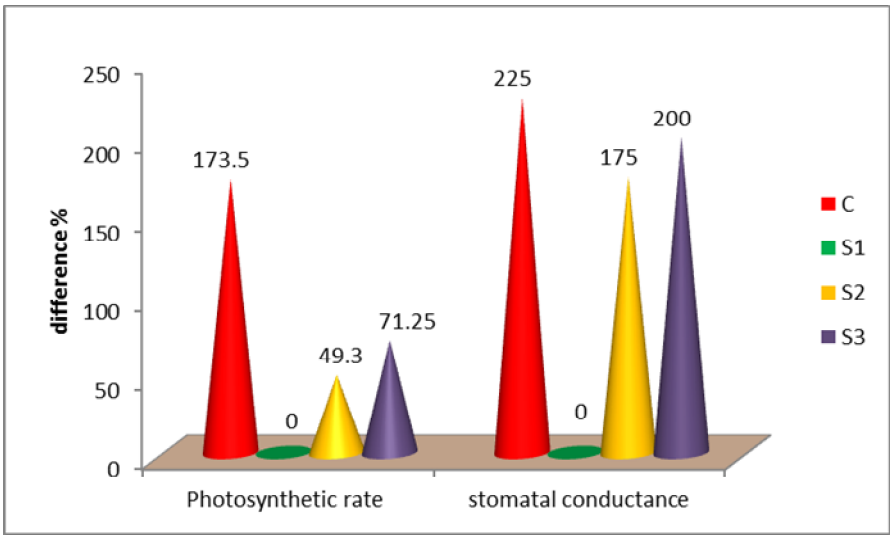

Fig.1.Percentage differences which reflect the effect of SA treatment on photosynthetic rate and stomatal conductance of wheat (*Triticum aestivum* cv. Crisana*)* plantlets under salt stress condition, compared with the control lot and with salt stressed lot marked with 0. The measurment were taken after the straw formation, in milk stage.

Khan et. al., 2010, obtained similar results after applying SA treatments to bean plants, with the purpose of growing their tolerance to salinity. Therefore, the photosynthetic rate and

stomatal conductance, significantly dropped at the lots which underwent saline stress. The SA effect over the photosynthetic parameters was a positive one, since this treatment returns the parameters to values that were almost similar with the ones belonging to the control lot of bean plants.

2. Biochemical parameters

Assimilatory pigments

Studying the *content of chlorophyllian pigment (chlorophyll a and b) and carotenoids* on the leaves of the wheat plantlets obtained from each experimental variant, we observed that salt stress decrease the assimilatory pigments content. Similar results were obtained by Kaydan et al 2007, they observed that under the influence of salinity the photosynthetic pigments greatly decreased.

A very significant increase of chlorophyll a, chlorophyll b and carotenoids contents in comparison with the salt stressed lot, was observed in the case of treatment with 0.05mM SA solution. The treatment with 0.1mM SA solution determined a significant or non-significant increase of assimilatory pigments in wheat plantlets leaves (fig.2).

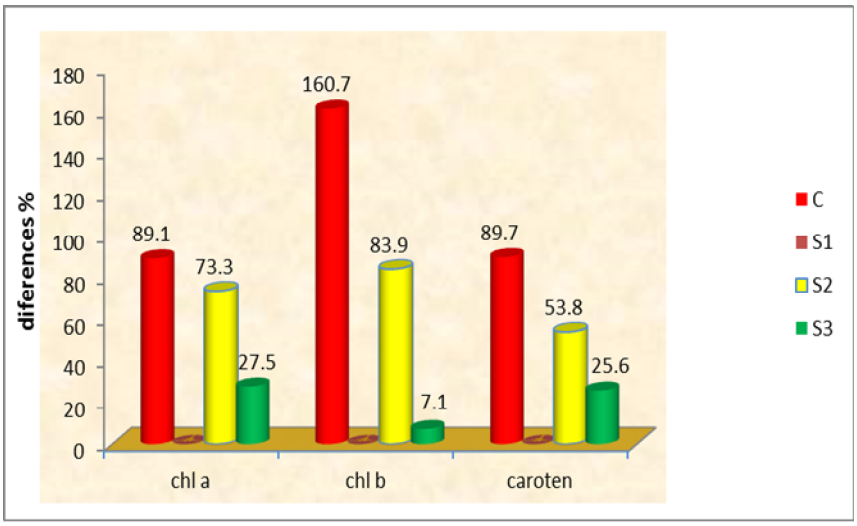

Fig.2. Percentage differences which reflect the effect of SA treatment on assimilatory pigments content of wheat (*Triticum aestivum* cv. Crisana*)* plantlets under salt stress condition, compared with the control lot and with salt stressed lot marked with 0. The measurment were taken after the straw formation, in milk stage.

Zhao et al., 1995, obtained similar results in soybean plants, so treatment with SA, increased the pigments content as well as the rate of photosynthesis. Sinha et al 1993, pointed out that chlorophyll and carotenoid contents of maize leaves were increased upon treatment with SA.

Antioxidative enzymes activity

It was studied the influence of salinity and salicylic acid on peroxidase and catalase activity, enzymes involved in reactive oxygen species (ROS) scavenging.

Salicylic acid applied to stressed plants, significantly increased the peroxidase activity, when treatments was made with 0.05 mM SA solution, and very significantly when the

weath plantlets was treated with 0.1 mM SA solution. Stress induced by salinity has increased very significantly the values of peroxidase activity obtained for the control group. In leaves, also, due to oxidative process caused by salinity, a very significant increase of peroxidase activity occurs. Treatments with SA causes a decrease in peroxidase activity in comparison with salt stressed lot (table 2).
In the case of samples treated with a 0.2M NaCl solution, there was a very significant decrease of the catalase activity (in the roots with 40% and in the leaves with 74% in comparison with the control lot).
Treatment with 0.05 mM and 0.1 mM SA solutions determined a significant and a very significant increase in catalase activity in roots and leaves of wheat plantlets (with 100% in roots and with 347% in leaves, in comparison with the salt stressed lot, table 2), the increases were higher for treatments with AS 0.1 mM solution.

Table 2

Estimative mean values for antioxidative enzymes activity of the salt stressed wheat plantlets leaves and roots with or without treatment with different concentration SA solutions in comparison with the same parameters of the control lot./salt stressed lot.

Treatment	Peroxidase		Catalase	
	roots	Leaves	roots	leaves
Control C	0.439±0.01	0.432±0.005	16.77±0.21	1.69±0.04
Salt (S_1)	0.577±0.01 ***	0.877±0.04 ***	10.0±0.15 ***	0.44±0.01 ***
Salt+ 0.05mM SA(S_2)	0.441±0.01 ns/*	0.522±0.02 */***	20.00±0.42 ***/***	1.89±0.03 ***/***
Salt+ 0.1 mM SA (S_3)	0.556±0.01 ***/***	0.695±0.009 ***/**	20.48±0.36 ***/***	1.97±0.02 ***/***

Data are presented as mean ± SD (n=3) ; $p>0.05$ = ns insignificant; $p<0.05$ * significant; $p<0.01$=** distinctly significant; $p<0.001$=*** very significant in comparison with control lot/ with salt stressed lot.

For sustain our results we mentioned the results obtained by other researchers, for example Noreen *et al.* (2009), also found that exogenous foliar applied SA enhanced antioxidant capacity in salt stressed sunflower. SA application was shown to increased POX activity in different plant species subjected to various abiotic stress (Janda, 1999; Popova et al, 2009).

Proline determination

Under stress condition, free proline level increased in the leaves of wheat plantlets. 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 wheat leaves the increase of proline content was higher than in untreated leaves. For the salt stressed leaves the increase was with 302.3% higher in comparison with 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 higher (with 148.7%) in comparison with salt stressed sunflower seedlings (Table 3, fig.3).

Table 3

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

Parameters	Treatment			
	Control (C)	Salt (S_1)	Salt+ 0.05 mM SA (S_2)	Salt+ 0.1 mM SA (S_3)
Proline µmoles proline/g FW	2.73±0.3	3.08±0.3 ***	4.43±0.3 ***/**	7.66±0.4 ***/***

Data are presented as mean ± SD (n=3); $p>0.05$ = ns insignificant; $p<0.05$ * significant; $p<0.01$=** distinctly significant; $p<0.001$=*** very significant in comparison with control lot).

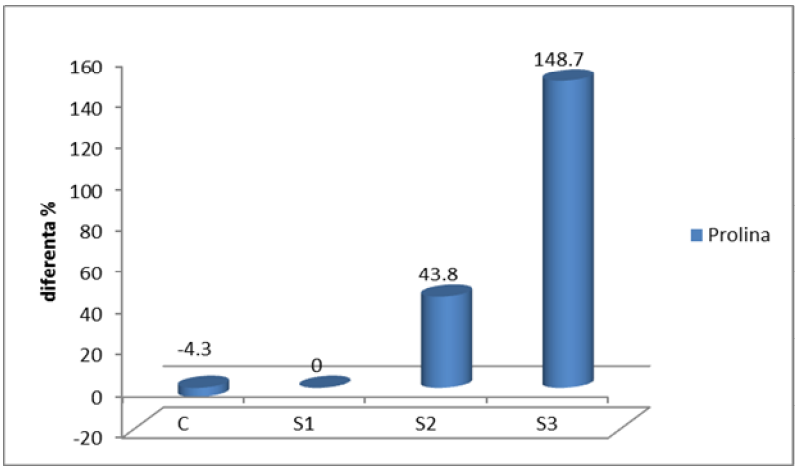

Fig. 3. Percentage differences of proline content measured in leaves of wheat seedlings in stressed conditions with or without SA treatment, in comparison with the same parameters measured in the leaves of wheat seedlings from the control lot soaked in water. The value for the control lot was marked with 0 on the chart.

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 stresed seedlings.

CONCLUSION

Salicylic acid treatment determined wheat plantlets growth and stimulated wheat salt tolerance by activating photosynthetic 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 results obtained after pre-treatment of wheat seeds with SA improved the antioxidative capacity of the plants, increasing the plant tolerance to salt stress induced in our experiment by 0.2M NaCl treatments.

The treatment with 0.05 mM and 0.1 mM SA significantly increased the proline content, a free aminoacid involved in ameliorating the salt stress induced damages.

As a final conclusion of our studies - the results showed that exogenous SA solution, administrated to the wheat seeds significantly ameliorated the negative effect of salt stress. Positive effects were more pronounced in the case of 0.1 mM SA solution.
The role of SA in the plant response to different abiotic stress factors might explain the possible use of this substance in future agrotechnical procedures. The controlled addition of SA can make it possible to use this substance as a growth regulator, which could result in increased crops

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