EFFECT OF STRIGOLACTONE ON POLAR AUXIN TRANSPORT AND PLANT ARCHITECTURE

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ABSTRACT

Physiologically, branching is regulated by a complex interplay of hormones including auxin, cytokinin and recently discovered strigolactone. The study is focused on the effect of strigolactone on shoot branching of pea (*Pisum sativum* L.) in relation with polar auxin transport, which has an essential role in apical dominance. After decapitation of the dominant apex lateral buds are released from growth inhibition and their outgrowth and elongation is initiated. Basipetal polar auxin transport system is realized by the downward movement of auxin from apical meristems towards the root system and strigolactone is transported acropetally. The polar auxin flow starts to be established in axillary buds, which is mediated by auxin efflux carriers - PIN1 proteins localized on the basal end of cells. Exogenous application of GR24 - synthetic analogue of strigolactone on the second or third bud of 7 day-plants led to partial growth inhibition of treated bud which is apparently associated with gene expression changes. Expression profiles of *PIN1* and *DRM1* genes and immunocytolocalizations of PIN1 proteins are studied, to reveal and understand how strigolactone interacts with polar auxin transport on transcriptional and translational levels. The gene expression and polarization of PIN1 proteins after short-time and long-time influence of strigolactone on the axillary bud was followed.

Key words: strigolactone, polar auxin transport, PIN1 proteins

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INTRODUCTION

We are interested in developmental growing processes in plants which are related with getting and formation shape of plant systems, with adaptive response plant on changing surrounding environment, viability and in the final consequence with high yield of crops. Study is focused on effect of strigolactone on the stem branching of Pea plants and in connection with polar auxin transport, which has a major role in apical dominance.

The process of stem branching is essential for a determination of plant shape. Bud outgrowth is regulated by the interaction of environmental signals and endogenous signals known like plant hormones (Ongaro & Leyser, 2008). Auxin, cytokinin and recently discovered strigolactone have a major influence on shoot system architecture. The phenomenon of the apical dominance is characterized by growing of main stem and repress of axillary branches growth currently the polar auxin transport in main shoot is maintained. After removal of shoot apex lateral buds are released from growth inhibition and their outgrowth is initiated. Into the downward movement of auxin flow a several protein families are involved such as influx facilitators AUXIN INFLUX CARRIER PROTEIN1 (AUX1) proteins (Parry et al., 2004) and PIN-FORMED auxin efflux carriers (PIN1) (Paponov et al., 2005). Corresponding genes *AUX1* and *PIN1* encodes this proteins. Decapitation of apex a major auxin source lead to synthesis of auxin in lateral buds and auxin stream is starts to be established thereby canalization of auxin from formed substitute auxin source to the stem is begun. Auxin export mediated by polarization of PIN1 efflux carriers is accompanied by formation of auxin transport pathways and vascular strands as well (Balla et al., 2011). On transcriptional level this process is demonstrated by change of *PIN1*, *AUX1* and *DRM1* (marker of dormant buds) gene expression (Stafstrom et al., 1998).

Strigolactone hormones are carotenoid-derived via carlactone (Adrian Scaffidi et al., 2013). Their existence was supported by the analysis of a series of shoot branching mutants in Arabidopsis, pea and petunia. Strigolactone have been identified as signalling molecules ´shoot multiplication signal´ that is transported acropetally from root to inhibit axillary branch outgrowth (Gomez-Roldan et al., 2008, Umehara et al., 2008). Additional various functions of strigolactone like control of secondary growth in cambium, root architecture (Ruyter-Spira et al., 2011), adventitious root formation and leaf development are related to auxin-dependent processes, and strigolactone acts at least in part by regulation auxin transport via the efflux carrier PIN1 (Sinohara et al., 2013).

MATERIAL AND METHODS

Pea plants (*Pisum sativum* L). cultivar Vladan are used for all experiments. This is a typical model plant for research approaches on Mendel University because of its strong apical dominance. Seeds were germinated on perlit and after approximately 5 days selected and replanted to plastic vessels where they were cultivated 5 to 7 days till the required plant length and numbers of buds was achieved. The vessels with selected plants were placed into growth cabinet with constant environmental conditions (temperature 18/20°C, photoperiod 16/8 h). Pea plants were treated with synthetic analog of strigolactone GR24 (Chiralix) in application solution. 0.5 µl of solution containing 50 µmol/l GR24 in 50 % ethanol, 2 % polyethylenglycol, 0.2 % aceton and 0.2 % dimethyl sulfoxid (Brewer et al., 2009) was applied on the second bud of plants. Ethanol provides efficient penetration into the tissue and polyethylengycol ensures better sticking on the surface of the buds. The rest of the decapitated/intact plants was treated by application of control solution (without addition of GR24). Buds from intact or decapitated plants were stored in the fixative solution in the case of immunolocalization of proteins or frozen by liquid nitrogen in the case of RNA isolation followed by RT-PCR.

For the assessment of gene expression of selected genes RNA was isolated from axillary buds by RNAeasy Plant Mini Kit (Qiagen, Germany). Reverse transcription and following amplification of obtained cDNA was realized with the Enhanced Avian HS RT-PCR Kit (Sigma-Aldrich, USA). For PCR reaction specific primers for selected genes - *PsPIN1, PsDRM1* were used. Obtained PCR product was separated by agarose electrophoresis, visualized under UV light and results were evaluated by the program GelWorks 1D. *β-tubulin* was used as a reference gene. immunocytolocalization was used according to Paciorek et al., 2006. This process involves sample fixation, dehydration, embedding to paraffin, cutting on microtome, deparaffination, blocking and binding of 1st and 2nd antibody and in final step lead to visualization of samples under confocal laser scanning microscope (Olympus Fluoview 300).

RESULT AND DISCUSSION

The obtained results of *PIN1* gene expression of the intact control variant in both times were not significantly changing according to our expectations. In comparison the application of GR24 caused a slight decrease of *PIN1* gene expression in intact plants. Decapitation of control plants lead to significant increase after 6 hours. Gene expression was increased more over than 100% while after decapitation and current application of strigolactone expression was going up slightly in the bud after 1 hr as well as 6 hr.

Taken together strigolactone reduced *PIN1* expression in intact plants in both times in contrast to decapitated plants where strigolactone stimulated the expression after 1 hr and remarkably decreased it after 6 hr. Expression was compared with *β-tubulin* as a reference gene, level of expression 100% was set according to expression in the bud of intact plants treated by GR24 in time 0 hour.

The data of *DRM1* expression have a declining trend in all variants except decapitated control variant after 1 hr, where the level of *DRM1* as an auxin inducible gene (Stafstrom et al., 1998) was slightly increased which is associated with faster depletion of auxin from the stem but after 6hr *DRM1* occurrence is almost insignificant.

The results of immunocytolocalization of PIN1 proteins confirm the same effect of strigolactone on translational level like on the transcriptional level. In the intact 0 hr control plants the signal of fluorescence were not intensive and polarized PIN1 proteins observable similarly like in intact plants after 1 hr where the PIN1 proteins are also all around the plasmatic membrane of the cells and do not concentrate just into the basal side of the cell membranes. After 1 hr of the application of GR24 increased presence of PIN1 proteins in central part of the apical meristem was demonstrated, but without polarization. Decapitation of plants with GR24 caused significant increase of polar auxin transport which was observed as elevated polarization of PIN1 proteins on the basal membrane of cells in apical meristem of axillary bud in contrast with control decapitated variant where the localization of the signal was unchanged.

In the case of long-term influence of strigolactone, similarly like in the work of Shinohara, 2013 where they observed that strigolactone addition results in a reduction in PIN1 levels in xylem parenchyma cells within 6 h, accompanied by a reduction in polar auxin transport, we have observed after 6 hr in intact and decapitated plants treated with GR24 the reduced signal of PIN1 compared with corresponding control variants. It corresponds with the idea that strigolactone acts by limiting PIN1 accumulation on the plasma membrane, dampening canalization and thus preventing bud activation (Crawford et al., 2010).

Obtained data from both methods – gene expression and immunolocalization confirm that strigolactone has stimulating effect after short time (1hr) and inhibiting effect after long time (6hr) on polar auxin transport. The reducing influence on bud outgrowth confirms the character of strigolactone as inhibitor of shoot branching (Umehara et al., 2008). In contrast to that short-term

influence of strigolactone lead to elevation of auxin export from the bud. This potential artifact is not resolved yet, but was observed also in experiments with BY2 tobacco suspension (Sibu, Petrášek, not published).

Our findings are in consistence with results of Ruiter Spira et al., (2011), that strigolactones are able to modulate local auxin levels and that strigolactone action is dependent on the auxin status of the plant. Strigolactone regulates growth redistribution in the shoot by modulating auxin transport. However recently here is the hypothesis that strigolactone is able to inhibit or promote shoot branching depending on the auxin transport status of the plant. Growth across the plant shoot system is balanced by competition between shoot apex for a common auxin transport path to the root and that strigolactones regulate shoot branching by modulating this competition (Shinohara, 2013).

CONCLUSION

The results of immunocytolocalization of PIN1 proteins show that strigolactone after short time (1 hr) causes increase of polar auxin transport what is observed as elevated presence of PIN1 proteins and strong signal of fluorescence in apical meristem of axillary buds of decapitated plants in contrast with control variant with steady signal. The same situation was proven in the case of gene expression, where the expression of *PIN1* gene was increased in buds after influence of strigolactone than in control decapitated plant.

Oppositely long-term effect of strigolactone has an inhibiting effect on PIN1 proteins in the lateral bud. It was identified as reduced occurrence and signal of auxin efflux carriers, and suppressed polar auxin transport and in the final consequence it leads to the arrest of lateral bud outgrowth.

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