

sorbitol), respectively as carbon sources for 24 hrs. The regenerated cell-wall polysaccharides were fractionated after remove of starch and the crude cell-wall materials were dissolved in 1 ml DMSO. The DMSO-soluble and DMSO-insoluble (cellulosic) fractions were methylated by methylsulfinyl anion and acetylated by acetic anhydride. The monomeric sugars (4-, 3,4-, 2,4-, 4,6-, 2,3,4-, 3,4,6-, 2,4,6- and 2,3,4,6-Glc) were analysed by a gas-chromatograph and a gas chromatograph-mass spectrometry. The results, in the cellulosic fractions, showed that the 2,4-Glc contents(%) of DCB-habituated protoplasts, grown in the four media were higher, while the 2,3,4,6-Glc contents were lower than the normal BY2 protoplasts, suggesting that DCB cell walls will have the loosen networks. On the other hand, sugar contents (μg) of each cell-wall fraction of the suspension-cultured cells were compared.

E205 Characterization of transgenic tobacco over-expressing ornithine decarboxylase gene from *Chlamydomonas reinhardtii*

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Ornithine decarboxylase (ODC) is a key enzyme in putrescine and polyamine biosynthesis. Our previous studies showed that polyamine level and tolerance to oxidative stress increased in transgenic cells of unicellular green algae, *Chlamydomonas reinhardtii*, over-expressing an ODC gene. To investigate the role of polyamine in oxidative stress and function of this gene in higher plant, we made transgenic tobacco plant using *Agrobacterium*-mediated transformation. We obtained 46 lines of transformants and selected several lines which showed higher ODC activities than that of wild type tobacco. Southern blot analysis showed that 11 transgenic lines were introduced with single copy gene. ODC activities of these 11 transgenic lines

were higher than those of other lines inserted with several copies. One of 11 transgenic lines (CrODC-40) was chosen and we analysed T1 generation of CrODC-40. In CrODC-40, ODC activity increased in comparison with that of wild type. Similar to ODC, ADC (arginine decarboxylase) activity increased, too. As ODC and ADC activities increased, spermine content in CrODC-40 was approximately 4-fold higher than that in wild type. The increase of endogenous spermine in transgenic tobacco is thought to be involved in tolerance to environmental stresses. So, we will research the tolerance of CrODC-40 to various stresses and the expression of genes related to tolerance to oxidative stress in this transformant.

E206 Effects of Micronutrient Boron on the Development of Roots in Sunflower seeding

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Three-day-old Sunflower(*Helianthus annuus*)seeding were grown in complete nutrient solution providing either deficient or sufficient boron supply and supplemented with aluminum or sulphonylurea herbicide chlorosulfuron. Increasing concentrations of aluminum and chlorosulfuron in the nutrient medium caused progressive inhibition of root growth. Although cessation of growth is the most apparent symptom of boron deficiency, elevated boron levels improved root growth under toxic aluminum and chlorosulfuron. conditions. When sunflower seedings were cultured hydroponically under acidic conditions, elongation growth of the primary root was inhibited depending on the lowering of pH in the range of 5.5 to 3.5. When the viability of sunflower roots exposed to low pH(3.0 or 3.5) solution that contained boron was examined, boron showed a strong ameliorative effect with Ca^{2+} . Ascorbate added to the medium

improved root growth in plants supplied with insufficient boron. These findings suggest that root growth inhibition resulting from boron deficiency, aluminum and chlorosulfuron toxicity may be a consequence of disrupted ascorbate metabolism.

E207 Salt Tolerance In Spinach Beet

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Growth and photosynthetic activity of spinach beet (*Beta vulgaris* var. *cicla*) and the patterns of glycinebetaine (GB) were studied under different salt conditions. Plants of forty-three days old were assessed by growing for 10 and 20 days at four NaCl concentrations (0, 100, 200, 300 & 400 mM). Shoot biomass was greatest for plants grown at control to 100 mM, and after 10d it was decreased slightly at salinities of 200 mM or higher. In spite of very low tissue K⁺:Na⁺ ratios, however, there were not significant differences in photosynthetic activity in response to NaCl. Salt stress led to a preferential accumulation of GB in shoot of spinach beet, especially at 200 mM NaCl treatment with 10 mM Ca²⁺. These findings suggest that high degree of NaCl tolerance of spinach beet is resulted from the accumulation of GB - known to act as osmoprotectant - in parts, and show that studies on solute compartmentation at the cellular level are required to elucidated the mechanisms by which this plant tolerates very low tissue K⁺:Na⁺ ratios.

E208 Does Transgenic Expression of a Grape UDP-glucose Flavonoid Glucosyl Transferase Gene Affect the Colour Development of Flowers in Tobacco ?

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Anthocyanin is flavonoid, a phenolic compound, responsible for most of the colours in various plant organs and the enzymes and genes involved in the biosynthetic pathway have been isolated and characterized in maize, petunia, snapdragon, apple and grape. However, the regulation of the anthocyanin biosynthesis has not been fully understood. Grape berry was chosen as a system to elucidate the mechanism of regulation of gene expression, in which the pigment accumulates only in berry skin. Of seven genes, only the UDP-glucose flavonoid glucosyl transferase(ufgt) -encoding gene was reported to be differentially regulated in grape berries: the ufgt gene was expressed only in berry skin, while the others in both skin and flesh. We isolated a ufgt cDNA clone from grape berries and analyzed its expression profiling in red and yellow grape varieties. The open reading frame of the ufgt cDNA was ligated in pBI121 vector in a sense or an antisense orientation under the control of the CaMV 35S promoter and the recombinant constructs were incorporated into tobacco. Several transgenic lines are being selected and characterized to test whether over-expression or repression of the ufgt gene affects the anthocyanin content. We also isolated a genomic clone and analyzed the nucleotide sequence of the promoter region in order to determine the regulatory elements involved in the environmental response and skin-specific expression of the ufgt gene.

E209 Molecular cloning and complementation of nif V gene from Frankia EulK1 strain

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