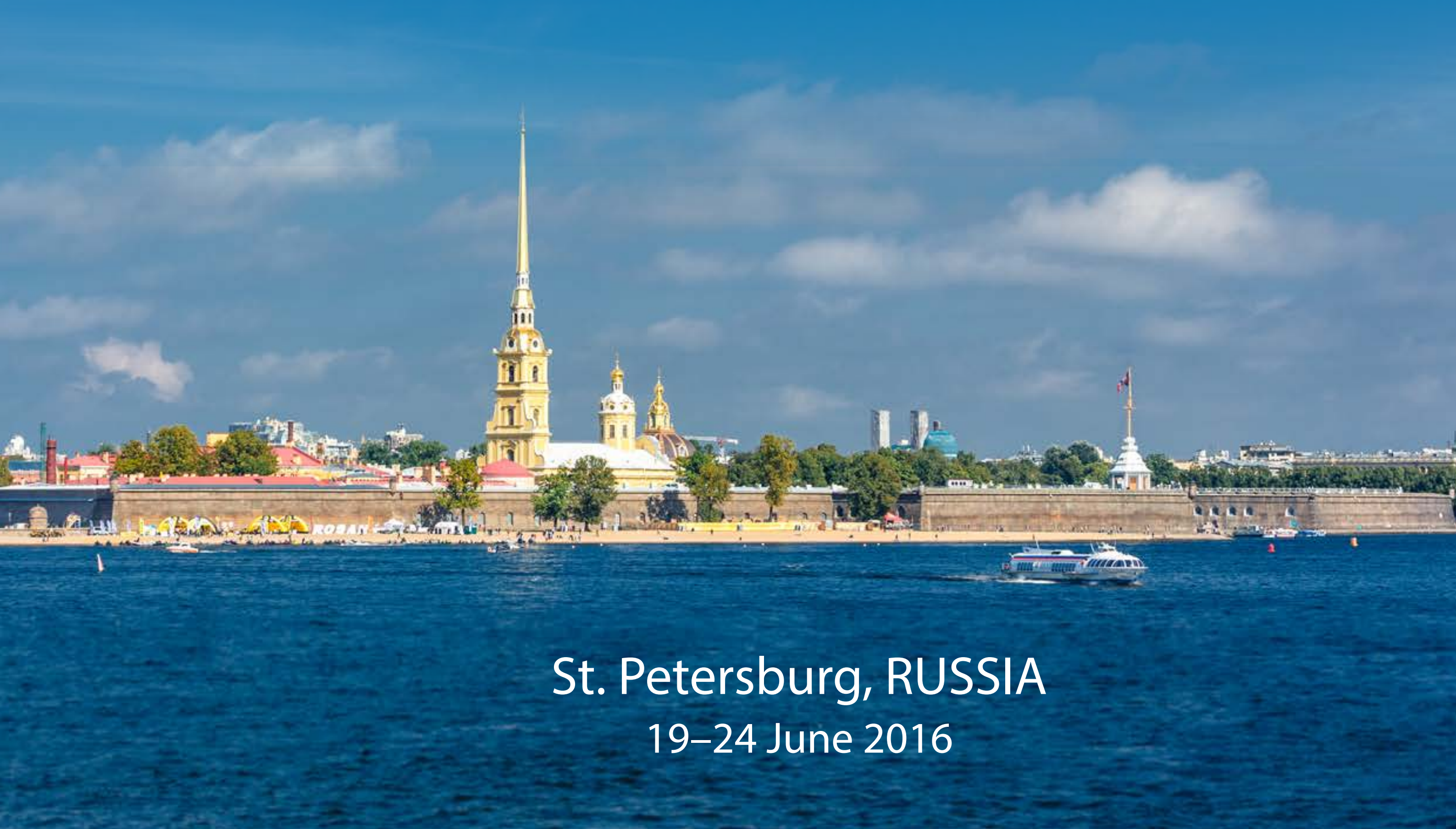
Plant Signaling & Behavior

4th International Symposium

Proceedings



St. Petersburg, RUSSIA

19–24 June 2016

fluorescence (*NPQ*). Comparison of average dynamics of the photosynthetic response and pH changes accompanying VP was performed to analyze the relation between these processes. It has been shown that dynamics of extracellular pH changes was quite similar to dynamics of the gas exchange response (correlation coefficient (*r*) was equal to –0.72). On the other hand, dynamics of intracellular pH changes differed significantly from that of the CO_2 assimilation changes (r = 0.31). On the contrary, high correlation coefficient between changes in intracellular pH and *NPQ* (r = –0.92) and considerable differences in dynamics of the extracellular pH and *NPQ* (r = 0.35) have been shown. These results can point to the presence of two components in the photosynthetic response caused by VP in pea seedlings. One of the components is determined mainly by the gas exchange changes and related to the extracellular pH shift; the second component is connected with the raise of the non-photochemical fluorescence quenching and the intracellular pH decline.
At the next stage of this work an additional theoretical analysis, based on a previously developed model of VP, was carried out. Analysis of the model showed that it describes the photosynthetic response near stimulation area, the disappearance of the response at long distance from stimulation area, as well as different shapes of dependences of CO_2 assimilation rate (ΔA_{CO2}) and *NPQ* (ΔNPQ) changes on VP magnitude. A monotone increase of ΔNPQ and two-stage changes (raise and subsequent decline) of ΔA_{CO2} with an increase in VP magnitude have been shown. This dependence corresponds with experimental data and confirms the presence of two components in photosynthetic response.
The obtained results reveal that VP-induced photosynthetic response in pea is connected with changes in the intra- and extracellular pH. Increase of the extracellular pH determines the component of response concerned with the inactivation of the dark stage of photosynthesis, while changes in the intracellular pH cause the development of the component independent from the dark stage activity.
This work was supported by the Russian Scientific Fund (Project No. 14-26-00098).

Brassinosteroids modify ion channel activities and induce elevation of cytosolic free Ca^{2+} in roots of higher plants

Straltsova D., Chikun P., Zhabinskii V.N., Zvanarou S., Khripach V.A., Demidchik V.
Belarusian State University, Minsk, Belarus
Institute of Bioorganic Chemistry NASB, Minsk, Belarus
dzemidchyk@bsu.by

Introduction. Brassinosteroids (BRs) are endogenous plant hormones essential for the proper regulation of multiple physiological processes required for normal plant growth and development. Exogenous BRs can improve the quantity and quality of crops and ameliorates effects of stresses. Using native and synthetic analogues of BRs as a tool to improve plant yield seems to have a great potential for agriculture and biotechnology (Khripach V., 2000). BRs have been intensively investigated for their biosynthesis, distribution and physiological functions using classical physiological tests, analyses of mutants and transgenic plants (*Arabidopsis thaliana* plants constitutively expressing aequorin). Recent data indicate that BRs are also sensed by the plasma membrane system catalyzing increase in the cytosolic free Ca^{2+} (in leaves of *Arabidopsis thaliana*). Zhao *et al.* (2013) have shown that the BR-induced elevation in the cytosolic free Ca^{2+} is abolished in knockout line lacking functional brassinosteroid receptor and after treatment with Gd^{3+} (blocker of Ca^{2+}-permeable nonselective cation channels) (Zhao Y., 2013). Zhang *et al.* (2005) using suspension culture cells of *Arabidopsis* have found that anion channel currents were inhibited by both 28-homobrassionolide and 28-castasterone and outwardly-directed K^+ conductance was stimulated by 28-homobrassionolide but inhibited by 28-castasterone (Zhang Z., 2005).
The aim of this study was to examine possible effects of brassinosteroids on the plasma membrane cation conductances in plant cells and related Ca^{2+} driven signalling events. Standard patch-clamp and aequorin chemiluminometry techniques were used (Demidchik V., 2011).
Results. Here, we report the first electrophysiological characterisation of brassinosteroid-activated Ca^{2+}-permeable channels in higher plants. Wheat root protoplasts (tested by patch-clamping) and whole arabidopsis plants expressing Ca^{2+}-reporting protein, aequorin (analysed by chemiluminometry), were used in this study.
In the whole-cell patches (wheat root protoplasts), 1 μM 24-epibrassonolide, 28-homobrassionolide or 24-epicastasterone were applied exogenously. Only 24-epicastosterone modified transmembrane cation currents while 24-epibrassonolide and 28-homobrassionolide did not cause any reaction. Addition of 24-epicastosterone at cytosolic side through the patch-clamp pipette increased Ca^{2+} influx conductance, which demonstrated characteristics of depolarisation-activated Ca^{2+} channels. The pharmacological analyses have shown that brassinosteroid-activated Ca^{2+}-influx conductance was sensitive to inhibitors of Ca^{2+}-permeable cation channels. Blockers of K^+ channels did not inhibit this conductance.The plasma membrane conductance, which was activated by an endogenous 24-epicastosterone, showed bell-like shape with maximal activation at depolarisation voltages (bath: 20 mM Ca^{2+}). Labelling castosterone

(and its derivates) with BODIPY (using castosterone-BODIPY conjugates which were synthesised chemically) showed that castosterone (and its derivates) can be transferred to the cytosol both in intact roots and protoplasts. This confirms that the effect of 24-epicastosterone at the cytosolic face can potentially be observed in real plants.
We also tested the effect of different brassinosteroids on cytosolic free Ca^{2+}, using *Arabidopsis thaliana* plants constitutively expressing aequorin. Six brassionosteroids including brassinolide, castosterone, 24-epibrassonolide, 28-homobrassionolide, 24-epicastosterone and 28-homocastosterone were tested. All six brassionosteroids induced elevation of the cytosolic free Ca^{2+} in arabidopsis root cells. In the present study we demonstrated that 24-epicastosterone being more potent than 24-epibrassonolide and 28-homobrassionolide. 10 μM of exogenous BRs was the minimal concentration at which statistically significant changes of the cytosolic Ca^{2+} were observed.
Conclusions. The obtained results suggest that the plasma membrane of root cells contains the brassinosteroid-activated cation-permeable channels, which can be involved in cell ion homeostasis and signalling.
References
1. Demidchik V *et al.* (2011) *Plant Physiol* 156: 1375-1385.
2. Khripach V *et al.* (2000) *Ann Bot* 86: 441-447.
3. Zhao Y. *et al.* (2013) *Plant Physiol* 163: 555-565.
4. Zhang Z. *et al.* (2005) *Plant Cell Physiol* 46: 1494-1504.

Variation potential in higher plants: mechanisms of generation and propagation

Vodeneev V.A., Katicheva L.A., Sukhov V.S.

Department of Biophysics, N.I. Lobachevsky State University of Nizhny Novgorod, Nizhny Novgorod, Gagarin Avenue, 23, 603950, Russia
v.vodeneev@mail.ru

Plant response to external wounding is based on activation of a number of signaling systems. In spite of the fact, that one of the leading line in the reaction coordination belongs to electrophysiological system, little is known about its functioning and in particular the process of generation and propagation of variation potential (VP) – the transitional depolarization of cell membrane extending out the zone of local wounding. It has been already shown that long membrane depolarization at VP is based on a temporary inactivation of the proton pump of plasma membranes. However, there are many signs of participation of passive fluxes of Ca^{2+}, Cl^- and K^+ in VP formation. The worth-noting one is the registered drop in the membrane input resistance at variation potential generation, that is probably based on the ionic channels opening. It has also been shown, that calcium ions can play a key role in the process of VP depolarization generation and transformation of the electric reaction to functional changes of plant cells. Such increase in intracellular concentration of free calcium leads to an inactivation of the proton pump and activation of anionic channels. Moreover, possible long-termed open time of calcium channels may be the reason of the long-lived inactivation of H^+-ATPase and long membrane depolarization and be also connected with the type of the channels participating in the reaction propagation, which is also still widely discussed. The prevalent hypothesis of VP propagation is hydraulic one relating to existence of induced by localized damage hydraulic waves, which propagation stimulates generation of electric response in plant cells. According to another hypothesis electrical reaction is induced by migration of wound chemical substance from the zone of damaging. There is also a suggestion that the transmission of the wound substance through the xylem occurs as a result of wound induced mass flow. The propagation of the wound substance could probably be connected with increase of diffusion velocity (turbulent diffusion), which is induced by hydraulic wave. Studying of the mechanisms of variation potential propagation with the processes of the electrical reaction formation on separate cells may reveal the way of the VP transformation to plant physiological response, i.e changes of photosynthesis activity, respiration rate, gene expression etc.