miRNAS FROM COTTON ROOTS INFECTED WITH *MELOIDOGYNE INCOGNITA*

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Abstract

The molecular activities associated with the resistance of Upland cotton (*Gossypium hirsutum* L.) to the root-knot nematode (RKN) are largely unknown. Small RNAs or microRNAs (miRNA), a well-conserved gene regulatory system, have an important role in plant development, stress responses, and epigenetic regulation primarily through transcriptional and post-transcriptional silencing of specific target genes. The objective of this work was to clone, characterize cotton micro-RNAs from root tissues, collected at 16 DPI (days post infection) and 24DPI respectively by comparing with uninfected root tissues (control) of RKN resistant (M-240) and susceptible (M-8) Upland cotton lines. We used the microRNA expression and cloning and kit developed at Integrated DNA Technologies Inc., (IDT, USA). The total RNA pool from root tissues was isolated and size fractionated to collect the small RNA pool. The 3' and 5' specific linkers were then ligated to both ends of small RNAs and ligated products concatamerized, cloned and sequenced. MicroRNA results showed that on an average 851 clones of 16DPI root samples had 24% miRNAs, 76% were other siRNAs and rRNAs. We also observed that 24DPI root tissues had 20% of clones of miRNAs and 80% were siRNAs and rRNAs. Results revealed that 24DPI root sample had an increase in miRNA percentage compared to the respective sample of 16DPI root tissue suggesting a change in miRNAs biological activities associated with the root development. The 24DPI resistant cotton line had the highest percentage of miRNAs among all of the samples. The sequencing of more than 1000 clones confirmed the presence of several root specific micro RNA families (e.g. miR-160, miR-164). Currently we are in the process of characterizing these miRNAs to identify their roles in RKN infection.