Alterations in Cell-Wall Composition of Transgenic Alfalfa Due to Expression of a Fungal Mn-Dependent Peroxidase Gene

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Introduction

Biochemical pulping of wood is desired as a method to reduce the environmental pollution associated with current chemical pulping technologies. Lack of supply and production costs of peroxidase enzymes, enzymes capable of both polymerizing and de-polymerizing lignin, needed in biochemical pulping is a major road-block to its development. Plants have been suggested as a vehicle to produce large amounts of industrial enzymes such as peroxidase through application of biotechnology. A Mn-dependent peroxidase from *Phanerochaete chrysosporium*, a lignin degrading aerobic fungus, has been successfully transferred into alfalfa. These transgenic plants are capable of producing the peroxidase; however, abnormal growth characteristics are observed in those plants which are high expressors of the peroxidase protein. The transgene protein product was targeted to be secreted out of the cell to the apoplast. Electron microscopy confirmed the presence of the enzyme in the apoplast and in the cell walls. We have examined the cell-wall composition of these transgenic plants for alterations induced by production of this fungal peroxidase.

Materials and Methods

Vegetatively propagated plants from eight alfalfa transformants were grown in the field in a replicated trial. Based on a Southern analysis, four of the transformants had zero, one or two copies of the transgene but did not produce the peroxidase protein product. Three of the other transformants had one copy of the transgene and one transformant had an unknown number of transgene copies. These latter four transformants all produced high levels of the peroxidase enzyme product of the transgene. The plants were harvested at flowering, separated into leaf and stem fractions, lyophilized, and ground to pass a 1-mm screen in a cyclone mill. Samples were analyzed for cell-wall concentration and composition (neutral sugars, uronic acids, Klason lignin, and esterified and etherified *p*-coumaric and ferulic acids).

Results and Discussion

The expression of the fungal Mn-dependent peroxidase had significant effects on the cell-wall concentration and composition of transgenic alfalfa plants (Table 1). Interestingly, leaves and stems responded differently to the presence of the peroxidase transgene product. Cell-wall concentration increased in leaves of plants expressing high levels of the transgene whereas the opposite effect was seen in the stems. Uronic acid content of the cell walls was increased in stems, but decreased in leaves, by expression of the transgene. Because peroxidases are needed for the polymerization of lignin during its biosynthesis, it was expected that lignin concentration would be impacted by the transgene. For leaves, an increase in Klason lignin content of the cell wall was observed for the high expressor plants, but no alteration occurred in high expressor stem tissue. The only consistent responses for both leaves and stems to the presence of the transgenic peroxidase were increases in ferulic acid esters in the cell wall and the molar proportion of arabinose among the neutral sugars. The increase in arabinose proportion was at the expense of glucose in the leaves, but in stems xylose was the neutral sugar which declined.

Conclusion

The presence of extra peroxidase in the apoplast and cell wall due to introduction of a transgene might be expected to alter cell-wall composition and structure because peroxidase is involved in cell-wall lignification and cross-linking. Why leaves and stems of the transgene expressing alfalfa plants reacted in opposite directions to the same genetic modification is not clear. Obviously the tightly controlled development of plant cell walls is extremely sensitive to manipulation and reacts in unexpected ways. The apoplast of plants may not be a suitable deposition site for transgenic production of reactive industrial enzymes.

Table 1. Cell-wall concentration and composition of transgenic alfalfa plants which were negative or high expressors of a fungal Mn-dependent peroxidase transgene.

	Leaves		Stems	
Trait	Negative	High	Negative	High
	Concentration, g kg^{-1} OM			
Cell wall	254	313*	666	609*
	Composition, g kg^{-1} CW			
Neutral sugars	501	474	632	617
Uronic acids	305	254*	134	143*
Klason lignin	191	270*	234	239
Esters				
p-Coumarate	0.31	0.27*	0.09	0.09
Ferulate	0.40	0.48*	0.10	0.13*
Ethers				
p-Coumarate	0.53	0.44*	0.13	0.13
Ferulate	0.44	0.52	0.27	0.29
	Neutral Sugar Composition, mol 100 mol^{-1}			
Glucose	56.2	44.8*	56.8	56.9
Xylose	4.9	7.6	29.5	27.1*
Arabinose	19.3	26.1*	7.2	8.7*
Galactose	13.7	15.3	4.0	4.7
Mannose	6.3	6.3	2.4	2.7

*High expressor transformants are different ($P < 0.10$) from negative expressors, within a plant part.