E202 Aluminum Stress in the Roots of Naked Barley

Mi-Sook Jeong and Sung-Ha Kim
Hamyeol High School and Korea National Univ. of Education

Phototoxicity of aluminum(Al) is the major limiting factor for the crops grown in the acid soils by rapid inhibition of root elongation. In this study, changes in root growth, total activity and isozyme patterns of antioxidant enzymes such as peroxidase, ascorbate peroxidase, catalase and *glutathione reductase* by Al stress were investigated in the roots of naked barley. As Al concentration increased up to 500uM, the rooting rate and root elongation were substantially decreased. But a slight increase at 50uM of Al was observed. Growth results suggested that this cultivar seemed to be the Al-sensitive species. Total activities of antioxidant enzymes extracted from the roots were determined. They generally increased at the lower Al concentration and then gradually decreased at the higher Al concentration. They also increased when the exposure time to Al was extended up to 48 hours. Changes in the isozyme patterns of antioxidant enzymes were investigated by in situ enzyme activity staining on the non-denaturing PAGE. They generally coincided with the changes in the total activities in parallel. Changes in the total activities of antioxidant enzymes also coincided with the changes of the root growth. It was also suggested that increased activities of antioxidant enzymes in the roots of naked barley would not be enough to block the severe Al stress. Since a growth reduction in the roots by Al stress could be related with the changes in the activities of antioxidant enzymes, these results suggested that Al may cause the oxidative stress in the roots of this cultivar of naked barley.

E203 지치(Lithospermum erythrorhizon)의 모상근 배양을 통한 shikonin 생산

황옥진*, 김옥태, 김광수, [1]안준철, 황백
전남대학교 자연과학대학 생물학과;[1]서남대학교 자연과학대학 생명과학부

지치(*Lithospermum erythrorhizon*)의 모상근 배양을 통한 shikonin 생산과 최적 배양조건을 조사하였다. 모상근의 생장은 B5 배지에서, shikonin의 생산은 SH배지에서 가장 좋았다. 탄소원으로서 sucrose를 9%로 첨가하였을 때 최대의 성장을 보였으며, 배지에 첨가되는 sucrose의 농도가 높아질수록 생장률은 증가하였으나, shikonin 생산량은 감소하였다. pH의 변화에 대한 모상근의 영향은 약산성(pH 5.8)~약알칼리(pH 8.8)일 때는 생장률의 변화가 없었으나, 그 이상 또는 이하에서는 생장율이 급격히 감소하였다. 배지에 첨가되는 질소원의 농도는 생장과 shikonin 생산성에 영향을 주었고, 최대의 성장은 B5기본배지에 50mM의 NO_3와 1mM의 NH_4가 첨가되었을 때, shikonin 생산은 5mM의 NO_3와 10mM의 NH_4가 첨가되었을 때 가장 좋았다. 생장조절물질로서 IAA, 2,4-D, NAA와 Kinetin을 처리한 결과, 0.1mg/L IAA가 첨가되었을 때 모상근의 성장률은 약 1.8배 증가해 가장 좋은 결과를 보였으나, shikonin의 생산성이 크게 감소되었다.

E204 Cellulose Biosynthesis from Protoplasts of DCB-habituated Cells of Tobacco (Nicotiana tabacum L. cv. BY2)

Up-Dong Yeo*[1] and Naoki Sakurai[2]
[1]Faculty of Biological Science, Chonbuk National University; [2] Department of Environmental Studies, Hiroshima University

To understand the *in vitro* regulation of cellulose biosynthesis of DCB-habituated cells and normal cells of tobacco, the protoplasts from the suspension-cultured cells were isolated in 0.6 M sorbitol plus CPW salts and cultured in four LS media containing sorbitol (0.4 M), sucrose (3% + 0.315 M sorbitol), glucose (1.5% 0.315 M sorbitol), UDPG (0.085 M + 0.315 M

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 acethylated 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 frations, 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 concents 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

Kook-hui Ryu* and SunHi Lee
Department of biology, Yonsei University

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 unicelluar 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 *Agrobactrium*-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

Eun-jung Go*, Sun-young Jin and Jung-hee Hong
Dept. of Biology college of Natural Sciences, Pusan National University Pusan 609-735

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, elougation 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 Ca2+. Ascorbate added to the medium