論　文　の　内　容　の　要　旨

生産・環境生物学　専攻
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氏　　名　胡　仲遠
指導教員名　堤　伸浩

論文題目　Studies on the roles of strigolactones in mesocotyl elongation during germination in rice

（イネの発芽過程のメソコチル伸長におけるストリゴラクトンの役割に関する研究）

Strigolactones (SLs) are plant hormones that regulate plant growth and development including shoot branching. They also trigger germination of seeds of parasitic plants of the genus *Striga* and stimulate plant symbiosis with arbuscular mycorrhizal fungi. In rice, it has been reported that at least 3 *Dwarf* (*D*) genes, *D10*, *D17*/*HTD* and *D27*, are involved in SL biosynthesis, and the *D3* gene is involved in SL signaling. The *D14*/*D88*/*HTD2* gene has also been thought to function in SL signaling or metabolism. The aim of this study was to understand the molecular mechanisms that regulate rice mesocotyl elongation during germination and growth in darkness, especially the antagonistic interaction between phytohormones SLs and cytokinins (CKs).

1. SLs negatively regulate mesocotyl elongation in rice during germination under darkness

Mesocotyl is a tissue located between the coleoptilar node and a basal part of the seminal root in young seedlings. Mesocotyl elongation of young seedlings grown under

darkness or submerged conditions contributes to survival in paddy fields. However, a long mesocotyl emerged from the soil surface may cause lodging of seedlings, so that mesocotyl elongation should be tightly regulated in response to environmental cues. After seeds were germinated and grown under darkness for 8 days, it was found that mesocotyl elongation was greater in rice mutants defective in SL-biosynthesis or signaling genes than in the wild-type. Exogenous application of a synthetic SL analog, GR24, rescued the normal mesocotyl phenotype in the SL-deficient mutants, *d10-1*, *d17-1* and *d27-1*, in a dosage-dependent manner, but did not affect mesocotyl lengths of the SL-insensitive mutants, *d3-1* and *d14-1*. It has been known that growth of mesocotyl is controlled not only by the lengthwise elongation of each cell, but also by an increase in the number of cells by cell division. No significant differences in the cell lengths of the mesocotyls were found between the *d* mutants and the wild-type, except for the short cell length observed at the lower half of the *d3-1* mesocotyl. On the other hand, the number of cells in the mesocotyls was 3-6 fold greater in the *d* mutants than in the wild-type. Treatment with GR24 reduced the number of cells in the *d10-1* mesocotyl to the wild-type level, but did not affect the number of cells in the *d3-1* and *d14-1* mesocotyls. These findings indicate that SL negatively regulates cell division, but not cell elongation, in rice mesocotyl during germination and growth under darkness.

2. Antagonistic interaction between SL and CK in regulating mesocotyl elongation

CKs are the only class of plant hormones known to promote cell division. To determine whether the longer-mesocotyl phenotype of the *d* mutants was associated

with increased CK levels in the mesocotyl, endogenous concentrations of several common natural isoprenoid CKs, namely N^6-(Δ^2-isopentenyl)-adenine (iP) and *trans*-zeatin (tZ), and their riboside derivatives, were measured. The tZ contents of the mesocotyls of both *d10-1* and *d14-1* were significantly higher than that in the wild type. Interestingly, upon treatment with GR24, the content of tZ-type CKs in the *d10-1* mesocotyl decreased dramatically to the wild-type level. However, the tZ-type CK contents in both the wild type and *d14-1* were not affected by GR24. Identification, by microarray analysis, of several SL-regulated genes helped us to understand the possible mechanism of SL and CK interaction in regulating mesocotyl elongation. Rice *cytokinin oxidase 9* (*OsCKX9*), which encodes a CK degradation enzyme, was found to be up-regulated by SL, suggesting that reduced expression of *OsCKX9* in the *d* mutants caused tZ-type CK accumulation in the mesocotyls and enhanced cell division. To confirm the interaction between SL and CK in the mesocotyl elongation, kinetin or cytokinin oxidase inhibitor, 1-(2-chloro-4-pyridryl)-3-phenylurea (CPPU), was applied to the seedlings. Treatment with kinetin or CPPU did not affect the mesocotyl elongation of the *d* mutants, but it enhanced the lengths of mesocotyls of the wild type in a dose-dependent manner. Treatment with kinetin or CPPU, together with GR24, confirmed the antagonistic function of these two hormones on mesocotyl elongation. Together, these results suggest that reduced expression of *OsCKX9* in the *d* mutants causes tZ-type CK accumulation in mesocotyl and enhances cell division, suggesting that SL and CK antagonistically regulate cell division of mesocotyl and its elongation during germination and growth under darkness.

In the microarray analysis, a TCP family transcription factor, *OsTCP2*, was identified as an SL up-regulated gene. Recently, several TCP family transcription factors,

including *ZmTB1* (maize), *AtBRC1* and *AtBRC2* (*Arabidopsis*), *SbTB1* (sorghum), *PsBRC1* (pea) and *OsTB1/OsFC1* (rice), were selected as downstream regulators of the SL pathway that controls bud activity. Both *OsTCP2* and all of the *TB1/BRC* genes belong to the class II TCP sub-family, whose members are known to repress organ growth by inhibiting cell proliferation. Expression of *OsTCP2* was decreased in the *d*-mutant mesocotyls compared to those in the wild-type mesocotyl. Application of kinetin or CPPU reduced the expression of *OsTCP2* in wild-type mesocotyl, but not in the *d*-mutant mesocotyls. Moreover, overexpression of *OsTCP2* clearly impaired the CK-induced mesocotyl elongation. Together, these results suggest that OsTCP2 negatively controls mesocotyl elongation and is antagonistically regulated by SL and CK in the mesocotyl.

Finally I proposed a model for the interactions between SL and CK in regulating rice mesocotyl elongation. In wild-type rice, endogenous SLs induce the expression of *CKX*, thus reducing CK levels. Thus, the inhibition of the expression of some *TCP* family genes, such as *OsTCP2* and *OsTB1*, is impaired. *OsTCP2* and *OsTB1* negatively regulate cell division, resulting in a shorter mesocotyl and less branching, respectively. In *d* mutants, a deficiency of SL or insensitivity to SL results in an increase in CK levels in the mesocotyl, which thus inhibits the expression of *OsTCP2*; cell division is therefore enhanced in the mesocotyls of the *d* mutants.