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EFFECT OF SALICILYC ACID ON ASSIMILATORY PIGMENTS AND AMINOACIDS CONTENT IN SALT STRESSED WHEAT (*TRITICUM AESTIVUM,* CV. CRISANA) SEEDLINGS

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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 some biochemical parameters modification like: assimilatory pigment contents, proline and other free aminoacid content in salt stressed wheat seedlngs. 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 cultivar Crisana, plantlets were recorded in the case of treatments with 0.1 mM SA solution.

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, growth rates of cereal yields have been falling. Bogdan et al [4] emphasized in their research, 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 such as developing new policies in applying dynamic action plans in agriculture, according to environmental factors climate change impact and tolerance degree of crop landcraces [1].

Salinity is one of the major abiotic stresses which decreased the contents of chlorophyll, soluble proteins and enhanced content of free amino acids on *Vicia faba* [6]. Proline, is a protective, free amino acid, one of the potential biochemical indicators of salinity tolerance in plants involved in plant protection [2].

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

responses to different abiotic stress factors, and plays a major role in the physiology of stress in plants.

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 [10]; SA also increased the chlorophyll and carotenoid content in maize plant [9]. 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 [7].

Proline, a protective free amino acid, contributes to membrane stability and mitigates the effect of NaCl on cell membrane disruption [2].

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 biochemical parameters determined in the roots or 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.

MATERIAL AND METHODS

The experiments were performed at the Agrifood Biochemistry Laboratory, University of Oradea and at the University of Debrecen in 2010. For the study we used wheat (*Triticum aestivum* cultivar Crisana), a cultivar created at the Agricultural Research and Development Station Oradea.

To study the action of SA treatments under laboratory conditions, the wheat 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 ($\mathbf{S_1}$) – 12 h soaked in water and germinated in 0.2M NaCl solution;
- sample 2 ($\mathbf{S_2}$) – 12 h soaked in 0.05 mM SA and germinated in 0.2M NaCl;
- sample 3 ($\mathbf{S_3}$) – 12 h soaked in 0.1mM SA and germinated in 0.2M NaCl.

Each recipient contained 50 seeds. The germination was made 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, and sprayed their primary

leaves each day with water or SA solutions. After 21 days we determined some biochemical parameters. The assimilatory pigments contents of the wheat seedling leaves were determined by using N,N-dimethylformamide (DMF) for the extraction [12] and an UV-visible mini-1240 Shimadzu spectrophotometer. The data obtained from the spectrophotometric determinations, were mathematically processed using the formulas proposed by Moran and Porath [14].

Proline was determined following Bates et al. [2]. 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**.**

The results represented the averages of 3 independent determinations and were statistically processed using the "t- test" - *Prisma 5 for windows*.

RESULTS AND DISCUSSION

Studying the content of chlorophyllian pigment (chl 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 chl a, 11.8% for chl b and with 37.5% for carotenoids). Similar results were obtained by Kaydan et al. [8], they observed that under the influence of salinity the photosynthetic pigments greatly decreased.

The content of chl a increased nonsignificantly (with 3.4% from the control lot considered) after seeds presoaking in 0.05 mM SA solution. A very significant increase of chl 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 chl b contents a nonsignificant increase could be observed, with 5.1% from control lot when using a 0.05 mM SA solution, and a very significant increase, with 47% in the case of treatment with 0.1 mM SA solution (Table 1).

Studying the carotenoid pigments content in the case of treatment with 0.05 mM SA solution, the results show that the accumulation of these pigments in the leaves of wheat seedling on the 21^{th} 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. Zhao et al., [15] obtained similar results in soybean plants, so treatment with SA, increased pigments content as well as the rate of photosynthesis. Sinha et al. [13] pointed out that chlorophyll and carotenoid contents of maize leaves were increased upon treatment with SA.

Under stress conditions, 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 wheat seedlings (Table 1).

Deef [5], 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. Taken together, the results of the previous authors support our findings.

Table 1

Estimative mean values for some biochemical 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		Treatment			
		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 **
Proline µmoles proline/g FW	**leaves**	0.85±0.02	3.42±0.05 ***	2.11±0.03 ***	8.77±0.04 ***

p>0.05= non-significant; p<0.05=* significant; p<0.01=** distinctly significant

In case of determination of amino acids, salt stress reduced significantly the content in amino acids. Treatement with SA solution determined an enhancement of these values in comparison with salt stressed lot differences from the control lot getting to be insignificant. The highest value of enhancement was registered in roots of salt stressed plantlets treated with 0.1mM SA solution (Table 2).

CONCLUSIONS

1. 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.
2. The treatment with 0.05 mM and 0.1 mM SA significantly increased the proline and other amino acids content. The highest value of enhancement was registered in roots of salt stressed plantlets treated with 0.1mM SA solution.

3. 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.

Table 2

Estimative mean values for amino acids content (g/100 g FW) 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

Aminoacid	Control (C)	Salt (S_1)	Salt+ 0.05 mM SA (S_2)	Salt+ 0.1 mM SA (S_3)
ASP	0.15±0.007	0.10±0.016 ***	0.23±0.01 ***	0.22±0.003 ***
THR	0.10±0.004	0.07±0.006 *	0.09±0.018 ns	0.09±0.010 ns
SER	0.10±0.012	0.071±0.002 ns	0.086±0.006 ns	0.10±0.006 ns
GLU	0.195±0.009	0.121±0.017 *	0.164±0.019 *	0.189±0.017 ns
GLY	0.10±0.031	0.071±0.002 ns	0.086±0.013 ns	0.096±0.005 ns
ALA	0.077±0.004	0.057±0.006 ***	0.07±0.015 *	0.078±0.007 ns
VAL	0.11±0.01	0.079±0.018 ***	0.095±0.016 *	0.104±0.019 ns
Aminoacid	**Control (C)**	**Salt (S_1)**	**Salt+ 0.05 mM SA (S_2)**	**Salt+ 0.1 mM SA (S_3)**
MET	0.03±0.001	0.017±0.009 ***	0.034±0.022 *	0.034±0.01 *
ILE	0.07±0.007	0.052±0.02 ***	0.062±0.008 ns	0.069±0.008 ns
LEU	0.16±0.073	0.112±0.013 ns	0.13±0.004 ns	0.147±0.006 ns
TYR	0.05±0.003	0.031±0.011 ***	0.041±0.002 *	0.044±0.005 ns
PHE	0.08±0.003	0.052±0.002 ***	0.061±0.011 ***	0.066±0.007 **
HIS	0.14±0.016	0.083±0.007 *	0.131±0.008 ns	0.132±0.011 ns
LYS	0.17±0.006	0.113±0.009 ***	0.13±0.006 ***	0.155±0.011 *

p>0.05= non-significant; p<0.05=* significant; p<0.01=** distinctly significant

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