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Brassinosteroid-induced Gravitropism in *Arabidopsis* Roots
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Exogenously applied brassinosteroids activated gravitropic curvature of *Arabidopsis* roots. The mechanism of the brassinosteroids-induced gravitropic response in *Arabidopsis* roots was thus examined by the use of wild-type and several mutants related to brassinosteroids signal transduction pathway. The *brl1-201* and *bak-1* mutant showed less gravitropic activity than that of wild-type. *BRI-GFP* exhibited much gravitropic response compared with that of wild-type. Application of brassinazole, a brassinosteroids biosynthetic inhibitor, did not decrease the gravitropic curvature in wild-type roots. Therefore it was thought that the brassinosteroids-induced gravitropic curvature was mediated not by increase of endogenous level of brassinosteroids but by enhancement of brassinosteroids signal perception in *Arabidopsis* roots. In addition, involvement of IAA in the brassinosteroids-induced gravitropic response of *Arabidopsis* roots was examined. In the presentation, the change of endogenous IAA level mediated by brassinosteroid signal transduction will be also discussed.

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Importance of C-26 Demethylation for Homeostatic Regulation of Brassinosteroids in Plants
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Conversions of [2H0] and [2H6]castasterone (CS) using an enzyme solution were carried out in maize, which brassinolide (BL) has not identified yet. GC-MS analysis of the metabolites revealed that [2H0] and [2H6]CS is converted into [2H0]26-norCS and [2H3]28-norCS, respectively. Because we previously determined that [2H6]BL to [2H3]28-norBL is an artificial reaction by isotope effect, the C-26 demethylation of CS was thought to be a natural reaction. Next, enzymatic conversions of [2H0] and [2H6]CS in *Phaseolus vulgaris* and *Marchantia polymorpha* were also examined. As enzyme product, not only [2H0] and [2H6]BL but also [2H0]26-norCS and [2H3]28-norCS were identified, providing the C-26 demethylation of CS simultaneously occurred with the conversion of CS to BL in the plants. Possibility for the C-26 methylation in CS biosynthetic precursors such as 6-deoxoteasterone, 6-deoxo-3-dehydroteasterone, and 6-deoxyphasterol was subsequently investigated. Based on interpretation of mass spectra, the metabolites were tentatively identified as 6-deoxo-26-nortasterone, 3-dehydro-6-deoxo-26-nortasterone, and 6-deoxo-26-nortyphasterol, which suggested that the C-26 demethylation is a common and an important reaction to maintain a steady-state level of brassinosteroids in plants.

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Nitric Oxide and Tumor Necrosis Factor- α Production by *Arctium lappa* in Mouse Peritoneal Macrophages

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Arctium lappa (AL) is a plant widely used in medicine for the treatment of a variety of pathologies. We tested immunomodulatory activities of AL in mouse peritoneal macrophages. Addition of AL caused an induction in nitric oxide (NO) production by macrophages stimulated with interferon- γ (IFN- γ). In the presence of AL, macrophages stimulated with IFN- γ produced higher levels of tumor necrosis factor- α (TNF- α). The increased production of NO from IFN- γ plus AL-stimulated peritoneal macrophages was decreased by the treatment with *N*G-monomethyl-L-arginine or *N*(α)-Tosyl-Phe Chloromethyl Ketone. Nuclear factor- κ B (NF- κ B) inhibitor, pyrrolidine dithiocarbamate was able to completely inhibit the production of NO and TNF- α . As NO and TNF- α play an important role in immune function, AL treatment could modulate several aspects of host defense mechanisms due to stimulation of the inducible nitric oxide synthase via NF- κ B activation.

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Generation of 5,469 Expressed Sequence Tags from the Root Nodule of *Glycine max*

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For the high throughput screening of useful genes, three root nodule specific cDNA libraries from soybean were constructed from three different stages of root nodules after inoculation with *Bradyrhizobium japonicum* USDA110. The nodule-specific EST database was constructed from these cDNA libraries. To eliminate redundancy, we performed the differential hybridization using high redundant clones as probes. As a result, 5,469 cDNA clones were sequenced, these EST sequences were clustered into 2,511 non-redundant sequences comprised of 769 non-redundant clusters and 1,742 singletons. Similarity searches were done against NCBI non-redundant protein databases, soybean EST database and MIPS database. From these results we could make the functional catalog of our ESTs, including 1) several nodule specific genes named nodulin, 2) various stress-responsive genes, 3) genes related to carbon and nitrogen metabolism, 4) putative kinase and phosphatase. Also to analyze expression patterns of some clones, we performed northern hybridization from various tissues and from root nodules of different developmental stages. cDNA clones and EST database obtained from this study will be used for cDNA microarray to study the genetic program for root nodule and general development of plant.