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Control of *Arabidopsis thaliana* shoot gravitropism at the level of cell wall biomechanics and metabolomics

Suslov D.[1]*, Pozhvanov G.[1, 2], Lipchinsky A.[1]
[1] *St. Petersburg State University, St. Petersburg, Russia*
[2] *Komarov Botanical Institute, RAS, St. Petersburg, Russia*
* *d.suslov@spbu.ru*

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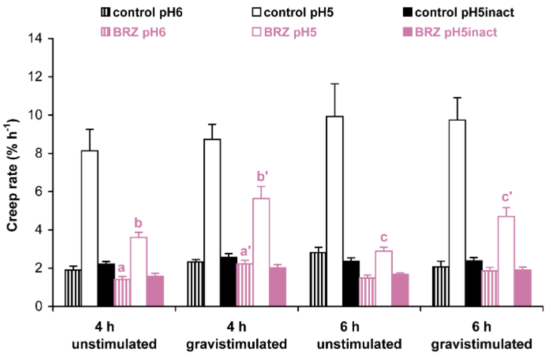
Motivation and Aim: Gravitropism is directed growth of a plant or plant organ in response to gravity, resulting in their bending. Plant shoots typically demonstrate negative gravitropism after inclination, i.e. bending upward in the direction opposite to the gravity vector. The bending results from different cell expansion rates on upper and lower flanks of the inclined shoot. It is controlled not only by cell wall extensibility, the main biophysical growth-limiting factor, but also by the flexibility of cell wall polymers and their compression resistance on the concave side of the gravistimulated shoot. Thus, plant shoot gravitropism is a convenient model to decipher the functions of cell wall polymers in plant growth and development. We have recently demonstrated that brassinazol (BRZ), an inhibitor of brassinosteroid phytohormone biosynthesis, stimulated Arabidopsis hypocotyl gravitropism, which was accompanied by a decrease in the level of mannans, minor hemicellulosic polysaccharides of cell walls [1]. Now we tried to establish if the effect of BRZ of shoot gravitropism was actually mediated by mannans. With this aim we compared cell wall biomechanics and metabolomics of hypocotyls in BRZ-treated Col-0 Arabidopsis plants and *csla2csla3csla9* (*csla2,3,9*) triple mutants, which are devoid of cell wall mannans.
Methods and Algorithms: Etiolated Arabidopsis seedlings were grown on vertical Petri plates with and without gravistimulation by a 90° counterclockwise rotation of the plates. Metabolites extracted from hypocotyls were analyzed by GC/MS. Cell wall biomechanics were studied by the creep method that measures creep, i.e. time-dependent deformation of the wall samples under a constant load. Creep of frozen/thawed hypocotyls was measured at pH 5 and 6 that activate different groups of cell wall proteins regulating its extensibility, and at pH 5 with heat-inactivation where cell wall proteins are heat-killed, and the wall extension is defined by the mechanics of its structural polymers and the character of static interactions between them.
Results: BRZ (0.2 μM) inhibited elongation of Col-0 Arabidopsis hypocotyls grown vertically, but greatly increased their bending after gravistimulation. In the presence of BRZ gravistimulated bent hypocotyls were significantly longer than unstimulated straight hypocotyls, suggesting that BRZ activates Arabidopsis shoot gravitropism via an overall increase in cell expansion rate. BRZ-treated gravistimulated hypocotyls had significantly higher cell wall creep rates than their unstimulated counterparts at pH 5 and 6 (Fig. 1). This indicates that cell wall loosening proteins active at pH 5 and pH 6 were involved in the mechanism of BRZ action on shoot gravitropism. BRZ changed the levels of many metabolites including the building blocks of cell wall structural polysaccharides (arabinose, xylose, galacturonic acid) and glycoproteins (oxyproline, proline). However, we observed no significant differences in metabolites between BRZ-

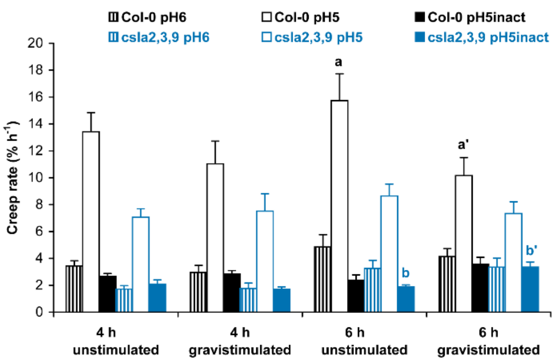
treated gravistimulated and unstimulated Col-0 hypocotyls. Apparently, the profound effect of brassinosteroid biosynthesis inhibition strongly outweighed and masked any changes in metabolites associated with gravistimulation.

Fig. 1. Effects of BRZ and gravistimulation on cell wall biomechanics



Hypocotyls of *csla2,3,9* mannan-deficient mutants had the same length as in wild type Col-0 plants. By analogy with BRZ, the triple mutation stimulated Arabidopsis shoot gravitropism, although to a lower extent than BRZ. Biomechanical studies of *csla2,3,9* hypocotyls revealed significant differences at pH 5 with heat-inactivation 6 h after gravistimulation (Fig. 2). Hence, the effect of *csla2,3,9* on shoot gravitropism is associated with a change in basic cell wall polymer structure. Metabolome analysis shows that structural cell wall glycoproteins could mediate this effect, as oxyproline, their hallmark component, was significantly decreased in gravistimulated *csla2,3,9* vs. gravistimulated Col-0 hypocotyls.

Fig. 2. Effects of csla2,3,9 and gravistimulation on cell wall biomechanics



Conclusion: We established that BRZ and mannan depletion had very different effects on the wall biomechanics and metabolome of Arabidopsis hypocotyls. This shows that the effect of BRZ on shoot gravitropism is not mediated by mannans.

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References

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