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

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Abstract

The anthropogenic activities and changed agricultural system, intense use of chemical fertilizers and artificial irrigation have increased abiotic stresses and caused yield losses annually to a greater extent. In our researches we studied the role of salicylic acid in, administrated in different concentrations by presoaking the seeds for 12 hour before germination, on some biochemical processes including: antioxidant enzymes, proline and assimilatory pigments content of the first leaves, of salt stressed wheat seedlings in comparison with the same parameters of the control lot which were treated with tape water. The germination was made 7 days in plastic recipients and for aditional 14 days in pots containing equal amounts of clay and sand. The results showed that exogenous 0.1mM SA solution, administrated to the wheat cariopses significantly ameliorate the negative effect of salt stress.

Keywords: SA, salt stress, *Triticum aestivum* cv Crisana., biochemical parameters.

INTRODUCTION

The anthropogenic activities and changed agricultural system, intense use of chemical fertilizers and artificial irrigation have increased abiotic stresses and caused yield losses annually to a greater extent. To overcome the yield losses due to abiotic stresses, plants need to possess mechanisms of avoidance and tolerance to stress. Abiotic stresses such as, heavy metals, salinity, drought, temperature, UV-radiation, ozone, cause drastic yield reduction in most crops (Khan et al, 2007).

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.

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).

Salinity decreased the contents of dry mass, chlorophyll, soluble proteins and enhanced content of free aminoacids 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).

Salicilyc acid (SA) is recognized as an endogenous signal molecule belongs to a diverse group of plant phenolics, involved in different physiological and biochemical processes and mainly involved in environmental stress tolerance in plants.

The aim of this work was to study the influence of the exogenous SA solution (aplied by soaking the seeds for 12 hour in the treatement solutions) on some biochemical parameters of plants, like antioxidative enzymes and proline content of the leaves of wheat *(Triticum aestivum* cv. Crisana*)* seedlings under salt stress, in pot experience, in comparison with the same parameters of the control lots which were treated with water.

The experiments was performed at the Agrifood Biochemistry Laboratory in the Faculty of Environmental Protection, University of Oradea in 2010.

MATERIAL AND METHODS

For study we used wheat (*Triticum aestivum* cv. Crisana), a cultivar created at 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).

In laboratory condition the seeds were soaked for 12 hours in 0.1 mM or 0.05 mM, SA solutions and in 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:

C control lot – 12 h soaked in water and germinated in water.

S_1 sample1– 12 h soaked in water and germinated in 0.2M NaCl solution;

S_2 sample 2–12 h soaked in 0.1 mM SA solution and germinated in 0.2M NaCl solution.

S_3 sample 3– 12 h soaked in 0.05mM SA solution and germinated in 0.2M 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 200 mM NaCl solution, and sprayed their primary leaves each day with water or SA solutions.

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, ε=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 acetic acid to chromatic acetate when heated in the presence of H_2O_2. We measure the absorbance 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 plant material 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

Antioxidative enzymes

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

Peroxidase activity

In the case of samples treated with a 0.2M NaCl solution, there was a very significant decrease of the peroxidase activity (with 20% in comparison with the control lot), in the roots of wheat seedlings.

Pre-soaking the seeds for 12 h in a 0.05 mM SA solution the increase of the peroxidase activity was nonsignificant (4%, in comparison with the control lot) but pre-soaking the seeds in 0.1 mM SA solution increased very significantly the peroxidase activity in the root of seedlings (39.1%, in comparison with control lot) (Table1, fig.1).

Table 1.

Estimative mean values for biochemical parameters 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		**Treatement**			
		Control (C)	**Salt (S_1)**	**Salt+ 0.05 mM SA (S_2)**	**Salt+ 0.1 mM SA (S_3)**
Peroxidase μmol guaiacol /min	**roots**	1.236±0.03	0.991±0.01 ***	1.286±0.02 ns	1.720±0.02 ***
Catalase **mmoles** H_2O_2/min/g FW	**roots**	13.33±0.2	21.43±0.35 ***	23.33±0.5 ***	23.81±0.3 ***
Proline μmoles proline/g FW	**leaves**	0.85±0.02	3.42±0.05 ***	2.11±0.03 ***	8.77±0.04 ***

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

Catalase activity

Studying the catalase activity in the case of samples treated with 0.05 mM or 0.1 mM SA solutions under salinity (irigation with 0.2M NaCl solution), a very significant increase was registered (60.7% for salt stressed lot, 75% and 78.5% for SA treated lots in comparison with the control lot), in the roots of wheat seedlings.

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 aplication was shown to increased POX activity in different plant species subjected to various abiotic stress (Janda, 1999; Popova et al,2009).

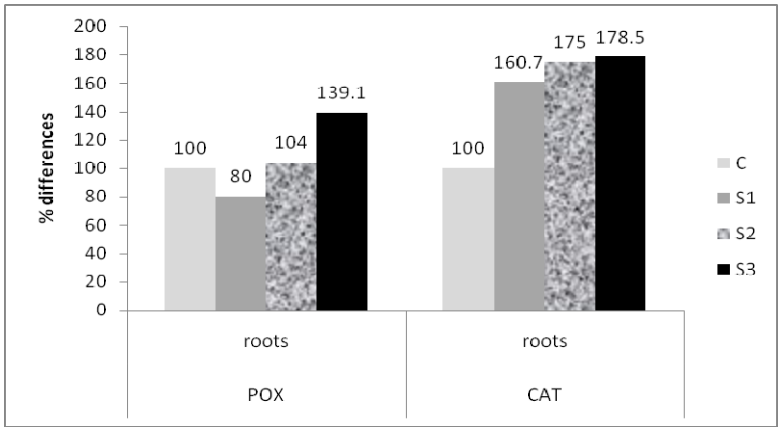

Fig 1**.** Differences of antioxidant enzymes, POX and CAT activity, measured in roots of wheat seedlings in stressed condition with or without SA treatment, in comparison with the same parameters measured in the roots of wheat seedlings from the control lot soaked in water. The value for the control lot was considered 100%

Proline content

Under stress condition, free proline level increased in the leaves of wheat seedlings, after 21 day's of germination. Studying the value after spectrophotometrycal determination of proline content, we observed that under salt stress, with or without SA treatement the proline content increased very significantly, but in case of SA treated seedling leaves the increase of proline content was higher that 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 205.3%) in comparison with salt stressed sunflower seedlings (Table1, fig.2).

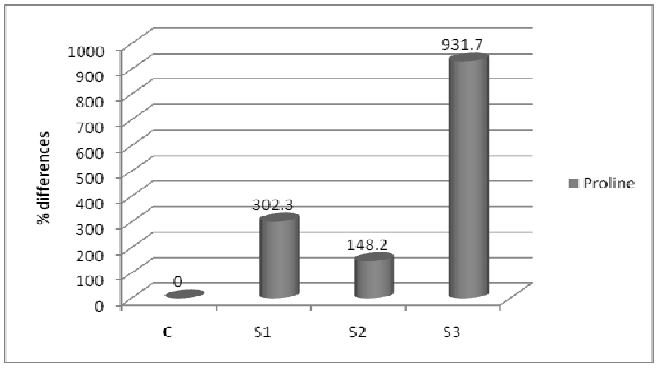

Fig. 2**.**Percentage differences of proline content measured in leaves of wheat seedlings in stressed condition with or without SA treatment, in comparison with the same parameters measured in the leaves of wheat seedlings from the control lot soaked in water. The value for the control lot was considered 100% (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

The results obtained after pre-treatment of wheat seeds with SA improving the antioxidative capacity of the plants, increasing the plant tolerance to salt stress induced in our experiment by 0.2M NaCl treatments.

The treatement with 0.05 mM and 0.1 mM SA significantly increased the proline content, a free aminoacid involved in ameliorate 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 ameliorate the negative effect of salt stress. Positive effects were more pronounced in the case of 0.1 mM SA solution.

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