**Additional file 4.** Supplementary methods for the identification of genes involved in primary metabolism and sugar transport.

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**Batch effect normalization for expression matrix**

To normalize potential batch effects between the three RNA-seq studies, gene expression data using RPKM values were prepared. Expression datasets using RPKM values for the first and second RNA-seq experiments were reported previously. For the third RNA-seq data set, raw reads were obtained and subjected to quality check according to Mizuno *et al.* [51]. Filtered reads were mapped to the sorghum reference genome (BTx623, Sbicolor\_v2.1\_255) using TopHat v2.0.14 (a maximum mismatch of 9 bp and default settings for other parameters) [13,73]. Read counts were calculated using ‘HTseq’ with uniquely-mapped reads and RPKM values were calculated for SIL-05 [74]. All the three datasets were normalized using RPKM. Genes with RPKM values ≥1 were considered as expressed genes. The distribution of gene expression levels (log2-transformed RPKM+1) were compared (Fig. S2a). Quantile normalization (calculated by the R package “preprocessCore”) was used to remove batch effects between the three datasets [75-77], resulting in similar distribution of gene expression levels between RNA-seq samples (Fig. S2b). Sorghum reference genes for qRT-PCR had smaller coefficient of variance after removal of batch effects (Additional File 3).

**Identification of genes involved in primary metabolism and sugar transport**

***Genes involved in cell wall metabolism.***

Genes related to cell wall metabolism were identified according to Rai *et al.* (2016). Several gene families in phenylpropanoid and monolignol pathways were characterized in sorghum with phylogenetic and functional approaches, including CAD (Saballos *et al.*, 2009; Jun *et al.*, 2017), CCR (Barakat *et al.*, 2011; Sattler *et al.*, 2017), CCoAOMT (Walker *et al.*, 2016), COMT (Green *et al.*, 2014), HCT (Walker *et al.*, 2013), PAL (Jun *et al.*, 2018) and 4CL (Saballos *et al.*, 2012). Categorization of the cellulose synthase (*Csl*) gene superfamily, including several families of Csl-like families, was identified according to a systematic phylogenetic-functional study (Little *et al.* 2018). The functional mixed-linkage (1,3; 1,4) β-glucan (MLG) synthase of the CslF6 family was reported elsewhere (Dimitroff *et al.* 2016). Other families of cell wall-related genes were collected elsewhere (Carpita *et al.*, 2001).

***Genes involved in starch and sucrose metabolism, and glycolysis***

Different sources, including Plant Metabolic Network (PMN, Chae *et al.* 2014), KEGG (Kanehisa *et al.* 2015), MapMan (Usadel *et al.* 2009) and Campbell *et al.* (2016), were integrated for annotating genes related to starch, sucrose metabolism and some steps of glycolysis. To increase gene annotation accuracy, maize gene models with proteomics evidence were used (Friso *et al.* 2010) and translated to sorghum gene models based on their orthologous relationships (Zhang *et al.* 2017). GWD (Sobic.010G143500) and PWD (Sobic.004G120100) with major roles in starch degradation were identified based on their homologs in maize. Genes encoding invertase (INV) were identified based on a recent manual curation in maize (Juarez-Golunga *et al.* 2018) and compared with an INV family study in sugarcane (Wang et al. 2017). The trehalose-6-phosphate synthase (TPS) and trehalose-6-phosphate phosphatase (TPP) families were described previously (Li *et al.* 2019).

***Genes involved in sucrose transportation.***

Genes encoding SUTs, SWEETs and TSTs were evaluated previously (Li *et al.* 2018). All the gene annotation information is shown in Table S3.

**Metabolic profiling.**

The samples were collected from a field with split plot design with three replicated blocks at the Waksman Institute, Rutgers, The State University of New Jersey. Each block consisted of three plots, with each sorghum genotype randomly planted in one plot. Each plot consisted of five rows which had 25 plants per row. To obtain the dynamics of soluble sugars in sorghum stem, five time points were chosen to collect stem tissues for detailed analysis: flag leaf stage (T1), 100% flowering (T2), 10 days after flowering (T3), 15 days after flowering (T4) and 30 days after flowering (T5). In each plot, the three central rows were further divided into five subplots containing nine plants per subplot, with each subplot (nine plants) randomly corresponding to one harvest time point. the upper internodes (internode No. 2, 3 and 4) were harvested from the nine plants per subplot at each of the five time points. Internodes were numbered from top to bottom. When sampling the internodes, two sections in the middle of each internode were used for RNA-seq (dataset1 used in the present study) and metabolome analysis. The six plants in each subplot were pooled together as one biological sample used for metabolic profiling. In total, six biological replicates (two samples multiplied by three blocks) were used for untargeted metabolomics analysis. The metabolome analysis was performed by Metabolon, Inc. under a service contract with Syngenta. Tyrosine and S-adenosyl-L-methionine (SAM) were identified and quantified in Rio, BTx406 and R9188 with untargeted metabolomics. The untargeted metabolomics platform is composed of four independent platforms: UHPLC-MS/MS optimized for basic species, UHPLC-MS/MS optimized for acidic species, polar LC platform (UHPLC(HILIC)-MS/MS) and GC-MS (Evans et al., 2009; Ohta et al., 2009). Briefly, each sample was extracted in methanol with recovery standards using an automated MicroLab STAR system (Hamilton). The resulting extract was divided into five fractions, with four fractions subjected to the four platforms, respectively, and one fraction reserved for backup. For LC methods, the analysis was carried out by using a Water Acquity UHPLC coupled to a Thermo Scientific Q-Exactive mass spectrometer equipped with an electrospray ionization source and Orbitrap mass analyzer. For GC-MS, samples were derivatized using bis-trimethyl-silyl-triflouroacetamide and then analyzed on a Thermo-Finnigan Trace DSQ fast-scanning single-quadrupole mass spectrometer. Known chemicals were identified by comparison to Metabolon’s library entries of purified standard compounds based on retention time/index (RI), mass to charge ratio (m/z), and MS/MS spectral data. A full description of the untargeted metabolomics method can be found under Supplemental Information. For data visualization, the raw area counts for each compound were median scaled. Detailed methods for sample collection and metabolomics analysis had been published elsewhere (Li et al. 2019)

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