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Prof. Touraev A (VIPCA, Austria)
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University of Vienna
Altes AKH, Courtyard 2.6,
Conference hall C1, Spitalgasse 2,
1030 Vienna, Austria



Plant Diseases and Resistance Mechanisms

International Conference
February 20-22th 2013, Vienna, Austria

International Conference "Plant Diseases and Resistance Mechanisms" to be held in Vienna on February 20-22th, 2013 will provide leading academy and industry scientists a platform to communicate recent advances in Plant-Pathogen Interactions and an opportunity to establish multilateral collaborations.

Approximately 300-350 participants are expected to attend this exciting scientific forum including almost 40 lectures delivered by worldwide known invited speakers and young, talented speakers selected from submitted abstracts.

Vienna is located in the heart of Europe on the banks of the Danube River, and considered one of the most important economic, cultural and touristic large cities of central Europe. Apart from providing top science, the Conference will capture the spirit of the city thanks to the central location of the venue, offering a multitude of cultural events just minutes away.



Invited speakers:

Asami T (Japan)	Höfte M (Belgium)
Batley J (Australia)	Loake G J (UK)
Boland W (Germany)	Miedaner T (Germany)
Collinge D B (Denmark)	Rivas S (France)
Ercolano M-R (Italy)	Staskawicz B (USA)
Grant M (UK)	Thomma B P (The Netherlands)
Arnold (UK)	Ton J (UK)
	Willems K A (Belgium)



Conference covers following topics:

- Ecology of Host-Parasite Interaction
- Plant-Parasite Coevolution & Evolution of Host Protection
- Genetics and Genomics of Plant Disease Resistance
- Plant Defence Activators
- Plant Response to Pathogens and Diseases: Immunity and SAR
- Plant Pathogen Effectors and their Action
- Computational Models for Elucidating Plant-Pathogen Interactions
- Plant Diseases, Resistance and Bioinformatics Platforms
- Elicitors & Hormone Signal Transduction of Plant Pathogenesis & Resistance
- Signal Transduction of Plant Pathogenesis and Resistance
- Natural and Induced Resistance Mechanisms Against Plant Pathogens
- Breeding and Biotechnology of Plant Diseases Resistance
- Engineering Pathogen Resistance in Crop Plants
- Detection and Molecular Diagnosis of Plant Disease

Contact

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N 65. Effects of salicylic acid on polyamine metabolism in tomato plants

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Salicylic acid (SA) is a hormone like secondary metabolite involved in biotic stress responses. Polyamines (PA) as small, organic polycations, found in all eukaryotic cells participate in various physiological processes such as programmed cell death. Polyamine oxidation is the main catabolic process of PAs which is an important factor in the regulation of 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 H₂O₂, which can act as a signaling molecule or can contribute to cell death induction and nitric oxide (NO) may also participate in this process. In our work we investigated whether SA-induced changes in polyamine metabolism are involved in triggering hypersensitive reaction (HR) in tomato tissues. Activities of diamine oxidase and polyamine oxidase were determined and the contents of main free polyamines, putrescine, spermidine and spermine were analyzed by HPLC. While higher concentration of SA (10⁻³ M SA) increased the activities of diamine and polyamine oxidases, 10⁻⁴ M SA treatment did not cause any significant change in these parameters. During the first 6 h of SA exposure the levels of reactive oxygen species (ROS) increased in the leaf and root tissues at 10⁻³ M SA concentration and the increase in ROS precedes NO maximum in root apices, while no similar changes can be detected in the root apices exposed to 10⁻⁴ M SA. It can be concluded that SA-induced nitric oxide along with hydrogen peroxide generated by polyamine catabolism can play a role in inducing cell death in tomato after SA treatment.

N 66. Late blight resistance in estonian potato cultivar ando

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Over a decade, the Estonian potato cultivar Ando, as well as the cv. Juku derived from Ando, have demonstrated high isolate-specific resistance to most of the local isolates of the oomycete *Phytophthora infestans*, the causal agent of potato late blight disease. In order to find functional marker(s) for the late blight resistance in Ando, an analysis of expressed sequences comprising the conserved NB-ARC domain of R-genes has been started. The NB-ARC domain is highly conserved between different R-genes and acts as a molecular switch of R protein activity upon pathogen perception. Based on the recently published potato genome sequence (DM1-3 516R44), we have designed a set of degenerate primers to amplify the 3' ends of the expressed R-gene sequences in Ando and in susceptible cvs Agra and Frila. By aligning the sequences generated by next generation sequencing technology, we aim to identify candidate sequence(s) specific to Ando which correspond to Ando's resistance. Cosegregation of candidate resistance genes with late blight resistance will be tested in two segregating populations obtained from crosses of resistant and susceptible parents Ando x Agra and Ando x Frila.

Effects of salicylic acid on polyamine metabolism in tomato plants



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Introduction

Salicylic acid (SA) is a hormone like secondary metabolite involved in biotic stress responses. Polyamines (PAs) as small, organic polycations, found in all eukaryotic cells participate in various physiological processes such as 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 and nitric oxide (NO) may also participate in this process (Papadakis and Roubelakis-Angelakis, 2005; Wimalasekera et al., 2011). Our previous studies proved that long-term pre-treatment with salicylic acid (SA) at low concentrations (10^{-4} and 10^{-5} 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). It has been revealed recently that exogenous application of SA from 10^{-3} M concentration caused the death of tomato plants in hydroponic system (Poór and Tari, 2011).

In the present study we investigated if SA-induced changes in polyamine metabolism are involved in triggering programmed cell death (PCD) in tomato tissues.

Materials and Methods

Solanum lycopersicum cv. Rio Fuego plants were grown in hydroponic culture in a controlled environment under $300 \mu mol m^{-2} s^{-1}$ 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).

Results and Discussion

Free polyamine contents

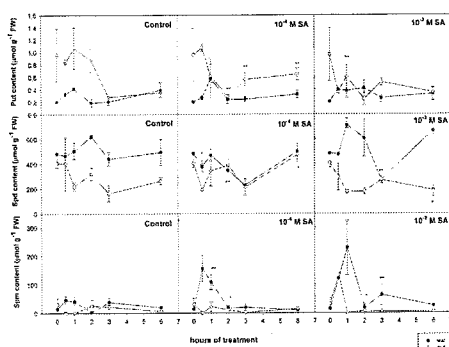
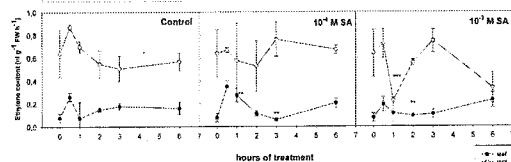


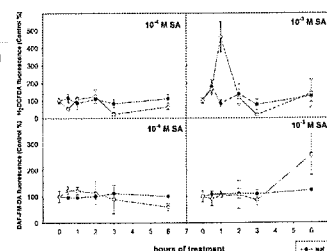
Fig. 1 Changes in the free polyamine content of tomato leaves and roots after treating the samples with 10^{-4} M or 10^{-3} M salicylic acid (SA). Results are the means $\pm$ SE from at least three or four independent experiments. *, **, *** indicate significance levels compared with the untreated control 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 of 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.

Ethylene content



Total ROS production



NO production

Fig. 3 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.

Polyamine catabolism enzymes

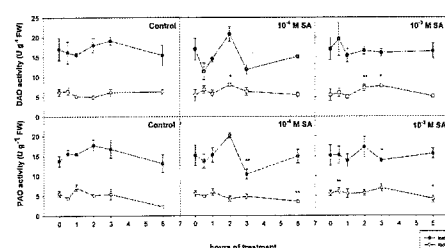


Fig. 2 Changes in the diamine oxidase (DAO) and polyamine oxidase activity (PAO) of tomato leaves and roots after treating the samples with 10^{-4} M or 10^{-3} M salicylic acid (SA). Results are the means $\pm$ SE from at least three or four independent experiments. *, **, *** indicate significance 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 with the exception of the 2-h samples of 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 Put levels in the root tissues exposed to lethal concentration of SA.

Hydrogen peroxide content

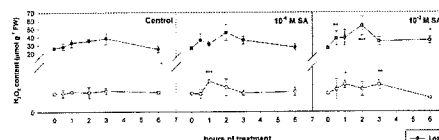
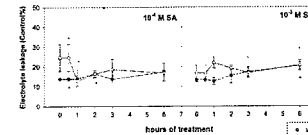


Fig. 4 Changes in the hydrogen peroxide production (H_2O_2) and electrolyte leakage of tomato leaves and roots after treating the samples with 10^{-4} M or 10^{-3} M salicylic acid (SA). Results are the means $\pm$ SE from at least three or four independent experiments. *, **, *** indicate significance 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.

Electrolyte leakage



Conclusion

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 5 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. Our results indicate that SA-induced polyamine catabolism can play a role in inducing cell death in tomato after SA treatment.

References

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