SL201 The Structure and Development of the Parasitic Angiosperm Cuscuta japonica

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Anatomical and ultrastructural features of embryos, seedlings, and haustoria were observed in the parasitic angiosperm *Cuscuta japonica* Choisy. Seeds with the spirally coiled mature embryos germinated at 30℃ in the dark. Most embryo cells possessed numerous protein bodies and lipid droplets and proplastids devoid of starch grains. However, the shoot apical cells of 3-day-old seedling grown in the dark did not have protein bodies and contained lots of lipid droplets, amyloplasts, and proplastids. After these seedlings were transferred to a lightened growth chamber, all the shoot apical regions of seedlings grown for 6, 8, and 10 days became greenish and hooked. The hooks opened only when one seedling made contact with another seedling and entwined their host seedling. In some seedlings, the massive root were circular or semi-circular. Most cells of the green shoot apices contained abundant amyloplasts and chloroplasts with starch grains and some thylakoids. Crystalline bodies within plastids were closely associated with grana. A granum was usually consisted of a few stacked thylakoids. After 6-day-old seedlings made conact with a host plant, haustoria, absorptive organ, were formed at the cantact side of the seedlings. Digitate cells consisting of the haustorium were remarkably large, and contained large nuclei with prominent nucleolei and various and abundant cell organelles. The features described above would be discussed in view of parasitic role of *C. japonica*.

SL202 Uptake and utilization of dissolved organic carbon by phytoplankton

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해양에서의 유기탄소원은 육상에서의 유입과 해양식물에 의한 생성 등으로 인하여 막대한 양이 침전되며, 침전된 유기탄소원 중 많은 양이 용출되어 나온다. 용출된 유기탄소원 중 탄수화물은 침전된 유기탄소원과 용출된 탄소원을 이루는 주요한 구성성분으로 알려져 있으나, 해양에서의 탄수화물에 관한 연구는 용출되는 유기탄소원의 총량 및 유기 질소원과의 연관관계 등의 연구에 초점이 맞추어져 있었을 뿐 해양생물에 의한 흡수 및 이용에 관하여는 거의 연구가 수행되지 않고 있다.

근래에 들어 식물플랑톤에 의해 발생되는 적조현상은 국내외적으로 연안에서 발생하여 커다란 경제적, 사회적 문제를 야기하고 있다. 적조는 다양한 환경요인에 의해 유발되는 것으로 알려져 있는데, 특히 육상에서 유입되는 다량의 영양염은 가장 중요한 요인으로 인식되고 있다. 영양염 중 최근까지 해양학자들에 의해 관심을 가지고 연구된 것은 주로 질산염과 인산염이다. 해양으로 유입된 다량의 질산염과 인산염은 수온, 염분도, 광도 등과의 복합적인 환경요인의 작용에 의해 식물플랑크톤 대발생을 유도하는 것으로 알려져 있다. 그러나 최근에 해양에서의 질소원의 고갈 후에 다량의 탄소원 소모가 이루진다는 연구보고는 여러가지 가설을 가능하게 하고 있다. 즉 해양에서의 질소원 흡수 후에 해양 저질로부터 용출되는 다량의 탄소원을 식물플랑크톤이 흡수하여 실질적이고도, 광범위한 적조발생에 기여할 수 있다는 것이다.

이와 같은 가설을 연구하기 위해서는 우선 식물플랑크톤에 의한 탄소원 흡수 및 이용에 관한 연구가 수행되어야 하지만, 아직까지 해

양식물플랑크톤을 이용한 탄소원 흡수 및 이용에 관한 연구는 거의 이루어지지 않고 있다. 따라서 본 연구실에서는 해양식물플랑크톤을 포도당이 공급된 배지에서 배양하면서 포도당 흡수 및 흡수기작을 연구하고 세포외 invertase의 작용 등을 연구함으써 적조현상에 미치는 탄수화물의 영향에 관하여 연구하고 그 가능성을 파악하고자 하였다.

SL203 Genetic competition between native and introduced species of *Taraxacum* (Asteraceae)

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Taraxacum officinale-laevigatum complex was introduced from Europe to Korea about a hundred years ago. This species complex occur widely on Korean penninsula and took over the habitats of native species of *Taraxacum* and increasingly common in open habitats. *Taraxacum officinale-laevigatum* complex (all triploids) produce more abundant apomictic seeds than native species, however, some populations also show facultative sexual reproductions. Native species of *Taraxacum* consist of diploids, triploids, and tetraploids and show a series of facultative sexual reproductions. In order to measure the degrees and directions of gene flow between native and introduced species and to identify the genetic reasons of the rapid decreasing of native species populations, the maternally inherited chloroplast DNA markers(RFLP patterns and nucleotide sequences of trnL-F regions) and biparentally inherited nuclear markers (ITS sequences) are analyzed from 130 populations of *Taraxacum*. Nuclear ITS tree shows the morphologically circumscribed species boundaries both in native and introduced species. However, the cpDNA trees were substantially different from that of nuclear rDNA and show mixed patterns of populations between European and Asian species. The DNA data indicate that diploid populations (*T. ohwianum-T. hallaisanense* complex) of native species are the most venerable to the genetic contaminations from the foreign species. The results also suggest that the introgressive hybridization or the nuclear displacement hybridization from introduced species into native species (or *vs.*) is one of major driving force to extinction of native populations. Hybridizations are also observed rarely among closely related native species complex of the *T. mongolicum - T. coreanum* complex.

SL204 Plant growth and brassinosteroids

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The term brassinosteroids collectively refer to the growth-promoting steroids found in plants. The chemical structure of brassinolide was determined more than 20 years ago (Grove et al., 1979). Since then, the first 16 years had been devoted to focus on physiological elucidation of the mode-of-action of brassinosteroids. Recently, more explicit data on the biological importance of these plant hormones were made from molecular genetic analysis of Arabidopsis *dwarf* mutants that are defective in brassinosteroid biosynthesis or signal transduction pathways. These mutants are characterized by distinguished phenotypes including short-robust inflorescences,

dark-green and round leaves, prolonged life cycle in the light, and abnormal development when grown in the dark. To date, we have isolated and extensively characterized more than 80 dwarf mutants from Arabidopsis. Out of the eight dwarf loci, we have shown that *DWF1*, *DWF4*, *DWF5*, and *DWF7* encodes BR biosynthetic enzymes, steroid C-24 reductase, C-22a hydroxylase, D^7 reductase, and C-5 desaturase, respectively (Choe et al., 1999a; Choe et al., 1998; Choe et al., 2000; Choe et al., 1999b). In addition, we have also found that a signaling mutant *dwf12* possesses dominant-negative mutations that are located in a conserved kinase (in preparation). Biochemical analyses, such as feeding tests with biosynthetic intermediates, detection of the endogenous intermediate levels, and metabolite tracing tests with deuterium-labeled precursors, synergistically contributed to characterizing the molecular mechanisms underlying the dwarf mutant phenotypes.

Engineering of the biosynthetic pathway by promoter swapping analysis of the rate-limiting *DWF4* gene with constitutive 35S promoter resulted in noticeably bigger plant phenotype in both Arabidopsis and tobacco. This suggests that the activity of DWF4 enzyme in BR biosynthetic pathway is primarily controlled at the transcriptional level. Due to its bigger plant phenotype, which could be attributable to increased endogenous BL effects, we hypothesized that overexpression of the key gene *DWF4* may have caused the pathway to accumulate the end product BL. However, the endogenous BL level in the *35S::DWF4* line is less that that of wild type, although the levels of the biosynthetic intermediates after the DWF4 step are significantly increased. Similarly, the end product BL was not detectable in the dark-grown seedlings that display active cell elongation pattern in the hypocotyls (Choe et al., 2001). Taken together, these findings led us to hypothesize that increased growth is reversely proportional to the levels of end product brassinolide, and this lower concentration is possibly due to rapid degradation of the end product right after exerting their effects. Therefore, we propose that metabolic engineering of the brassinosteroid biosynthetic pathway such that overexpression of the *DWF4* gene in the insensitive mutant background *bri1/dwf2* may lead to great accumulation of the end product BL. This hypothesis is based on our finding that *bri1/dwf2* accumulates a significant amount of brassinolide (Noguchi et al., 1999), and *35S::DWF4* transgenic plants greatly enhance metabolic flux from the step mediated by DWF4 in the biosynthetic pathways (Choe et al., 2001).

Now it is clear that lesions both in brassinosteroid biosynthetic and signaling pathways result in characteristic dwarf phenotype due to a lack of sufficient cell growth. In addition to the genes that we have isolated previously, we are identifying brassinosteroid-related genes by performing a larger scale isolation and characterization of the *dwarf* mutants as well as using microarray analysis in Arabidopsis. These approaches will lead us to identity all of the genes involved in the biosynthetic and signaling pathways, and eventually enable us to manipulate the metabolic and signaling pathways to meet specific demands in crop trait enhancement projects.

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SL205 Brassinosteroids Action in Arabidopsis thaliana

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Plant steroid hormone brassinosteroids (BRs) play important roles during plant growth and development. Unlike animal steroids that predominantly use nuclear receptors to directly activate target gene expression, genetically identified BR receptor BRI1 is a membrane-bound receptor kinase. To expand our knowledge of the molecular mechanisms of plant steroid signaling, additional BR-insensitive mutants *bin3* and *bin5* with many characteristics of *bri1* mutant were identified and characterized. The mutants are partially insensitive to BR treatments, suggesting that the underlying gene products may either transduce or modulate BR signaling. Bin5 shares significant homology with achaeon topoisomerase VI subunit A and yeast SPO11 while Bin3 is the only eukaryotic homolog of the archaebacterial topoisomerase VI subunit B. Our microarray data show that many BR-regulated gene expression are down regulated in the mutants, suggesting that Bin3 and Bin5 may constitute a potential *Arabidopsis* topoisomerase VI and modulate BR-regulated gene expression. Another approach would be to screen for pathway active mutants. Activation tagging is a power method to obtain gain of function mutations and a very useful complement to simple loss of function mutations most often created by the insertion of T-DNA into genes. We have performed an activation tagging screen in the bri1-5 background, and T1 plants were screened in basta for long hypocotyls phenotype. Out of about 20,000 independent T1 plants, we identified several plants with longer hypocotyls than bri1. These plants ware genotyped to be homozygous for the bri1-5 mutation. Further genetic analysis of these bri1 suppressor mutants will elucidate their functions in BR signaling. The reduced fertility of BR mutants presents a difficulty in suppressor screens. In addition, a suppressor mutant with no phenotype in the wild type background might also be difficult to map. A BR biosynthesis inhibitor named brassinazole (Brz) was shown to block specifically the BR biosynthesis at steps from campestanol to teasterone catalyzed by cytochrome P450s

encoded by CPD and DWF4. As such, wild type plants grown on medium containing brassinazole display a phenotype similar to BR-deficient mutants, namely short hypocotyls and open cotyledons in dark, and dwarfism in the light. We performed a genetic screen in which activation tagged col-7 seeds were grown in the dark on medium containing Brz, and mutants with longer hypocotyls and elongated petioles and expanded leaf blades. Some of mutants also have apical hooks and closed cotyledons. Further phenotypic characterization and genetic mapping of these mutations are underway.

SL206 Determination of Phytochrome A Specific Degradation Domain Using PhyA/PhyB Chimera

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The ubiquitin/26S proteasome pathway is a well known proteolysis pathway of many signaling proteins. Recent studies have shown that this pathway is also connected to the degradation of some proteins in higher plants. The well known photoreceptor phytochrome A (phy A) from its inactive Pr form to its biologically active Pfr form initiates the rapid degradation of this protein. The ubiquitin/26S proteasome pathway has a main role for Pfr specific phytochrome A degradation. Previous kinetic and biological studies implicated that multiple domains within the chromoprotein are involved in the ubiquitin binding and its specific degradation. To further resolve the essential domains, we constructed a series of chimeric photoreceptors from potato PHYA and PHYB. Resulting chimeric photoreceptors were over-expressed in transgenic plant and analyzed the ubiqitination and Pfr-specific degradation. N-terminal ubiquitination was surprising result because it was contradictory to the previous report that the ubiquitination was on the C-terminal domain. Several N-terminal domains were exchanged and swapped between potato phytochrome A and B. Data analysis showed that phytochrome A amino-acid sequence from 364 to 423 was mainly involved in the Pfr specific degradation of phytochrome A. There may be ubiquitination sites in this degradation domain (364 ~ 423) where two lysines are highly conserved.