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담배 (*Nicotiana tabaccum*) 잎 절편 배양 시 묘조 형성 결정 시기에 발현되는 유전자의 분리

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본 실험에서는 담배(*Nicotiana tabacum* line No.1)의 leaf disk로부터 기관분화를 위한 determination time을 확인하고, suppression subtractive hybridization(SSH) 방법으로 묘조 분화 시기에 나타나는 특이 유전자를 분리하고자 하였다.묘조 분화를 위한 식물생장조절물질의 조성으로 benzyl adenine(BA) 1.0 ㎎/l로 결정하고, 이를 이용해 묘조 형성이 결정되는 determination time을 확인한 결과 묘조 형성 배지에서 10일간 배양한 leaf disk에서 묘조의 형성이 이루어졌다. 묘조 형성 시기에 나타나는 유전자를 분리하기 위하여 식물생장조절물질이 처리되지 않은 leaf disk를 대조구로 사용하고, 묘조 형성이 결정되는 시기의leaf disk를 실험구로 이용하여 SSH를 수행한 결과 총 100여개의 clone을 선별하게 되었다. 이를 tobacco shoot clone(TSC)이라 명명하였다. 분리된 각각의 TSC를 대상으로 northern blot 분석을 수행한 결과, TSC-7, 9, 22, 47, 54가 묘조 형성이 결정되는 시기의 재료에서 높은 발현을 나타내는 것을 확인하였다. GenBank의 BLAST를 이용하여 각 TSC들의 homology 검색한 결과, oxygen evolving 23 kDa polypeptide (TSC-7), glycine rich protein(TSC-9), plastidic aldolase (TSC-47), chloroplast glyceradehyde-3-phosphate dehydrogenase(TSC-54)가 확인되었고, TSC-22는 일치하는 유전자가 없는 것으로 나타났다.

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Development of Specific Fruit Promoters From *Citrus cinensis*

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It is necessary to apply molecular and biotechnological approaches, such as gene and promoter cloning, transformation and breeding, in order to improve citrus fruit quality. We have tried to clone a fruit specific promoter from *Citrus cinensis* as the first step of the research. Six cDNA clones specific to fruit were obtained from differential screening between leaf and fruit cDNA libraries. After northern blot analyses, one clone, *Lea* 5, was selected whose expression was increased with the fruit development. We screened a genomic library with *Lea*5 cDNA as a probe and obtained ca. 3 kbp genomic clone which contains a promoter region of 1.5 kbp. We subcloned the promoter region into 3 parts, ligated in the front of GUS reporter gene, and transformed into Arabidopsis plants.

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An Arabidopsis Semi-dwarf Mutant is Resistant to Typhasterol-mediated Root Growth Inhibition

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Brassinosteroids (BRs) are growth-promoting plant steroid hormones. Isolation and characterization of Arabidopsis dwarf mutants has been instrumental in characterization of BR biosynthetic enzymes and further validation of the biosynthetic pathways. However, 3 downstream steps including an ultimate one converting castasterone to brassinolide has been left without corresponding dwarf mutants. Near complete analysis of allavailable BR dwarfs indicated that mutants for these steps may not show dwarfism or the mutations are lethal. Thus we employed an alternative method to isolate mutants. Based on assumption that BR intermediates such as typhasterol (TY) and castasterone (CS) should be converted to an end product prior to becoming bioactive BRs, we plated EMS mutant population on TY-containing media and screened for phenotypes displaying unaffected root growth in spite of concentrated TY. After primary screening of >100,000 M2 seeds, 57 lines were identified. The 57 lines were subject to secondary screening to isolate 11 lines possessing more obvious phenotypic difference. One of these mutants was named semidwarf1 (sdf1) for its relatively weak dwarfism as compared to previously described BR dwarf. sdf1 is characterized to have round leaves with slightly reduced stature relative to a wild-type control. Results of various genetic and biochemical analyses will make it clear to define the function of this gene product in BR biosynthetic pathways.

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Control of the DWARF4 Gene Expression in the BR Biosynthetic Pathways

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The plant steroid hormones Brassinosteroids (BR) control many different aspects of plant growth and development, such as cell elongation, cell division, senescence, stress tolerance, and vascular system differentiation. Mutants defective in BR biosynthesis or signaling display severely retarded growth patterns regardless of their growing in the light or dark, indicating that BRs are generalized growth promoters. Of the enzymes involved in BR biosynthesis, DWF4 mediates a rate-limiting step which is the 22-hydroxylation in the biosynthetic pathways. Thus, it was hypothesized that DWF4 expression pattern could represent the tissues of BR biosynthesis in Arabidopsis. Different methods including an RNA gel blot analysis, RT-PCR, and DWF4::GUS histochemical analysis consistently revealed that the DWF4 transcripts differentially accumulate in the actively growing tissues, such as root tips, bottom of floral organs, axilary buds, collets, and elongating zone of dark-grown seedlings. Consistently, examining the endogenous levels of BR biosynthetic intermediates showed that metabolic flux after DWF4-mediated step increased in these tissues. Histochemical analysis of DWF4::GUS transgenic plants displayed that exogenous application of BRs and 2,4-D antagonistically regulate the DWF4 expression: decrease by BRs but increase by 2,4-D. Similarly, DWF4::GUS expression was upregulated in the BR-deficient dwarf mutant background, however, the change was not noticeable or even weaker in auxin resistant mutants. When the DWF4::GUS construct was introduced into two brassinazole-resistant mutants bzr1 and bes1, the GUS expression was effectively eliminated, suggesting that the BZR proteins act as negative regulator of the DWF4 gene expression.