Plant Signaling & Behavior

4th International Symposium

Proceedings

St. Petersburg, RUSSIA

19–24 June 2016

Polyamines induce programmed cell death in *Arabidopsis thaliana* roots

Chychko A.[1], Mackievic V.[1], Voitsekhovskaja O.V.[2], Demidchik V.[1,2]

[1] Belarusian State University, 220030, 4 Independence Ave., Minsk, Belarus
[2] Komarov Botanical Institute RAS, Saint Petersburg, Russia
dzemidchyk@bsu.by

Introduction. Polyamines are water-soluble aliphatic compounds containing several amine groups and having fundamental importance in plant stress biology. Putrescine (diamine), spermidine (triamine) and spermine (tetramine) are the most widespread and physiological important polyamines in plants. The level of these polyamines in tissues increase in response to almost any abiotic stress. Some studies showed that the symptoms of oxidative stress decrease in plants overexpressing enzymes of polyamine biosynthesis. However polyamines also act as substrates for biosynthesis of ROS in apoplast by polyamine oxidase. Perhaps, pro- or antioxidant effects of polyamines depend on exact physiological state and environmental conditions. It is difficult to predict how exogenous polyamines can modify cell viability and whether they can induce the programmed cell death (PCD). PCD is a crucial reaction in plant stress responses and its regulation by polyamines may have fundamental significance.
The aim of this study was to determine symptoms of PCD in *Arabidopsis thaliana* root cells in the presence of exogenous spermine, spermidine and putrescine.
Materials and methods. Roots of *Arabidopsis thaliana* L. *Heynh* ecotype WS-0 (Wassilewskija) were used. They were grown vertically on Petri dishes using standard sterile conditions. PCD symptoms were assessed in control and after treatment with 0,01-1 mM spermine, spermidine and putrescine. The effect of polyamines on PCD was also examined in the presence of 0.3% dimethyl sulfoxide (DMSO), 1 mM thiourea, 600 units ml^{-1} superoxide dismutase (SOD) and 1000 units ml^{-1} catalase. Morphological symptoms of PCD were identified and compared in atrichoblasts and trichoblasts. Generation of ROS (superoxide) was measured using a fluorescent probe dihydroethidium (10^{-6} M; Sigma, USA).
Results. All polyamines significantly inhibited elongation of Arabidopsis roots at 0,03-0,3 mM. 1 mM spemine and spermidine stopped root growth. The amount of root cells with PCD symptoms (protoplasm shrinkage, plasma membrane damages, dark areas, etc.) in control did not exceed 10%. Quantity of trichoblasts with PCD symptoms increased significantly when seedlings were exposed to 0,03 mM spermine, 0,1 mM spermidine, and 0,1 mM putrescine. Similar effect was observed in atrichoblasts. The addition of ROS scavengers inhibited the development of PCD caused by 0,3 mM putrescine, but did not change the influence of spermine. The effect of spermidine was sensitive to SOD and DMSO. Tests with dihydroethidium showed that 0,3 mM spermine stimulated superoxide generation in root cells while spermidine did not change superoxide productions. Intriguingly, putrescine significantly inhibited superoxide generation.
Conclusions. Treatment with exogenous polyamines triggers PCD. Polyamine-induced PCD is inhibited by antioxidants, such as SOD and DMSO. Polyamines have contrasting effects on superoxide production: spermine stimulates it, spermidine does not affect this process while causes its inhibition.
This study was supported by Russian Science Foundation grant #15-14-30008 to VD.

Phytaspases: role in plant cell death and beyond

Chichkova N.V., Beloshistov R.E., Galiullina R.A., Trusova S.V., Vartapetian A.B.

Belozersky Institute of Physico-Chemical Biology, Moscow State University, Russia
varta@genebee.msu.ru

Phytaspases are subtilisin-like plant proteases exhibiting peculiar aspartate ('caspase-like') substrate specificity. Being synthesized as inactive precursor proteins, phytaspase proenzymes are constitutively and autocatalytically processed to generate mature enzymes which are secreted into the apoplast [1]. Induction of programmed cell death (PCD), however, triggers phytaspase re-localization into plant cells. In accord with this behavior, phytaspases are involved in the accomplishment of plant PCD induced by biotic and abiotic stresses [1,2]. We will discuss this plant strategy to control PCD, which is distinct from that of animals. Emerging evidence for phytaspase-mediated fragmentation of protein targets and possible consequences of these cleavage reactions will also be presented.
1. Chichkova NV, Shaw J, Galiullina RA, Drury GE, Tuzhikov AI, Kim SH, Kalkum M, Hong TB, Gorshkova EN, Torrance L, Vartapetian AB, Taliansky M (2010) Phytaspase, a relocalisable cell death promoting plant protease with caspase specificity. EMBO J. 29, 1149-1161.
2. Chichkova NV, Kim SH, Titova ES, Kalkum M, Morozov VS, Rubtsov YP, Kalinina NO, Taliansky ME, Vartapetian AB (2004) A plant caspase-like protease activated during the hypersensitive response. Plant Cell 16, 157-171.