Title: Leaf Carbon and Nitrogen Isotope Composition in Diverse Sorghum Lines Under Differential Water and Nitrogen Treatments

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Project Goals:
The *overall project goal* is to establish a foundational, systems-level understanding of plant, microbial, and environmental interactions that will lead to strategies for enhancing growth and sustainability of sorghum through genetic and microbial adaptations to water and nitrogen limited environments.

The ***specific objectives*** of the research presented here are to:
1. Conduct phenotypic characterizations of a diverse panel of sorghum genotypes to define photosynthetic and isotope response under water and nitrogen limited environments.
2. Test sorghum genotype by water and nitrogen limited environment interactions in both controlled environment and field growth conditions.
3. Determine if measurements of leaf carbon and nitrogen isotope composition can screen for differences in water and nitrogen use efficiency in diverse sorghum genotypes.

Towards achieving our project goals, we have conducted several phenotyper, greenhouse and field experiments. Our initial phenotyper experiment in 2016 was to screen 30 diverse sorghum lines under controlled environment growth conditions in the Bellweather Phenotyping System at the Danforth Center. This population included 18 energy, 2 grain and 10 sweet sorghum lines. Under the phenotyping system two separate experiments were conducted using a random block design to study the growth, photosynthetic and stable isotope response of this diverse sorghum panel to changes in nitrogen and water availability. Additionally, whole plant nitrogen and water use efficiency were estimated from the phenotyping data. Towards the end of both experiments the upper most fully expanded leaf from individual plant was used for gas exchange measurements with a LI-6400XT open gas exchange system (Li-COR Biosciences, Inc. Lincoln, NE). A portion of the same leaf was sampled for nitrogen and carbon isotope composition ($\delta^{15}N$ and $\delta^{13}C_{leaf}$, respectively).

For these controlled environment experiment data will be presented on differences in nitrogen and water use efficiency, rates of CO_2 assimilation, stomatal conductance, leakiness, intrinsic TE_i and photosynthetic nitrogen use efficiency (PNUE). Measurements of total leaf C/N content and $\delta^{13}C_{leaf}$ and $\delta^{15}N$ will also be presented.

We also collected leaf samples for $\delta^{15}N$ and $\delta^{13}C_{leaf}$ analysis from field experiments in Nebraska in both 2016 and 2017. The western Nebraska field site (Scottsbluff) was used to study response to water stress conditions and the eastern location (Central City) was used to characterize response to low nitrogen. Initial characterization of these data sets suggests significant variation between the sorghum genotypes in their responses to nitrogen and water availability.

To follow up on this previous work, we used a greenhouse experiment to determine the relationship of whole plant water use efficiency (WUE_{plant}) with intrinsic transpiration efficiency (TE_i), defined as the rate of CO_2 assimilation (A_{net}) relative to water loss *via* stomatal conductance (g_s), and $\delta^{13}C_{leaf}$ in sorghum. The TE_i has been considered as a major component of WUE_{plant}. Further, theoretical models in C_4 plants have demonstrated that $\delta^{13}C_{leaf}$ is related to TE_i, when efficiency of CO_2 concentrating mechanism (leakiness) remains constant. Accordingly, $\delta^{13}C_{leaf}$ has been proposed as a high-throughput phenotyping tool for TE_i in C_4 plants. However, there is inadequate information about how leakiness responds to water stress and therefore limits the application of $\delta^{13}C_{leaf}$ for TE_i and thereby WUE_{plant} screening in C_4 crops. The aim of these experiments was to determine response of leakiness to short- or long-term water stress and to revisit the relationship of $\delta^{13}C_{leaf}$ with TE_i and WUE_{plant} in the C_4 bioenergy sorghum line (Grassl). Our results demonstrated that the leakiness is not responsive to short- or long-term water stress. Yet, $\delta^{13}C_{leaf}$ was uncorrelated with TE_i under short- and long-term water stress conditions, whereas $\delta^{13}C_{leaf}$ showed a significant negative relationship with WUE_{plant}. This suggests that in contrast to WUE_{plant}, the steady-state measurements of TE_i do not capture time-integrated responses to water stress. The fact that leakiness is not responsive under water stress suggests that the time-integrated signal of $\delta^{13}C_{leaf}$ can be used as a phenotyping tool for $\delta^{13}C_{leaf}$ in this bioenergy sorghum.

To scale up our results on the relationships between $\delta^{15}N$ and $\delta^{13}C_{leaf}$ with leaf nitrogen and water use efficiency, respectively, we conducted a nitrogen limitation and water stress experiment with three diverse genotypes at Greenhouse Innovation Complex Phenotyper Facility, UNL. The goal of this work was to establish mechanistic link between $\delta^{15}N$ and nitrogen use efficiency (NUE) and scale $\delta^{13}C_{leaf}$ from additional sorghum lines to WUE_{plant}. Our results suggested significant variation between the sorghum genotypes and their responses to nitrogen uptake and use efficiency as well as differences in WUE_{plant}.

Future directions

Leaf level and whole plant traits will be assessed across genotypes in response to both changes in nitrogen and water availability. This information will be analyzed in comparison to field grown material to help identify and select for genomic traits and potentially elite lines for enhanced nitrogen and water use efficiency in sorghum.

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