



Title	Analysis of two host genes required for induction of root-knot nematode feeding sites [an abstract of dissertation and a summary of dissertation review]
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## 学位論文審査の要旨

博士の専攻分野の名称	博士 (農学)	氏名	Arshana Nor Noorul Amin
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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)

The PhD thesis under examination was comprised of 130 pages, 31 figures, 8 tables and 1 appendix that were divided into 6 chapters. The thesis also included one first-author research paper in publication.

One of the major challenges in achieving a sustainable agriculture is overcoming the yield loss caused by plant pathogens. Root-knot nematodes are of particular concern as they infest many different crop species and disrupt both the function and morphology of plant root systems, yet control options are extremely limited. The parasitic process of root-knot nematodes is unique as they establish permanent feeding sites inside plant roots by transforming host plant cells into a different type of nutrient acquiring cell, known as giant cells. It is thought that this is achieved by hijacking specific plants genes/proteins to manipulate endogenous signaling pathways in the host, however these remain elusive and yet to be experimentally confirmed. Discovery of such host factors will enable development of new control strategies. Studies to date have been limited by the absence of a consistent and reliable method for measuring root-knot nematode-plant interactions. This research project now provides such high-quality methods and the subsequent discovery of a host protein with an essential role in nematode parasitism.

#### **1) Functional study of the FIE Polycomb subunit during feeding site formation**

Despite intensive study by many groups, there had been no reports of plant genes or mutations essential for nematode infestation. This research project therefore took a hypothesis-based approach and proposed a role for the plant *FIE* gene in developmental reprogramming of the host cells. This was based on well-established knowledge that the FIE protein is part of a key complex important in almost all developmental transitions in plants. To overcome challenges with the critical role for *FIE* in general plant growth, this project utilized an inducible gene knockdown system to specifically deplete *FIE* at the stage when root-knot nematodes are expected to trigger host cell transformation. It was shown that this approach was effective in temporal reduction of *FIE* and the effect could persist during early infestation stages. Using a transgenic tomato hairy root system, a

reduced ability of root-knot nematodes to progress through the early induction stage was demonstrated. Although infestations in the hairy root system was found to differ slightly from that of intact whole plants, the results were consistent with a role for FIE in mediating the transformation of plant cells into specialized giant cells.

## **2) A robust system for quantitative analysis of root-knot nematode infestation**

The first part of this project highlighted a problem experienced by many groups in this field - suboptimal plant systems for studying root-knot nematode infestation and lack of consistent assays. This research project developed a new, robust and highly reproducible assay method to solve such problems that have persisted in this field of research. A new approach was developed for preparation of high quality nematode inoculum that contained juveniles synchronized to a similar infective stage and free of any other biological or non-biological contaminants that typically introduce variability. In addition, the host plant *Lotus japonicus* was identified as enabling consistent and highly reproducible quantification of plant infestation levels. This was not only restricted to intact whole plants, a markedly improved hairy root transformation protocol was also developed for *Lotus japonicus* that was compatible with root-knot nematode infestation and showed the same infestation progress as that in intact plants.

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

The third part of this research project represented the highly significant discovery that the *ASTRAY* host gene is essential for the early infestation step of root-knot nematode parasitism. This was identified in a screen of publicly available *Lotus japonicus* plant mutants. Traditional genetic approaches were combined with the newly developed hairy root transformation assay system, revealing that the wild-type *ASTRAY* protein likely functions to facilitate both host compatibility and also the initial giant cell transformation. This gene has been implicated in beneficial symbiotic interactions under specific conditions, whereas it was revealed that the requirement in root-knot nematode infestation is present in a much wider range of conditions including open growth in soil. Analysis of a similar genetic lesion in a different plant ecotype also suggested the presence of parallel pathways that would explain why this key host factor remained undiscovered until now.

The research in this PhD thesis has provided novel information on two host genes implicated in the early initiation of root-knot nematode infestation, an area that has been traditionally very difficult to study and had seen little advance in recent years. The new methods and assay systems described will also help propel the field forward and facilitate rapid subsequent discovery not previously possible. Therefore, we acknowledge that the author, Arshana Nor Noorul Amin, is qualified to be granted the Degree of Doctor of Philosophy in Agriculture from Hokkaido University.