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SALICYLIC ACID INVOLVEMENT IN SALT STRESS ALLEVIATION IN WHEAT (*TRITICUM AESTIVUM* CV CRISANA) SEEDLINGS

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ABSTRACT
Salt stress affects around 20% of the world's cultivated areas. Many crops species are sensitive to salinity. Soil salinity causes reduction in crop productivity, because plants may suffer four types of stress: osmotical conductance, specific ion toxicity, ion imbalance and oxidative stress with production of reactive oxygen species. Salicylic acid (SA) plays an important role in plants response to biotic and abiotic stress. Pre-treatment of wheat seeds with SA may cause a low level of oxidative stress, improving the antioxidative capacity of the plants. Salicylic acid can increase the plant tolerance to salt stress induced by NaCl treatments. In our experiment we determined the effect of pre-treatment of wheat seeds with 0.05 mM and 0.1 mM concentration of SA solution on growth, relative water content, on photosynthesys and on assimilatory pigments content of the wheat seedlings in vegetative stage stressed with 0.2M NaCl solution. The results obtained showed that exogenous application of SA induced an increase in growth parameters of wheat seedlings and improved photosynthetic capacity of wheat seedlings against salt induced stress. The SA treatement also ameliorate the total clorophyllian pigment content of wheat seedling leaves under salt stress.
KEYWORDS: wheat, salt stress, salicylic acid, growth, photosynthesis, assimilatory pigments.

INTRODUCTION

Biodiversity is important at the level of ecosystems, species, populations, individuals and genes. There is a need for better management to safeguard biodiversity at all these interacting levels. While some losses in biodiversity over time are inevitable through both natural and human induced causes, diversity can be conserved and managed through a wide range of actions, from the establishment of nature reserves and managed resource areas, to the inclusion of conservation (FAO, 2003).

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).

Local food is a principle of sustainability relying on consumption of food products that are locally grown. It is part of the concept of local purchasing; a preference to buy locally produced goods and services. Food and farming play a key role in creating our culture, landscape and health. Local food production brings people together to make this possible and also benefit local economies (Ipate, 2010).

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 (Antofie, 2010).

In vitro conservation is an important method of germplasm conservation, as traditional conservation of crop both plants of agricultural interest (Petruş, in press 2011), as well as the medicinal (Pop, 2011).

The soil-plant-animal relationship is an ecopathological indicator in a farm located near a highly polluted area. (Ioniţă,2010).

Cereals are still by far the world's most important sources of food, both for direct human consumption and indirectly, as inputs to livestock production. What happens in the cereal sector is therefore crucial to world food supplies (FAO, 2003).

The anthropogenic activities and changed agricultural system, intense use of chemical fertilizers and artificial irrigation have increased temperature, ultraviolet radiation, drought, salinity and heavy metals stresses and caused yield reduction in most plants crops (Nafees and Sarvajeet, 2007). To overcome the yield losses due to this abiotic stresses, plants need to possess mechanisms of avoidance and tolerance to stress. For sustainable agriculture development, future crops should have abiotic stress resistant traits and the mechanism for stress tolerance. The tolerance mechanisms can also be improved by the development of new techniques employing plant physiology and plant molecular biology tools (Khan et al., 2003).

Salicylic acid (SA) belongs to a diverse group of plant phenolics, is a natural signaling molecule involved in the regulation of different physiological and biochemical processes, including membrane

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permeability (Barkosky and Einhellig, 1993), ion uptake, enzymes activities, photosynthesis (Hayat et al., 2005), growth and development of plants (Hayat and Ahmad, 2007), and may function as a plant growth regulator (Arberg, 1981). Quiroz-Figueroa et al., in 2001 suggested that it is possible as these phenolic compounds act as a signal, which induce the differentiation processes. An explanation is the possibility that, due to the chelating properties of these compounds, some inhibitors present in the embryogenic cultures are inactivated.

Salicylic acid play an important role in the plant response to adverse environmental conditions such as low temperature stress (Janda et al., 1999; Tasgin et al, 2003), salt and osmotic stress (Senaratna et al., 2000, Borsani et al., 2001).

The application of SA, or of acetylsalicylic acid (ASA) or other analogues of salicylic acid, to leaves of corn and soybean accelerated their leaf area and dry mass production, but plant height and root length remained unaffected. However the leaves of corn and soybean treated with ASA or gentisic acid exhibited no change in their chlorophyll contents (Khan et al., 2003).

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 (Moharekar et al., 2003).

The soaking of wheat (*Triticum aestivum* L.) seeds in 0.05mM SA also reduced the damaging effects of salinity on seedlings growth and accelerated the growth processes (Shakirova et al., 2003).

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 (Tari et al., 2002;2004; Szepesi et al., 2005).

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 *Triticum aestivum* and about 128% in *Hordeum vulgare*) in the seedlings subjected to saline stress.

Purcărea and Cachiță (2008 a,b) studying the influence of SA and ASA on the growth of sunflower (*Helianthus sp*.), seedling roots, on their total absorbtion capacity and on content of assimilatory pigments, in they primary leaves, observed that on the 6-th day of germination the diluted ASA solutions, with concentrations of 0.01, 0.1 and 0.5 mM had greater effects on the growth and determined the highest increase of the total absorption capacity of sunflower root system, with 159.6% compared to the control lot being recorded in case of the treatment with 0.1 mM ASA solution. The diluted ASA solution, with 0.01 mM the 0.1 mM concentration, determined an increase in the total chlorophyllian and carotenoid pigments content in the primary leaves of sunflower plantlets especially for 0.01 mM and 0.1 mM concentration. Higher concentrations than 0.5 mM decreased the same parameter, the greatest inhibitions being obtained for the SA or ASA solutions of a 5.0 mM concentration.

The aim of this study was to determine the effect of pre-treatement of wheat seeds with 0.05 mM and 0.1mM concentration SA solutions on growth (plantlets height, dry matter, leaf area), relative water content, on photosynthesys and on assimilatory pigments content of the 21 days old wheat seedlings stressed by 0.2M NaCl solution.

For the future we will continue to study the role of salicylic acid and its derivatives, like acetylsalicylic acid (ASA) in the response of the plants to other abiotic stress factors or on other agricultural plant.

MATERIALS AND METHODS

The experiments were performed at the Agrifood Biochemistry Laboratory, University of Oradea. 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.

In field conditions, accuracy of results is influenced by a complex random factors: one in connection with the climatic conditions, some associated with soil type. Experiences growing in pots under controlled greenhouse or laboratory condition, removes some of the unpredictable variations in the pedoclimatic factors. 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.

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Experimental variants were as follows:

- control lot (S_1) – 12 h soaked in water, sown in pots and irrigated with water;
- sample 1 (S_2) - 12 h soaked in water, sown in pots and irrigated with 0.2M NaCl solution;
- sample 2 (S_3) – 12 h soaked in 0.05 mM SA, sown in pots and irrigated with 0.2M NaCl;
- sample 3 (S_4) – 12 h soaked in 0.1mM SA, sown in pots and irrigated with 0.2M NaCl;

Plant growth measurement:

For the biometrical determination the measurment were taken in march (vegetative stage). The length of the roots and leaves plus shoots, of 10 wheat seedlings were measured, and it were taken 3 independent repetitions for each determination. Plant growth was estimated measuring accumulation of roots and leaves plus shoot weight, after drying the plants material at 60°C for 72h.

Relative water content was also measured and express according to the following equation:

$$RWC(\%) = \frac{Wf - Wd}{Ws - Wd} \times 100$$

RWC = relative water content; Wf = Fresh leaf Weight; Wd = Dry leaf weight; Ws = Saturated leaf weight

Photosynthetic rate(PR) and stomatal conductance(SC)

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). The wheat seedling leaves area were measured with Leaf area-meter. Three measurements/plot were undertaken.

Assimilatory pigments:

The content of chlorophyllian pigments of the wheat plantlets primary leaves, using *N,N-dimethylformamide* (DMF), 99.9%, (Moran and Porath, 1980) for the extraction. The extraction of assimilatory pigments in higher plant tissue using DMF expedites the process and enables the determination of small samples with low pigment level (Moran, 1982). There is a vast array of solvents used for the extraction and determination of the chlorophyllian pigments, but most of them necessitate grinding and centrifuging of material with or without heating. The use of DMF renders the process simpler and faster, since the pigments can be extracted from intact tissue. For extraction, 50 mg fresh weight of primary leaves, were collected separately from each sample, and were blended with 5ml DMF and then cooled at 4˚C for 72 hours. The supernatant was separated and the content of the pigment was determined using a UV-visible mini–1240 Shimadzu spectrophotometer, at 664nm wave length for chlorophyll a, 647 nm for chlorophyll b, and 480 nm for carotenoids.

The data obtained after the spectrophotometrycal determination, was mathematically processed using formulae proposed by Moran and Porath (1980).

$$\text{Chlorophyll a (mg/g sp)} = (11.65\ a_{664} - 2.69\ a_{647}) \bullet V/sp$$
$$\text{Chlorophyll b (mg/g sp)} = (20.81\ a_{647} - 4.53\ a_{664}) \bullet V/sp$$
$$\text{Carotenoids (mg/g sp)} = (1000\ A_{480} - 1.28\ \text{chloroph.a} - 56.7\ \text{chloroph.b})/\ 245 \bullet V/sp$$

The results obtained for all parameters are averages of 3 determinations and were statistically processed using the "t- test" using *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

Plant growth

Studying the length 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 in length of root, leaves (between 33.8% for the leaves, and 34.7% for entire plant) in compartion with the control lot, evaluated as 100% also the dry weight content of the wheat plantlets was very significantly reduced, between 43.1% and 47.9%, as compared 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. Dry weight of roots and leaves were significantly reduced under salt stress (between 43.16 and 47.9%) (Tables 1 and 2, and Fig.1). We can observed that treatement with 0.05 mM SA solution determine a more intense improvement of roots growth parameters and the 0.1 mM SA solution determine increasing of growth parameters of wheat seedlings leaves.

Table 1.

Estimative mean values for plant characteristic of the salt stressed wheat (*Triticum aestivum* cv Crisana) 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 length (cm)	Root Length (cm)	Leaf Area mm²/plant	Photosynthetic rate (PR) (µmol CO_2 m^{-2} s^{-1})	Stomatal conductance (SC) (mol m^{-2} s^{-1})
Control (S_1)	21±3	10.33±0.76	10.83±2.84	2148±136.5	12.09±0.95	0.19±0.002
Salt 0.2M NaCl (S_2)	13.33±0.57 *	6.83±1.89 ***	6.66±1.52 ns	722±3 ***	5.73±0.67 ***	0.08±0.001 ***
Salt+ 0.05mM SA(S_3)	20.33±2.08 ns	10±1 ns	10.33±2.46 ns	1513±45 **	7.57±0.22 **	0.13±0.004 ***
Salt+ 0.1 mM SA (S_4)	18.33±1.52 ns	10.66±2.08 ns	7.66±0.57 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.

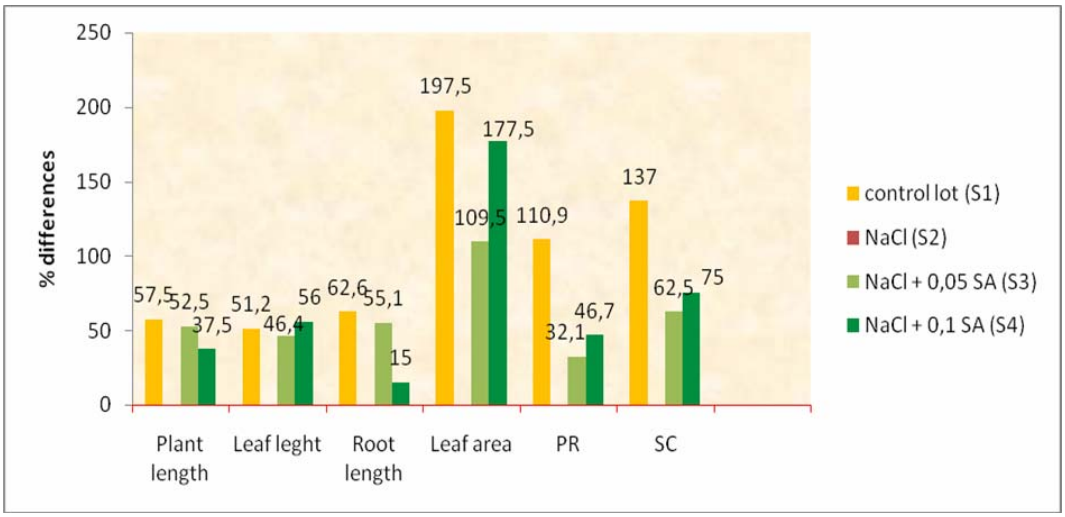

Fig.1. Percentage differences which reflect the effect of *salicylic acid* pretreatment on some physiological parameters of wheat (*Triticum aestivum* cv. Crisana*)* seedlings under salt stress condition, treated or untreated with SA as compared with the salt stressed lot marked with 0. The measurment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

The lowest leaf area and leaf relative water content 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 (2001), 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. Salicylic acid (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 2.

Estimative mean values for fresh and dry matter of root and leaves and for relative water content of the salt stressed wheat (*Triticum aestivum* cv. Crisana*)* 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	Root fresh weight/plant (g)	Root dry weight /plant (g)	Leaves fresh weight/ plant (g)	Leaves Saturated weight/plant (g)	Leaves Dry weight/ plant (g)	RWC %
Control (S_1)	0.0838±0.005	0.0273±0.004	0.6287±0.02	0.7082±0.03	0.278±0.01	84.77±0.5
Salt 0.2M NaCl (S_2)	0.0473±0.006 ***	0.0131±0.001 **	0.2897±0.007 ***	0.3249±0.008 ***	0.120±0.009 ***	82.82±0.7 **
Salt+ 0.05mM SA(S_3)	0.1272±0.003 ***	0.034±0.002 * ***	0.4113±0.007 *** ***	0.4546±0.01 *** ***	0.1395±0.007 *** *	86.24±0.3 *** ***
Salt+ 0.1 mm SA (S_4)	0.0797±0.001 ns	0.0285±0.002 Ns ***	0.4671±0.005 *** ***	0.5001±0.01 *** ***	0.224±0.008 *** ***	88.04±0.1 *** ***

p>0.05= non-significant; p<0.05 * significant; p<0.01=** distinctly significant; p<0.001=*** very significant in comparison with control lot. Semnification in comparison with control lot marked with black and in comparison with the salt stressed lot marked with red.

Assimilatory pigments

Studying the *content of chlorophyllian pigment (chlorophyll a and b) and carotenoids* on the 4rd 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.

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 the Table 3, and in the Fig.2. 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 4rd leaves.

Table 3.

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			
		Control (S_1)	Salt (S_2)	Salt+ 0.05 mM SA (S_3)	Salt+ 0.1 mM SA (S_4)
Assimilatory pigments mg/g FW	chl a	1.403±0.001	1.348±0.003 ***	1.426±0.012 ***	1.453±.0.004 ***
	chl b	0.662±0.002	0.632±0.008 ***	0.633±0.006 ***	0.643±0.002 ***
	carotenoids	0.512±0.01	0.473±0.01 *	0.506±0.01 *	0.498±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.

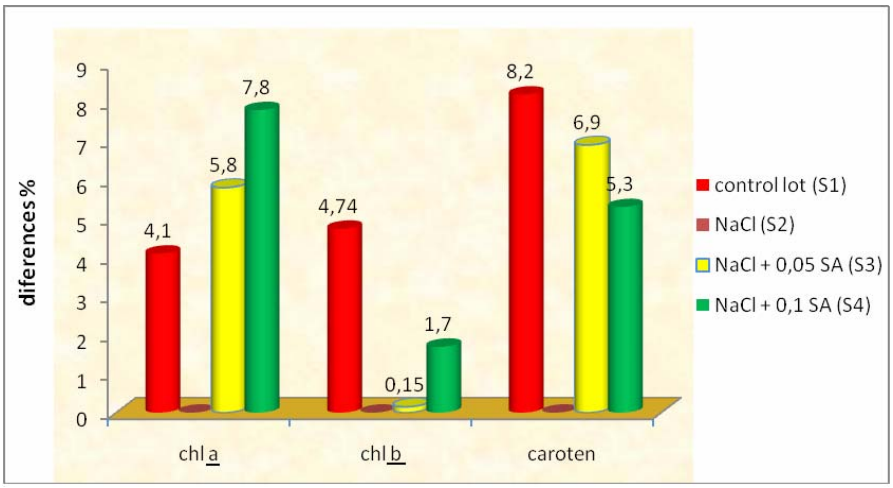

Fig.2 Percentage differences which reflect the effect of *salicylic acid* pretreatment of wheat (*Triticum aestivum* cv. Crisana*)* seedlings on assimilatory pigments content estimative mean value under salt stress condition. treated or untreated with SA. as compared with the salt sressed lot. The measurment were taken in march (vegetative stage) before the straw formation.

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.

Stomatal conductance and photosynthetic rate always declined with salinity but, SA treatement alleviate salt stress induced negative effects, and increased both parameters, photosynthetic rate and stomatal conductance.

According to obtained results. there is a clear correlation between the treatement applied and the content of assimilatory pigments (chlorophyllian and carotenoid pigments) in the 4rd leaves of wheat seedling in vegetative stage.

Salicylic acid was involved in salt stress alleviation in studied wheat (*Triticum aestivum* cv. Crisana L) seedlings in vegetative stage of field experience. The highest enhancements of the tolerance to salinity were recorded in the case of treatments with 0.1 mM SA solution.

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