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Pectin May Hinder the Unfolding of Xyloglucan Chains during Cell Deformation: Implications of the Mechanical Performance of *Arabidopsis* Hypocotyls with Pectin Alterations

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ABSTRACT **Plant cell walls, like a multitude of other biological materials, are natural fiber-reinforced composite materials. Their mechanical properties are highly dependent on the interplay of the stiff fibrous phase and the soft matrix phase and on the matrix deformation itself. Using specific *Arabidopsis thaliana* mutants, we studied the mechanical role of the matrix assembly in primary cell walls of hypocotyls with altered xyloglucan and pectin composition. Standard microtensile tests and cyclic loading protocols were performed on *mur1* hypocotyls with affected RGII borate diester cross-links and a hindered xyloglucan fucosylation as well as *qua2* exhibiting 50% less homogalacturonan in comparison to wild-type. As a control, wild-type plants (Col-0) and *mur2* exhibiting a specific xyloglucan fucosylation and no differences in the pectin network were utilized. In the standard tensile tests, the ultimate stress levels (~tensile strength) of the hypocotyls of the mutants with pectin alterations (*mur1*, *qua2*) were rather unaffected, whereas their tensile stiffness was noticeably reduced in comparison to Col-0. The cyclic loading tests indicated a stiffening of all hypocotyls after the first cycle and a plastic deformation during the first straining, the degree of which, however, was much higher for *mur1* and *qua2* hypocotyls. Based on the mechanical data and current cell wall models, it is assumed that folded xyloglucan chains between cellulose fibrils may tend to unfold during straining of the hypocotyls. This response is probably hindered by geometrical constraints due to pectin rigidity.**

Key words: ***Arabidopsis thaliana*; mutants; cellulose; xyloglucan; pectin; cyclic loading tests.**

INTRODUCTION

The primary cell wall of plants is a unique engineering structure that combines conflicting characteristics such as rigidity as well as plasticity and compliance (Rose and Bennett, 1999; Whitney et al., 1999; Cosgrove, 2000). Rigidity is needed to withstand the osmotic pressure of the living cell (Taiz, 1984) and to cope with external loads, whereas sufficient plasticity and compliance are needed for cell wall expansion during growth (Baskin, 2005). Furthermore, the primary wall is specifically designed to provide a rigid barrier against pathogenic intrusions (Creelman and Mullet, 1997) and, at the same time, performs dynamic tasks in absorption, transport, and secretion of substances throughout plant growth and development (Eckardt, 2003).

In dicotyledonous plants, the primary wall consists of approximately 30% cellulose, 30% hemicelluloses, 35% pectin,

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and 1–5% structural proteins on a dry weight basis (Vorwerk et al., 2004). The structures of the individual polymers are well known; however, their specific arrangement and bonding patterns in the entire cell wall are not fully understood yet.

The complex assembly of stiff cellulose fibrils and pliable matrix components can be characterized in the same way as fiber-reinforced composites (Kerstens et al., 2001; Fratzl et al., 2004). Based on deep-etching methods and NMR spectroscopy, several cell wall models have been proposed (Keegstra et al., 1973; Hayashi, 1989; Fry, 1989a; Talbott and Ray, 1992; Ha et al., 1997; Cosgrove, 2000). Generally, the cellulose microfibrils are thought to be tethered by xyloglucan mainly through hydrogen bonding (Hayashi, 1989). Xyloglucan attaches itself on the fibril surface as well as in between fibrils (Pauly et al., 1999). Thereby, it coats the fibrils serving as a spacer that prevents direct hydrogen bonding between cellulose chains (Carpita and Gibeaut, 1993). Pectic polysaccharides form a co-extensive network that interpenetrate this network (McCann et al., 1990; Talbott and Ray, 1992) and interact to a certain degree with hemicelluloses via both non-covalent and covalent bonding (Fry, 1989b; Thompson and Fry, 2000).

From the 1960s onwards, mechanical measurements have been performed on plant cell walls using extensometers to elucidate factors that influence the extensibility of the cell walls as well as the mechanical role of the cell wall components and their interaction in the entire cell wall (Cleland, 1967, 1984; Cleland and Rayle, 1977; Cosgrove 1988, 1989). Uniaxial tensile tests have been also performed on various *Arabidopsis* mutants (Köhler and Spatz, 2002; Ryden et al., 2003; Peña et al., 2004; Cavalier et al., 2008). Ryden et al. (2003) compared stiffness and strength of *Arabidopsis* hypocotyls of GDP–fucose biosynthesis mutant *mur1*, the xyloglucan fucosyltransferase mutant *mur2*, and the xyloglucan galactosyltransferase mutant *mur3*. The results were interpreted in a way that the mechanical performance of primary walls depends on both galactosylated xyloglucan side chains and borate-complexed rhamnogalacturonan II. However, a mechanistic model that proposes how xyloglucan and pectin influence the stiffness and strength of *Arabidopsis* hypocotyls has not been proposed yet.

The approach reported here aims to gather further insight into the principle deformation mechanisms of the primary cell wall and the mechanical interactions of the polymer networks, particularly the interactions of xyloglucan and pectin. To elucidate the mechanical role and the interplay of the structural networks in the primary cell wall, standard tensile tests and cyclic loading experiments were carried out on *mur*-mutants (*mur1* and *mur2*) and *qua2*. The latter mutant contains 50% less homogalacturonan in comparison to the wild-type (Mouille et al., 2007; Ralet et al., 2008). Based on the mechanical responses of the various hypocotyls, a simple structural model is proposed that extends existing models on the deformation of the cellulose fibril–xyloglucan network (Passioura, 1994; Passioura and Fry, 1992; Veytsman and Cosgrove, 1998) by a possible interplay of xyloglucan chains with the pectin network. This model shows some analogies to the so called 'hidden length mechanism', which, for instance, explains the high toughness of bone by an additional deformability of matrix polymers due to their specific structural alignment in the assembly and polymer interactions by means of ionic sacrificial bonds (Fantner et al., 2005; Gupta et al., 2007).

RESULTS

Figure 1A shows a representative stress–strain curve of a 4-day-old *Arabidopsis* wild-type (Col-0) hypocotyl illustrating its mechanical behavior and how the mechanical parameters used in this study were determined.

The stress–strain curve of the standard tensile test shows an initial phase, which is followed by an almost linear phase and a regime of non-linear deformation after yield. The curve ends at the point of rupture. Stiffness was calculated from the slope of the curve in segment 2 and the ultimate stress value can be taken as an approximate measure of the strength of the hypocotyl.

In Figure 1B, an exemplary stress–strain curve of a 4-day-old *Arabidopsis mur1* hypocotyl in a cyclic loading experiment is shown. Cyclic loading tests can further provide important information on the deformation behavior of a sample. Stiffness was calculated for the upward loading phases. Stiffness 1 equates to the stiffness in the standard loading experiment presented in Figure 1A. Stiffness 2, stiffness 3, and so forth reflect the material response when the sample is re-loaded after unloading in the cycles. Cyclic loading experiments also allow the distinction between the elastic and the plastic fraction of a material response (Cleland, 1984). In a pure elastic deformation, all energy is returned after unloading, which means that the unloading curve should hit the abscissa in the initial point of the experiment. The 'plastic strain', as indicated in Figure 1B, was calculated as a qualitative measure of irreversible deformation. The given example also shows that the initial slope (stiffness 1) of a hypocotyl does not necessarily reflect a pure elastic material response (Young's Modulus).

Standard tensile tests according to Figure 1A were carried out on the hypocotyls to determine their ultimate stress levels (~strength) and the stiffness (stiffness 1). Figure 2 shows the mechanical behavior of the 4-day-old *mur* hypocotyls (Fig. 2A) and the 6-day-old qua2 hypocotyls (Fig. 2B). Ultimate stress levels and the stiffness of Col-0 hypocotyls of both respective ages are shown for reference.

In the ultimate stress-versus-stiffness plots (Figure 2), only *mur2* shows a significant difference in the ultimate stress level from Col-0, whereas no significant differences between Col-0 and the mutants with pectin alterations, *mur1* and *qua2*, were observed. However, in terms of stiffness, all mutants were significantly different from Col-0. While *mur2* showed only a moderate reduction in stiffness (~13%), the stiffness of the two mutants with pectin alterations was noticeably decreased. The stiffness of *mur1* was reduced by ~40% and the stiffness of *qua2* by ~34% compared to the Col-0