Plant Signaling & Behavior

4th International Symposium

Proceedings

St. Petersburg, RUSSIA

19–24 June 2016

УДК 581.1(063)
ББК 28.57я43
P71

Proceedings of Fourth International Symposium on Plant Signaling and Behavior /
Project management: Vadim Demidchik, Olga Voitsekhovskaja, Elena Tyutereva, Gregory Pozhvanov.-
Saint Petersburg: SINEL Co.Ltd., 2016.-208 p.
ISBN 978-5-9908187-9-8

4th International Symposium on Plant Signaling and Behavior will be held from June 19 to June 24, 2016, at Komarov Botanical Institute of the Russian Academy of Sciences under the auspices of the Society of Plant Signaling and Behavior and the Russian Science Foundation. The scientific program includes plenary lectures by international speakers from Society of Plant Signaling and Behavior and Komarov Botanical Institute of the Russian Academy of Sciences. For early career and young scientists the School "Plant Stress Signaling" will be organized during the Symposium.

УДК 581.1(063)
ББК 28.57я43

Publisher: SINEL Co.Ltd.
Design, typesetting, cover and map design: Gregory Pozhvanov
Photographs on p. 10, 12–13: Kulgaviy V.Ya. and Tkachenko K.G.
Illustrations and design at p. 30–40 (inset) are property of respective sponsoring companies
Logotypes of BIN RAS, PSB, RSF are property of respective owners
Map background: © MapBox, Inc. St. Petersburg
Metro Map: © SUE St. Petersburg Metropoliten
On the cover: Peter and Paul Fortress, St. Petersburg
The 4th PSB2016 Symposium is supported by the Russian Science Foundation (Project #15-14-30008).

ISBN 978-5-9908187-9-8

SESSION 9

Response to Radiation and Gravity

Effect of microgravity conditions on germination of *Brassica napus* seeds

Bilova T.E.[1,2], Didio A.[2,3], Ihling C.[4], Chantseva V.[1], Smolikova G.N.[1], Grishina T.V.[3], Sinz A.[4], Frolov A.[2], Medvedev S.S.[1]

[1] Department of Plant Physiology and Biochemistry, St. Petersburg State University, Universitetskaya nab. 7/9, 199034, St. Petersburg, Russia

[2] Leibniz Institute of Plant Biochemistry, Department of Bioorganic Chemistry, Weinberg 3, 06120, Halle/Saale, Germany

[3] Department of Biochemistry, St. Petersburg State University, Vasil'evsky ostrov, Sredniy pr. 41, 199178, St. Petersburg, Russia

[4] Martin-Luther Universität Halle-Wittenberg, Institute of Pharmacy, Wolfgang-Langenbeck-Str.4, 06120, Halle/Saale, Germany

bilova.tatiana@gmail.com

The strength and direction of gravitational field is homogenous in terrestrial environment. This feature makes it the most important factor defining the polarity of plant growth and development, and underlying the phenomenon of gravitropism, i.e. polarized growth on gravity field. Indeed, plants are able to distinguish the vector of gravity force and adjust the position of root and shoot in the way optimal to access light, water and mineral salts. However, the mechanisms of growth polarity maintenance are still not completely understood. Moreover, the impact of individual proteins in establishment of growth polarity by seed germination is still to be characterized. To address these mechanisms, we characterize here the changes in *B. napus* seedling proteome in response to the loss of gravity vector by application of microgravity conditions by rotation in a 2D clinostat. All experiments (n = 3) were performed twice, and were accompanied with comprehensive biochemical and physiological characterization of plant state. Our analytical strategy relied on LC-based bottom-up proteomic approach employing high-resolution data-dependent acquisition experiments (Thermo Fisher Scientific Orbitrap Fusion Tribrid mass spectrometer).

Although biochemical analyses revealed no essential oxidative stress, the application of microgravity conditions triggered significant changes in abundances of individual proteins. The overall numbers of up- and down-regulated proteins (approximately 1.5-3.3-fold changes, ANOVA test: $p \leq 0.05$) found in at least one experiment, were 160 and 100, respectively. An essential part of affected proteins were involved in energy metabolism and ribosome biogenesis. The patterns of the proteins obtained after one day of clinostat rotation just partially overlapped with that observed one day later. Thus, glyceraldehyde-3-phosphate dehydrogenase (cytoplasmic and chloroplast isoforms), phosphoenolpyruvate carboxykinase, 6-phosphogluconate dehydrogenase and cytoplasmic ribosomal protein L3A were up-regulated during the both observation days. These results indicate higher degree of energy metabolism activation and protein biosynthesis level in the absence of constant vector of gravity field.

Unexpected turns in gravitropic curvatures

Gorshkova T.A., Ibragimova N.N., Ageeva M.V., Mokshina N.E., Gorshkov O.V.

Kazan Institute of Biochemistry and Biophysics Kazan Scientific Center of the Russian Academy of Sciences, Lobachevsky str., 2/31, Kazan, Russia, 420111

gorshkova@kibb.knc.ru

Gravitropic reaction – the process of plant organ orientation in accordance with gravity vector direction – belongs to the most important factors in plant organism development. The widely spread version of gravitropic response, which is accompanied by the formation of stem curvature, is the restoration of stem vertical position after plant inclination. The major mechanism of gravitropism that is discussed for herbal plants is based on the non-uniform elongation of cells located on the opposite stem sides, occurring in the growing zone of an organ. However, gravitropic response may be well-pronounced in

72 and 109-244). K.V acknowledges the university of Antwerp and the Research Foundation Flanders (G009412N, G.0.602.11.N.10 and 1.5.091.11.N.00).

Genome stability in plants from Chernobyl zone is facilitated by DNA-repair pathways

Shevchenko G.[1], Talalaiev O.[1], Doonan J.H.[2]

[1] Institute of Botany, Cell Biology Department, 2, Tereshchenkivska St., Kiev, Ukraine

[2] Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, Aberystwyth, Ceredigion, SY23 3DA, UK

g_shevchenko@botany.kiev.ua

Despite of high contamination for almost 30 years, flora in Chernobyl zone continues to flourish, evidencing the adaptation of plants to chronic radiation and heavy metals in the soil. One of the immediate targets of radiation is the genetic material, DNA which can be irreparably damaged. DNA damage activates DNA-damage response (DDR) which mediates radiation-dependent cell cycle arrest and triggers the induction of DNA repair. In plants, DDR pathways must be extremely efficient since plants are sessile organisms and have to accommodate their life to the contaminated environment. However, the genetic basis of the adaptation to the extreme environment is still not fully understood. Such knowledge is of special interests because it provides basis for biotechnology for crop improvement and soil remediation. For our investigations, seeds of *Arabidopsis thaliana* have been collected in contaminated sites of Chernobyl zone. Recently developed micro-phenotyping system has been used for assessment of natural *Arabidopsis* tolerance (root length and nucleus integrity) to radiomimetic and heavy metal impact. A certain degree of variation with regard to their ability to tolerate heavy metal stress and DNA damage by radiomimetic agents has been observed among *Arabidopsis* plants collected from different sites. More tolerant *A. thaliana* lines from Chernobyl zone were selected as experimental models for further QTL analysis. Treatments with radiomimetic revealed increasing expression of genes involved in DDR pathways and cell cycle control. In particular, expression of *ATR* kinase gene and downstream expression of *CycB1;1* was increased after bleomycin and zeocin treatments suggesting role of ATR-dependent pathway in DNA repair and cell cycle control in genome stabilization in Chernobyl plants. We continue investigations on other mechanism of DDR including cell cycle regulation and PCD hallmarks in order to compose the complete picture on the mechanism of plant tolerance to anthropogenically contaminated environment.

Simulated microgravity induces specific alterations in the metabolite profiles of germinated *Brassica oleracea* L. seeds

Smolikova G.N.[1], Nyukalova M.[1,2], Chantseva V.[1], Medvedev S.S.[1]

[1] Department of Plant Physiology and Biochemistry, St. Petersburg State University, Russia

[2] Komarov Botanical Institute of Russian Academy of Sciences, St. Petersburg, Russia

g.smolikova@spbu.ru

Gravitation is one of the most important polarized environmental factor defining the development of plant organisms in space. Every plant can 'estimate' its position with respect to the gravity vector and correct it, when necessary, by means of polarized growth. However, plant organisms at the space stations are growing in the microgravity, which is leading to the altered physiology in comparisons to the plants on the planet.

On the Earth microgravity can be closely simulated using clinorotation or random positioning – rotation of the plant around the horizontal axis (or several axes), which is disorients studied object in the gravity field. Microgravity simulation using the continuous changes of object´s orientation relative to the gravity vector can generate the effects comparable to the effects of true microgravity.

Experiments in space biology have provided a wealth of knowledge pertaining to physiology, genomics and proteomics of plants grown under the microgravity conditions. Nevertheless it should be mentioned that plant growth and development changes under microgravity also associated with the alterations in plant metabolome. We report the metabolic and developmental changes that occur with the germinated *Brassica oleracea* L. seeds under the simulated microgravity.

In this study, we used clinostat with two axes, which is often referred to as 'random positioning machine' or three-dimensional (3-D) clinostat. 3D random rotation could provide a better simulation of the weightlessness conditions as compared to the classical 2D clinostat. Our experiments show that the 3-D

clinorotation leads to the disorientation of root growth with respect to the gravity vector and affect the following development of seedlings, causing the larger number of abnormally developed seeds.
The content of low-molecular-weight substances in excised embryonic axes and cotyledons of *B. oleracea* seeds germinated for 24 h and 48 h under the simulated microgravity has been evaluated using the gas chromatography– mass spectrometry. For every metabolomic profile, we estimated 129 target substances, and 34 of them were unambiguously identified. These compounds included amino acids, organic and fatty acids, tocopherols, phytosterols.
To keep the data assay within the context of multivariate statistics, we used the principal component analysis (PCA). The analysis revealed a significant difference between the metabolomic profiles of excised embryonic axes in 24 h germination group, with the primary role of the amino acids and organic acids. Noticeably, the 1st and 2nd principal components showed 68 % of the explained variance and, according the cross-validation test, 74 % accuracy of identification. The predictive squared correlation coefficient (Q_2) was 61 %. The significant difference between the metabolomic profiles of the cotyledons appeared only in 48 h germination group with explained variance of 64 % and Q_2 equal to 67 %.
Thus, the embryonic axes were sensitive to the disorientation in space already on the seed germination stage. Metabolomes of the cotyledons demonstrated the difference in the stage of seedling development.
This work was carried out using the equipment of the Resource Center of St. Petersburg State University "Development of Molecular and Cellular Technology" and supported by the St.Petersburg State University (grant 1.38.233.2014) and Russian Foundation for Basic Research (grant 14-04-01-624).

Multiple cell wall-related mechanisms mediate the effect of brassinosteroids on Arabidopsis hypocotyl gravitropism

Suslov D.[1], Vandenbussche F.[2], Ivakov A.[3], Funke N.[4], Ruprecht C.[4], Vissenberg K.[5], Van Der Straeten D.[2], Persson S.[6]

[1] St. Petersburg State University, 199034 Universitetskaya emb. 7/9, St. Petersburg, Russia
[2] Laboratory of Functional Plant Biology, Ghent University, K.L. Ledeganckstraat 35, B-9000 Gent, Belgium
[3] Australian National University, GPO Box 475, Canberra, ACT 2601, Australia
[4] Max-Planck Institute of Molecular Plant Physiology, Am Muehlenberg 1, 14476 Potsdam, Germany
[5] University of Antwerp, Groenenborgerlaan 171, 2020 Antwerpen, Belgium
[6] University of Melbourne, Parkville, VIC 3010, Melbourne, Australia
suslov.dmitry@gmail.com

Gravitropism can be defined as the directed growth of plant organs with respect to the gravity vector. Plant shoots normally demonstrate negative gravitropism growing upright, in the direction opposite to the gravity vector.
In the present work we studied the role of cell walls in the control of the negative gravitropism of hypocotyls from etiolated *Arabidopsis thaliana* seedlings using brassinosteroid-related treatments as experimental tools. We found that hypocotyls of plants grown on horizontal Petri plates in the presence of 24-epibrassinolide (EBL, 100 nM), one of the most active natural brassinosteroids, lay flat on the agar surface, thus demonstrating compromised negative gravitropism. On the contrary, brassinazole (BRZ, 1 μM), an inhibitor of brassinosteroid biosynthesis, increased the negative gravitropism of hypocotyls. EBL had no effect on the gravitropic bending of hypocotyls in gravistimulated plants grown on vertical Petri plates, while BRZ increased it. EBL and BRZ treatments had opposite effects on cell wall mechanics estimated by the creep method. EBL increased the wall creep at pH 6, which is consistent with the weakening of cell walls and their disability to support the weight of hypocotyls against gravity. BRZ decreased creep under these conditions, thus increasing the wall strength. EBL did not influence the wall monosaccharide composition, cellulose and uronic acid content but led to disordered cellulose microfibril orientation in the outer epidermal wall as revealed with the Pontamine S4B dye. This could explain the effect of EBL on gravitropism, because wild type plants treated with oryzalin (250 nM) and *pom2-4* mutants, both having disordered microfibrils, also demonstrated a decrease in the percentage of standing hypocotyls. BRZ stimulated gravitropism independently of cellulose orientation, as it increased the percentage of standing hypocotyls in oryzalin-treated wild type and *pom2-4* plants to 100%. The BRZ effect on gravitropism was accompanied by a decrease in cellulose and mannose content, and an increase in non-cellulosic glucose, which is consistent with changes in cell wall mannans and/or cellulose crystallinity. The involvement of mannans in the control of gravitropism was confirmed using a triple *csla2csla3csla9* mutant deficient in this class of polysaccharides that demonstrated increased gravitropic bending. BRZ also changed the pattern of epidermal cell expansion along the axis of hypocotyls greatly diminishing the growth rate of three most basal epidermal cells. This effect may result