The Genetic and Biochemical Frameworks Underlying the Energy Value of Sorghum

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Introduction

- Sorghum [Sorghum bicolor (L.) Moench] is a cereal that produces grains rich in carbohydrates, predominantly starch.
- Sorghum grain is important for providing the necessary calories to both humans in Sub-Saharan Africa and Southern Asia, and livestock in the United States.
- Grain yield, composition, and digestibility in cereals all contribute to the amount of usable energy available for food, feed, and fuel uses.
- Genome-wide association studies (GWAS) are currently being utilized to elucidate genomic regions associated with grain number, size, and composition.
- Data show significant variation in grain number per panicle (63-4024), thousand-grain weight (5.36-40.56g) and starch content (53.80-74.07% dry matter) among accessions.
- Understanding the genetic and biochemical frameworks of these complex, quantitative traits will prove beneficial by creating a platform to boost cereal grain yield and value to help compensate for the steady-increasing food demand.

Figure 1. 2013 Sorghum Association Panel (Florence, SC)

Objectives

1. Characterize the natural diversity in traits directly associated with total energy value of grain sorghum per unit area.

2. Identify genomic regions that are significantly associated with these traits.

3. Investigate these regions of the genome for functional candidate genes in high linkage disequilibrium with the single nucleotide polymorphism.

Experimental Design

- Approximately 400 individuals from the Sorghum Association Panel (SAP) developed by Casa et al. (2008) was planted in 2013 (SC2013) and 2014 (SC2014) in Florence, South Carolina.
- These individuals were planted in a complete randomized design (CRD), with each individual planted in a 2 row, 6.1m (20 ft) plot with a row spacing of 0.762m (2.5 ft).
- Three main culm panicles per plot were randomly bagged at date of flowering to prevent bird and insect predation.
- Bagged panicles were harvested at physiological maturity, dried down to constant weight, and threshed by hand to allow for accurate measurement of grain number per panicle.
- The grain was cleaned using an air aspirator (AT Ferrell Company, Inc.) and wheat dehuller, and then run through a Model 900-2 seed counter (Old Mill) to obtain grain number per primary panicle (GNP).
- Thousand-grain weight (TGW) was calculated from total weight of grains divided by total grain number per panicle.
- To determine grain composition, a DA 7250 NIR Analyzer (Perten Instruments) was used on both whole and ground grain to determine starch, amylose, amylopectin, and in vitro starch digestibility percentages. Grain was ground into 1mm particles using a CT 193 Cyclotec (Foss).

Results

SC2013	Maturity	GFD	GNP	Grain Weight	TGW	Head Mold	Starch	Amylopectin	Amylose	Prolamin	IVSD
Maximum	131.5	59.5	4024	72.52	40.56	2.5	74.07	89.92	21.85	6.61	64.49
Minimum	78	15	63.17	1.82	5.36	1	53.8	79.43	4.78	3.22	46.74
Range	53.5	44.5	3960.83	70.7	35.2	1.5	20.27	10.49	17.07	3.39	17.75
Mean	105.16	37.16	1323.34	30.87	22.52	1.23	67.21	83.4	16.94	5.35	49.08

Table 1. Ranges of traits showing the natural diversity that exists in sorghum. Data taken from the average values across two replications in SC2013.

Maturity - days after planting
GFD – grain fill duration, days from mid-anthesis to physiological maturity
GNP – grain number per primary panicle
Grain Yield – total weight of grains per panicle, grams
TGW – thousand-grain weight, grams
Head Mold – visual observation of mold on the panicle, 1=no mold and 5=severe mold
Starch/Amylopectin/Amylose/Prolamin – percent dry matter basis
IVSD – In vitro starch digestibility, percent starch digested using enzymatic assay

SC2013	Maturity	GFD	GNP	Grain Yield	TGW	Head Mold	Starch	Amylopectin	Amylose	Prolamin	IVSD
Maturity	1	0.631	0.124	0.14	0.023	0.132	0.172	0.006	-0.073	-0.092	-0.031
GFD	-	1	-0.041	0.084	0.242	0.257	0.063	-0.009	-0.026	-0.148	0.181
GNP	-	-	1	0.728	-0.226	0.032	0.318	-0.045	-0.1	-0.298	-0.165
Grain Yield	-	-	-	1	0.45	0.058	0.414	-0.169	-0.122	-0.352	-0.138
TGW	-	-	-	-	1	0.11	0.166	-0.173	-0.061	-0.109	0.05
Head Mold	-	-	-	-	-	1	0.022	0.134	-0.06	0.017	0.357
Starch	-	-	-	-	-	-	1	-0.072	-0.216	-0.485	-0.26
Amylopectin	-	-	-	-	-	-	-	1	-0.438	0.345	0.122
Amylose	-	-	-	-	-	-	-	-	1	0.04	-0.171
Prolamin	-	-	-	-	-	-	-	-	-	1	-0.101
IVSD	-	-	-	-	-	-	-	-	-	-	1

Table 2. Correlations among traits using the Pearson method.

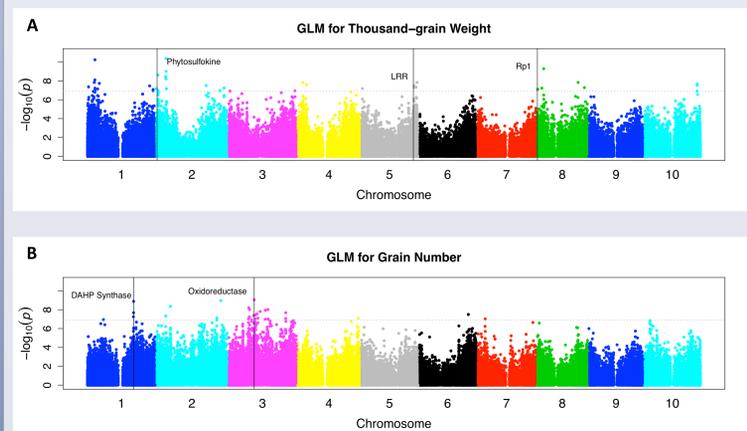**A** GLM for Thousand–grain Weight
B GLM for Grain Number

Figure 2. (A) Manhattan plot displaying associations for thousand-grain weight across the sorghum genome using general linear modeling which does not take into account population structure. Vertical lines correspond to locations of gene candidates. The horizontal dashed line denotes the Bonferroni correction for statistical significance used when performing simultaneous, independent statistical analyses. LRR, leucine-rich repeat. (B) GWAS for grain number per panicle that includes nearly 400 accessions from the SAP. Each dot in the figure represents a SNP. A top candidate gene, DAHP synthase, is the first enzyme in the Shikimate pathway and is critical in regulating the flow of carbon for grain macronutrient production.

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Results

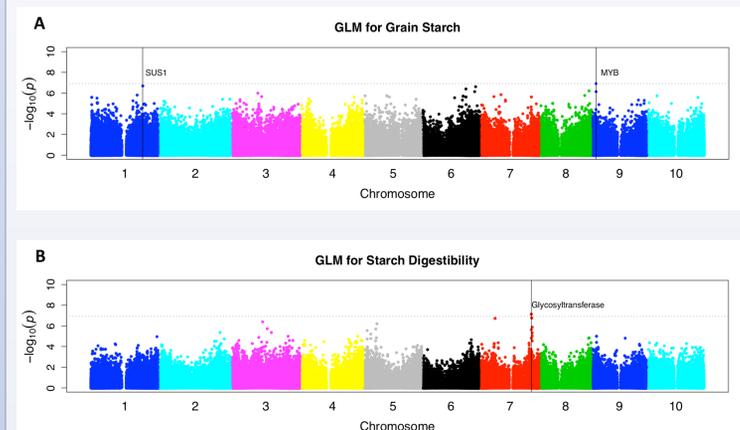**A** GLM for Grain Starch
B GLM for Starch Digestibility

Figure 4. Additional Manhattan plots displaying overall grain starch content and starch digestibility. (A) The SNP with highest significance with grain starch is near a putative MYB-like transcription factor. The second most significant marker on chromosome 1 was in high LD with a gene orthologous to sucrose synthase 1 (SUS1) in maize. SUS1 is responsible for breakdown of sucrose and controls carbon allocation throughout the plant. (B) GWAS for in vitro starch digestibility shows a strong peak on chromosome 7. The peak is near a glycosyltransferase putative transcript which is involved in carbon fixation.

Conclusions

- Ranges from raw data for traits directly influencing energy value reveal enormous natural diversity exists and can be exploited for crop improvement.
- A weak negative correlation found between grain number per panicle and thousand-grain weight along with differences in candidate genes suggest that the biological pathways underlying these yields components can be manipulated independently. This work provides evidence that increasing grain number through proper selection and breeding can be done without compromising weight of the grain, and vice versa.
- Starch content is positively correlated with overall grain yield as well as yield components: grain number per panicle and thousand-grain weight. If the sink of carbon fixation into starch in the grain drives total yield, then it leads to the possibility of increasing starch content and total grain yield simultaneously to generate greater energy yields per unit area.
- Genome-wide association mapping tagged a SNP marker in high linkage disequilibrium with SUS1, suggesting that this gene may be responsible for the fixation of simple sugars into starch and play a role in total grain starch content in sorghum.

Future Work

- Collection and evaluation of the 2014 grain diversity panel data are in progress to provide corroborating evidence toward the trait relationships and candidate gene significance.
- The same phenotypes are also being evaluated in multiple biparental mapping populations that have lines which are segregating for grain number, size, and starch composition. The aim is to properly eliminate false positives produced from the GWAS in the diverse panel (SAP) and provide additional support toward candidate genes.
- Once confirmation of peaks across years is complete, candidate gene sequencing in selected individuals will commence to determine allelic diversity in the germplasm.

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