Impact of CCR1 silencing on the assembly of lignified secondary walls in Arabidopsis thaliana

Katia Ruel1, Jimmy Berrio-Sierra1, Mohammad Mir Derikvand2, Brigitte Poller3, Johanne Thévenin2, Catherine Lapierre3, Lise Jouanin2 and Jean-Paul Joseleau1

1Centre de Recherches sur les Macromolécules Végétales (CERMAV-CNRS), UPR 5301, Associée à l’Université Joseph Fourier (UJF), Grenoble, France; 2Biologie Cellulaire, IJPB, INRA, 78026 Versailles cedex, France; 3UMR 206 Chimie Biologique AgroParisTech-INRA, AgroParisTech Centre de Grignon, 78850 Thiverval-Grignon, France

Summary

• A cinnamoyl-CoA reductase 1 knockout mutant in Arabidopsis thaliana was investigated for the consequences of lignin synthesis perturbation on the assembly of the cell walls.
• The mutant displayed a dwarf phenotype and a strong collapse of its xylem vessels corresponding to lower lignin content and a loss of lignin units of the noncondensed type. Transmission electron microscopy revealed that the transformation considerably impaired the capacity of interfascicular fibers and vascular bundles to complete the assembly of cellulose microfibrils in the S2 layer, the S1 layer remaining unaltered. Such disorder in cellulose was correlated with X-ray diffraction showing altered organization.
• Semi-quantitative immunolabeling of lignins showed that the patterns of distribution were differentially affected in interfascicular fibers and vascular bundles, pointing to the importance of noncondensed lignin structures for the assembly of a coherent secondary wall.
• The use of laser capture microdissection combined with the microanalysis of lignins and polysaccharides allowed these polymers to be characterized into specific cell types. Wild-type A. thaliana displayed a two-fold higher syringyl to guaiacyl ratio in interfascicular fibers compared with vascular bundles, whereas this difference was less marked in the cinnamoyl-CoA reductase 1 knockout mutant.

Abbreviations: CCR, cinnamoyl-CoA reductase; CMFs, cellulose microfibrils; CP/MAS, cross-polarization/magic angle spinning; G, guaiacyl unit; GS, mixed guaiacyl–syringyl structures; H, p-hydroxyphenyl unit; ifs, interfascicular fibers; KL, Klassen lignin; LCM, laser capture microdissection; S, syringyl unit; SEM, scanning electron microscopy; TEM, transmission electron microscopy; TFA, trifluoroacetic acid; vbs, vascular bundles.

Introduction

The precise contribution of lignins in the assembly of plant cell walls has not been elucidated completely. However, it is widely accepted that, during differentiation, the composition and structure of lignins have a specific impact on the assembly and structural cohesion of the secondary walls according to cell type (Joseleau & Ruel, 1997). The study of mutant plants altered in the biosynthesis of any one of the wall polymers offers promising opportunities to obtain an insight into plant cell wall macromolecular assembly and organization. The monolignols originate from the phenylpropanoid pathway, which includes many steps that are potential targets for the modification of lignin biosynthesis (Baucher et al., 1998; Dixon et al., 2001). Many transgenic or mutant plants affected in the expression of the corresponding genes have been studied (reviewed in Anterola & Lewis, 2002). Cinnamoyl-CoA reductase (CCR) is the first enzyme specific to the monolignol pathway (Lacombe et al., 1997). This enzyme has been shown to catalyze the NADPH-dependent conversions of p-coumaroyl-CoA, feruloyl-CoA and sinapoyl-CoA into the corresponding aldehydes. By analyzing CCR-down-regulated tobacco plants...