Legumes can enter into symbiotic relationships with rhizobia resulting in the formation of nitrogen fixing root nodules. Nodule numbers are internally regulated by the plant via phytohormones, Nod Factor (NF) perception and a long-distance signalling network termed Autoregulation Of Nodulation (AON). In parallel, nodulation is externally regulated by factors such as nitrate, water-availability, and soil pH. Our research aims to determine the interplay between external pH stress and the internal regulation of nodulation. It also aims to identify the root-derived elicitor (Q) and shoot derived inhibitor (SDI) of AON in soybean.

Characterizing Acid Tolerance and Identifying Autoregulation Receptor Kinase (NARK) in the shoot

- Upwardly transported signal produced in roots
- Perceived by Nodulation Autoregulation Receptor Kinase (NARK) in the shoot

The AON Pathway

Q

- Developed bioassay to detect Q in xylem sap
- Sap samples include Q+ (from Bradyrhizobium japonicum inoculated supernodulating Gmnark mutant plants) or Q- (from inoculated non-nodulating Gmnfr5x mutant plants)
- Identified a leaf molecular marker for the presence of Q
- Measure marker expression in leaves of WT soybean shoots dipped in sap samples for 24 h

SDI

- Downwardly transported signal produced in shoots following NARK perception of Q
- Inhibits continued nodule development in roots

- Developed bioassay to detect SDI in leaf extracts
- Feed extracts into petioles of supernodulating Gmnark mutants for 9 d, inoculate with B. japonicum 1 d after initiating feeding and count nodule numbers 8 d after inoculation

SDI activity can be diluted out but can not be amplified

To identify SDI, we are currently using Mass Spectrometry to examine candidate peaks following HPLC fractionation of leaf extracts

- Extracts from WT (Bragg) plants suppress supernodulation
- Extracts from nark mutant plants fail to suppress, indicating SDI is NARK-dependent
- Only inoculated-Bragg extracts suppress nodulation, indicating SDI is B. japonicum-dependent
- SDI is small (<1 kDa) and heat stable
- SDI is Proteinase K-resistant

• Hairy-root overexpression of the NF receptor, GmNFR1a, in the non-nodulating Gmnfr1a mutant resulted in increased nodule numbers, even in acidic conditions

- Currently planning a transcriptome analysis using a deep-sequencing approach to identify pH-regulated genes required for nodulation

- Low pH is inhibitory to nodule development in soybean

Transferring soybean plants from pH 7 to pH 4 within 12-48 h of B. japonicum inoculation inhibited nodulation; after 48 h nodule numbers were not significantly affected