

Analysis of early-stages of root-knot nematode infection site formation in plant roots using an *in vitro* system

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Background & Aim: Root-knot nematodes (RKNs) are parasites that are capable of infecting nearly all plant species. RKN infection takes place deep inside the host plant root, where infective RKN juveniles invade the root tip and induce specific cells to become enlarged multinucleate giant cells (GCs). These GCs then act as permanent feeding sites. It is essential to understand how RKNs are able to reprogram the function and structure of normal plant cells. The aim of this research was to explore the hypothesis that host chromatin machinery (the Polycomb complex) is utilised for infection site development.

Approach: Micro-tom (*Solanum lycopersicum* cv. Micro-tom) and RKN (*Meloidogyne hapla* line VW9) were used in this study. The FIE protein is a key component of plant Polycomb complexes, and thus the tomato homolog (*LeFIE*) was cloned from Micro-tom genome. Infection sites were studied using a transgenic hairy root approach and root sectioning. To manipulate gene expression, a DEX-inducible RNAi hairpin vector was utilised. All root treatments and RKN infections were carried out *in vitro* under specifically controlled conditions.

Result & Discussion: A transgenic hairy root strategy was successfully established, compatible with both the inducible system and RKN infection assays. The *LeFIE* gene was shown to be expressed in non-infected Micro-tom root tip and this expression remained during early RKN infection when roots undergo strong activation of nuclear and cell division (Fig 1). A reduction in RKN infection was observed in transgenic roots containing the *LeFIE* RNAi hairpin vector. This reduction was specific to induction treatment, whereas equivalent non-treated transgenic roots showed normal infection (Fig 2). These results suggest a role for the host Polycomb complex in development of RKN infection sites.

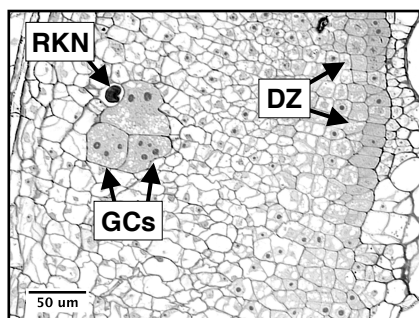


Fig 1: Sectioning analysis of an early RKN infection site in Micro-tom. RKN, head of the infecting root-knot nematode; GCs, giant cells with multiple nuclei; DZ, zone of rapid cell division.

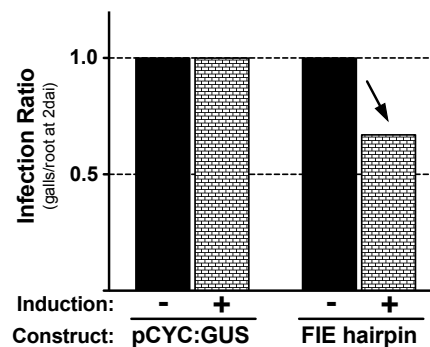


Fig 2: RKN infection in transgenic hairy roots containing either a control or FIE hairpin construct. Induction was carried out using 10mM DEX 30 min prior to RKN infections. Infections were scored as gall formation at 2 dai.