# 学 位 論 文 要 旨

Involvement of programmed cell death in suppression of the number of root nodules formed in soybean induced by *Bradyrhizobium* infection

ダイズにおける根粒着生数の抑制へのプログラム細胞死の関与

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Leguminous plants form root nodules, within which soil bacteria called rhizobia symbiotically fix atmospheric nitrogen gas into ammonia, and provide the legumes with organic nitrogen compounds, such as ureides or asparagine, in exchange for the energy derived from photosynthates. This symbiotic relationship enables the legumes to grow in nitrogen-deficient soils. However, excessive nodulation damages the host growth by over-consuming nutrients in the plant. It can be assumed that legumes have evolved diverse mechanisms to regulate root nodule number according to the host's own energy demands.

Autoregulation of nodulation (AON) is a mechanism that regulates root nodule number, which is systemically controlled by roots and shoots via long-distance signaling factors. In AON, it is thought that a root-derived signal, generated upon infection by rhizobia, is transported to the shoot, eliciting a shoot-derived signal, which is translocated down to the roots to inhibit root nodulation. In addition to systemic regulation via long-distance signals derived from roots and shoots (AON), local regulation via short-distance signaling that occurs only in the root has been postulated as another mechanism for controlling root nodule formation. Plants also defend themselves against bacteria and other pathogens by the induction of localized acquired resistance (LAR) surrounding local lesions formed by hypersensitive response (HR) in the infected areas, and of systemic acquired resistance (SAR) or/and induced systemic resistance (ISR) to survive. Herein, we show that the number of root nodules is suppressed by programmed cell death (PCD), and is simultaneously controlled by SAR and ISR in soybean (*Glycine max* [L.] Merr.).

We observed that both the numbers of root nodule primordia (Stage1 + Stage2) and the ratios of Stage2

to (Stage1 + Stage2) in wild-type soybean Williams 82 were noticeably fewer than in hypernodulation mutant NOD1-3 on d4, d5, and d6, indicating that root nodule formation and development are suppressed in wild type during d4-d6, which also suggests that these suppression phenomena are genetically and biochemically regulated during d0-d4 after inoculation of the wild-type soybean roots with rhizobium. We also discovered characteristics of PCD accompanied by accelerated DNA degradation at 3.5 (d0), 26.5 (d1), 37 (d1), 66 (d2), 78 (d3), 97.5 (d4), and 122 h (d5), enhanced generation of reactive oxygen species (visualized by 3, 3′-diaminobenzidine staining) on d2, and markedly more cell death (detected on staining with trypan blue) and fewer root nodule primordia on d3 after rhizobia inoculation in wild-type soybean Williams 82 than hypernodulation mutant NOD1-3. These findings suggest that the number of root nodules in wild-type soybean is suppressed by PCD. In addition, we conducted microarray, gene ontology and pathway analyses to detect the transcriptional response controlling the number of root nodules at rhizobia infection sites. Numbers of up-regulated genes associated with defense responses in wild type were several times larger than those in hyper-nodulation mutant (incidentally, it is shown that many genes were upregulated on d0 and/or d5, and were downregulated on d2 and/or d4 in Williams 82 compared with NOD1-3, suggesting that many genes are probably involved in the suppression of formation and development of root nodule primordia on d0 and/or d5, whereas these genes possibly participate in delaying the formation and development of root nodule primordia on d2 and/or d4 in wild type compared with the hypernodulation mutant.); essential factors for HR or disease resistance such as resistance genes, proteins generating $H_2O_2$, mitogen-activated protein kinase cascade, SAR, salicylic acid, jasmonic acid, ethylene etc., were activated in wild-type plants; the total numbers of sequences and enzymes participated in the pathways involved in defense responses including primary and secondary metabolisms in wild type were obviously larger than those in hyper-nodulation mutant. The data obtained from these analyses also corroborate the above finding that soybean controls its root nodule number by PCD suggesting simultaneously through SAR and ISR. We further show that most nodulins do not move in tandem with the regulatory mechanisms of defense response except for 2 nodulins. These findings provide new insight into the control of nodulation to balance nutritional requirements and energy status in legumes.

Like SAR and ISR, AON is induced by bacterial infection, and thus, exhibits systemic resistance against bacteria. In the present study, we elucidated the mechanisms underlying the suppression of root nodule formation in soybean using wild-type cv. Williams 82 and hypernodulation mutant NOD1-3. In the present study, we show that the containment of root nodule number in soybean is associated with PCD and HR, and is simultaneously controlled by SAR and ISR, suggesting that AON or local regulation is a part of SAR and ISR. It seems that LAR is induced around the local lesions in the infected areas by HR from pathogen infection, and shows a high degree of resistance in wild-type soybean Williams 82; and that the reason why it exhibits strong resistance in LAR is that the LAR around the HR site includes genes expressed both in the SAR and ISR.