

Abstract of Ph.D. thesis

**EFFECT OF LONG-TERM SALICYLIC ACID PRE-
TREATMENT ON SALT STRESS ACCLIMATION OF
TOMATO PLANTS**

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INTRODUCTION AND AIMS

Salinity in soil or water is one of the major stresses and, especially in arid and semi-arid regions, can severely limit crop production. Therefore it is very important to investigate the mechanisms of salt tolerance, selection or production biotechnologically of stress-tolerant species. There are some traditional breeding methods to improve the salt tolerance of tomatoes. There were some attempts with genetically modified plants and with changes of plant growth conditions in order to make the genotype more salt tolerant. This means so called hardening process that can induce abiotic stress tolerance of plants. Before our experiments the application of SA as pre-treatment or chemical priming in order to improve salt stress acclimation was not successful, because it sometimes caused inhibition of seedling growth or the vegetative development. The protective effect of exogenously applied SA against salt stress is well characterized in the literature but the results were contradictory. Effect of SA depends on the mode of application, the developmental phase of plants and the applied concentration of SA. High ($> 10^{-3}$ M) SA concentrations cause high level of oxidative stress for the plants but lower concentrations of SA (10^{-7} M és 10^{-4} M SA) which we used in this study can induce hardening process which can improve the tolerance to salt stress (100 mM NaCl) in tomatoes grown in hydroponic cultures.

Improvement the salt tolerance of tomato (*Solanum lycopersicum* Mill. L.) plants

In my thesis I present the results which intend to answer the following questions:

- How can SA concentrations affect the salt stress resistance of tomato plants? What is the mechanism of the SA effect?
- Can long-term pre-treatment with SA cause a decrease in water potential as written in the literature?
- What changes can occur in the parameters of water status, water potential and osmotic potential of tomato plants?
- Can SA promote osmotic adaptation during pre-treatment period and the following salt stress in tomato plants? Can SA induce the accumulation of inorganic osmolytes and possibly the synthesis of organic osmolytes during pre-treatment period? Which are the organic osmolytes or osmoprotective compounds that can provide osmotic adaptation during salt stress?

- What is the role of SA in the induction of a stress hormone, abscisic acid (ABA) biosynthesis and changes of ABA contents which determines the success of the acclimation to high salinity? Can SA pre-treatment affect the activity of abscisic aldehyde oxidase, one of the regulating points of ABA biosynthesis? How does ABA concentration change during salt exposure?
- How do the markers of stress resistance like Chl-*a* fluorescence induction parameters, Chl-*a*/Chl-*b* ratio and lipid peroxidation change in the pre-treated tomato plants during 100 mM NaCl treatment?
- What is the effect of SA pre-treatment on the activation of ABA-induced protective mechanisms during salt stress? How can the concentrations of polyamines and proline change in the pre-treated plants?
- How can SA affect the enzymatic and non-enzymatic antioxidant protective mechanisms like the amounts of reduced and oxidized forms of ascorbate and glutathione and activities of enzymes involved in the detoxification? We also investigated the effect of SA pre-treatment on the parameters mentioned above in the salt stressed plants.
- Proline that can accumulate after SA pre-treatments under salt stress, is an important compatible osmolyte and antioxidant. We investigated how the different proline concentrations can affect the cell viability of mesophyll protoplasts derived from tomato leaves. By microscopic staining we also investigated the effect of salt stress on generation of NO and H₂O₂ in protoplasts treated with different concentrations of proline. The question was how different concentrations of proline affect the viability of cells under salt stress and how it is connected to H₂O₂ and NO production of cells?
- One of our aims was to compare the antioxidant responses of drought-tolerant bean genotypes with those of tomato plants during salt stress.
- The other goal of our study was to reveal the common points of the SA-induced acclimation processes of cultivated tomato plants and responses of the wild, salt tolerant species, *Solanum pennellii* during salt stress.

MATERIALS AND METHODS

Plant material:

Tomato (*Solanum lycopersicum* Mill. L. cvar Rio Fuego) plants and mesophyll protoplasts

Green bean (*Phaseolus vulgaris nanus* L.) genotypes

Applied treatments:

Long-term (3 weeks) SA pre-treatment with two concentrations (10^{-7} M SA, 10^{-4} M SA)

Salt exposure for 1 week by 100 mM NaCl

Drought stress by irrigation withdrawal

Plant water relation and photosynthetic parameters:

The water potential was measured by pressure chamber, the osmotic potential by digital osmometer, the stomatal conductance by steady-state porometer. The Chl-a fluorescence induction parameters were measured by pulse amplitude modulated fluorimeter. In order to detect the K^+ uptake, we applied $^{86}Rb^+$ isotope. Determination of ion contents was done by atomic absorption spectroscopy, the determination of chlorophyll and carotenoid pigment contents was measured by spectrophotometer.

Abscisic acid:

We investigated the abscisic acid content by competitive ELISA method, the enzyme activity of abscisic aldehyde oxidase was determined in native activity gel.

Compatible osmolytes:

The soluble sugar content, sucrose, glucose and fructose, proline, sorbitol and related aldose reductase enzyme activities were determined by spectrophotometer.

Polyamine analysis:

We measured polyamine contents by HPLC.

Protoplast cell viability, NO and H_2O_2 content:

We investigated the cell viability of mesophyll derived protoplasts by staining with FDA (fluoresceine diacetate), the relative NO content by DAF-2 DA (4,5-diaminofluoresceine

diacetate) and the H₂O₂ generation by DC-FDA (2,7-dichlorofluoresceine diacetate). Samples were analysed by Zeiss Axiowert 200 M-type fluorescent microscope.

Enzymatic and non-enzymatic antioxidant system:

We determined the activities of antioxidant enzymes and levels of non-enzymatic antioxidants by spectrophotometer.

RESULTS

Our aim was to study the effect of long-term SA pre-treatment on salt stress acclimation of cultivated tomato plants.

We stated that the exogenously applied 10⁻⁴ M SA was capable to improve the tolerance for ionic osmotic stress in tomatoes; however pre-treatment with 10⁻⁷ M SA was not successful.

The present study provides the following new results in this topic:

1. It was established that the 10⁻⁷ M and 10⁻⁴ M SA pre-treatment inhibited the uptake of potassium (K⁺/⁸⁶Rb⁺), one of the most important inorganic osmolytes. Both applied concentration caused significant decrease of water potential and resulted in stomatal closure on the first week of the pre-treatment in tomato plants. However, growth inhibition can be observed only in case of 10⁻⁴ M SA pre-treatment. We can conclude that SA at 10⁻⁴ M in this case caused an allelopathic growth inhibition independently of plant water status.
2. Roots can uptake water from the environment only if the cell content is concentrated by accumulation of osmolytes or volumetric adaptation, and the water potential of root cells decreases below the water potential of nutrient solution. This osmotic stress can activate the synthesis of organic osmolytes. After 3-week of SA pre-treatment in the shoot tissues an osmotic and/or volumetric adjustment and then a water potential increase occurred. This occurred principally due to the accumulation of carbohydrates. After pre-treatment the content of soluble sugars increased both in the roots and shoots. While in the roots sucrose, in the shoots hexoses, glucose and fructose accumulated and when the plants were exposed to 100 mM NaCl, the tissues had already been osmotically adapted in 10⁻⁴ M SA pre-treated plants.

3. It was proven that despite of osmotic adjustment SA pre-treatment also induces the synthesis of ABA and ABA concentration significantly increased in the roots and shoots of 10^{-4} M SA pre-treated plants at the end of pre-treatment. So the ABA signal pathway and ABA-induced gene expression could be activated before the second stressor.
4. It can be stated that the enhanced synthesis of ABA is a consequence of the activation of abscisic aldehyde oxidase, AO1 isoenzymes whose activities increased moderately after 10^{-7} M SA pre-treatment and significantly increased during the pre-treatment with 10^{-4} M SA in roots and shoots. We can conclude that SA evolved its protective effect against osmotic and salt stress partly by activating previously the ABA synthesis and accumulation.
5. With some important stress marker it was proven that physiological parameters of 10^{-4} M SA pre-treated plants after 100 mM NaCl exposure showed better values than salt stressed control. The primary photochemical processes of photosynthesis (F_v/F_m , Φ_{PSII} , qP) functioned equally or better than that of the control, the Chl-*a*/Chl-*b* ratio and contents of carotenoids increased and the lipid peroxidation decreased in these plants.
6. Accumulation of Na^+ in mesophyll cells of SA pre-treated plants, particularly at 10^{-4} M SA and there act as inorganic osmolyte functioned to increase the water potential like in some halophyte species.
7. SA pre-treatment significantly increased the activity of aldose reductase at both concentrations and the sorbitol contents in the shoots formed in the reaction catalysed by AR. Since the content of sorbitol is not enough to play a role in the osmotic adjustment, the increase of AR activity may have greater importance in the recycling of NADPH.
8. In the upper leaves of SA pre-treated plants we measured significant proline accumulation during salt stress. This may be an adaptive process but the excess of proline can cause programmed cell death depending on its concentration. The exposure to 100 mM Pro, 100 mM NaCl salt stress combined with 100 mM Pro

decreased the cell viability of mesophyll protoplasts. 1 mM Pro application improved the cell viability during salt stress. Although the hydrogen peroxide contents did not decrease significantly in the cells, NO content was not enhanced in the 1 mM Pro treated cells. The decrease of cell viability can be observed at those treatments where the relative amount of NO was high along with the hydrogen peroxide content of tissues.

9. In the shoots of 10^{-4} M SA pre-treated plants during salt exposure the level of putrescine was enhanced significantly while in the roots concentrations of spermidine and spermine were high. These polyamines not only can inhibit ROS-generating NADPH oxidase but directly protect the integrity of macromolecules (DNA, proteins and membranes) in the presence of sodium ions. High spermine concentrations characterize the halophyte species during salt stress.
10. To improve abiotic stress acclimation SA has to affect antioxidant systems. This is based on the fact that depending on its concentration SA can enhance tissue hydrogen peroxide concentration. We were the first who described the improvement of salt stress acclimation by long-term SA pre-treatment. It can be stated that in those treatments when the salt stress induced damage, high SOD activity in the upper leaves and roots (C, 10^{-7} M SA), transiently (10^{-7} M SA, upper leaves, root) or permanently decreased CAT activity (C, upper leaves) can be observed during salt stress. In these samples (C, 10^{-7} M SA upper leaves, roots) POD activity was maintained at high level which can also contribute to the elimination of hydrogen peroxide. In the 10^{-4} M SA pre-treated plants high POD activities decreased to the end of the experiments which indicates a decrease of oxidative stress.
11. Different SA pre-treatments affect in a distinct way the enzyme activities of APX, the most important enzyme of ascorbate-glutathione cycle. 10^{-7} M SA pre-treated plants were not so efficient to eliminate cellular hydrogen peroxide than plants treated with 10^{-4} M SA due to constant low APX enzyme activities. In contrast of this, the 10^{-4} M SA pre-treatment enhanced an APX activity which along with high concentrations of non enzymatic antioxidants was capable to successfully detoxify the reactive oxygen species.

12. GR is essential for the function of antioxidant enzymes and for the effective reduction of non enzymatic antioxidants. The high GR basic activity and its induction by salt stress can cause the successful stress acclimation of 10^{-4} M SA pre-treated plants.
13. SA pre-treatments increased the contents of ascorbate and glutathione in the different parts of plants, mainly in the upper leaves. We can state that contrary to other treatments, 10^{-4} M SA pre-treated plants accumulated non enzymatic antioxidants like ascorbate and glutathione in their roots during salt exposure which caused efficient protection against reactive oxygen species. This is very important because in the roots exposed high salinity, efficient ROS inactivation is very important in order to maintain the normal plant homeostasis.
14. SA pre-treatment induced some similar physiological changes in the cultivated tomatoes which are functioning in the halophyte wild tomato species, *S. pennellii* under salt stress. It can promote the transport of sodium ions to the shoots and the utilization of Na^+ as inorganic osmolyte, which occur as in the halophyte wild species of tomato. Na^+ accumulation contributed to an increased relative growth rate during salt stress in these plants. Similarly, in 10^{-4} M SA pre-treated plants the GSH and Asc were accumulated and represented the main protective line against oxidative stress.

LIST OF PUBLICATIONS

(* Present thesis is based on articles marked by an asterisk)

- * 1. **Szepesi Á**, Csiszár J., Gémes K., Horváth E., Horváth F., Simon L.M., Tari I (2009) Salicylic acid improves the acclimation to salt stress by stimulating abscisic aldehyde oxidase activity and abscisic acid accumulation, and increases Na⁺ contents of the leaves without toxicity symptoms in *Solanum lycopersicum* L. J. Plant Physiol. 166 (9): 914-925. **IF:2.239**
- * 2. Tari, I., D. Camen, G. Coradini, J. Csiszár, E. Fediuc, K. Gémes, A. Lazar, E. Madosa, S. Mihacea, P. Poor, S. Postelnicu, M. Staicu, **Á. Szepesi**, G. Nedelea, L. Erdei (2008) Changes in chlorophyll fluorescence parameters and oxidative stress responses of bush bean genotypes for selecting contrasting acclimation strategies under water stress. Acta Biol. Hung. 59: 335-345. **IF: 0,447**
- *3. **Szepesi, Á.**, Csiszár, J., Gallé, Á., Gémes, K., Poór P., Tari I. (2008) Effects of long-term salicylic acid pre-treatment on tomato (*Lycopersicon esculentum* Mill. L.) salt stress tolerance: changes in glutathione S-transferase activities and anthocyanin contents. – Acta Agronomica Hungarica 58:(2) 129-138.
4. Tari, I. Kiss, Gy., Deér, AK, Csiszár, J., Horváth, F., Poór, P., **Szepesi, Á.**, Simon, LM. (2009 or 10) Salicylic induced increases in aldose reductase activity and sorbitol accumulation in tomato under salt stress.-Submitted to Biol. Plantarum, **IF: 1,259**
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- *6. **Á. Szepesi**, J. Csiszár, Sz. Bajkán, K. Gémes, F. Horváth, L. Erdei, A. Deér, LM. Simon, I. Tari (2005) Role of salicylic acid pre-treatment on the acclimation of tomato plants to salt- and osmotic stress. – Acta Biol Szeged, 49: 123-125.

Conference abstracts published in scientific journals:

1. Gémes K, Poór P, Sulyok Z, **Szepesi Á**, Szabó M, Tari I (2008) Role of salicylic acid pre-treatment on the photosynthetic performance of tomato plants (*Lycopersicon esculentum* Mill. L. cvar. Rio Fuego) under salt stress. Acta Biol Szeged 52 (1): 161-162.
- *2. **Szepesi Á**, Poór P, Gémes K, Horváth E, Tari I (2008) Influence of exogenous salicylic acid on antioxidant enzyme activities in the roots of salt stressed tomato plants. Acta Biol Szeged 52 (1): 199-200.

Conference abstracts published in other journals:

1. Gémes, K., **Szepesi, Á.**, Guóth, A., Tari, I. (2007) Role of photosynthetic performance in salt stress acclimation of tomato after salicylic acid pre-treatment. 2nd World Conference of Stress, 23-26 August, Budapest, Hungary, Book of Abstracts, pp. 213.
2. **Szepesi, Á.**, Gémes, K., Tari, I. (2007) Salicylic acid pre-treatment induced antioxidant defence processes in tomato (*Lycopersicon esculentum*) during salt stress. RoS in Plants 2007, September 12-14, Gent, Belgium
3. **Szepesi, Á.**, Csiszár, J., Gémes K., Tari I. (2006) Salicylic acid improves the acclimation of tomato to high salinity by stimulating abscisic acid biosynthesis and accumulation. XV FESPB Congress of European Societies of Plant Biology, 17-21 July 2006, Lyon, France, Book of Abstracts, pp. 174.
4. **Szepesi, Á.**, Csiszár, J., Gémes, K., Tari I. (2006) Salicylic acid improves the acclimation of *Lycopersicon esculentum* to high salinity by approximating its salt stress response to that of the wild species *L. pennellii*. - 3rd EPSO Conference, „Plant Dynamics: from Molecules to Ecosystems”, Visegrád, Hungary, 28 May – 1 June, 2006 Book of Abstracts, pp.163.
5. I. Tari, J. Csiszár, Á. Gallé, B. Bartha, F. Horváth, A. Pécsváradi, **Á. Szepesi**, D. Zeller, L. Erdei (2005) The role of ABA and NO in the drought stress acclimatisation mechanisms of wheat genotypes. XVII. International Botanical Congress, Vienna, Austria, Europe, 17-23 July 2005, Abstracts, pp. 253.
6. Tari, I., Csiszár, J., Horváth, F., Pécsváradi, A., **Szepesi, Á.**, Zeller, D., Erdei, L. (2004) Drought stress resistance of bean genotypes II.: Do drought-hardened plants suffer from fever?- Innováció és szakemberképzés az alkalmazott növénybiológiában. Final report. 19-36. pp. Hungary – Romania PHARE CBC Programme (projekt number: HU 2002/000,627,03-14). Romanian-Hungarian minisymposium.
7. Csiszár, J., Tari, I., **Szepesi, Á.**, Gallé, Á., Bartha, B., Bajkán, Sz., Zeller, D., Vashegyi, Á., Pécsváradi, A., Horváth, F., Lazar, A., Dorin, C., Staicu, M., Petolescu, C., Gabor, L., Erdei, L. (2004). Az antioxidáns védőmechanizmus egyes elemeinek vizsgálat a zöldségfélékben szárazságstressz hatására. – Final report. 37-43. pp. Hungary – Romania PHARE CBC Programme (projekt number: HU 2002/000,627,03-14). Romanian-Hungarian minisymposium.

8. Tari, I., Csiszár, J., Gallé Á., Bajkán Sz., **Szepesi Á.**, Vashegyi Á. (2004) Élettani megközelítések gazdasági növények szárazságtűrésének genetikai transzformációval történő javítására. Bot. Közlem. 90: 139-158.
9. Tari, I., Simon, LM:, Deér, KA, Csiszár, J., Bajkán, Sz., Kis, Gy., **Szepesi, Á.** (2004) Influence of salicylic acid on salt stress acclimation of tomato plants: oxidative stress responses and osmotic adaptation. . – Acta Physiol. Plant. , The 14th FESPB Congress, August 23-27, 2004, Cracow, Poland, Book of Abstracts, pp. 237. (**IF: 0.379**)
10. **Szepesi Á.**, Tari I. (2003) A fotoszintetikus hatékonyság szerepe a szalicilsavval előkezelt paradicsom növények sóstressz akklimatizációjában. – V. Magyarországi Fotoszintézis Konferencia és Fotoszintézis Iskola, Noszvaj, 2003. szeptember 15-16. Abstracts, P15.

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