

Directed Plant-Microbiome Evolution for Improved Crop Drought Tolerance

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Background/Objectives. Microbiome optimization is increasingly seen as a solution to improve the performance of biological systems from humans to plants and ecosystems. Yet, owing to challenges in finding and cultivating microbiomes that maintain their function in field conditions, use of microbes to improve plant productivity or crop stress tolerance has not become widespread despite years of trials. Directed evolution is a method used to in biochemistry to control protein and enzyme production of microbes or microbial consortia to fit human industrial needs, as well as for studying fundamental evolutionary principles in controlled conditions. Based on the strong interactions and interdependency of rhizosphere microbes and plants, directed evolution has been suggested as a means for developing microbiomes that influence plant traits in a desired way. It is assumed that the plant will apply selective pressure to the rhizosphere microbiome, enriching the microbes that induce desired traits in the plant when propagated. So far, directed evolution approaches have been successfully applied to alter flowering time in *Arabidopsis thaliana*, delay wilting in wheat grass, and improve salt tolerance of *Brachypodium distacyon*. In this study, our objective was to develop a directed evolution protocol for producing microbiomes that can improve water use efficiency and alter stomatal closure point of maize. We designed an experiment to test the hypotheses that 1) natural soil microbiomes can be altered to affect plant traits in a desired way, and 2) microbiomes can be developed to influence plant physiological traits such as water use efficiency (WUE) and stomatal control point (SCP) in addition to structural traits such as root growth, or plant height. WUE and SCP were selected because they are plant functional traits related to water use and plant productivity, and modifying them would allow growing productive crops with reduced irrigation.

Approach/Activities. To test our hypotheses, we cultivated maize plants from seed (B73 variety) in an artificial soil (Fritted clay). In the first generation, the plants were divided into three treatment groups. The first group was inoculated with a microbiome originating from a ponderosa pine forest in Los Alamos, New Mexico, the second, inoculated with a microbiome from an USDA-ARS maize field in Akron, Colorado, and the third, a control group, with no inoculation. The plants were grown in a greenhouse under mild drought to impose selective pressure for drought tolerance for 9 weeks (9th leaf), at which point functional traits (WUE and SCP), and structural traits (height, leaf number, stem diameter, root biomass and leaf mass per area) were determined, and samples of the soil microbiome were collected for analysis. After two generations of propagation of the microbiomes with no selection, the microbiomes of three plants demonstrating the best and worst WUE and SCP values for each microbiome were selected as “parents” of the microbiomes of the initial generation of directed evolution. These microbiomes were then used to inoculate two plants in a subsequent generation of plants under a directed evolution protocol. These plants were harvested after 8 weeks of growth, microbiomes of three best and worst SCP and WUE plants from each soil type were selected as parents for the next generation to propagate the lineage of best or worst SCP and WUE microbiomes. This protocol was repeated for three generations.

Results/Lessons Learned. Our results show that in three generations, the microbiome originating from the forest soil was able to evolve to consistently influence the SCP of the plants, while the microbiome from the agricultural field had no significant effect on either WUE or SCP. The forest and agricultural microbiomes remained distinct from one another throughout the evolutionary process, but the microbiomes adapted to the greenhouse experiment such that the parent and child microbiomes became progressively more similar in subsequent generations. Interestingly, we also found that the microbiome originating from forest soil consistently produced faster growing plants than the microbiome from the agricultural field. This microbiome contained more bacteria related to the nitrogen cycle than the agricultural microbiome, while the agricultural microbiome was more abundant in bacteria commonly found in dry soils. We were unable to consistently identify microbial taxa related to plant traits between generations when using direct correlations between bacterial abundance and plant traits. However, we were able to identify consortia of bacteria related to the plant traits using a dimension reduction method called Latent Dirichlet Allocation (LDA). Our experiment demonstrates that in only a few generations (three as opposed to 6-10 in the previous studies), directed evolution can be used to produce soil microbiomes that influence SCP, an important functional drought tolerance trait in maize, but not all soils may have the microbial diversity or species structure needed for optimizing plant traits.