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Funding Source: Plant Genomics Internship @ MU

Localization of fw2.2 mRNA expression in soybean root

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Soybean and Rhizobia soil bacteria have a symbiotic relationship that results in nitrogen fixation, the process in which nitrogen is converted from N_2 to ammonia (NH_3). The bacteria induce the expression of genes responsible for the organogenesis of root nodules, the organ in which fixation occurs. Nitrogen fixation only occurs in a nitrogen-poor soil environment. The *fw2.2* soybean gene was found by microarray and qRT-PCR to be upregulated during nodule development. The function of soybean *fw2.2* is unknown. In tomato, the *fw2.2* gene was found to negatively regulate cell division, resulting in fruit size changes of up to 30 percent. In the current studies, *in situ* hybridization was used to develop a time course of spatial expression of *fw2.2* in soybean root. The technique used a digoxegenin (DIG)-labeled RNA probe recognized by a DIG-specific antibody coupled to alkaline phosphatase. Colorometric detection of alkaline phosphatase activity was then used to visualize the cellular location of *fw2.2* mRNA. Both antisense RNA (T3 polymerase) and sense RNA (T7 polymerase) probes were used to differentiate between specific and non-specific staining. Further work will be done to determine the expression of soybean *fw2.2* in roots over time as well as in seeds and root tips. We are currently optimizing the *in situ* hybridization technique to better illustrate the dynamics of *fw2.2* expression in soybean and to further understand the biological function of *fw2.2.*