



UMEÅ UNIVERSITET

Characterization of adventitious root formation in *Populus* species and Norway spruce

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Akademisk avhandling

som med vederbörligt tillstånd av Rektor vid Umeå universitet för
avläggande av filosofie doktorsexamen framläggs till offentligt
försvar i KBF301, KBC huset,

Torsdagen den 3 juni 2021, kl. 10:00.

Avhandlingen kommer att försvaras på engelska.

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Department of Plant Physiology

Organization

Umeå University
Department of Plant Physiology

Document type

Doctoral thesis

Date of publication

7 Maj 2021

Author

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Title

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Abstract

Adventitious root (AR) formation is a form of post-embryonic development and is a key adaptive trait in plants. *De novo* adventitious root regeneration represents an elegant evolutionary innovation that allows many plant species to multiply through vegetative propagation; it is widely used in forestry and horticulture to multiply elite genotypes. However, several tree species with high economic and ecological value are difficult to root, and the genetic and molecular bases underlying AR regeneration remain largely elusive. Recently our laboratory showed that jasmonate (JA) and cytokinins (CK) act cooperatively to repress AR initiation (ARI) in *Arabidopsis* hypocotyls, while auxin positively controls ARI by repressing this negative effect. With the recent availability of the reference genomes of *Populus* spp. and Norway spruce (*Picea abies*), the aim of this thesis is to explore the molecular and mechanistic foundations of AR formation in woody species and check whether or not there is conservation of the molecular mechanisms identified in *Arabidopsis*. First, physiological, molecular and hormonal approaches coupled with extensive anatomical analysis were combined to explore the role of light spectral quality in the control of ARI in *P. abies* de-rooted seedlings. We showed that constant red light (cRL) promotes ARI by reducing the content of the wound-induced phytohormones JA and JA-isoleucine and repressing the accumulation of the isopentyl-adenine-type cytokinins. These results suggest that the cooperative role of JA and CK signaling in the repression of ARI is evolutionarily conserved. Next we compared transcriptomic data from the cambium tissue of woody stem cuttings of the hybrid aspen T89, which is difficult-to-root, and from the hybrid poplar OP42, which is easy-to-root, under hydroponic conditions. The analyses revealed high transcriptional activity in OP42, with twice as many transcription factors differentially expressed in OP42 24 hours after cutting compared to T89. Although we did not observe significant differences in the expression of Auxin response factor (ARF) genes between the two genotypes, the production of transgenic plants downregulating or over-expressing *ARF6*, *8* or *17* confirmed that *PtARF6* and *PtARF8* positively and *PtARF17* negatively regulate AR development in transgenic hybrid aspen. Interestingly, the expression of *MYC2* orthologs as well as the expression of several genes involved in JA signaling increased more in T89 than in OP42, suggesting that JA could be a negative regulator of ARI in *Populus* spp. We also showed that overexpressing *PtMYC2* led to a reduced number of ARs in hybrid aspen cuttings. In addition, many genes encoding ROS scavenging proteins such as peroxidases or GSTs were significantly differentially expressed in OP42 24 h after cutting but not in T89, which is interesting since peroxidase activity has often been positively correlated with ARI. In parallel to this research, we characterized the rooting phenotype of clones from the Swedish Aspen (SwAsp) collection using *in vitro* cuttings. We observed a significant variation in the rooting ability as well as different root system establishment between the clones. We analyzed the expression of some genes known to be involved in AR development in selected clones with contrasting AR phenotypes but could not identify any correlation between gene expression and rooting phenotype. A transcriptomic analysis of selected clones, with contrasting AR phenotypes, could be a useful tool in the identification of marker genes, which can be used for future selection of the best rooting clones of *Populus* or other economically important trees in breeding programs.

Keywords

Adventitious root, Conifers, *Picea abies*, auxin, cytokinins, jasmonate, red light, *Populus* spp., hybrid poplar, hybrid aspen, cambium, stem cuttings, *P. tremula*, Swedish Aspen (SwAsp) collection.

Language

English

ISBN

Print: 978-91-7855-538-3
PDF: 978-91-7855-539-0

Number of pages

108 + 3 pages