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Polyamine metabolism under salt-induced cell death in tomato plants*Péter Poór, Ágnes Szepesi, Zoltán Takács, Judit Kovács, Irma Tari*

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Programmed cell death (PCD) plays a critical role in response to changing environments. PCD is induced by various abiotic stressors such as high salinity. To investigate the role of salt stress in cell death induction, 100 and 250 mM NaCl were added into the nutrient solution of tomato plants. PCD was identified within 6 h in the root. The essential mediator of PCD, H_2O_2 increased significantly and showed two maximums at 30 min and 2 h after the treatment with 250 mM NaCl both in roots and leaves. This lethal NaCl concentration induced high production of reactive oxygen species (ROS) and NO after 30 min. In contrast, treatment with 100 mM NaCl increased the ROS production and H_2O_2 level after 1 h in the roots and only 3 h in the leaves. 100 mM NaCl increased NO production after 3 h. H_2O_2 can be generated among others by the activation of diamine and polyamine oxidases, which catalyses the oxidation of polyamines. The activity of these enzymes changed in parallel with the H_2O_2 in case of 250 mM NaCl. Moreover, spermidine content decreased but spermine increased in the leaf. 250 mM NaCl caused significant ethylene production only in the leaves, 100 mM NaCl increased ethylene evolution both in roots and leaves. It is concluded that the salt stress induced PCD is associated with rapid increased in H_2O_2 and NO levels, with ethylene production and changes in polyamine metabolism and content as a function of time. This work was supported by OTKA K101243.

P-2-104

Impact of silver nanoparticles on important food crops and rhizobacteria

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Use of nanoparticles as carriers of insecticides and fertilizers has improved plant protection. These particles as carriers enhance the efficacy of chemicals by providing higher surface area for absorption. However, side effects of the leftover nanoparticles are unknown. Besides plants, rhizobacteria may also be affected. These microbes are free-living, root colonizing, soil-borne bacteria that promote plant growth and maintain soil health. The present report therefore attempts to examine impact of a metal nanoparticle on two economically important plants, *Vigna radiata*, *Brassica juncea* and associated rhizobacteria.

Thoroughly characterized, chemically synthesized spherical silver nanoparticles of 16-20nm were used for study. Seeds of *V. radiata* and *B. juncea* were germinated in pots containing 200g soil. The plants were exposed to different concentrations of nanoparticles at regular intervals. For studying the effect on rhizobacteria, first, culture-based method was adopted to visually score the bacteria. Additionally, DNA from treated and untreated soil samples was estimated by direct, colorimetric and culture-independent Diphenylamine method. Significant reduction in the root and shoot length ($p < 0.05$) was found in nanoparticle treated plants. Additionally, stunting, leaf curling, distortion and etiolation were observed. Particles also affected the formation of root hair. Also, less number of colonies was observed in treated vs. untreated soil. Total DNA content in untreated soil was 8.46µg/ml whereas in the treated soil it was 3.84µg/ml. Results indicated that both plant growth and soil microbes were adversely affected by residual silver nanoparticles. Molecular studies elucidating the mode of action of nanoparticles on plant and microbes are underway.



Polyamine metabolism during salt-induced cell death in tomato plants

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INTRODUCTION

Programmed cell death (PCD) is an active cellular suicide which plays a role in plant development and in response to abiotic or biotic stressors. Salt stress induces strong oxidative-, ionic- and osmotic stresses. The disturbance in the redox status, the production of reactive oxygen species (ROS) and the failure of the ROS-scavenging mechanisms caused by salinity can induce the lethal damage of proteins, lipids and DNA and leads to PCD (de Pinto et al. 2012). ROS can be synthesized in various cell compartments, among others by diamine-(DAO) and polyamine-(PAO) oxidases (Mittler et al. 2011). The roles of PAs in salt stress response are rather contradictory. PAs may inhibit the ethylene (ET) biosynthesis and are effective inducers of anti-senescence mechanisms. PAs have positive effect on the maintenance of ion balance, membrane stabilization and ROS scavenging in plants. Alternatively, PAs can also act as prooxidants because the catabolism of PAs produces H_2O_2 and can induce PCD (Groppa and Benavides 2008). Moschou et al. (2008) showed that spermidine (Spd), was secreted to the apoplast and here it was oxidised by PAO. This activity increased the apoplastic oxidative stress through the generation of H_2O_2 and induced PCD. The nitric oxide (NO) can interplay with ROS in a variety of ways and participates in signaling and in defence responses under salt stress (Poór et al. 2012; Siddiqui et al. 2011). Gémes et al. (2011) found that exogenous spermidine (Spd) and spermine (Spm) enhanced both ROS and NO levels and decreased the viability of tomato leaf protoplasts. NO has both promoting and suppressing effects on PCD through protein S-nitrosylation. In this work, the common features and differences in the plant responses to sublethal- (100 mM) and lethal (250 mM) concentrations of NaCl were studied in tomato plants. The putative role of PA and ET in ROS and NO production and their correlation with the survival or damage of tissues were investigated during this process. It was also of interest whether PA and ET production might affect the balance of ROS and NO and could control the cell death program in tomato.

MATERIALS AND METHODS

- Tomato (*Solanum lycopersicum* L. cv. Rio Fuego) plants were grown hydroponically in a controlled environment (300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity, 12/12-h light/dark period, a day/night temperatures of 24/22°C and a relative humidity of 55–60%). The plants were treated for 6 hours with 100 and 250 mM NaCl in the culture medium.
- Electrolyte leakage was determined with conductivity meter (Radelkis, Hungary) (Sun et al. 2010).
- Determination of H_2O_2 contents of intact tissues was performed according to Velikova et al. (2000).
- ROS was visualized using 2',7'-dichlorofluorescein diacetate (H_2DCFDA) and NO using 4-amino-5-methylamino-2',7'-difluorofluorescein (DAF-FM DA) (Poór et al. 2012). Fluorescence intensity was detected with Zeiss Axiowert 200M type fluorescence microscope (Carl Zeiss, Jena, Germany). The fluorescence intensity was measured with AXIOVISION REL. 4.5 software.
- PAO and DAO assay were described by Federico et al. (1985) and modified by Moschou et al. (2008).
- PAs were determined by the method of Flores and Galston (1982) with HPLC System (JASCO, Japan).
- Ethylene production was measured with a Hewlett-Packard 5890 Series II gas chromatograph with a flame ionization detector (Tari et al. 2006).
- Data presented are mean values from at least three independent experiments. Statistical analysis was carried out with Sigma plot 11.0 software (SPSS Science Software). Statistical analyses were performed using Student's t test and differences were considered significant if $P < 0.05$. **, *** indicate significance levels compared to the untreated control at $P < 0.05$, 0.01, 0.001, respectively, in each time point.

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RESULTS

250 mM NaCl enhanced the electrolyte leakage (EL) from the roots which indicated serious damage to the plasma membrane. EL also increased from the leaf tissues after the 6-h-long salt exposure. Treatment with 100 mM NaCl caused only reversible changes (Fig. 1.).

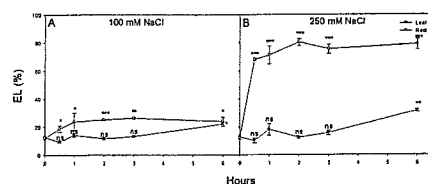


Fig. 1 Disintegration of plasma membrane was determined by electrolyte leakage (EL) in tomato leaves and roots treated with 100 mM (A) and 250 mM (B) NaCl for 6 hours. Mean $\pm$ SE, n=3.

The level of H_2O_2 , the essential inducer and mediator of PCD increased significantly and showed two maxima at 30 min and 2 h after treatment with 250 mM NaCl in the leaf tissues. In contrast, H_2O_2 production showed only one peak in the leaves after the treatment with 100 mM NaCl (Fig. 2.).

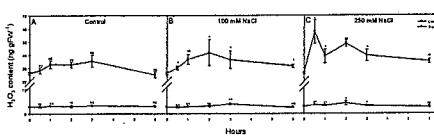


Fig. 2 H_2O_2 content in tomato leaves and roots (A) treated with 100 mM (B) and 250 mM (C) NaCl for 6 hours. Mean $\pm$ SE, n=3.

The lethal NaCl concentration induced a strong increase in ROS after 30 min and two peaks in NO production at 1 h and 3 h in the root tips. In contrast to the leaves, the ROS decreased after 3 h in plants exposed to 250 mM NaCl in the root apex. At sublethal salt concentration the enhanced ROS production was followed by a significant increase in NO after 3 h in the root tips (Fig. 3., 4.).

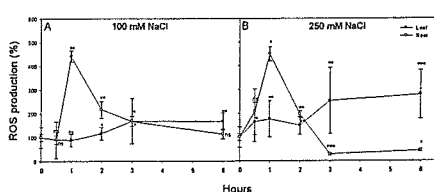


Fig. 3 Kinetics of reactive oxygen species (ROS) production in tomato leaves and root tips treated with 100 mM (A) and 250 mM (B) NaCl for 6 hours. Mean $\pm$ SE, n=3.

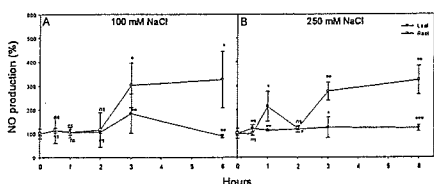


Fig. 4 Kinetics of nitric oxide (NO) production in tomato leaves and roots treated with 100 mM (A) and 250 mM (B) NaCl for 6 hours. Mean $\pm$ SE, n=3.

H_2O_2 level can be generated among others by the activation of DAO and PAO, which catalyses the oxidation of polyamines. The activity of these enzymes in the leaf significantly increased compared with the untreated control and changed in parallel with the H_2O_2 content of tissues in case of 250 mM NaCl treatment (Fig. 5.).

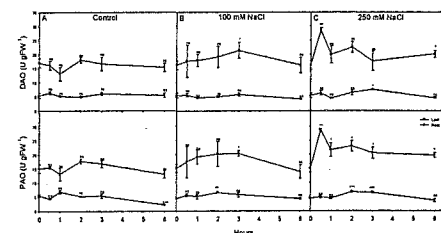


Fig. 5 Diamine oxidase (DAO) and polyamine oxidase (PAO) activity in tomato leaves and roots (A) treated with 100 mM (B) and 250 mM (C) NaCl for 6 hours. Mean $\pm$ SE, n=3.

Spd and Spm contents increased in the leaf after salt treatment but the accumulation of these PAs especially that of Spm was much lower in leaves exposed to 250 mM NaCl, which corresponds well with increased PAO activities of tissues (Fig. 6.).

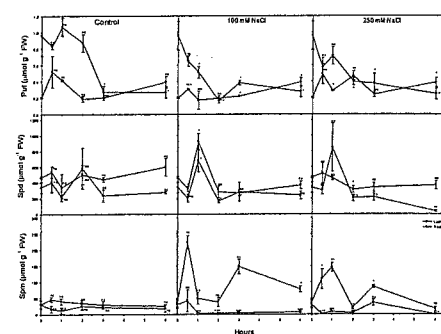


Fig. 6 Polyamine content in tomato leaves and roots treated with 100 mM (A) and 250 mM (B) NaCl for 6 hours. Mean $\pm$ SE, n=3.

250 mM NaCl caused significant induction of ET production only in the leaves, 100 mM NaCl enhanced the evolution of ET both in roots and leaves.

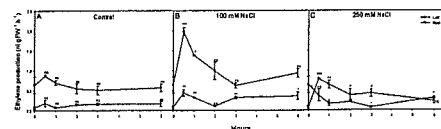


Fig. 7 Ethylene production in tomato leaves and roots (A) treated with 100 mM (B) and 250 mM (C) NaCl for 6 hours. Mean $\pm$ SE, n=5.

CONCLUSIONS

The effect of sublethal (100 mM) and lethal (250 mM) concentrations of NaCl was compared in tomato plants with special emphasis on polyamine metabolism. It was concluded that the salt stress induced PCD is accompanied with rapid increase in H_2O_2 content in the leaves, in ROS and NO levels in the apical root tissues and decreased levels of Spd, Spm and ET in plants exposed to lethal salt concentration. The increased generation of H_2O_2 in the leaves may be partly PAO (or DAO) dependent under the lethal salt stress. It seems to be a combination of different mechanisms, and it is not a PCD trigger in plants exposed to sublethal concentration of NaCl.