A study on RNA molecules from parasitic nematodes that may contribute to infection success

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Background & Aim: Root-knot nematodes (RKNs) are plant parasitic nematodes (PPNs) that cause severe crop loss worldwide. RKNs hatch from eggs as infective stage juveniles (J2s), undergoing their first molt from J1 to J2 inside egg. After J2s invade plants, they induce rediffentiation of plant cells and stay inside roots for the remainder of their life-cycle. At present, the only effective methods for RKN control target juveniles in soil but lack specificity. miRNAs have been discovered to play an important role for correct development of the non-parasitic *C. elegans* nematode and proteins for miRNA biogenesis were also reported to be critical for RKN juvenile development. However data on miRNAs in RKNs has not been available. The aim of this study is to identify miRNAs in RKNs and find RKN-specific miRNAs compared to *C. elegans.*

Approach: We have identified miRNAs in RKN using next-generation sequencing (NGS) and bioinformatics analysis. A subset of these were experimentally validated by northern blots. To confirm wether these miRNAs are absent in non-parasitic nematodes and other PPNs, *C. elegans* and two genus of PPNs were used in addition to RKN. From the finding of RKN specific miRNAs and their potential of targeting plant genes, we hypothesized that miRNAs may be effectors to induce the redifferentiation. RNAs from secretion was investigated by 3' end labeling using [alpha-32P] cordycepin and polyA polymerase.

Results & Discussion: 56 miRNAs were identified though NGS and subsequent bioinformatic analysis. 19 examined miRNAs were confirmed to be present in RKN J2. Surprisingly, only 4 of these were also detected in *C. elegans*, and remaining 15 were absent in *C. elegans* (Figure A-1). Further northern blot analysis on seven specific miRNAs using two genus of PPNs identified one PPN-specific miRNA. The presence of RKN-specific miRNAs, especially PPN-specific miRNAs confirmed a potential for these as new control targets that could overcome unfavourable secondary effects. As a separate experiment, RNA detection confirmed the presence of a distinct 85 nt RNAs in RKN secretion although miRNAs were not detected clearly (Figure A-2). It is possible that 85 nt RNAs may be a new type of RNAs that regulate plant cells and cloning of this RNAs is underway.

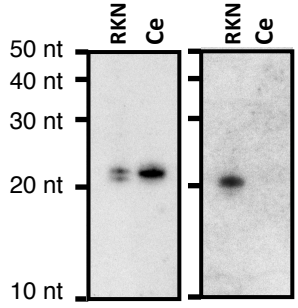

Figure A-1 :
Northern blot analysis showed small RNAs that were detected both in C. elegans and RKN, and specific in RKN. Ce; *C. elegans*

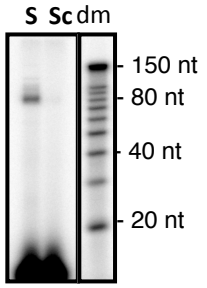

Figure A-2 :
85 nt RNAs were identified by 3' end labeling to RKN secretion samples. S; Secretion, Sc; Secretion control.