Biological nitrogen fixation on the aerial roots of sorghum enhances the sustainability of feedstock production

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Modern agriculture is heavily dependent on fertilizer to ensure high crop yields. Chemical fertilizers contain ammonium produced from nitrogen (N_2) and hydrogen (H_2) gas via the Haber-Bosch process at high temperature and pressure. The environmental footprint of ammonium fertilizer is huge due to the large input of fossil energy during synthesis and subsequent fertilizer run-off that contaminates ground- and surface water. A sustainable alternative is biological nitrogen fixation (BNF), which can provide plants with ammonium as a result of interactions with diazotrophic microbes, which use nitrogenase to convert nitrogen from the air into ammonium in a low-oxygen environment. BNF is commonly associated with legumes, but indigenous landraces of maize (*Zea mays* L.) in Oaxaca, Mexico and select landraces of sorghum (*Sorghum bicolor* (L.) Moench) can support nitrogen-fixing bacteria in the mucilage of their so-called aerial roots, adventitious nodal roots that do not penetrate the soil and that can be formed as high as 2 m above the ground. We have shown that sorghum can obtain as much as 40% of its nitrogen needs via BNF. We are using a systems biology approach to investigate the mechanisms enabling BNF in sorghum. A genome-wide association study identified several sorghum loci associated with the number of nodes forming aerial roots. The formation of aerial roots appears to be initiated by modulating the expression of the same genes associated with the formation of sorghum brace roots, which have a similar architecture but are located at the base of the stem and, unlike aerial roots, do penetrate the soil. Even though the diazotrophic bacteria *Klebsiella variicola* and *Azospirillum brasilense* appear to be primarily responsible for the nitrogen fixation, the complexity of the bacterial communities residing on the roots and the variation in bacterial communities between geographic locations implies successful BNF depends on interactions between the plant and a variety of microbial species. In addition to the mechanistic studies, we have developed breeding populations derived from crosses between landraces that produce aerial roots and bioenergy sorghums. Selections are being made on plots that receive only half of the standard level of fertilizer to generate regionally adapted high-biomass cultivars that can be cultivated commercially with reduced nitrogen inputs. These combined approaches are expected to substantially enhance the sustainability of biomass production from both an environmental and economic perspective. *Funded by the U.S. Department of Energy Office of Biological and Environmental Research grant no. DE-SC0021052.*