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Stress-induced Rb^+ efflux from roots of *Arabidopsis thaliana* plants lacking functional K^+ outwardly-rectifying channel GORK

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Introduction. Potassium (K^+) is the most abundant macronutrient, which has electrical, osmotic and metabolic functions in plants. K^+ uptake in roots is crucial for plants; however, K^+ efflux also occurs in some physiological conditions. Loss of K^+ from roots is often induced by stresses, such as pathogens, salinity, freezing, oxidants and heavy metals. At the cellular level, K^+ efflux is caused by K^+ efflux channels which in roots of *Arabidopsis* are encoded by GORK or SKOR ion channels. These channels are activated by both depolarization and reactive oxygen species (ROS). Here we have designed an assay based on measurement of the radiorubidium efflux, which can be used in studies of the structure-function relationship of K^+ channels and determining of the channel's ROS-sensitive moieties.
The main aim of this study was to develop a system for measurement of $^{86}Rb^+$ efflux and investigate effects of NaCl, hydroxyl radicals and H_2O_2 on $^{86}Rb^+$ efflux in *Arabidopsis thaliana* L. roots.
Materials and methods. We used radiotracer $^{86}Rb^+$ ($t_{1/2}$=18.64 d) as chloride (Radioisotope Centre POLATOM, Poland). Labeling solution activity was 40 kBq ml^{-1}. Ten to fourteen-day-old seedlings of *Arabidopsis thaliana* L. 'WS-0' and KO lines of K^+ channel GORK were used. The activity accumulated by seedlings was measured using gamma counter with a large detector (diameter of 7 cm). Seedlings were placed in holders and transferred in the labeling solution. After 30 min, the $^{86}Rb^+$-loaded seedlings were removed from the solution and placed in isotope-free solution. Loss of $^{86}Rb^+$ was registered every 1-5 minutes. Stresses were applied to roots 5 min after beginning of $^{86}Rb^+$ efflux registration. The following stresses were examined: 1) 200 mM NaCl; 2) 1 mM Cu^{2+}, 1 mM L-ascorbic acid, 1 mM H_2O_2 (Cu/a); 3) 10 mM H_2O_2. Root weight was measured at the end of each experiment. Curves of time course of $^{86}Rb^+$ efflux were plotted and analysed.
Results. $^{86}Rb^+$ efflux revealed three distinctive phases: rapid phase (5 min), slow phase I (5-10 min) and slow phase II (10-25 min). First one corresponded to efflux of $^{86}Rb^+$ from cell wall (apoplastic space). Slow phases I and II were associated with the isotope efflux from intracellular stores (symplastic phases). $^{86}Rb^+$ efflux rates in control and under tested stresses were calculated for the slow phase I (mostly cytosolic). In WT plants, $^{86}Rb^+$ efflux rates increased by 5, 3 and 2,5 times in response to salinity, Cu/a stress and H_2O_2, respectively (as compared to control). In GORK KO lines, stress-induced $^{86}Rb^+$ rates were approximately half that of WT. Thiourea (specific scavenger of hydroxyl radicals) decreased NaCl- and hydroxyl-induced $^{86}Rb^+$ efflux rates by 15-20% while in WT this agents did not cause any changes in fluxes.
Conclusions. We have designed a system for an accurate measurement of the stress-induced $^{86}Rb^+$ efflux from roots of higher plants. We have found that NaCl, hydroxyl radicals and H_2O_2 significantly stimulate the $^{86}Rb^+$ efflux from root cells in *Arabidopsis thaliana.* GORK channels catalyse at least a half of this efflux.
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VP-induced changes in intra- and extracellular pH influence the light and dark stages of photosynthesis in two different pathways

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A system response to a local action of adverse factors includes generation and propagation of electrical signals in higher plants. Damaging stimuli cause variation potential (VP) that can induce a number of functional responses, including photosynthetic changes, which is possibly connected with proton signal in a cell. Analysis of the role of intra- and extracellular pH changes in VP-induced photosynthetic response in pea seedlings was the aim of this work.
It has been shown that leaf burning induced VP propagation which was accompanied with an increase of extracellular pH and decrease of intracellular pH. Furthermore, VP caused the photosynthetic changes including decline of CO_2 assimilation rate and raise of non-photochemical quenching of