102(F125)

A MEMBRANE-BOUND ENDO-1, 4-β-GLUCANASE IS REQUIRED FOR CELLULOSE BIOSYNTHESIS IN *ARABIDOPSIS*

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Previously, we reported that the temperature-sensitive cell elongation mutant *"acw1"* is a mutant of a membrane-bound endo-1,4-β-glucanase gene and the 31°C-grown mutant has a reduced amount of cellulose. In this study, the effects of the *acw1* mutation on the cell elongation and the cellulose biosynthesis were analyzed in detail.

We analyzed the effect of the mutation on cell wall architecture by FE-SEM. FE-SEM demonstrated well the architecture of the cellulose bundles on the cell wall surface. The bundles of the wild type and the 21°C-grown mutant plants represent to be straight, but the bundles of 31°C-grown mutant plant are wavy.

To see the initial effect of the mutation in the cell wall synthesis, we examined the cell wall regeneration process from the mutant protoplasts. As a result, the cell wall regeneration is delayed in the 31°C-cultured mutant protoplast. These results suggest that an *Arabidopsis* membrane-bound endo-1,4-β-glucanase is required for cellulose biosynthesis.

103(F126)

ELUCIDATION OF XYLEM FORMATION MECHANISM WITH *EUCALYPTUS* EST DATABASE

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The *Eucalyptus* is composed of about 500 species or more, and contains a lot of inherited varieties. This is reflected and use over the log processing, the fuel materials, and the pulp raw materials and many topics is admitted in industry. We are improving the *Eucalyptus* by the genetic engineering technique, and have already established transformation systems of several *Eucalyptus* species. Additionally, constructions of the EST database at several Eucalyptus tissues have been started to acquire effective genes. Using the *Eucalyptus* as both a useful biomass and a model system in the woody plants, we will elucidate mechanism of dilative growth of stem and the biosynthetic pathways of the secondary metabolic products in near future. In this meeting, we will show mainly the homology research results (BLAST) of *Eucalyptus* xylem origin EST data obtained.

104(F127)

LOCALIZATION OF THE YIELDIN, A WALL-BOUND PROTEIN REGULATING THE YIELD THRESHOLD TENSION OF THE CELL WALL.

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To study the tissue- and subcellular-localization of yieldin, a novel wall-bound protein regulating the yield threshold tension[1,2], in the stem of cowpea seedling, anti-yieldin antibody was purified from rabbit antiserum raised against recombinant yieldin. The immunocytochemical study using the antibody showed that yieldin localized in the cell wall of cortex cell in the region from 1mm to 15 mm below the cotyledonary node containing the rapidly elongating region.

[1]Okamoto-Nakazato, A., Nakamura, T. & Okamoto, H. (2000) *Plant Cell & Environ.***23** : 145-154.

[2]Okamoto-Nakazato, A., Takahashi, K., Kido, N., Owaribe, K. & Katou, K. (2000) *ibid.* **23** : 155-164.

105(F128)

EXPRESSION OF ENDO-1,3-β-GLUCANASE GENES IN RICE PANICLES AND ROOTS: cDNA CLONING AND EXPRESSION IN Escherichia coli

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To find new members of the rice endo-1,3-β-glucanase gene family, a cDNA library made from rice seedlings (Oryza sativa L. cv Yukihikari) was screened with barley GII endo-1,3-β-glucanase cDNA as probe. Out of 32 clones isolated, two clones, OsGLN1 and OsGLN3 appeared to contain the full-length cDNA based on the sequence analysis. Comparison of the deduced amino acid sequence of OsGLN 1 and OsGLN3 with GenBank database revealed that OsGLN1 and OsGLN3 are similar to gsn4 (GenBank accession no. U72250) and glu3 (GenBank accession no. AF030167), respectively. Northern blot analysis of RNA indicated that OsGLN1 is preferentially expressed in panicles before heading whereas OsGLN3 is strongly expressed in mature roots. To analyze enzymatic properties of OsGLN1 and OsGLN3 these proteins were expressed in E. coli as GST (glutathione S-transferase) fusion proteins. The expressed GST-OsGLN1 and GST-OsGLN3 fusion proteins rapidly hydrolyzed 1,3;1,6-β-glucan laminarin and produced oligosaccharides. However, no enzyme activity was detected towards barley 1,3;1,4-β-glucan. This suggests that proteins encoded by both OsGLN1 and OsGLN3 are endo-1,3--β-glucanases. Other biochemical characteristics on GST-OsGLN1 and GST-OsGLN 3 fusion protein are also discussed.