Brassinosteroids as modulators of signaling, ion transport, growth and development in higher plants

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Brassinosteroids (BRs) are phytohormones with a multitude of fundamental functions, which are critical 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, 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, 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, 2005). First part of our 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, 2011). 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. 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. Apart from determination of molecular nature of the brassinosteroid action on plants, in our second part of the study, we have investigated BR effect on growth and development of plant species that have not yet been tested for their BR sensitivity. BR effects in orchid plants have never been tested although *Orchidaceae* is one of the two largest families of flowering plants. Six BRs, belonging to two main BR classes, were examined here for their effects on growth rate and development of *Phalaenopsis* × hybridum Blume protocorm-like bodies. the influence of 10^{-10}-10^{-6} M brassinolide (BL), castasterone (CS), epicastasterone (EC), homocastasterone (GC), epibrassinolide (EB) and homobrassinolide (GB) was measured and analysed. Our data demonstrated that all BRs significantly stimulated orchid growth *in vitro*. The greatest effect on length was caused by castasterone, while maximal increase of weight was induced by brassinolide and epibrassinolide. Orchid microclones, grown in the presence of BRs revealed twice bigger length that control plants. Weight gain also increased 2 and 3.5 times when plants were cultivated on media containing BRs. Overall, we have demonstrated for the first time that BRs stimulate growth of representative of *Orchidaceae* and that this stimulation exceeds effect of auxins.

Видоспецифичность действия экзогенной салициловой кислоты на синтез антоцианов *in vivo*

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Салициловую кислоту (СК) рассматривают как сигнальную молекулу гормонального типа, включающую программы синтеза антиоксидантов при стрессе. Целью работы являлось исследование влияния экзогенной СК на синтез пигментов полифенольной природы в однодольных и двудольных растениях. Объектом исследований служили проростки озимой ржи (*Secale cereale L.*) и гречихи (*Fagopyrum esculentum Moench*). СК вводили в интактные растения через корни. Содержание пигментов определяли спектрофотометрически. Инкубация 3-дневных проростков ржи, в которых лист еще не вышел из колеоптиля, на растворе СК в течение 2 сут приводила к увеличению общего содержания полифенольных компонентов (в 1,5 раза по сравнению с контролем), но не влияла на синтез антоцианов. Такой же эффект наблюдали и в более зрелых проростках при увеличении продолжительности инкубации на растворе СК (с 6 до 10 сут). В листьях и стеблях молодых проростков гречихи обнаружено стимулирование накопления антоцианов после 3-х суток инкубации интактных 4-дневных растений на растворе СК (в 1,7 и 1,3 раза). В более старых проростках такая картина не наблюдалась. Содержание полифенолов при этом существенно не изменялось. Таким образом, в работе обнаружено отсутствие сцепленности в изменении общего содержания полифенолов и антоцианов как