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Short Communication

Gene expression studies of the soybean-nematode interaction

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Soybeans [*Glycine max* L. (Merr.)] are a major source of protein for animal feed and are also an increasingly important component of the diets of consumers. Soybean cyst nematode (SCN), *Heterodera glycines,* is the major pest of soybean and over the last decade accounted for more than 50% of the crop lost to disease. Due to improved crop management and breeding, the loss to SCN decreased to some extent, but it has been remained a great challenge. Therefore, further improvement to control nematode infection is indispensable to reduce the crop losses. Root-knot nematode (RKN, *Meloidogyne incognita*) is also a pathogen of soybean. Although RKN does not cause as great a loss to the soybean crop as SCN, RKN has a very broad host range and is particularly damaging where soybean is rotated in with other susceptible crops (1). Comparison of the pathogenicity of the cyst and root-knot nematodes is also needed to create strategies to broaden resistance to nematodes in soybean and other crops.

Parasitism of plants by SCN is initiated by penetration of the nematode into the root, often near the root cap and lateral branches. Then the nematode migrates to edge of the vascular bundle, where the nematode induces the formation of a feeding structure which is called a syncytium. This structure is formed by partial hydrolysis of contiguous cell walls and fusion of plasma membranes to form a large multinucleated cell (2). Moreover, the SCN feeding site is metabolically hyperactive in susceptible soybean roots (3) and the nematode may feed for up to two months. Several changes occur to form the feeding cell, including nuclei and nucleoli hypertrophy, cytoplasmic organelle proliferation, and reduction or loss of the central cell vacuole. Scientists have been trying to develop the strategy to prevent the entry of the nematode by the production and accumulation of toxic substances in the area of infection (4), and production of a hypersensitive response in the host (5).

Over the past several years our lab and others have contributed to the enormous increase in data available for gene expression during nematode infection in both susceptible and resistant soybean genotypes to develop new approaches to improve resistance to nematodes (6, 7). In these studies, *G. max* cv. Kent seedlings were inoculated with *H. glycines*, second stage juveniles (J2s) then grown for 8 days (Fig. 1). Laser capture microdissection (LCM) is a new technique used to collect syncytial cells and to identify genes expressed in these cells upon which the nematode feeds. First we identify the highly infected regions in soybean roots by Fuchsin staining (8). Then we dissect the tissue by LCM to collect

syncytia, extract RNA, and analyze the gene transcripts by deep-sequencing using RNA-Seq according to our published procedures (9). All techniques for growing soybean, infecting roots with nematodes, sample preparation, laser capture microdissection, RNA extraction and analysis have been published by the Matthews laboratory (Fig. 1), (7, 9, 11). Our bioinformatics analysis of expression levels of genes encoding enzymes and proteins are represented in the KEGG biochemical pathway database (http://www.genome.jp/kegg/pathway.html) for easier understanding of expression patterns. We examined gene expression in susceptible and resistant interactions of SCN with soybean at 3, 6 and 9 days after inoculation, so we can follow the progression of gene expression as the nematode feeds and as the soybean root responds to the infection in a compatible and an incompatible interaction. The combination of LCM, deep-sequencing and bioinformatics analysis and visualization, provides us with a picture of what is happening at the gene expression level over time and provides us with clues as to which genes are important to resistance and susceptibility of soybean to nematodes. One-hundred of these genes were cloned and over-expressed in transgenic soybean roots challenged with SCN to determine their role in resistance and susceptibility (10). Nine genes reduced the number of mature female SCN by more than 50% when overexpressed, while four genes increased the number of mature SCN females by over 200% when overexpressed. These data help us understand which genes are expressed by the host plant in response to SCN invasion to provide resistance and which genes may be the result of nematode effector proteins commandeering the metabolic machinery of the host to provide a compatible environment for its own growth. Furthermore, some of these genes are good candidates for developing genetically engineered resistance in soybean against SCN.

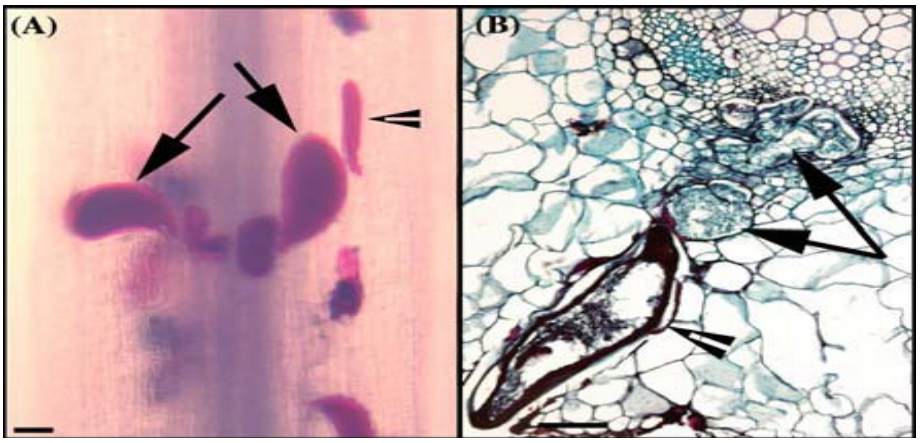

Fig 1. **A.** Acid fuschin staining reveals enlarged nematodes (arrows) at the vascular cylinder. A smaller nematode not associated with the vascular cylinder has also stained (arrowhead) (bar=500 lm).
B. Transverse section showing the syncytium (arrows) with a closely associated nematode (arrowhead) (bar=100 lm).

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