Exogenous ascorbate as a signalling molecule in plants Demidchik V.<sup>1,3</sup>\*, Vaitsiakhovich M.<sup>1</sup>, Svistunenko D.<sup>2</sup>, Navaselsky I.<sup>1</sup>, Hryvusevich P.<sup>1</sup>, Mackievic V.<sup>1</sup>, Samokhina V.<sup>1</sup>, Pozhvanov G.<sup>4</sup>, Straltsova D.<sup>1</sup>, Smolikova G.<sup>4</sup>, Medvedev S.<sup>4</sup>, Sokolik A.<sup>1</sup>

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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  $\mu$ M) induces a transient increase of the cytosolic free Ca<sup>2+</sup> activity ([Ca<sup>2+</sup>]<sub>cvt</sub>). 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 [Ca2+]cyt. elevation while cation channel blockers, free radical scavengers, low extracellular [Ca<sup>2+</sup>], transition metal chelators and removal of the cell wall inhibited this reaction. These data show that the apoplastic redoxactive transition metals are involved in the ascorbate-induced  $[Ca^{2+}]_{cvt}$  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 Demidchik V.<sup>1, 2,\*</sup>

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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

