

## Salt stress and salicylic acid induced programmed cell death in tomato

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Programmed cell death (PCD) is an integral part of the plant development and in the response to changing environments. PCD is induced by various abiotic stressors such as high salinity. A special type of PCD in plants is the hypersensitive response during biotic stress which is mediated by salicylic acid (SA). Salt stress results in the disturbance in ion homeostasis, water status and redox equilibrium of plant cells. SA can also cause changes in the water status of plants and can result in oxidative stress but it is not known how SA affects the ion homeostasis of cells. The aims of our work were to study the effects of different concentrations of NaCl and SA on the cell death initiation in tomato in order to highlight the common features or differences in the NaCl- and SA-induced PCD.

Supraoptimal concentrations of NaCl and SA increased the amount of reactive oxygen species (ROS) and H<sub>2</sub>O<sub>2</sub> content of leaf tissues. The inhibition of photosynthetic electron transport can significantly contribute to the generation of ROS in chloroplasts. The photosynthetic performance can be controlled by the limitation of CO<sub>2</sub> diffusion through stomatal pores. The addition of NaCl at 100-250 mM and SA at 10<sup>-7</sup>-10<sup>-2</sup> M to the hydroponic culture of tomato plants for 6 hours resulted in stomatal closure on intact leaves. In parallel with stomatal closure 100-250 mM NaCl and 10<sup>-3</sup>-10<sup>-2</sup> M SA decreased the maximal CO<sub>2</sub> fixation rate (A<sub>max</sub>), and the initial slopes of the CO<sub>2</sub> (A/C<sub>i</sub>) and light response (A/PPFD) curves and relative electron transport rate (Rel. ETR) in intact leaves. Those concentrations of SA which decreased the photosynthetic performance in intact leaves led later to PCD.

Our work aims to investigate whether SA has direct control over stomatal movement by increasing the levels of ROS and nitric oxide (NO) in guard cells or by changing the photosynthetic activity of stomata. In contrast to leaves, stomata on the abaxial epidermal peels closed in buffers containing 10<sup>-7</sup> and 10<sup>-3</sup> M SA but remained open at 10<sup>-4</sup> M. At those concentrations which induced stomatal closure ROS and NO levels raised which could be prevented by ascorbic acid, catalase and diphenyleneiodonium or cPTIO, the scavengers of ROS and NO, respectively. In contrast, the lack of a permanent ROS accumulation and the decrease in NO production in guard cells promoted stomatal opening at 10<sup>-4</sup> M SA. SA at higher concentrations inhibited the Rel. ETR in guard cell chloroplasts suggesting a decrease in photosynthetic performance of guard cells.

The specific genes involved in cell death program were induced by NaCl and SA and their expression levels were analysed by RT-PCR. The expression of both the inhibitors (e.g. BAX-Inhibitor) and effectors (e.g. cysteine proteinases) of PCD were significantly enhanced at lethal concentrations of NaCl and SA. These results suggest that the PCD in these tissues can be triggered in spite of the high expression level of PCD-inhibiting genes.

Signal transduction pathways induced by salt stress and SA have been compared at cell level in tomato cell suspension culture. 250 mM NaCl and 10<sup>-3</sup> M SA caused the death of tomato suspension cells within 6 hours which was accompanied by DNA fragmentation. Our results show that supraoptimal concentration of NaCl induced cell death by generating ionic- and oxidative stress and inducing ethylene production but SA induced cell death by generating oxidative stress.

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## The Type II restriction endonuclease MvaI has dual specificity

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Type II restriction endonucleases (REases) are sequence-specific endonucleases that recognize short DNA sequences and cut the DNA at defined positions within or close to the recognition sequence. In the producer cell the host DNA is protected by specific methylation of the recognition sequence. Methylation is established by DNA methyltransferases, which methylate a cytosine or adenine to produce C5-methylcytosine, N4-methylcytosine or N6-methyladenine. This huge group of enzymes shows great diversity. Members are classified into subgroups according to the symmetry of their recognition sequence, the position of the cut site relative to the recognition sequence, the number of target sites the enzyme interacts with, etc.

From the perspective of our study, two subgroups of Type II REases are especially interesting. Enzymes in the Type IIM subgroup (methyl-directed REases) break the general rule of protection by DNA methylation; unlike most restriction endonucleases, they require methylated substrate site for activity. The other interesting subtype are nicking REases, which cut only one strand of the substrate DNA. Such enzymes include natural nicking REases, e.g. N.BstNBI, isolated subunits of heterodimeric REases (Nb.BsrDI, Nb.BtsI, and mutant REases engineered to cut only one strand of the substrate (Nt.A1wI).

The MvaI REase recognizes the sequence CC↓WGG (W stands for A or T) and cuts both strands as indicated, generating one nucleotide 5'-overhangs. The cognate DNA-methyltransferase M.MvaI modifies the internal cytosines to produce N4-methylcytosine: (C<sup>m4</sup>CAGG/C<sup>m4</sup>CTGG). MvaI was shown to recognize its pseudosymmetric target site as a monomer. An interesting feature of the enzyme is its toler-