Project Title: "Genomics of Energy Sorghum's Water Use Efficiency/Drought Resilience"
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ACCOMPLISHMENTS:
AIM 1: Screen energy sorghum for variation in traits that have the potential to improve water use efficiency, soil water extraction, and drought resilience.
1.1 Development of rhizotron lysimeters.
Rhizotron lysimeters were developed to measure variation in plant water use efficiency, root traits, and responses to water deficit. A range of rhizotron diameters (15-40cm), depths (50-150cm) and soil volumes were tested to identify rhizotron dimensions that replicate to the extent possible soil conditions and root growth responses observed in the field. It was found that rhizotrons (20-25cm x 75cm) filled with field soil allowed replication of root growth and responses to water deficit observed in the field for the developmental phase from 21DAE to 60DAE (post juvenile, early vegetative phase). Significant genotypic variation in sorghum root growth response and water use rates following imposition of water deficit (WD) were detected using this system. A gantry system was installed to enable rhizotron weighing (water use) and for deployment of optical sensors (cameras, thermal sensors) above plants to improve the detection of changes in plant responses such as stomatal closure during the day and leaf growth rate variation. Sensors for measuring soil temperature and oxygen were deployed in the field and in rhizotrons to better explain variation in rooting depth and root growth responses to intermitted irrigation/rainfall followed by periods of soil water drying and deficit. Rhizotrons were also developed that allow control of soil temperature and oxygen at specified depths in the soil profile. These capabilities enaled sorghum genotypes to be screened for variation in root and shoot growth under conditions of water deficit, temperature constraint, and hypoxia.
1.2 Root system growth and phenology analysis (field and controlled environment rhizotron)

A time course of root system development from germination through 20 days post anthesis (87 DAE) was obtained for two of the parental genotypes (BTx642, Tx7000) used to produce RILs for QTL analysis. Root system growth stopped earlier in Tx7000 compared with BTx642, coincident with earlier anthesis. Root system development of these early flowering genotypes under field conditions was assayed through grain maturity. A similar developmental time course of root system development was collected from the energy sorghum R07020 in the field. The analysis showed that the size of energy sorghum root systems is much larger that root systems of grain sorghum. Early in the season energy sorghum root systems were located in the top 20-30cm of the soil profile while in rhizotrons, root systems could reach 150cm depth by 52 DAE. Experiments revealed that soil temperature and oxygen levels inhibit deep rooting early in the growing season.

1.3 Root system growth response to temperature

Sorghum is not planted until soil temperatures increase above 60F. Sorghum root growth in soils of different temperature (under ambient air temperatures >70F) confirmed that root growth is increasingly inhibited at soil temperatures below 65F. This is a constraint on root system development in the early portion of the season in many locations.

1.4 Root system growth under hypoxic conditions

Sorghum root systems remain in the upper portions of soil profiles for 40-60 days in College Station Texas in some seasons. We found that in some fields/seasons oxygen levels were low in soil more than 30cm below the surface and following rainfall oxygen levels decreased throughout the soil profile. Analysis of root growth in rhizotrons engineered with poor drainage (and hypoxic soils) at specific depths in the soil profile showed that sorghum roots do not grow into these hypoxic soils. This discovery helps explain the distribution of roots in field soil profiles early in the season and provides a way to screen for genotypes that can grow to greater soil depths in soils with poor drainage. Once soil profiles drain, energy sorghum root systems can reach 2m in depth.

1.5 Root system growth response to water deficit

Diverse sorghum genotypes were grown for 21-32 days under well-watered (WW) conditions in rhizotrons then with no additional water for an additional 27 days (WD). Some genotypes continued rapid development and increased root system size and branching (lateral roots) in response to WD whereas other genotypes slowed growth and used water at a slower rate increasing the time before symptoms of water deficit (wilting, leaf rolling, leaf senescence) appeared.

1.6 Root transcriptome analysis

Roots (lower growing zone, upper portion of roots) were isolated from plants during the juvenile, vegetative, and reproductive phases of development from BTx623, the genotype used to construct the reference genome sequence. RNAseq transcriptome profiles were collected and analyzed in collaboration with JGI (McCormick et al., 2018). Transcriptome profiles from a developmental progress of nodal roots was also collected and RNAseq data will be generated by JGI and added to the sorghum RNA Atlas (Phytozome).

1.7 Analysis of root hairs of sorghum seedlings grown in aeroponics.

The Libault lab uses an aeroponic system to analyze variation in the density and morphology of sorghum root hair cells among genotypes and in response to drought stress. To enhance the microscopic observation and measurement of root hair cells, the sorghum root system is being stained in a 4% trypan blue solution to maximize contrast. Root hair cell density and morphology was recorded from 3 different locations on the root system (i.e., proximal to the shoot, median to the root system, and proximal to the root tip) using ImageJ software. *Sorghum bicolor* (L.) Moench BTx623 plants were used to establish a water deficit treatment using the ultrasound aeroponic system.

Root architecture analysis: After 35 days under well-watered (WW) conditions in the aeroponic system, we induced drought stress by progressively decreasing the production of nutritive mist. Plants growing under WW (638 ml/24 hours) and water deprivation (WD; 392 ml/24 hours) conditions were then grown for 14 days. Using Winrhizo-Pro, we analyzed the architecture of the root system of the two parental lines of the BTx642/RTx7000 population as well as 50 different RILs (i.e., on average 5 seedlings per RIL or parental line, and per growing condition). We collected information about the total root system length, surface area, root average diameter, length per volume, root volume, lateral root length, and number of tips on the root system. First, our results indicate that morphological root traits as root volume, root surface and diameter could be advantageous traits for sorghum plants when resisting to drought conditions. Our results also showed significant variation of root length between the RILs under WW and WD conditions (pairwise t-test, p-value < 0.05). Notably, RILs 12CS4136, 12CS4145 and 12CS4158 clearly show an improvement of the size of their root system in response to drought stress (Figure 3, orange dots). Oppositely, the root system of 12CS4187 is less developed when grown under DR condition compared to WW condition.

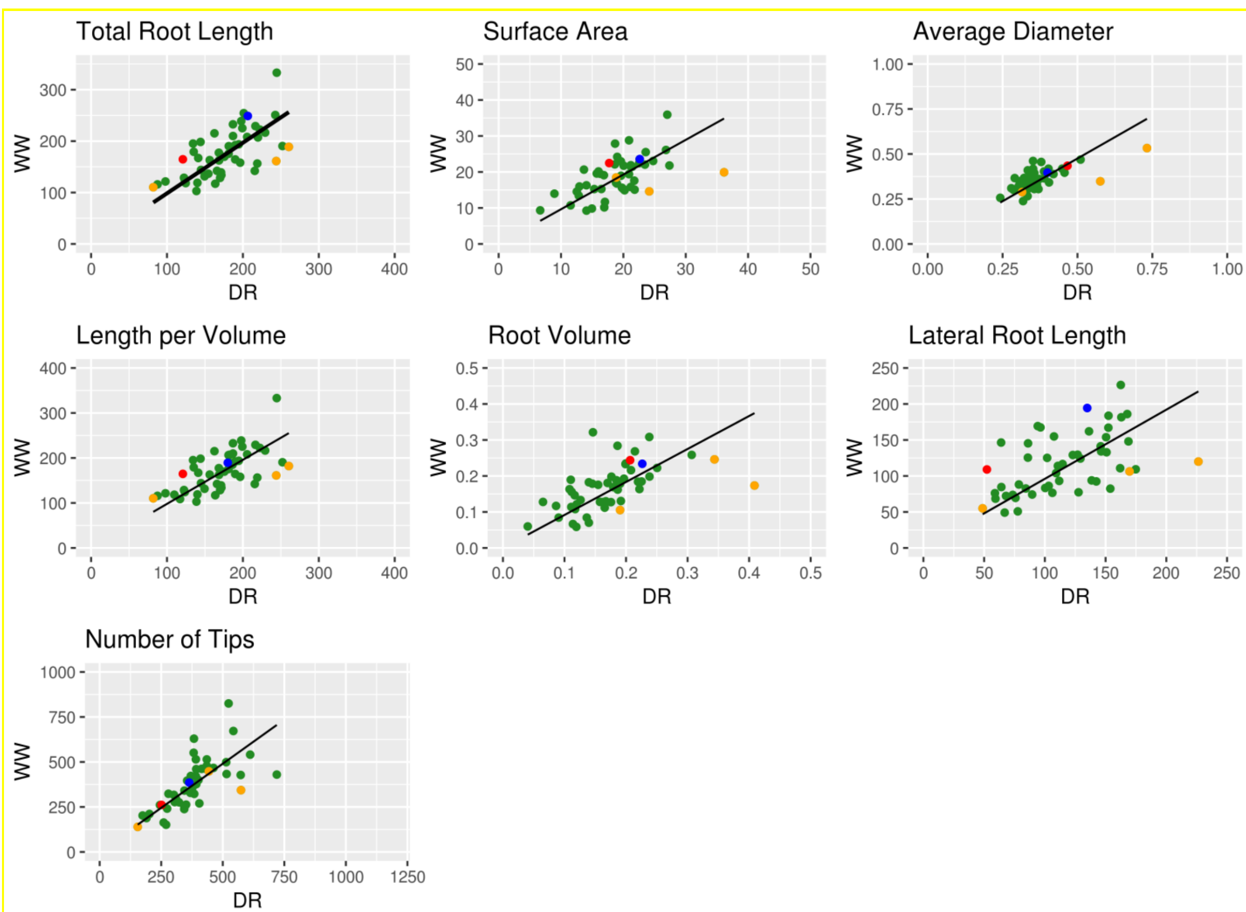

Figure 1. Correlation between the DR (x axis) and WW (y axis) values of each RIL for 7 different morphological root traits. The black line represents the regression line. The parental lines are indicated in red (BTx642) and blue (RTx7000). RILs are represented with green and orange dots.

Root hair length and density analysis: Upon microscopic observation of the RILs' root hairs and ANOVA analysis of their morphology with ImageJ software (http://imagej.net), we observed that the root hair density of the 2 parental lines (i.e., BTx642 and RTx7000) increases in response to WD. In addition, the root hair lengths of the 12cs4148 and 12CS4176 RILs are significantly different compared to 12CS4205, 12CS4204, and 12CS4169 RILs. Root hair density is also different between the 10 RILs analyzed. Taking in consideration drought treatments, we noticed that root hair density and root hair length fluctuate between genotypes (e.g., 12CS4185 is lacking root hairs when growing under WD while root hair density of 12CS4138 increases in response to WD. 12CS4188 have shorter root hairs when plants grow under WW condition compared to WD. 12CS4149 RH are showing an opposite trend: longer root hair cells in plants growing under WW condition). The root architecture and root hair length/density measurement of BTx642/RTx7000 RILs' population will continue with the objective to record root phenotypic variations between the entire RILs populations. These data are currently analyzed.

Establishment of the sorghum root hair transcriptomic and epigenomic responses to drought stress: In collaboration with the JGI (Community Science Program), we are establishing the transcriptional and epigenomic profiles of the sorghum root hair cell under well-watered and water deficit [(*Sorghum bicolor* (L.) Moench BTx623]. RNA-seq, Methyl-seq and smRNA-seq technologies are being used to analyze the transcriptome, methylation pattern of the gDNA and the qualitative and quantitative analysis of the root hair smallRNAs of the sorghum root hairs. The sorghum root hair transcriptional profile has also been established. It will be soon compared with the transcriptome of sorghum root hair cells growing under drought conditions. These transcriptomic datasets will also be analyzed in an evolutionary context by performing comparative genomic and transcriptomic analysis between plant root hair cells isolated from various mono- and di-cotyledons. The gDNA methylome is also currently analyzed. The set of root hair smallRNAs is currently sequenced by the JGI. We are expecting that at least 2 to 3 manuscripts relevant to this project will be generated.

AIM 2: Identify QTL and characterize gene regulatory networks that modify traits that improve water use efficiency, soil water extraction, and drought resilience.

2.1 Genetic analysis of root morphometric traits: QTL for morphometric root traits segregating in several sorghum RIL populations grown in rhizotrons were identified using root phenotypes generated by analysis of roots after washing using WINRHIZO. One of these populations was grown in the field and root systems excavated, imaged and the data used for QTL analysis.

2.2 Root angle QTL mapping: Root angle QTLs were identified in 3 sorghum populations by measuring root angles of plants grown for 21 DAE. Fine mapping of a major QTL is in progress.

AIM 3: Analyze the impact of sorghum traits that enhance water use efficiency, soil water extraction, and drought resilience under field conditions.

3.1 Genetic variation for WUE, SWE and DR in energy sorghum RILs under field conditions. An energy sorghum RIL population (R07018*R07019) was grown in the field and root systems excavated and analyzed using WinRhizo. The results were used to identify QTL analysis of root traits under field conditions. Several main effect QTL for root depth, root branching, and root size (nodal root number, biomass, length, surface area) were identified.

3.2 APSIM modeling of energy sorghum growth, water use, and root systems: The APSIM sorghum crop modeling framework developed by Graeme Hammer and his colleagues was adapted to help understand the response of energy sorghum to water deficit and to identify traits that could enhance biomass yield of this crop. APSIM was modified using parameters for energy sorghum. The modified model was able to predict the time course of biomass accumulation under field conditions with reasonable accuracy. The APSIM for energy sorghum was used to model the value of VPD limited transpiration trait (Truong et al., 2017).

Publications:

Libault M and Chen S **(2016)** Toward Plant Single Cell Type Systems Biology. **Frontiers in Plant Science**. 7:35. doi: 10.3389/fpls.2016.00035.

Libault M, Pingault L, Zogli P, and Schiefelbein J **(2017)** Plant Systems Biology at the Single-Cell Level. **Trends in Plant Science.** doi: 10.1016/j.tplants.2017.08.006.

McCormick, R.F. Sandra K. Truong, Avinash Sreedasyam, Jerry Jenkins, Shengqiang Shu, David Sims, Megan Kennedy, Mojgan Amirebrahimi, Jane Grimwood, Brock Weers, Brian McKinley, Ashley Mattison, Daryl Morishige, Jeremy Schmutz and John Mullet (**2018**) The *Sorghum bicolor* reference genome: improved assembly and annotations, a transcriptome atlas, and signatures of genome organization. **The Plant Journal** 93: 338-354.

Nourbakhsh-Rey M, and Libault M **(2016)** Decipher the molecular response of plant single cell types to environmental stresses. **BioMed Research International.** 2016:4182071. doi: 10.1155/2016/4182071.

Pingault L, Zogli P, Brooks J, and Libault M **(2018)** Enhancing phenotyping and molecular analysis of plant root system using ultrasound aeroponic technology. **Current Protocols in Plant Biology**. 3(4):e20078.

Truong, S.K., McCormick, R.F., Mullet, J.E. (**2017**) Bioenergy Sorghum Crop Model Predicts VPD-Limited Transpiration Traits Enhance Biomass Yield in Water-Limited Environments. **Frontiers in Plant Science 8:** doi: 10.3389/fps.2017.00335.

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