Project Title: Using soybean microbes to enhance the growth and resilience of soybeans

Objective 1. Evaluate microbial isolates and microbial consortia for improving soybean stress tolerance

In this reporting period we developed and deployed a drought screening protocol for microbes. The protocol involved subjecting a drought-sensitive soybean genotype to a two-phase drought treatment consisting of (i) reduction of soil moisture to 40% field capacity (FC) and (ii) complete withholding of irrigation once plants reached the unifoliate stage. Microbial strains were screened that had been isolated from soybean rhizosphere communities under drought and represented microbial taxa that were known to be enriched in soybean roots under drought. After introducing microbes as seed inoculants, the impact of the microbes was evaluated based on plant shoot height, shoot weight, and two measures of photosynthetic performance, quantum yield (photosystem II efficiency) and electron transport rate, all of which were significantly lower for drought-stressed than non-stressed plants in the assay.

Among the 12 microbial isolates tested, a single *Streptomyces* isolate showed promise at mitigating drought stress based on modest increases in shoot weight and both measures of photosynthetic performance compared with uninoculated, drought-stressed control plants. However, this strain did not provide consistent improvement in plant performance in subsequent repeated experiments. In addition to individual isolates, 12 mixtures of microbes, termed microbial consortia, were constructed, with 11 consortia constructed based on their relatedness at the genus and family levels and one consortium with >30 diverse microbes. None of these consortia provided significant improvement in plant performance under drought. Given the labor-intensive nature of the assay and the lack of promise based on these results, we shifted subsequent efforts to other aspects of the project.

Objective 2. Explore the influence of reactive oxygen species (ROS) on interactions within the root microbiome

We hypothesize that stress-induced systemic ROS in soybean enrich for specific microbes by killing those that are not ROS tolerant. To evaluate this hypothesis, we characterized collections of microbes isolated from drought-stressed and non-stressed soybean roots for their tolerance to ROS using hydrogen peroxide as a model ROS compound. Based on measurements of the minimum inhibitory concentration (MIC) of hydrogen peroxide for each strain, we classified 39 strains as ROS tolerant and 55 strains as ROS sensitive. The majority of the ROS tolerant strains were in the class of bacteria that was most strongly enriched in drought-stressed soybean roots, namely the *Actinomycetes* (Fig 1 in attached report). These results support the possibility that bacterial ROS tolerance enhances persistence in drought-affected rhizosphere environments, and highlights the potential for antioxidant strategies to improve the growth, persistence, and/or performance of microbial inoculants, such as *Bradyrhizobium* spp., in roots with accumulated ROS.

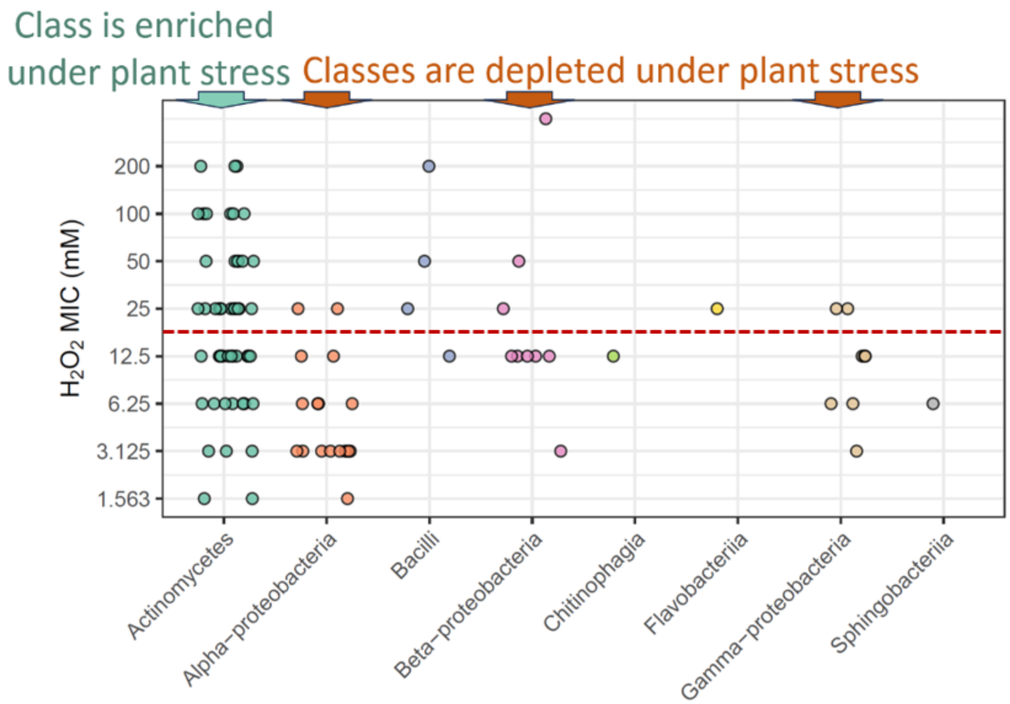

Fig. 1. The ROS tolerance of >90 soybean root isolates based on their minimum inhibitory concentration (mM H_2O_2) when grown in liquid medium with hydrogen peroxide. The dashed red line indicates the demarcation for classifying bacteria as ROS tolerant versus ROS sensitive (above and below the line, respectively).

Objective 3. Use of ROS-based strategies to improve the efficacy of *Bradyrhizobium* inoculants under stressful environmental conditions.

Nodulation of soybean is known to be negatively impacted by drought stress. We quantified this impact by evaluating the growth and nodulation properties of soybean plants with and without inoculation with *Bradyrhizobium diazoefficiens* USDA110. The plants were subjected to two levels of drought, modest (40% FC) and severe (20% FC), and also to well-watered conditions (80% FC). Although inoculation significantly increased the shoot weight of the plants under each of the conditions as compared to the uninoculated plants, the number of nodules was reduced and the nodule mass was dramatically reduced under the drought conditions as compared with well-watered inoculated plants (Fig 2, see attached report). The negative impact of water deficits on nodule development highlights the opportunity to improve nodulation under these conditions, and this could be done by exploiting the ability other microbes to improve inoculant performance.

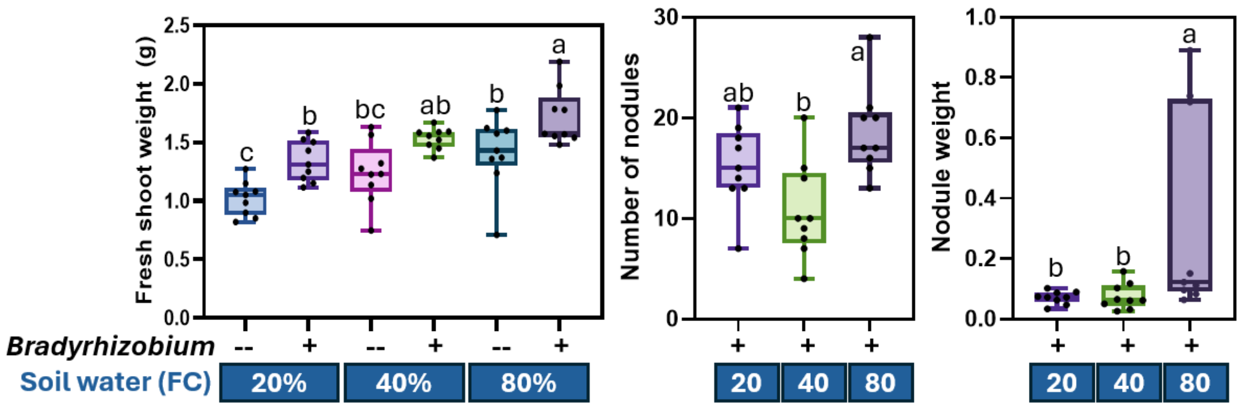

Fig. 2. Impact of seed inoculation with *B. diazoefficiens* USDA110 on soybean plant growth (fresh shoot weight) and nodulation (number of nodules and nodule weight) in plants grown with two levels of soil water deficits (20 and 40% FC) and water replete conditions (80% FC). FC, field capacity.

We evaluated the potential for antioxidants to improve the growth of *Bradyrhizobium* strains. We first tested six *Bradyrhizobium* strains for their sensitivity to oxidative stress and found that they were all highly sensitive (MIC values for hydrogen peroxide of 3 to 6 mM). We then evaluated the impact of exogenous antioxidants on *Bradyrhizobium* growth at various levels of stress, finding that the antioxidants glutathione and Trolox provided some protection, whereas the antioxidant ascorbic acid did not.

Microbes can confer protection to other microbes against oxidative stress via a variety of mechanisms beyond antioxidant production. We are currently developing a screen to evaluate the direct impact of microbes on the growth of *Bradyrhizobium* using an engineered *Bradyrhizobium diazoefficiens* (previously *B. japonicum*) strain that exhibits fluorescence based on expression of a green fluorescent protein (Ledermann et al. 2015). We are optimizing this screen to identify microbes that can enhance either the growth of *B. diazoefficiens* in the absence of stress, or its growth and/or stress tolerance in the presence of oxidative or salinity stress. The assay is rapid, as *B. diazoefficiens* growth is monitored based on fluorescence, overcoming the necessity of differentiating *B. diazoefficiens* from the candidate helper strains when co-cultured in microplates. By screening ROS-tolerant and/or potential antioxidant-producing *Actinomycete* strains from drought-stressed soybean roots, along with a broad collection of other soybean root isolates, our goal is to identify microbial helper strains that can enhance the resilience of *Bradyrhizobiu*m inoculants on roots under soil water deficits and potentially other stressful conditions.

References

Ledermann, R., I. Bartsch, M.N. Remus-Emsermann, J.A. Vorholt and H.-M. Fischer. 2015. Stable fluorescent and enzymatic tagging of *Bradyrhizobium diazoefficiens* to analyze host-plant infection and colonization. *Mol Plant-Microbe Interact* 28:959-967.