COMPETITIVE CANALIZATION OF AUXIN IN PEA CAN BE INVOLVED IN INITIATION OF AXILLARY BUD OUTGROWTH

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ABSTRACT

In many plants the growing shoot apex inhibits the outgrowth of axillary buds, a phenomenon termed apical dominance. Removal of the shoot apex, the starting point of polar auxin transport (PAT), leads to release of inhibited or dormant axillary buds to form branches. PAT system is necessary to establish various developmental processes in plants and has an essential role also in apical dominance. One of the latest theories explaining the mechanism of apical dominance is the theory of competitive auxin canalization, by which canalization from the lateral auxin source is possible only if the primary source is removed or weakened. After decapitation axillary buds in pea (*Pisum sativum* L.) establish directional auxin export by subcellular polarization of PIN1 auxin efflux transporters. In this work, initiation of bud outgrowth based on the polar auxin export was characterized by expression profiles of *PsPIN1* and *PsAUX1* genes coding auxin transmembrane carriers, and of the dormancy marker gene *PsDRM1*. Application of auxin efflux (NPA, TIBA) or protein synthesis (cycloheximide) inhibitors to the second axillary bud of decapitated plants reduces bud outgrowth. Inhibition of outgrowth of the second axillary bud in these plants caused outgrowth of the first bud. This competition between the second and first axillary buds as new potential sources of auxin after decapitation is associated also with changes in expression profiles of *PsAUX1*, *PsPIN1* and *PsDRM1* genes. The obtained results are in accordance with the competitive auxin canalization theory.

Key words: polar auxin transport, *Pisum sativum* L., bud outgrowth

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INTRODUCTION

The dynamic process governing plant architecture is regulated by developmental factors and environmental conditions. Whether or not axillary meristems grow to form branches is one key component of plant architecture. The axillary meristems have the same predispositions as the primary shoot apical meristem (SAM). Thus the formation of axillary meristems and the subsequent regulation of their activity contribute greatly to variation in shoot architecture (Müller and Leyser, 2011). In many plants the growing shoot apex inhibits the outgrowth of axillary buds; a phenomenon termed 'apical dominance' (Cline, 1997). It is best demonstrated via shoot tip removal (decapitation), which leads to the release of dormant axillary buds below it to form branches. It has been known for a long time that the phytohormone auxin plays a major role in apical dominance. Auxin is mainly synthesized in young expanding leaves at the shoot apex (Ljung et al., 2001) and subsequently transported basipetally in the polar auxin transport stream by membrane-located proteins regulating the rates of efflux from and influx into the cells (Krupinski and Jonsson, 2010). Auxin is actively distributed within the plant by cell-to-cell movement that is facilitated by auxin influx carriers (AUX1/LAX proteins) and by auxin efflux carriers (PIN and PGP protein families) (Friml et al., 2003; Vieten et al., 2007). The direction of auxin flow is largely determined by the asymmetric cellular localization of the PIN proteins (Wisniewska et al., 2006).

Despite its long history, the mechanism by which auxin inhibits the growth of axillary meristems is not fully understood. One of the latest theories explaining the mechanism of apical dominance is the theory of competitive auxin canalization, by which auxin export from axillary buds and subsequent canalization is possible only if the primary source of auxin is removed or weakened. In plants with genetically-based strong apical dominance, the presence of the primary auxin source prevents auxin export and canalization from secondary sources during plant development (Balla et al., 2011). Removal of the primary apex results in activation of axillary buds below the decapitated stump due to the withdrawal of auxin. Bud activation can also be triggered by the efflux of auxin produced in the buds. The sustained export of auxin from buds induces PIN expression and polarity in competent tissues, which is then followed by differentiation of new vasculature along the PIN1-marked auxin channel (Sauer et al., 2006). Enhanced auxin levels can directly increase the auxin transport capacity via modulating the expression of *PIN1* (Dun et al., 2006). Initiation of axillary bud outgrowth is accompanied by changes in expression of *PsAUX1*, *PsPIN1* coding auxin transmembrane carriers and by decrease in expression of dormancy-associated gene *PsDRM1* (Stafstrom et al., 1998).

To prove whether auxin efflux inhibitor can inhibit bud outgrowth, subsequent polar auxin transport from the buds and influence competition for outgrowth of axillary buds, auxin efflux inhibitor (NPA, TIBA) and furthermore protein synthesis inhibitor cycloheximide were applied on the following model system.

MATERIAL AND METHODS

Pea plants (*Pisum sativum* L.) cv. Vladan were grown in perlite in a growth chamber at 20/18 °C, photoperiod 16-h day/8-h night and light intensity 150 $\mu mol.m^{-2}.s^{-1}$. Seven-day-old pea seedlings were used for all experiments. Lanolin paste containing 1 % polar auxin transport inhibitor NPA (naphtylphtalamic acid), TIBA (2,3,5-triiodobenzoic acid) or 1 % protein synthesis inhibitor (cycloheximide) was applied onto 2nd axillary bud. After NPA/TIBA/cycloheximide application plants were decapitated 10 mm above the 2nd bud. Axillary buds of intact plants not treated with lanolin paste containing inhibitors were analyzed as the control treatment. Expression of *PsPIN1*, *PsAUX1* and *PsDRM1* genes were studied in the 2nd treated and 1st not treated axillary buds. Furthermore, influence of length of the decapitated stem stump on changes in gene expression in the 2nd axillary buds and therefore on the bud outgrowth initiation was studied. Seven-day-old pea

seedlings were decapitated 90 mm above the 2nd axillary buds or just above the 2nd axillary buds. Tissue samples were harvested and ground in liquid nitrogen. Total RNA was isolated from 25 to 30 buds using the RNAeasy Plant Mini Kit (Qiagen) following the manufacturer's protocol. Total cDNA was synthesized from 0.5 µg of total RNA using AMV reverse transcriptase (Life Technologies). Real-Time PCR (qPCR) was performed using LC 480 SYBR Green I Master Mix (Roche) with the specific primers for *PsPIN1*, *PsAUX1* and *PsDRM1*. Expression levels of *Psβ-tubulin*, *PsActin* and *PsEF1-α* genes was used as normalization control. Two biological repeats were analyzed in duplicate. From replications of each variant the mean value and standard deviations were assessed. For statistical analysis Student's t-test was performed to test the significance of differences between the individual variants.

RESULTS AND DISCUSSION

Expression of dormancy associated gene *PsDRM1*

In 2nd axillary buds of all variants the expression of dormancy-associated gene *PsDRM1* decreased during 3 hours after decapitation, but at about 24 hours the expression of *PsDRM1* rapidly increased in buds treated with inhibitors compared with the control treatment. *PsDRM1* expression is tightly linked to the non-growing state in tissues (Stafstrom et al., 1998). By contrast, in not treated axillary buds of the 1st node was observed that the expression of *PsDRM1* disappeared completely within 2 hours. Auxin-repression of *PsDRM1* is consistent with what is known about the auxin content of axillary buds. For example, dormant axillary buds of *Phaseolus* contain less auxin than growing buds (Gocal et al. 1991). The changes in expression profiles were in accordance with 1st axillary bud outgrowth, while 2nd bud was inhibited. Based on the results more effective in bud outgrowth inhibition seems to be TIBA than NPA application. On the contrary, in axillary buds of not treated plants 2nd axillary buds were growing, whereas outgrowth of 1st buds was as a consequence of competition between two auxin sources suppressed. After 24 hours, the expression of *PsDRM1* in the 2nd buds treated with cycloheximide rapidly dropped again. This can be caused by the cycloheximide, an inhibitor of protein synthesis that induces the expression of early auxin-inducible genes by depleting short-lived transcriptional repressors (Soeno et al., 2010).

Expression of *PsPIN1* and *PsAUX1* genes coding auxin membrane carriers

NPA or TIBA application onto 2nd axillary buds induces increase in the expression of *PsPIN1* gene coding auxin carriers. This can be caused by accumulation of auxin in buds, which cannot be transported out from cells as a result of application of the auxin efflux inhibitors. NPA and TIBA strongly inhibit basipetal transport (Guerrero et al., 1999) and TIBA induces an increase in IAA content of stems and leaves (Peng et al., 2013). In buds treated with 1% TIBA was observed a decline after 12 hours in *PsPIN1* expression. In contrary, in buds treated with 1% NPA the *PsPIN1* expression gradually increased. This difference can be as a consequence that TIBA itself may be polarly transported in a basipetal direction (Guerrero et al., 1999). In 2nd buds treated with cycloheximide was also observed an increase in the expression of *PsPIN1* at first, and then was found a rapid drop after 12 hours after protein synthesis inhibitor application and decapitation. In these axillary buds, similar expression profile of *PsAUX1* gene was observed, presumably as a result of inducible effect of cycloheximide on the expression of early auxin-responsive genes (Soeno et al., 2010). Application of efflux inhibitor TIBA resulted in a larger increase of *PsAUX1* gene expression than application of NPA, probably due to possible polar transport of TIBA (Guerrero et al., 1999). In treated variants in 1st not treated axillary buds a rapid increase or decrease in the expression of *PsPIN1* and *PsAUX1* was not observed. In axillary buds of decapitated not treated control plants significant differences between 2nd and 1st bud in expression of *PsPIN1* or *PsAUX1* genes were not observed. Based on these data, it is possible to say that it is not conclusively clear if 1st not treated or 2nd treated bud is activated in early time intervals after inhibitors application and decapitation. Decapitation of garden pea caused bud outgrowth within 4 to 6 hours after shoot tip removal (Morris et al., 2005), and the outgrowing shoot can be the new

auxin source. These data show that in early time intervals 1st bud and 2nd bud as new potential auxin sources apparently compete with each other. Based on the results it is possible to propose that despite the application of inhibitors onto 2nd bud presumably a signal on the removal of the shoot tip (primary auxin source) can be transmitted into this bud as well as into the 1st bud. However, in later time intervals 2nd bud is inhibited as a result of inhibitors application and 1st bud is growing out and becomes the new primary auxin source. Balla et al. (2011) observed that after decapitation activated axillary buds rapidly polarize PIN1 proteins and establish directional auxin export from the bud. Subsequently, the buds induce formation of PIN1-expressing auxin channels delineating future vascular connections between the activated bud and the main stem.

CONCLUSIONS

Pea (*Pisum sativum* L.) has a genetically-based strong apical dominance. In intact plants, the shoot apex grows predominantly and inhibits outgrowth of axillary buds. Decapitation leads to initiation of axillary buds outgrowth, what is associated with changes in gene expression. One of the bud takes over the function of decapitated shoot tip and becomes a new auxin source that on the basis competition inhibits auxin export from secondary sources.

Application of auxin efflux (NPA, TIBA) or protein synthesis (cycloheximide) inhibitors onto the second axillary bud of decapitated plants reduces bud outgrowth. This inhibition caused outgrowth of the first bud, which is associated with changes in expression profiles of *PsPIN1, PsAUX1* genes coding auxin membrane carriers and dormancy associated gene *PsDRM1*. Given that shoot tip as the primary source of auxin was removed by decapitation and second bud was inhibited, the first bud took over the role of primary auxin source. These results support the competitive auxin canalization theory, by which canalization from the lateral auxin source is possible only if the primary source is removed or weakened.

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