Exogenous ascorbate as a signalling molecule in plants

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Plant cell signaling relies on a multitude of primary and secondary messenger molecules. Exogenous L-ascorbic acid (ascorbate) has not been considered as a signaling molecule in plant cells. However we have shown that, in *Arabidopsis thaliana* L. root cells, exogenous ascorbate (>30 μM) induces a transient increase of the cytosolic free Ca^{2+} activity ($[Ca^{2+}]_{cyt.}$). This phenomenon is fundamental to plant signaling because it underlies transmission of most important plant signals, such as hormones and stress. Exogenous copper and iron stimulated the ascorbate-induced $[Ca^{2+}]_{cyt.}$ elevation while cation channel blockers, free radical scavengers, low extracellular $[Ca^{2+}]$, transition metal chelators and removal of the cell wall inhibited this reaction. These data show that the apoplastic redox-active transition metals are involved in the ascorbate-induced $[Ca^{2+}]_{cyt.}$ elevation. Exogenous ascorbate also induced moderate increase in programmed cell death symptoms in intact roots, but it did not activate Ca^{2+} influx currents in patch-clamped root protoplasts. Intriguingly, replacement of gluconate with ascorbate in the patch-clamp pipette revealed a large ascorbate efflux current, which showed sensitivity to anion channel blocker, anthracene-9-carboxylic acid (A9C), indicative of the ascorbate release via anion channels. EPR spectroscopy measurements demonstrated that salinity (NaCl) triggered accumulation of root apoplastic ascorbyl radicals in A9C-dependent manner, confirming that L-ascorbate leaks through anion channels under depolarisation. This mechanism can underlie ascorbate release, signaling phenomena, apoplastic redox reactions, iron acquisition and control of membrane ionic and electrical equilibrium (together K^+ efflux via GORK channels). Financial support of the Russian Science Foundation (grant#15-14-30008 to VD) is gratefully acknowledged.

Ion channels as sensors for reactive oxygen species in plants

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The reactive oxygen species (ROS) are involved in all major aspects of plant physiology. ROS are produced by intracellular and extracellular mechanisms and accumulate in the cell wall (apoplast), where the antioxidant capacity is much lower than in cytosol. The moderate generation of ROS is involved in normal plant physiology and adaptation needs

but their overproduction, for example during the environmental stress, results in irreversible oxidative damage and dysfunction of cell components (Demidchik 2015, Environ Exp Bot). The question of sensing ROS is still debated in plant physiology. Here, it is demonstrated that the plasma membrane ion channels transporting cations, such as Ca^{2+} and K^+, function as prime targets of ROS in plants. These systems can catalyse early and rapid sensing of ROS in plants involved in a multitude of physiological reactions, such as adaptation to stresses, control of photosynthesis, cell elongation and gravitropic responses. In the plasma membranes of lower and higher plants, ROS instantaneously activate two major classes of ion channels: Ca^{2+}-permeable nonselective cation channels (NSCCs) and K^+ outwardly-rectifying channels (KORs encoded by GORK). Activation of cation channels by ROS leads to dramatic influx of Ca^{2+} for signaling, developmental and nutritional needs and K^+ loss (electrolyte leakage) inducing autophagic and necrotic cell death. Ca^{2+} entry also rearranges actin cytoskeleton and modifies vesicular transport. ROS-activated ion channels reveal complex nature of activation, depending on the developmental stage and oxidative capacity of tested ROS. The transition metal binding centres have recently been identified in some members of cyclic nucleotide-gated channels, a subclass of NSCCs (Demidchik *et al*. 2014, JXB). These centers potentially produce hydroxyl radicals from H_2O_2 (Haber-Weiss reaction) directly in the channel's macromolecule. Mutations in ROS-sensitive moieties in K^+ efflux GORK channel leads to the decrease of ROS-sensing capacity, suggesting that distinct molecular groups are responsible for ROS sensing by ion channels. These moieties probably confer physiological properties related to ROS, such as programmed cell death and autophagy. This study was supported by Russian Science Foundation grant#15-14-30008 to VD.

Salinity stress and improving salt tolerance in crops via regulation of Na^+ and K^+ transport

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Soil salinity is a main type of abiotic stresses that leads to considerable crop yield losses, affecting millions of hectares of land around the world. The scale of this problem is expected to increase due to global climate change and expansion of irrigation practices in agriculture. The negative impact of salt stress on agricultural productivity is significant due to growth inhibition, reduced tillering and development of reproductive organs. The mechanisms of plant salt tolerance of plants rely on tight coordinated regulation of hundreds of genes and depending from them physiological programs. The significant progress in gene discovery, gene delivery and genome editing provide an excellent tool to improve salt tolerance of crops. The negative effects of high salinity are divided into two distinct phases. The first, it is independent from salt tissue accumulation - "osmotic phase". The second is "ionic phase". This type of phase is related to toxic effect of ions, mainly Na^+ and Cl^-, during salt accumulation in plant tissues. Due to similar physicochemical properties, the Na^+ is a main K^+ competitor in key metabolic processes in the cytoplasm. The one of main strategies employed by plants during salinity stress is maintenance of high cytosolic K^+/Na^+ ratio. The enrichment of plant tissues by K^+ and restriction of Na^+ uptake and accumulation are very promising approaches for plant salt tolerance improvement. The one of major mechanism of plant salt tolerance rely on regulation of Na^+ and K^+ transport.