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Short term effect of salicylic acid on polyamine metabolism in tomato plants

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Salicylic acid (SA) and polyamines (PAs) are plant growth regulators with multifunctional role in plant development and plant defense. Enzymes of PA catabolism, such as diamine oxidase (DAO) and polyamine oxidase (PAO) can generate the secondary messenger H_2O_2 , which is associated with plant defense, including programmed cell death, and with abiotic stress responses. We carried out time course experiments in tomato exposed to SA to determine the metabolism of PAs. Plants were treated with 10^{-4} M and 10^{-3} M SA for 24 h in a nutrient solution. Higher SA concentration triggered a decrease in PA and increase in H_2O_2 content, which showed two peaks at 1 and 3 h in roots, while 10^{-4} M SA-treated roots showed just one peak at 1 h. An increase in activities of DAO and PAO were observed in 10^{-3} M SA-treated roots but nitric oxide level did not change. Ethylene production remained close to the control in 10^{-3} M SA-treated roots. Along with increased electrolyte leakage, these changes may be accompanied with the cell death processes in these roots. The H_2O_2 generated by DAO and PAO activity in 10^{-4} M SA-treated leaves correlated also with the decrease in free PAs. It is concluded that sublethal concentration of SA maintained the level of free PAs in the roots which can contribute to a successful cell survival while lethal SA level can trigger oxidative burst involving polyamine catabolism, which leads to cell death in tomato tissues. Supported by OTKA K 101243.

P-14-038

A yeast chemical proteomics approach to identify targets of small bioactive molecules

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The identification of protein targets of small bioactive compounds is of great interest in many aspects of plant science, ranging from understanding the mode of action of signaling molecules inside plants, deciphering the mode of action of phytotoxins, to the study of off-target effects of agrochemicals and drugs. A powerful tool to study these questions is the yeast three-hybrid screening technology, which is based on the well-established yeast two-hybrid system. A derivatized small molecule is used as bait to identify interacting proteins from a library of preys.

One limiting factor of protein screening techniques is the need for exhaustive cDNA libraries. We have therefore developed an improved yeast three-hybrid method whose relevance relies on highly complex protein fragment libraries that are screened to saturation using an optimized mating procedure. Currently, seven libraries from plant species are available for screening (e.g. *Arabidopsis thaliana*, *Nicotiana benthamiana*, *Solanum lycopersicum*, *Oryza sativa* and *Triticum aestivum*).

Here, we illustrate this new approach with screening results obtained with our *A. thaliana* seedlings library, using the broad-range kinase inhibitor Purvalanol B as bait molecule. In one single experiment we identified 28 different kinases specifically targeted by the molecule. Thanks to our domain-based strategy, we were able to determine the kinase domain as minimal interacting region in all of these partner enzymes.

These results demonstrate the power of the tool in the chemical proteomics arsenal. New ways are now open for mechanistical studies and the improvement of pest management of agronomically important crops and vegetables.

Short-term effect of salicylic acid on polyamine metabolism in tomato plants



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Introduction

Polyamines (PAs) as small, organic polycations, found in all eukaryotic cells are involved in various physiological processes such as cell proliferation, growth, morphogenesis, differentiation and programmed cell death (Kumar et al., 1997). Polyamine oxidation is the main catabolic process of PAs which is an important factor in regulating their intracellular levels. The main PA catabolic enzymes, the diamine oxidase(s) (DAO, EC 1.4.3.6) and polyamine oxidases (PAO, EC 1.5.3.3), generate H_2O_2 , which can act as a signaling molecule or can contribute to cell death induction. H_2O_2 produced by polyamine catabolism in the apoplast may participate in stress responses (Papadakis and Roubelakis-Angelakis, 2005). Our previous studies proved that long-term pre-treatment with salicylic acid (SA) at low concentrations (10^{-7} and 10^{-4} M SA) can induce the synthesis of polyamines in tomato plants (Szepesi et al., 2009). Moreover, exogenous polyamines, mainly spermidine (Spd) and spermine (Spm), enhanced both ROS and NO levels and decreased the viability of tomato leaf protoplasts (Gémes et al., 2011). In the present study we would like to reveal how short-term SA treatment can influence the polyamine contents and activities of enzymes in polyamine catabolism. We are interested in how these changes can contribute to the cell death processes or stress acclimation mechanisms at lethal (10^{-3} M) or sublethal (10^{-4} M) SA concentrations, respectively.

Results

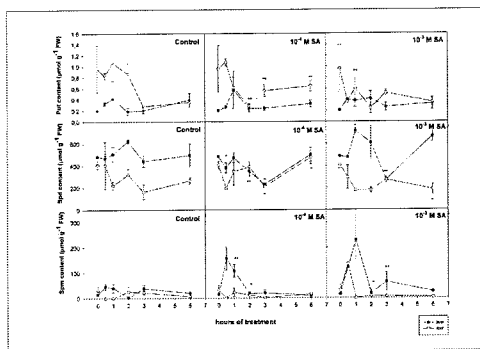


Fig. 1 Changes in the free polyamine content of tomato leaves and roots after treating the samples with 10^{-4} M or 10^{-3} M salicylic acid (SA). Results are the means $\pm$ SE from at least three or four independent experiments. *, **, *** indicate significance levels compared with the untreated control at $P < 0.05$, 0.01, 0.001, respectively, in each time point (Student's t test; ns: not significant).

Compared to the control, 10^{-4} M SA treatment caused an increase in free putrescine (Put) and Spd contents in the roots, while Spm content just showed a slight early increase in the leaves. In the case 10^{-3} M SA Put content declined below control level, Spd and Spm contents exhibited a short, transient increase in the leaves, but in the root tissues PA levels remained low.

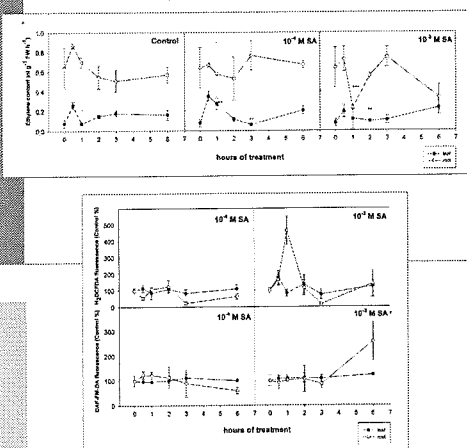


Fig. 4 Changes in the ethylene, total ROS and NO production of tomato leaves and roots after treating the samples with 10^{-4} M or 10^{-3} M salicylic acid (SA). Results are the means $\pm$ SE from at least three or four independent experiments. *, **, *** indicate significance levels compared with the untreated control at $P < 0.05$, 0.01, 0.001, respectively, in each time point (Student's t test; ns: not significant).

Ethylene production remained close to the control levels in the leaves, while in the roots treated with 10^{-3} M SA the ET evolution exhibited a slight decrease compared to the control. NO and ROS contents did not change significantly in the leaves but in the case of 10^{-3} M SA treatment there was a sharp increase at 1 hr in the total ROS content and at 6 hrs in NO content of the root apical segments.

Materials and Methods

Solanum lycopersicum cv. Rio Fuego plants were grown in hydroponic culture in a controlled environment under $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity with 12/12-h light/dark period, a day/night temperatures of 24/22°C and relative humidity of 55-60 %. Plants were treated for 6 hours with 10^{-4} M and 10^{-3} M SA added to the nutrient solution. Samples were prepared from the second, fully expanded leaves and roots in 3 replicates. Experiments were repeated 3-5 times. Data of time-course analysis are means $\pm$ SE of 3 replicates. Free polyamines were determined as described by Flores and Galston (1982). Hydrogen peroxide levels were measured according to Velikova et al. (2000). Enzyme activities of diamine oxidase (DAO, EC 1.4.3.6) and polyamine oxidase (PAO, EC 1.5.3.3) were estimated by the method of Federico et al. (1985) with some modifications (Moschou et al., 2008). Detection of total ROS occurred by staining with fluorescent dye H_2DCFDA , and NO was stained with DAF-FM-DA and these samples were visualized with fluorescence microscopy. Ethylene was detected by gas chromatography (Tari et al., 2006). Disintegration of plasma membrane was determined by measuring electrolyte leakage as described by Sun et al. (2010).

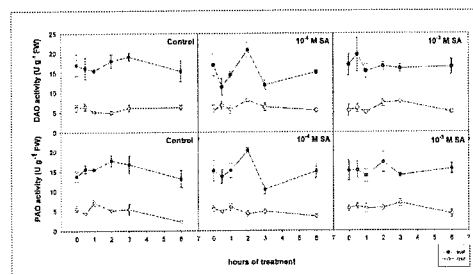


Fig. 2 Changes in the diamine oxidase (DAO) and polyamine oxidase activity (PAO) of tomato leaves and roots after treating the samples with 10^{-4} M or 10^{-3} M salicylic acid (SA). Results are the means $\pm$ SE from at least three or four independent experiments. *, **, *** indicate significance levels compared with the untreated control at $P < 0.05$, 0.01, 0.001, respectively, in each time point (Student's t test; ns: not significant).

DAO enzyme activities did not show significant increases compared to the control except in 10^{-4} M SA and 10^{-3} M SA-treated roots at 2 hrs and 3 hrs, while in the case of PAO activities a decrease can be seen in the leaves at 3 hrs. It can be concluded that increased DAO activities may contribute by Put degradation to low polyamine levels in the root tissues exposed to lethal concentration of SA.

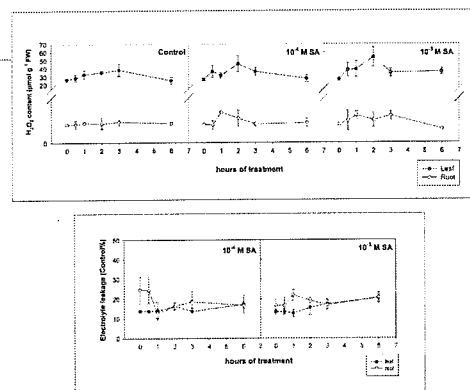


Fig. 3 Changes in the hydrogen peroxide production (H_2O_2) and electrolyte leakage of tomato leaves and roots after treating the samples with 10^{-4} M or 10^{-3} M salicylic acid (SA). Results are the means $\pm$ SE from at least three or four independent experiments. *, **, *** indicate significance levels compared with the untreated control at $P < 0.05$, 0.01, 0.001, respectively, in each time point (Student's t test; ns: not significant).

Leaf H_2O_2 contents slightly increased in the case of 10^{-3} M SA treatment, while this increase was only transient in the intact roots. The electrolyte leakage increased by about 15-20 % at both SA concentrations, suggesting that in short-term experiments the disintegration of plasma membrane was small and reversible.

Discussion

In this work the effect of sublethal (10^{-4} M) and a lethal (10^{-3} M) concentration of SA on PA catabolism was compared. In our experiments it was found that in short-term treatment SA induced changes in free endogenous PA levels or NO and ROS contents in the root apices of intact tomato plants. During the first 6 h of SA exposure H_2O_2 levels increased only slightly in the leaf and root tissues at both SA concentrations and the increase in ROS precedes NO maximum by 6 hours in root apices. 10^{-4} M SA treatment increased free Put and Spd levels in roots and Spm content in the leaves, while 10^{-3} M SA caused a decrease of free PAs in roots. The extractable DAO and PAO activities exhibited only small changes and their contribution to H_2O_2 generation presumably is small. In the case of lethal, 10^{-3} M SA treatment the sharp increase in total ROS and the delayed NO maximum along with decreased free PA levels in the roots may contribute to the initiation of the cell death processes, while sublethal SA (10^{-4} M) concentration caused an increase in free Put and Spd levels in the roots which have protective role under SA-induced oxidative stress.

References

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