

論文の要旨 (Thesis Summary)

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Characterization of tobacco plants with the hyper-galactosylated cell wall components

(高ガラクトシル化された細胞壁をもつタバコ植物の性状解析)

Introduction: Plant cell wall (CW) is a dynamic and complex structure composed of carbohydrates and proteins that influencing plant morphology, defense, and growth. Therefore, studying of CW composition is fundamental for understanding CW biosynthesis and remodeling effects on plant life cycle via biological function of CW such as signaling properties. UDP-Galactose (UDP-Gal) is one of the most important nucleotide-sugar precursors in CW galactosylation. Among cell wall materials, pectin (rhamnogalacturonan-I and -II) and hemicellulose (xyloglucan) as non-cellulosic part of cell wall (so called cell wall matrix components) are galactosylated in Golgi body. The aim of my study is to investigate the hyper-galactosylation of CW components and its influence on plants phenotypic and physiological characteristics which might result from signaling activity of CW components.

Influence of *human UDP-Gal transporter1 (hUGT1)* expression on plant CW composition: UDP-Gal transporter is responsible for transporting UDP-Gal from cytosol to Golgi lumen to be used for assembling CW matrix components. It has been reported that in tobacco plant transformed with *hUGT1*, hUGT1 mainly localized in the trans-Golgi and displayed UDP-Gal transporter activity. I examined a hypothesis that extra transportation of UDP-Gal from cytosol to Golgi by *hUGT1* expression might lead to a increased galactosylation CW matrix polysaccharides. The monosaccharide composition of CW matrix components of leaf and stem in *hUGT1*-transgenic and control plants was analyzed. HPLC analysis revealed the alteration of monosaccharide composition in pectin and hemicellulose. In particular, Gal ratio in total sugar composition of CW dramatically elevated in the hemicellulose II and pectin fraction in the *hUGT1*-transgenic plants. Furthermore, oligosaccharide mass profiling (OLIMP) revealed that xyloglucan (XG) is the main acceptor of Gal in hemicellulose II. Since the degree of galactosylation in XG would be related to the mechanical strength of primary CW, the hyper-galactosylation of XG might elevate the rigidity of tobacco plants. Moreover, an increased Gal tolerance was shown in the *hUGT1*-transgenic plants. The enhanced stream of Gal from cytosol to Golgi body by hUGT1

and consequent incorporation of this extra Gal into CW matrix components seemed to lead to the increased Gal tolerance.

Influence of *hUGT1* expression on plant hardness: Polysaccharides in plant CW are not only Physical support substances but also signaling molecules. Thus, I proposed hyper-galactosylation of CW matrix components via expression of *hUGT1* might have an important impact on plant growth and development such as plant hardness. Strength test for stem and leaf of *hUGT1*-transgenic and control plants determined by breaking and bending tests revealed an increased rigidity in leaf and stem of *hUGT1*-transgenic plants. This result suggested that the hyper-galactosylated side chains of XG were involved in the increased strength of CW. Subsequent transmission electron microscopic analysis showed the greater CW thickness in leaves palisade cells and those of cortex cells and xylem fibers in the stem of *hUGT1*-transgenic plants. Besides, to explore the further possibility, the biomass and total CW content were measured. The biomass and total CW materials extracted from the leaves and stems of *hUGT1*-transgenic plants were higher than those of control plants. These results supported the increased CW thickness. In addition, the CW of the *hUGT1*-transgenic plants showed increased lignin contents, which was supported by the up-regulation of some genes encoding enzymes which are rate-limiting in lignin biosynthesis. From these results, although a possibility of hyper-galactosylation of XG is ruled out, the increased rigidity of *hUGT1*-transgenic plants are mainly caused by the increased CW thickness and the enhanced lignin accumulation.

Conclusion: Altogether, changes in the Gal composition of CW and its thickness because of *hUGT1* expression have a remarkable impact on plant growth and development via XG and pectin structure and their probable signaling activity. These results may suggest a new novel plant modification strategy that is useful for plant improvement in the future.