

Role of salicylic acid pre-treatment on the acclimation of tomato plants to salt- and osmotic stress

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ABSTRACT The alleviation of oxidative damage and increased resistance to salt stress and to non-ionic osmotic stress induced by 100 mM NaCl or 100 mOsm polyethylene glycol (PEG) treatments, respectively, often correlate with a more efficient antioxidative defence systems and detoxification mechanisms. Pre-treatment of plants with salicylic acid (SA) enhanced antioxidant enzyme activities in concentration dependent manner and increased the stress tolerance of seedlings. Improved acclimation of SA-pre-treated plants to salt stress depended on the previous activation of the antioxidative enzymes and accumulation of ionic and non-ionic osmolytes, but increased resistance to non-ionic osmotic stress could be achieved in those plants which showed reduced antioxidative enzyme activities at the initial stage of the stress. The present work deals with the possible role of the osmotic adaptation and oxidative defence mechanisms during long-term pre-treatment of tomato plants with low concentrations of SA.

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KEY WORDS

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fluorescence induction
osmotic adaptation
osmotic stress
oxidative stress
salt stress

Salicylic acid (SA), a plant phenolic is now considered as a hormone-like endogenous regulator, and its role in the defence mechanisms against biotic and abiotic stressors has been well documented (Yalpani et al. 1994; Szalai et al. 2000). It was found that inhibition of catalase, a H₂O₂ scavenging enzyme, by SA plays an essential role in the generation of reactive oxygen species (Horváth et al. 2002). By increasing H₂O₂ concentration of the tissues, moderate doses of SA may activate the antioxidative mechanisms. Application of exogenous SA enhanced the drought and salt stress resistance of plants (Senaratna et al. 2000; Tari et al. 2000), but the results were contradictory and depended on the developmental phase of plants (Borsani et al. 2001) or on the experimental conditions (Németh et al. 2002). Both high salinity and drought give rise to ionic and osmotic effects combined with oxidative damage in tissues. The aim of present work was to reveal whether tomato plants pre-treated with low concentrations of SA could acclimate to 100 mM NaCl-induced salt stress or to 100 mOsm polyethylene glycol (PEG)-induced non-ionic osmotic stress by osmotic adjustment and/or by increasing their antioxidant metabolism in response to stress.

Materials and Methods

Tomato (*Lycopersicon esculentum* Mill. L. cv. Rio Fuego) plants were grown hydroponically in a greenhouse, under 180 μmol m⁻² s⁻¹ light intensity and at 12/12 day/night photoperiod. The temperature was maintained at 25°C and the relative humidity was 55-60%. The plants were treated with 10⁻⁷-10⁻⁴

M SA for three weeks. The salt stress induced by 100 mM NaCl treatment and osmotic shock, elicited by 100 mOsm polyethylene glycol (PEG 6000) were applied for 1 week. Metal contents of tissues, relative water content (RWC), water potential (ψ_w) and osmotic potential (ψ_π) of the youngest expanded leaves were measured and pressure potential (ψ_p) was calculated as described earlier (Tari 2003/2004). *In vivo* chlorophyll fluorescence parameters in light and dark-adapted samples were determined with a pulse amplitude modulated fluorometer (PAM-2000, Walz, Effeltrich, Germany). After 15 min of dark adaptation the maximum quantum efficiency of PSII primary photochemistry (F_v/F_m) was determined by application of a 1 s saturation flash to the apical leaflet of completely expanded leaf. The leaves were then exposed to actinic light (165 μmol photons m⁻² s⁻¹) to determine the quantum yield of electron transport at PSII ((F'_m-F_s)/F'_m) in light-adapted samples (Genty et al. 1989).

Results and Discussion

Hundred mM NaCl and especially 100 mOsm PEG treatments significantly reduced the relative water content of tomato leaves. The osmotic shock caused by a single-step PEG treatment resulted in a dramatic inhibition of growth, early senescence and desiccation of plants, while a treatment with 100 mM NaCl corresponding two times higher osmotic concentration, reduced the relative growth rate to 61.2% of the control. The 10⁻⁷ M SA pre-treatment decreased the osmotic stress-induced reduction in relative water content (RWC), but this alleviating effect was not so pronounced at 10⁻⁴ M SA concentration (Fig. 1). A small increase could be

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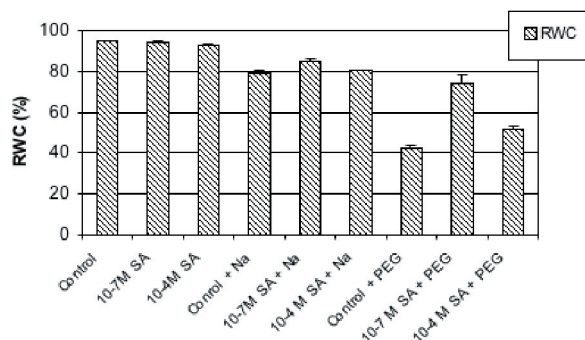


Figure 1. Effect of salt and non-ionic osmotic stress induced by 100 mM NaCl or 100 mOsm PEG 6000, respectively, on the relative water content in leaves of 7-week-old tomato plants pre-treated with 10^{-7} or 10^{-4} M salicylic acid (SA) for three weeks. Means \pm SE, n=4.

observed in the water and osmotic potential of SA-pre-treated samples, but in case of the pressure potential (ψ_p) the changes were higher.

Under the influence of salt stress the osmotic potential (ψ_π) greatly decreased and the SA pre-treatments moderated it at both concentrations. Comparing the effect of two different SA concentrations during non-ionic osmotic stress, we can conclude, that SA pre-treatment was more effective at 10^{-7} M than at 10^{-4} M. (Table 1). The increased water potential values in SA pre-treated samples under ionic and non-ionic osmotic stress suggest that accumulation of inorganic or organic osmolytes makes the surplus of water uptake possible as it can also be seen from the increased relative water contents of tissues.

SA pre-treatments reduced K^+ contents of leaves under salt- and non-ionic osmotic stress. In case of salinity stress, the pre-treatment enhanced the translocation of Na^+ from roots to shoots and compared to the NaCl-treated plants, SA decreased the Na^+/K^+ ratio in the roots and increased it significantly in the leaves. Na^+ , accumulated in the leaf tissues, functioned as an inorganic osmolyte, and resulted in an increased water potential and water content (Fig. 2).

The photosynthetic light reactions and electron transport proved to be one of the main sources of reactive oxygen species (ROS). Since SA improved the photosynthetic performance of plants under stress conditions (Ananieva et al. 2002), and chlorophyll *a* fluorescence could give insight into the ability of a plant to tolerate environmental stresses, we determined the fluorescence induction parameters in SA-pre-treated samples under salt and osmotic stress. SA alone did not influence the maximal (F_v/F_m) and effective quantum yield ($(F'_m-F_s)/F'_m$) of primary photochemical reactions at PSII (Fig. 3). A decrease in F_v/F_m in dark-adapted leaves indicates a photoinhibitory damage in response to stress. This parameter was significantly reduced by PEG 6000 treatment indicating that a sudden drop in water potential of the hydroponic medium resulted in a photoinhibitory damage of the photosynthetic apparatus. At low photosynthetic light intensity ($165 \mu\text{mol m}^{-2} \text{s}^{-1}$) the effective quantum yield was only slightly affected in NaCl-treated samples, but it was significantly reduced under non-ionic osmotic stress. This can be partially overcome if plants are pre-treated with SA. Since under non-photorespiratory conditions the effective quantum yield of PSII provides useful information concerning photosynthetic performance of C3 plants, these results suggest that SA-pre-treatment may improve the gross rate of carbon assimilation during osmotic stress.

In the presence of SA the leaves accumulated different compatible osmolytes, such as sugars, sugar alcohol and proline. SA pre-treatment decreased the CAT activity both in the roots and leaves, but the activity of other enzymes associated with the antioxidative defence, superoxide dismutase (SOD), peroxidase (POD), ascorbate peroxidase (APX) and glutathione reductase (GR) exhibited different changes at 10^{-7} M SA or 10^{-4} M SA. As a general rule, the activity of these enzymes (CAT, SOD, POX and APX) decreased compared to the control in the leaves of tomato plants at 10^{-7} M SA pre-treatment, while at 10^{-4} M their activity could be enhanced (SOD, POX, GR). Moreover, in some cases different tendencies were found in the shoot or root tissues.

We can conclude that the salt tolerance induced by 10^{-4} M SA was associated with the previous activation of the

Table 1. The effect of long-term salicylic acid (SA) pre-treatments on water potential (ψ_w), osmotic potential (ψ_π) and calculated pressure potential (ψ_p) of fully expanded, youngest leaves of tomato plants exposed to 100 mM NaCl or 100 mOsm PEG 6000 for one week. (Means \pm SE, n=5).

| Treatment | Control | | | 100 mM NaCl | | | 100 mOsm PEG | | |
|----------------|----------|------------|----------|-------------|------------------|----------|--------------|------------|----------|
| | ψ_w | ψ_π | ψ_p | ψ_w | ψ_π (MPa) | ψ_p | ψ_w | ψ_π | ψ_p |
| -SA | -0.436 | -0.503 | 0.067 | -0.398 | -1.875** | 1.477 | -2.300** | n.d. | n.d. |
| 10^{-7} M SA | -0.363 | -0.484 | 0.122 | -0.355 | -0.727* | 0.372 | -0.243** | -1.095* | 0.853 |
| 10^{-4} M SA | -0.336 | -0.448 | 0.112 | -0.310 | -1.030* | 0.720 | -0.336 | -0.818 | 0.482 |

Means of ψ_w and ψ_π denoted by * or ** are significantly different from the values of SA-free Control at $P \leq 0.05$ and 0.01 level, as determined by Student's t-test (n.d. = not detectable).

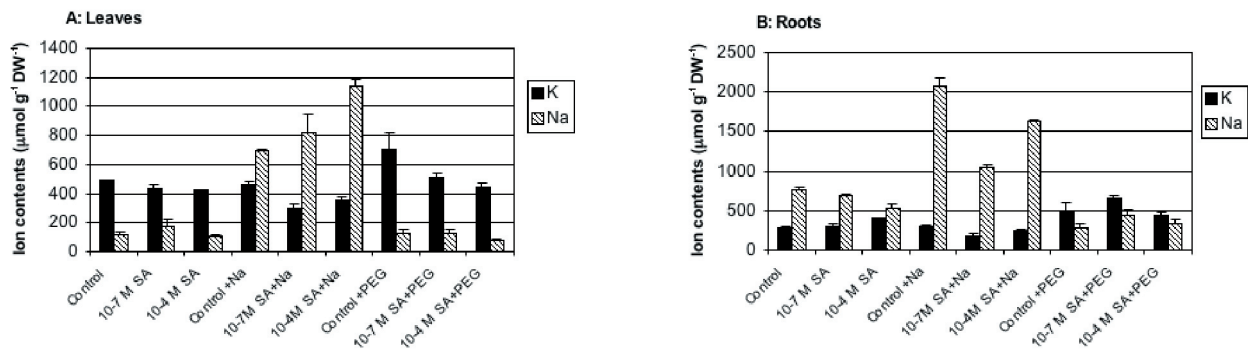


Figure 2. Effect of salt and non ionic-osmotic stress induced by 100 mM NaCl or 100 mOsm PEG 6000, respectively, on the K⁺ and Na⁺ contents in the shoots (A) or roots (B) of 7-week-old tomato plants pre-treated with 10⁻⁷ or 10⁻⁴ M salicylic acid (SA) for three weeks. Means±SE, n=4.

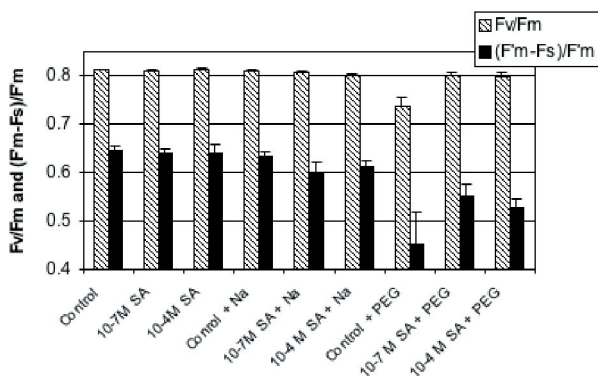


Figure 3. Effect of salt and non-ionic osmotic stress induced by 100 mM NaCl or 100 mOsm PEG 6000, respectively, on the maximal (Fv/Fm) and effective quantum efficiency ((Fm-Fs)/Fm) of 7-week-old tomato plants pre-treated with 10⁻⁷ or 10⁻⁴ M salicylic acid (SA) for three weeks. Means±SE, n=5.

oxidative defence mechanisms and with the accumulation of osmolytes but in case of PEG-induced osmotic stress the acclimation of plants pre-treated with 10⁻⁷ M SA successfully occurred at highly reduced antioxidative enzyme activities at the initial stage of the stress.

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