学位論文内容の要旨

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学位論文題名

Analysis of two host genes required for induction of root-knot nematode feeding sites

（ネコブセンチュウが栄養摂取部位の誘導に要する二つの宿主遺伝子の解析）

Root-knot nematodes are plant parasites that cause widespread loss in agriculture worldwide. Host infestation by root-knot nematodes is comprised of three stages. During the first invasion stage, nematode juveniles penetrate the root and migrate to target cells in the root tip. This is followed by the induction stage, when the nematode induces host cells to adopt a new enlarged “giant cell” morphology specialized for nutrient transfer. The nematode then feeds permanently from these giant cells for its remaining life-cycle. The induction stage is the key step that determines parasitic success and thus the development of effective control strategies requires an in-depth understanding of this process. However, the molecular mechanism(s) by which root-knot nematodes induce feeding sites remains unknown. In this study, two host genes were analysed to provide insight on the molecular mechanisms for feeding site induction. The first gene, *FIE*, was selected in a hypothesis-based approach for genes that may drive the global reprogramming process, whereas the second gene, *ASTRAY*, was selected based on infestation phenotype observed in mutant plants.

1. Functional study of the FIE Polycomb subunit during feeding site formation

In plants, the Polycomb Repressive Complex 2 (PRC2) regulates global gene expression during developmental transitions. This study therefore tested the hypothesis that the plant PRC2 complex is used by root-knot nematodes to direct the reprogramming of giant cell fate during the induction stage. The role of PRC2 was tested by perturbing expression of the complex core subunit gene, *FIE*. An inducible RNAi vector system was used for temporal knockdown of *FIE* expression during root-knot nematode invasion and induction stage. Inducer treatments were established for use with nematode infestation assays and the inducer vector was confirmed to be effective at depleting *FIE* levels in a temporal manner. A transgenic hairy root system

was successfully implemented for testing and optimisation, although a high degree of variability was observed when quantifying nematode infestation. Nevertheless, one line was isolated that showed a consistent phenotype and a defect in the induction stage following inducer treatment. This was consistent with a role for FIE in feeding site development and stable independent transgenic plant lines have now been successfully generated that will facilitate future in-depth studies.

2. A robust system for quantitative analysis of root-knot nematode infestation

One limiting factor in understanding the molecular mechanisms of feeding site development has been the lack of a standardised quantitative assay, which should also enable downstream analyses and be free of any other biological sources of variability. As part of this study, a new robust and highly reproducible assay method has now been developed that solves these problems. This includes a new approach for preparation of high quality axenic nematode populations and optimised infestation conditions for the selected host plant *Lotus japonicus*, a model legume. In addition, an improved compatible hairy root transformation protocol was established facilitating mechanistic studies. The strategies developed here are also readily applied to different plant hosts.

3. Characterisation of the *astray* mutant defective in feeding site induction

The absence of a plant loss-of-function mutation that disrupts feeding site development has been a major factor in studying the associated molecular mechanisms. During this project a *L. japonicus* symbiosis mutant, *astray* (Gifu ecotype), was identified as having a possible defect in root-knot nematode infestation and the genetic nature of this mutation was characterised. Using a combination of genetic crosses and the above-described hairy root assay strategy, it was found that the *astray* mutation was a recessive loss-of-function trait that restricts infestation prior to completion of feeding site induction. This was associated with reduced invasion, indicating that ASTRAY has an essential function during both the invasion and induction stages. Unlike that observed for symbiosis, this phenotype was constitutively displayed in both plate and soil culture. A similar loss-of-function *astray* allele was isolated from the MG-20 ecotype by targeted mutagenesis screening, however, surprisingly the *astray* mutation in this different genetic background did not display the nematode phenotype. These results suggested that *ASTRAY* is an essential host gene for root-knot nematode infestation and that this role may have remained hidden due to the presence of parallel genetic pathways for root development.