The Host Genetics and Systems Biology of AMF and other Sorghum Root Microbes

Jeff Bennetzen

Department of Genetics
University of Georgia

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Arbuscular Mycorrhizal Fungi (AMF) - Host Interactions

Developing a model systems biology dataset and framework for understanding their interactions in an agricultural environment, with a focus on host genetics
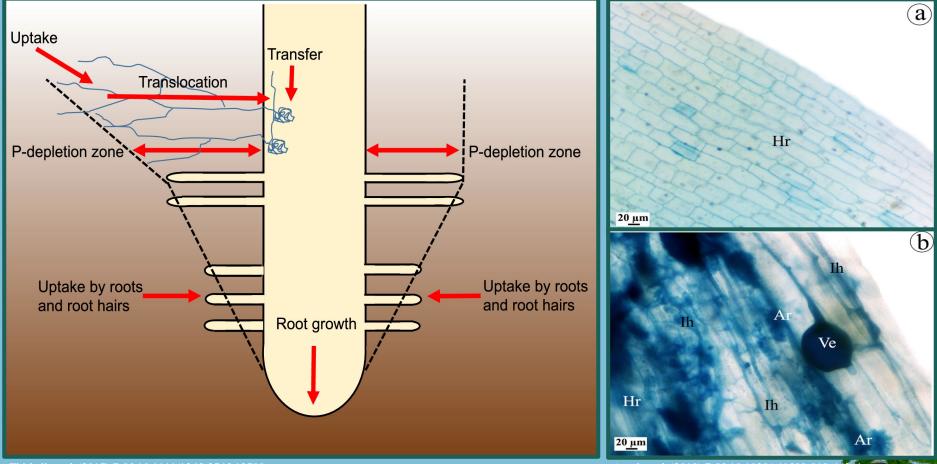
AMF

Diverse members of phylum Glomeromycota, including at least 21 genera with probably many more than the current ~3700 species hypotheses

Ancient association, co-evolving with plant roots over the last ~410 million years

Provides host plants with improved access to soil nutrients, including phosphate and water

Provides resistance to some biotic and abiotic stresses

Arbuscular Mycorrhizal Fungi (AMF)

Uptake

Transfer

Translocation

P-depletion zone

P-depletion zone

Uptake by roots and root hairs

Uptake by roots and root hairs

Root growth

a

Hr

20 μm

b

Ih

Ar

Ve

Ih

Hr

Ih

Ar

20 μm

AMF

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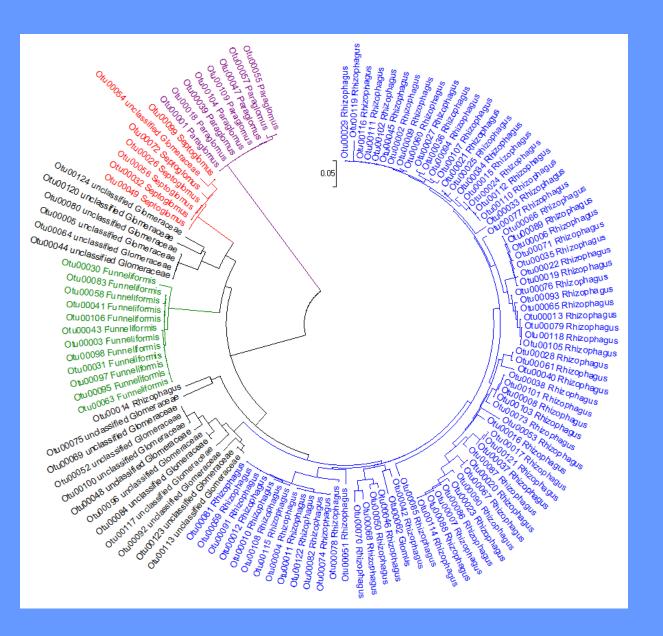
Ancient association, co-evolving with plant roots over the last ~410 million years

Provides host plants with improved access to soil nutrients, including phosphate and water

Provides resistance to some biotic and abiotic stresses

Contributions are context-dependent, varying from wholly beneficial to wholly parasitic, depending on the abiotic and biotic environment

Mycorrhizal diversity in switchgrass roots

AMF-Host Associations

Are host-species specific

Our previous switchgrass results indicate genotype-specificity within a species

Relative abundance of mycorrhizal phylotypes in selected switchgrass genotypes

Genus	317										458											
	1.BC	1.BF	1.TC	1.TF	2.BF	2.TC	2.TF	3.BC	3.TC	3.TF	1.BC	1.BF	1.TC	1.TF	2.BC	2.BF	2.TC	2.TF	3.BC	3.BF	3.TC	3.TF
Paraglomus	3	17	4	31	28	38	3	90	46	6	623	553	928	61	869	644	402	350	914	898	950	176
Rhizophagus	1354	1348	1361	1314	114	214	132	1099	1300	1326	489	436	260	13	307	190	144	502	242	189	203	57
unclassified_Glomeraceae	0	0	0	0	1280	1282	1354	3	0	0	0	66	26	0	77	152	1	0	35	37	62	0
Funneliformis	2	3	3	0	51	14	1	116	3	0	142	232	110	1241	106	277	782	431	105	238	95	1037

AMF-Host Associations

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Our previous switchgrass results indicate genotype-specificity within a species

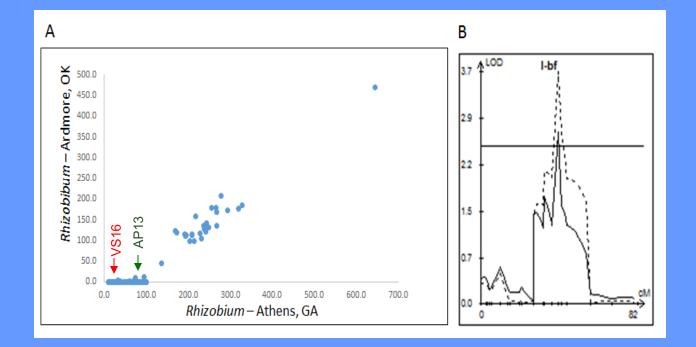**These host variations can be used to map QTL for abundance of particular genera of AMF**

Seven switchgrass QTL mapped, non-overlapping, for five genera of AMF (8%-23% contributions)

No QTL were detected by measurement of total AMF levels

This host genotype specificity is also true for other microbial associations

Some associations show excellent penetrance that is not highly affected by the environment: Mapping a QTL for rhizobium association with switchgrass roots using field data from Georgia and Oklahoma

Sorghum is an important target for lignocellulosic biomass production

High yield under low input on poor soils

Diploid

Highly diverse

Excellent genetic tool set (including genome sequences, mapped populations, diversity panels, RNAseq data, mutagenized stocks)
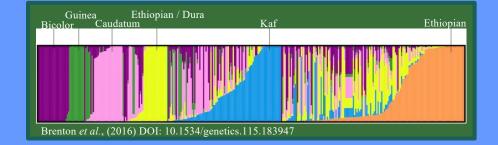
Active research community

Exists in both perennial and annual forms

Also includes sweet-stalk sorghum

Sorghum head type and seed diversity

Will investigate RILs between wild and domesticated sorghum and a 385 member GWAS panel from Kresovich and colleagues

Goals:
Ongoing DOE-Funded Project on Sorghum Germplasm

Elucidate the genomic basis by which sorghum genotypes cultivate specific interactions with AMF and rhizosphere communities, and how microbes develop mutualistic relationships with plants over time and environments.

Detailed Goals

Utilize genetic diversity and comparative genomics to identify key genes/alleles that confer key plant traits to optimize biofuel sorghum for two very different ecoregions of the US – Georgia's piedmont uplands and Arizona's Sonoran desert.

Elucidate the genomic basis by which sorghum genotypes cultivate specific interactions with rhizosphere communities and how microbes develop mutualistic relationships with plants over time and environments.

Measure the influence of plant-associated microbiome composition and dynamics on the fitness and productivity of bioenergy sorghum.

Test hypotheses about how plants differentiate between mutualists and pathogens, and how beneficial communities can be host-created and utilized to deal with abiotic stress.

Hypotheses

A series of integrated field and greenhouse experiments will be used to test four hypotheses:
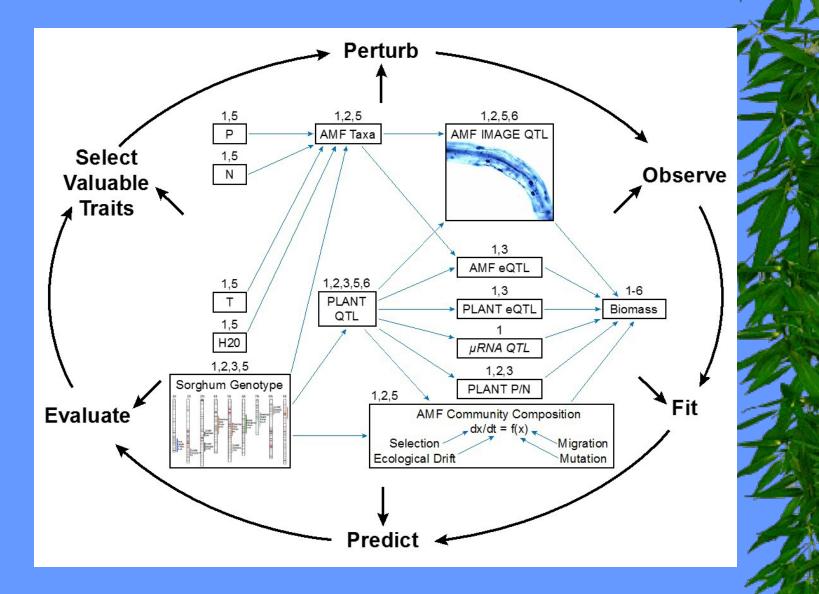
H1: There are sorghum genes whose allelic variation determines AMF abundances and performance, and we can map these genes.

H2: The microbial environment, especially the AMF community composition, influences which sorghum alleles generate beneficial AMF symbioses.

H3: Abiotic variables (e.g., N, P, soil type and water availability) help determine which sorghum genotype-AMF taxa-microbial community interactions are most beneficial to sorghum biomass productivity.

H4: Systems models can be generated to predict which sorghum genotype-AMF-microbiome-environment combinations will provide sorghum farmers the greatest biomass return on investment.

Systems Analysis

Years 1-3 are descriptive and model building

Years 4 and 5 will take identified correlations and test them with hypothesis-based analyses

(*E.g*., does planting of a specific genotype support predicted AMF populations and performance <u>or</u> does mutation of an identified QTL candidate gene lead to predicted changes in AMF associations and functions.)

Experimental Variables

**Sorghum Genotype
(including perennial versus annual)**

Field Location (Georgia vs Arizona)

Fertilizer Input

Drought

Proposed Experiments

To answer the four hypotheses described above, we have developed six subprojects.

To identify sorghum genotypes and genes (both mRNA- and miRNA-encoding) in a sorghum biomass genome-wide association study (GWAS) panel that influence AMF abundances, gene expression from the eukaryotic root community, and sorghum biomass performance, under conditions of variable biotic and abiotic environments.

To investigate the role of perenniality in how sorghum genotypes and genes influence AMF abundances, gene expression from the eukaryotic root community and sorghum biomass performance, under conditions of variable biotic and abiotic environments and over multiple years.

To scan mutagenized and sequenced sorghum populations for mutations in the genes associated with AMF benefits and other traits identified from the GWAS studies, and then test these mutants for the degree to which they confirm observed phenotypic contributions in the GWAS and controlled environment experiments.

Proposed Experiments (continued)

To iteratively build systems models, including structural equation models (SEMs), using the results from GWAS, host performance, microbiome, morphological, microscopic and transcriptome studies, and to iteratively test these models on field data.

To investigate microbiome assembly mechanisms and validate modeled effects on plant performance under specific input regimens using a subset of sorghum and AMF genotypes.

To develop, test and utilize imaging tools for high throughput visualization of root structural properties and nutrient flow in the AMF-sorghum root interaction.

Ultimate Applied Goal

Identify sorghum genotypes that attract and maintain the optimal AMF and overall microbiome, thus decreasing need for inputs (e.g., fertilizer, irrigation) without any added inoculum.
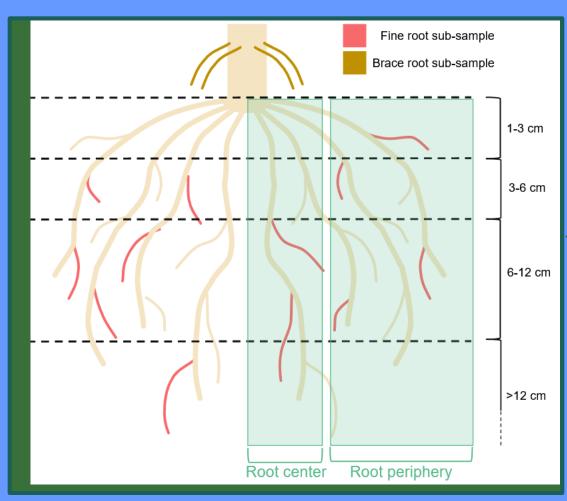
2021/2022 Root Data Being Generated

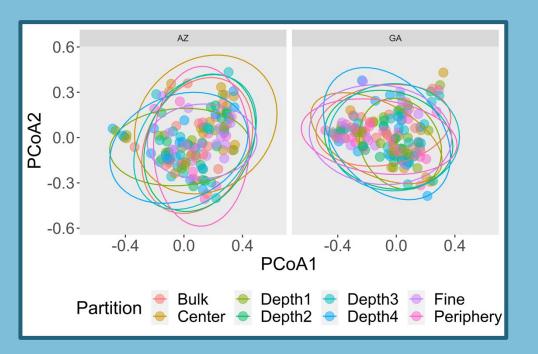Conduct time course for AMF/full microbiome establishment

Measure full microbiome associations with AMF and sorghum under all conditions

Describe plant and microbiome (especially AMF) expression patterns during interactions by RNAseq analysis

Automation/implementation of microscopic scoring of AMF in root

Root-Specific Differences in Microbiome (including AMF) Abundances and Functions

Principal Co-ordinates Analysis (PCoA) plots of AMF communities separated by partition.

Single points represent an aggregate value for each sample based on Bray-Curtis dissimilarity values (distance matrix) calculated pairwise between every sample. Points which are further away from each other are *more dissimilar*.

Ellipses represent 95% confidence intervals of each partition.

There is no visual clustering of samples by partition

Model: dist ~ (Partition x Genotype) + (Partition x Timepoint) + (Partition x Site)

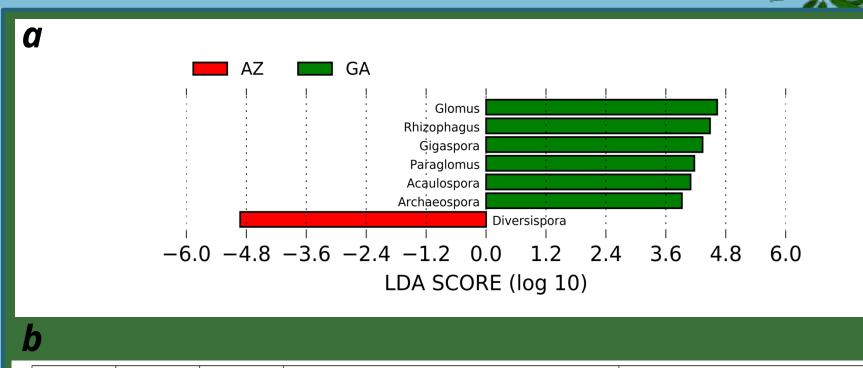| Compar-ison	Df	SumOfSqs	R²	F	P-value
Partition	7		0.018		0.88
Genotype	2		0.017		**0.01 ****
Site	1		0.064		**0.01 ****
Timepoint (50 – 90 days post planting)	2		0.010		0.08
Partition x Genotype	14		0.040		0.89
Partition x Site	7		0.027		0.07
Partition x Timepoint	14		0.039		0.90
Residual	241		0.784	-	-
Total	288		1	-	-

Permutation Analysis of Variance (PERMANOVA) analysis was performed on the Bray-Curtis dissimilarity matrix calculated from all samples with >500 reads.

The constructed model considers only interactions between partitions and genotypes and not the fourway interaction of partition x genotype x timepoint x site because there is not adequate replication for this analysis due to sample loss in curation.

The R^2 value shows the ***proportion of variation*** that can be attributed to the model parameters. This is important as even if a parameter is "significant" at p<0.05 it may not have a very strong explanatory power.
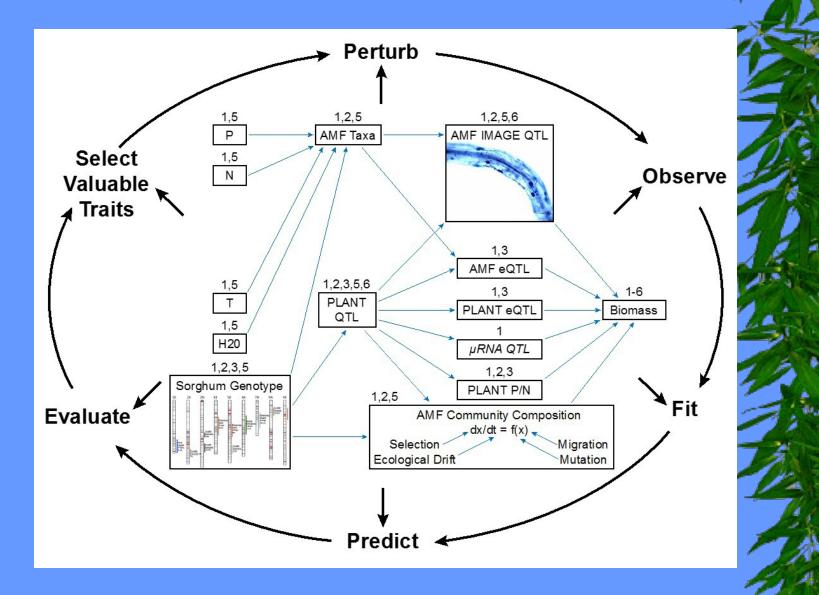
Differential Abundance Analysis

a

b

	Paraglomerales	Archaeosporales	Claroideoglomerales	Diversisporales				Glomerales		
	Paraglomus	Archaeospora	Claroideoglomus	Acaulospora	Diversispora	Scutellospora	Gigaspora	Glomus	Rhizophagus	Funneliformis
AZ	0.019	0.00002	0.029	0.00043	0.291	0.207	0.029	0.194	0.206	0.024
GA	0.034	0.00155	0.027	0.00301	0.13	0.18	0.068	0.264	0.278	0.015

Georgia 2022 field to test N and P effects: 4 treatments, 12 plots, >4000 samples

Systems Analysis

Collaborations

UGA Crew

Philip Brailey-Jones
Srini Chaluvadi

Olivia Asher
Josue Fernandez
Ahmad Kabir
Ben Long
Tom Pendergast

Katrien Devos – Univ. Georgia

Jonathan Arnold – Univ. Georgia
Anny Chung – Univ. Georgia
Nancy Collins Johnson – Northern Arizona Univ.

U.S. DEPARTMENT OF ENERGY