SELECTED TALKS

Lignin deposition and synthesis in the internodes during barley (*Hordeum vulgare* L.) development

OP2-1

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In monocotyledons, especially grasses (*Poaceae*), process of lignification is still unexplored in many of its aspects. Lignin deposition in cell wall is temporally and spatially specific and differs between species and tissues. The aim of this study was to investigate the dynamics of lignin synthesis and deposition, by using anatomical, physiological and molecular approach, in the stem of three spring barley cultivars (*Hordeum vulgare* L.) during four developmental stages: heading, anthesis, grain filling and ripening. Lignin localization and histochemical changes related to lignin deposition, total lignin content and gene expression of cinnamoyl CoA reductase (HvCCR) and cinnamyl alcohol dehydrogenase (HvCAD) were studied. Analyses showed that most of the lignin was deposited in the sclerenchyma ring in all three internodes. Total lignin content increased with stem development, especially in the first internodes and was highest in ripening, but it was also relative to the cultivar and developmental stage. Genes of the lignin biosynthetic pathways HvCCR showed highest expression in anthesis in the third internode of all three cultivars whereas gene HvCAD, in cultivars Astor and Scarlett, showed highest expression in anthesis and in cultivar Jaran in heading stage. Results obtained in this study will contribute to the comprehensive description of the biochemical and molecular changes that accompany lignin deposition in the cell wall during barley development.

Keywords: lignin, barley, CCR, CAD

DUF1070 is a conserved signature domain of some arabinogalactan peptides

OP2-2

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Most proteins are composed of functional domains, of which >20% are "domains of unknown function" (DUFs). We have recently identified an arabinogalactan (AG) peptide (AGN92423) with DUF1070 domain in *Centaurium erythraea* transcriptome. AGPs are ubiquitous plant cell wall hydroxyproline-rich glycoproteins with diverse functions. Since classical AGPs and AG peptides contain no signature motifs, the presence of highly conserved DUF1070 caught our attention. DUF1070 is present in 479 NCBI plant protein sequences, of which

271 are non-redundant: 126 AG peptides and 145 unknown/hypothetical proteins. All DUF1070-containing sequences are short (71 aa on average). With few exceptions, these sequences are comprised of N-terminal signal sequence, followed by an AG-II glycomodule (typically PAPAPT). DUF1070 is up to 37 aa long, encompassing (a part of) AG-II glycomodule and a highly conserved C-terminal stretch of 26 aa, typically "SDGTSIDQGIAYVLMLVALVLTYLIH". This motif is a textbook example of glycosylphosphatidylinositol (GPI) anchor signal peptide, containing 1) three relatively small aliphatic amino acids at the ω (GPI attachment site), ω+1 and ω+2 positions (SDG in 74% sequences); 2) a relatively polar spacer (often TSIDQG), followed by 3) a hydrophobic domain (typically IAYVLMLVALVLTYLIH). When GPI is attached to ω (S), the C-terminal peptide is cleaved, while the hydrophobic domain remains embedded in the ER membrane. Since most of the DUF1070 is predicted to be cleaved and discarded, it is worth wondering what selective pressure kept it so conserved (unlike other GPIsp sequences) among AG peptides from unrelated plant species. We propose that DUF1070 is a signature of some AG peptides.

Keywords: arabinogalactan proteins, AG peptides, DUF1070, glycosylphosphatidylinositol anchor, GPI signal peptide

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Phosphoproteomics profiling of tobacco mature pollen and pollen activated *in vitro*

OP2-3

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Angiosperm mature pollen represents an extremely desiccated, metabolically quiescent structure surrounded by a tough cell wall. Upon re-hydration it becomes metabolically active and later on pollen tube growth starts. These changes in cytoplasm hydration and metabolic activity are accompanied by protein phosphorylation, which is one of the most dynamic post-translational modifications. In order to identify the phosphoproteins playing role during tobacco pollen activation *in vitro*, the following experiments were performed. Mature pollen, 5-min-activated pollen, and 30-min-activated pollen were subjected to TCA/acetone protein extraction, trypsin digestion and phosphopeptide enrichment by titanium dioxide. The enriched fraction was then subjected to nLC-MS/MS. We identified 471 phosphopeptides that carried 432 phosphorylation sites, position of which was exactly matched by mass spectrometry. These 471 phosphopeptides were assigned to 301 phosphoproteins, since some proteins carried more than one phosphorylation site. Of the 13 functional groups, the majority of proteins were put into these categories: transcription, protein synthesis, protein destination and storage, and signal transduction. We also presented quantitative data; the identified phosphopeptides were divided into seven groups based on the regulatory trends; the major group comprised mature