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学 位 論 文 題 目	Studies on systemic regulatory mechanisms of root nodulation in *Lotus japonicus*
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論文内容の要旨
Summary of thesis contents

To establish symbiotic associations with rhizobia, a group of nitrogen-fixing bacteria, leguminous plants form nodules on their roots in response to rhizobial infection. The rhizobia colonize these nodules, supplying host plants with fixed atmospheric nitrogen while receiving photosynthates in return. Although such a symbiotic relationship is generally beneficial to both partners, the formation of excessive numbers of nodules inhibits growth of the host plants. To avoid this effect, plants perform autoregulation of nodulation (AON), which systemically controls the number of nodules. AON is a long-distance negative feedback system involving root–shoot communication. In *Lotus japonicus*, the initial step of AON is the production of mobile CLE-RS1 and CLE-RS2 peptides in roots during nodulation; the perception of the signal by the receptor kinase HAR1 in the shoots presumably induces the production of an unidentified shoot-derived inhibitor (SDI) that translocates to the roots and blocks further nodule development. Although these results provide some insight into signaling mechanisms between root and shoot, the mechanism and regulation of AON-inhibition of nodule development remain mostly unclear. In particular, the identification of the shoot-derived inhibitor, SDI, is primarily required.

The *har1* mutant shows a hypernodulation phenotype possibly due to defects in SDI production. In addition, the constitutive expression of *CLE-RS1* or *CLE-RS2*, which encode peptides acting as ligands of HAR1, inhibits further nodulation, most likely due to increased SDI production in the shoot. Consequently, I hypothesized that SDI levels in the shoots should differ between *har1* mutants and *CLE-RS1* or *CLE-RS2* constitutive expression lines. If so, candidates of SDIs should be identifiable by comparing the transcriptome and metabolite profiles between these different lines.

In this study, *L. japonicus* was used as a leguminous plant. First, I compared the transcriptome profiles and phytohormone compositions in the shoots of the wild type with those of the *har1-7* mutants and *CLE-RS1* and *CLE-RS2* overexpression lines. These results suggest that the production of cytokinins (CKs) is induced in shoots through activation of the CLE-RS1/2-HAR1 signaling pathway.

Second, to investigate whether the CKs accumulating in the shoots have SDI-like activities, I applied CKs to seedlings via cut surfaces of cotyledons, inhibiting nodulation in the roots of the wild type and *har1* mutant. Additionally, this inhibition was not observed in too much love (*tml*) mutants, a component of AON acting in the roots. Thus, these results suggest that the inhibition of nodulation caused by CKs is under the control of AON. I further demonstrated the shoot-to-root

long-distance transport of CKs in *L. japonicus* seedlings using labeled CKs; specifically, the labeled CKs that were applied to shoots were detected in the root tips. Therefore, I concluded that shoot-derived CKs have a SDI-like capacity to systemically suppress nodulation.

Third, I focused on the production mechanism of shoot-derived CKs during nodulation. Isopentenyl transferases (IPTs) mediate the initial step of the CK biosynthetic pathway and significantly affect the production of CKs. In this study, I indicated that the expression of *LjIPT3* genes was induced by rhizobial infection in a HAR1-dependent manner. Additionally, grafting experiments using *LjIPT3* overexpression lines and *Ljipt3* mutants indicated that the LjIPT3 of the shoots acts as a negative regulator of nodulation. Taken together, I indicated that the LjIPT3 of the shoots is involved in CK production during nodulation and that the produced CKs systemically inhibit nodulation.

Fourth, the results of this study suggest that AON is likely to inhibit nodulation by suppression of *NSP2* expression, a positive factor for nodulation. Additionally, grafting experiments showed that the site of AON action may be downstream of LHK1, which encodes a putative CK receptor. Thus, these results suggest that AON seems to suppress the expression of *NSP2* downstream of LHK1.

Finally, I indicated that AON is likely to also regulate the lateral root number. The lateral root number of transgenic plants with an activated AON pathway was decreased, while that of the *har1* mutants was increased

Since AON was reported in 1990, the molecular mechanisms of AON and Nod factor signaling have been actively studied. However, the mechanism regulating root nodulation in the shoots remains unknown. In this study, I indicated that the shoot-derived CKs systemically regulate nodulation on AON and that inhibition is regulated by LjIPT3 in the shoots. In addition, the results of this study suggest that AON is likely to regulate not only nodulation but also the formation of lateral roots. The findings of this study add essential components to understand how legumes control the nodule and lateral root numbers and will be valuable for understanding the molecular mechanism involving systemic regulation by CKs.