MiR397 overexpression, a strategy to engineer laccase activity and lignification in model plants and in crop plants and trees

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Role of rhamnogalacturonan-II in pollen germination and pollen tube elongation

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Rhamnogalacturonan-II (RG-II) is the most complex pectic polysaccharide present in the primary cell wall of all land plants. Despite its highly complex structure, RG-II is evolutionarily conserved in the plant kingdom suggesting that this polymer has fundamental functions in the primary cell wall organization. To date, very little is known about the biosynthesis of RG-II. Recently, a bioinformatic study identified 24 new putative glycosyltransferases possibly involved in the Arabidopsis RG-II biosynthesis [1]. Among them, two sialyl-like transferases, At1g08660 and At3g48820, were selected and proposed to be involved in the transfer of Kdo (3-deoxy-D-*manno*-octulosonic acid) and/or Dha (3-deoxy-D-*lyxo*-heptulosaric acid) on the homogalacturonan backbone of RG-II [2] because sialic acid is absent in plants [3] and these three acidic monosaccharides share common structural features. Mutation in At1g08660 was previously shown to induce defects in pollen germination [4]. As a consequence, we focused our study on At3g48820 using Arabidopsis pollen tubes as experimental model. Homozygous mutant line is not available for this gene. Analyses of two heterozygous lines revealed a strong reduction in pollen tube germination both *in vitro* and *in vivo*. Moreover, pollen tubes exhibited abnormal swollen tubes by comparison with the wild-type pollen. These data suggest that sialyl-like transferases are important for the cell wall formation and stability during pollen germination and pollen tube growth.

[1] A. Voxeur *et al*., (2012) *PLoS One*, **7**, e51129.; [2] M. Séveno *et al*., (2010) *Glycobiology*, **20**, 617-628; [3] M. Séveno *et al*., (2004) *Nat. Biotechnol.*, **22**, 1351-1352. [4] Y. Deng *et al.,* (2010) *J. Integr. Plant Biol.*, **52**, 829-843.

P2-08

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We have recently established that several laccases are involved in the lignification of Arabidopsis stems [1]. To substantially reduce their lignin level and therefore improve their enzymatic degradability, it was necessary to down-regulate the two main laccases expressed in Arabidopsis stems. This was made possible by the production of double knockout Arabidopsis mutants, a strategy not available for all plant species and particularly for dedicated energy crops.

Laccases are the endogenous targets of the highly conserved microRNA miR397 in plants. This microRNA is not expressed in normal growth conditions, but its expression is induced by various stresses (including copper deficiency [2]).

In this work, the overexpression of miR397 was considered as a strategy to simultaneously silence the laccases putatively involved in the lignification of various plants (*Arabidopsis thaliana*, *Brachypodium distachyon*, rice, maize, poplar). Constructs for the expression of miR397 under the control of constitutive or lignin-specific promoters were introduced in these target plants. We selected transgenic plants displaying a high miR397 expression in stems and obtained some homozygous lines. We evaluated the impact of this miR397 overexpression on the transcripts of laccases considered as lignin-specific and on stem lignification.

[1] Berthet *et al.* (2011) *Plant Cell*, **23**, 1124-1137; [2] Sunkar and Zhu (2004) *Plant Cell*, **16**, 2001-2019.