*Frankia*, a gram-positive actinomycete, can establish a nodule symbiosis with eight families of angiosperms, collectively called actinorhizal plants. Among the genes involved in the nitrogen fixation, *nif* V encodes the homocitrate synthase and its catalytic product, homocitrate, is associated with the FeMo-cofactor, the component of the nitrogenase complex. Although many *nif* genes from *Frankia* EuIK1 strain, a symbiont of *Elaeagnus umbellata*, were cloned and characterized, *nif* V was not. So, in this study,*nif* V from *Frankia* EuIK1 strain was cloned and functional complementation was designed. The *nif* V ORF,consisting of 1245bp,started at ATG and terminated at TGA. Unusually, it wasn't organized in clusters with other *nif* genes and a putative promoter, the Shine-Dalgarno sequences were not found, either. The deduced amino acid sequence revealed high similarities with NifV proteins from other organisms available in database, with values ranging from 60.43% (*Azospirillum brasilense* ) to 80.42% (*Frankia* sp. FaC1) and also had two conserved regions of NifV proteins. The phylogenetic tree based on amino acid sequence similarities supported the possibilities that three *Frankia* species would be closely related and be grouped with other -class Proteobacteria. To verify its function, the complementation test using *Klebsiella pneumoniae* mutants is in progress.

## E210 Expression patterns of two -3 fatty acid desaturases from hot pepper and overexpressed plants analysis under cold and heat stresses.

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-3 fatty acid desaturases (FAD) are enzymes that increase the content of trienoic fatty acid by introducing double bonds into fatty acyl chains. Trienoic fatty acids have been known to be important not only for low temperature acclimation, but also for precursors of plant pathogen defense-related signaling molecules. These imply that regulation of trienoic fatty acid level is involved in the defense response of higher plant cells to environmental stresses. In this study, To know the expression patterns of chloroplast -3 fatty acid desaturase (chFAD) and microsomal -3 fatty acid desaturase (mFAD), isolated from *Capsicum annuum*, we treated environmental stresses such as cold and heat. Benzyl alcohol (membrane fluidizer) and dimethyl sulfoxide (membrane rigidifier) were also used to examine the relationship between membrane fluidity and induction of the desaturase synthesis. ChFAD transcription level was decreased under heat and benzyl alcohol treatments but increased under light. mFAD transcription level was not changed under heat and light. ChFAD and mFAD was not affected by cold (4?) treatment during short times. Moreover, To know the functions of two genes, we overexpressed two fatty acid desaturase genes in *Arabidopsis thaliana* and will investigate the responses to cold or heat of transgenic plants.

## E211 Salt 스트레스가 근대의 내생 Gibberellins, Abscisic acid 및 Jasmonate 함량에 미치는 영향

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Salt 스트레스에 대한 식물체의 적응 메카니즘을 구명하기 위한 일환으로 내염성 식물로 알려져 있는 근대(*Beta vulgaris* subsp. cicla L.)를 이용하여 식물체내의 내생호르몬 생합성{Gibbrellins(GA), Abscisic acid(ABA), Jasmonic acid(JA)}에 미치는 salt의 영향을 조사하였다. Salt처리를 한 근대의 지상부 생체시료에 내부표준물질로 ()-3,5,5,7,7,7-$d_6$ ABA와 [9,10-$^2H_2$]JA 및 [$^2H_2$ $GA_1$, $^2H_2$ $GA_{12}$, $^2H_2$ $GA_{19}$, $^2H_2$ $GA_{20}$, $^2H_2$ $GA_{53}$]를 일정량 첨가하여 각각의 호르몬을 분리한 후

GC-MS-SIM을 이용하여 정량하였다. ABA 함량은 salt 처리후 시간이 경과할수록 증가하는 경향을 보였으며, salt 처리 농도에 비례하여 증가속도가 빠른 것으로 나타났다 (400mM 처리의 경우 처리 후 1시간내에 2-3배 증가). JA 함량의 경우도 ABA와 비슷한 경향을 보여 처리 후 1시간 이내에 급격히 증가하는 경향을 보였다. ABA와 동일 전구체에서 생합성되는 GA 함량은 salt 처리 농도가 증가할수록 감소하는 것으로 조사되었다. 특히 3-hydroxylase의 활성정도를 추정할 수 있는 $GA_1/GA_{20}$의 비율은 salt농도가 증가함에 비례하여 감소하는 것으로 나타나 salt 스트레스하의 식물생장의 둔화는 식물체내의 일차 또는 이차물질의 교란뿐만 아니라 식물호르몬의 감소와도 밀접히 연관되어 있음을 보여주었다.

## E212 Differential Expression of AtTPS Suggests a Regulatory Role of Trehalose

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Trehalose is a disaccharide of two glucose units. It is synthesized by sequential action of TPS (trehalose-6-phosphate synthase) and TPP (trehalose-6-phosphate phosphatase) and is degraded by trehalase. However, it is rarely found in higher plants and its role is unknown. We have shown that transgenic tobacco plants producing trehalose exhibited improved tolerance against dehydration and high temperature. But minute amounts of trehalose detected in these plants suggests that it is not likely to act as osmoprotectants. Recently, functional homologs of *TPS* and *TPP* were found in *Arabidopsis*. To look into the role of trehalose in *Arabidopsis* we generated transgenic *Arabidopsis* plants overexpressing *E. coli* TPS gene (*ots A*) or carrying -glucuronidase (*GUS*) as reporter gene to examine *AtTPS* expression by vacuum infiltration and in the process of making antisensor plants. Overexpressor plants manifested severe dwarfism and extended generation time as in transgenic tobacco in varying degree, but morphological alterations in leaf shape or branching patterns were not observed. Contrastingly, GUS plants looked normal. Histochemical analysis of GUS plants revealed that *AtTPS* is not constitutively expressed implying that trehalose is not a mere metabolic molecule. *AtTPS* is mainly expressed in stems and leaves on vascular bundle area, but is not expressed in the roots and flowers at all. It was also strongly expressed on stalk of silique. Furthermore, the expression of *AtTPS* was increasingly induced by drought or heat stress, but not by chilling stress.

## E213 Phosphorylation of BRs in Suspension Cultured Cells of Phaeolus vulgaris

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We first investigated phosphorylation of castasterone(CS) and brassinolide (BL) in suspension cultured cells of *P. vulgaris* using cell-free system by addition of ATP and $Mg^{2+}$ as a substrate and cofactor. Enzyme products of CS and BL were analyzed by GC-MS. Bismethaneboronate (BMB)-trimethylsilly(TMSi) of two polar CS metabolites gave a molecular ion at *m/z* 664 identical to BMB-TMSi of CS phosphate ester. Analysis of mass spectra revealed that phosphate is incorporated into a hydroxyl at C-22 or C-23 of CS. BMB-TMSi of a BL metabolite showed a molecular ion at *m/z* 680 which is identical to a BL phosphate ester. Mass fragmentation pattern indicated that phosphorylation also occurred at either C-22 or C-23 of BL. Studies of biological activity and determination of the position of BRs phosphates are under investigation.