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Screening for Arabidopsis seed coat mutants using an EMS population

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Relatively little is known about synthesis and regulation of pectin biosynthesis. One way to gain insight into pectin synthesis and modification is to use model systems. One such system is the Arabidopsis seed mucilage, which is produced in the Arabidopsis seed coat epidermal cells and consists of rhamnogalacturonan (RGI), which is easily extracted, and contains only small amounts of cellulose and xyloglucan [1], [2], [3].

Mutants with seed coat mucilage defects were identified by screening 1700 M2 EMS plants for alterations in sugar composition. Mutants with altered mucilage composition are expected to have changes in pectin biosynthesis and/or modification. More than 10 mutants with heritable and profound changes were identified, and according to the observed chemical alteration divided in five phenotypic groups. Candidates within each phenotypic group were tested for complementation. Furthermore, all candidate lines were analysed for changes in seed surface morphology and mucilage release. Three lines from different phenotypic groups, along with the Col-2 wild type as a reference genome, were sequenced using deep sequencing. Using SNPs detection tools, we confirmed the sensitivity of our approach by identifying a known mucilage biosynthetic gene RHM2/MUM4. In the other two sequenced lines we detected candidate genes for mucilage synthesis/modification and currently we are working on the confirmation of the phenotype by looking in the potential knock-out lines. The rest of the candidate lines are cleaned up by crosses to the Col-2 WT and are prepared for Hiseq Illumina sequencing.

[1] B. Usadel *et al*. (2004) *Plant Physiol*., **134(1)**, 286-95; [2] T.L. Western *et al*. (2004) *Plant Physiol*., **134(1)**, 296-306; [3] A.A. Arsovski *et al.* (2010) *Plant Signal Behav*., **5(7)**, 796-801.

P5-18

Identification of proteins involved in cell wall assembly and remodeling by a proteomic approach on *Brachypodium distachyon* grain.

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A major part of the daily caloric intake of human societies around the world is derived from cereal grain. The major components in domesticated cereal grains are starch and proteins. Cell wall polysaccharides only account for about 3-8% of grain but have major effects on the use of grain. Cell walls are mainly composed of polysaccharides (pectins, cellulose and hemicelluloses) and smaller proportion of glycoproteins.

Except for cellulose, precursor oligosaccharides are synthesized in the Golgi apparatus, then transported to the cell wall where they are presumably assembled to larger polysaccharides. In addition, the polysaccharide remodeling occurs to meet the plant needs during its different stages of development. The actors required for these processes are poorly understood, especially in cereal plants. Glycosyl hydrolases (GH) may play a crucial role in the reorganization of cell wall polysaccharides.

To identify the enzymes involved in the assembly and remodeling mechanisms of cereal cell wall, we performed a proteomic analysis of grain cell wall from the cereal plant model *Brachypodium distachyon*. Grains were collected at three stages of development and subjected to cell wall fractionation. Then cell wall proteins (CWPs) were extracted by successive steps using $CaCl_2$ and LiCl buffers, according to a protocol derived from [1]. Identification of proteins was performed using mass spectrometry (LC-MS/MS). The results revealed CWPs potentially involved in remodeling or re-arrangement of cell wall polysaccharides. These proteins will be compared with other sets of data obtained from other *B. distachyon* organs.

[1] Feiz *et al.* (2006) *Plant Methods* **27**, 2-10.