ABSTRACT

"Identification of novel regulators of lignification and programmed cell death of xylem elements"

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Xylem maturation involves lignification and programmed cell death (PCD) that are interlinked through the action of the NAC family transcription factors. The downstream regulators and the executioners are poorly known. We have identified two metacaspase genes as candidates for such key regulators in *Populus* trees on the basis of specific upregulation during the xylem cell death phase of wood formation. The closest *Arabidopsis* homologue to these two *Populus* genes is *METACASPASE9* (*AtMC9*, At5g04200). Also *AtMC9* shows xylem specific expression pattern throughout the plant, suggesting involvement in xylem cell death. In order to characterise the function of AtMC9 in xylem development, a reverse genetic approach was taken in *Arabidopsis*. *AtMC9* T-DNA knock-out lines showed rather normal progression of protoxylem differentiation and PCD in *in vitro* grown seedling roots by analysis of genetic crosses to various xylem reporter lines. Interestingly, *AtMC9* RNAi lines revealed increased overall growth of the plants, which together with double mutant analyses in the metacaspase family suggest involvement of AtMC9 together with other type II metacaspase genes in growth control. We are currently performing proteomic analyses to identify targets of the AtMC9 action. Upstream regulators have been sought by screening an EMS mutagenised pro*AtMC9*::*GFP* reporter line.

We have also utilized the *Zinnia elegans in vitro* tracheary element (TE) differentiation system to identify novel regulators of lignification and PCD. In this system, application of silver thiosulphate (STS) blocked both lignification and PCD of TEs resulting in prolonged lifespan of the TEs, while TE secondary cellulose formation was not affected. This allowed us to identify novel genes specifically involved in lignification and/or PCD by analyzing differential gene expression patterns between cell cultures treated with or without STS. One of the identified

genes was a *PIRIN* gene that was previously implicated in transcriptional regulation in mammals. Expression of the *Zinnia PIRIN* gene was strongly upregulated during lignification and/or PCD of normally developing TEs, whereas no upregulation was present in non-lignifying TEs after STS treatment. Functional analysis of the *PIRIN* gene family was undertaken in *Arabidopsis thaliana* which contains four PIRIN (PRN) homologs: AtPRN1 (At3g59220), AtPRN2 (At2g43120), AtPRN3 (At3g59260) and AtPRN4 (At1g50590). Promoter activity assay by using GUS reporter gene showed that, out of the four *PIRIN* genes, *AtPRN2* was most specifically expressed in the vascular tissues. Pyrolysis-GC/MS analysis of the various *PIRIN* gene family mutants suggests involvement of AtPRN2 in control of lignification. However, *in vitro* and *in vivo* analyses demonstrated interaction of AtPRN2 with several different types of proteases, which suggests involvement of AtPRN2 primarily in regulation of hydrolytic processes during xylem cell death. We can therefore link together the two processes of lignification and PCD and provide evidence on a novel player in this interplay.