**Final report**

DE-SC0002259

Characterization of nitrogen use efficiency in sweet sorghum

We have successfully graduated a Ph.D. student in August 2013 Dr. Malleswari Gelli who has accepted a sorghum genomics with Pioneer Hybrids in Johnston, Iowa.

We were able to generate three as a result of completing the projects:

1. Malleswari Gelli, Yongchao Duo, Anji Reddy Konda, Chi Zhang, David Holding, Ismail Dweikat. 2014. Identification of differentially expressed genes between sorghum genotypes with contrasting nitrogen stress tolerance by genome-wide transcriptional profiling. In press. Journal: BMC Genomics.

## Abstract

### Background

Sorghum is an important cereal crop, which requires large quantities of nitrogen fertilizer for achieving commercial yields. Identification of the genes responsible for low-N tolerance in sorghum will facilitate understanding of the molecular mechanisms of low-N tolerance, and also facilitate the genetic improvement of sorghum through marker-assisted selection or gene transformation. In this study we compared the transcriptomes of root tissues from seven sorghum genotypes having differential response to low-N stress.

### Results

Illumina RNA-sequencing detected several common differentially expressed genes (DEGs) between four low-N tolerant sorghum genotypes (San Chi San, China17, KS78 and high-NUE bulk) and three sensitive genotypes (CK60, BTx623 and low-NUE bulk). In sensitive genotypes, N-stress increased the abundance of DEG transcripts associated with stress responses including oxidative stress and stimuli were abundant. The tolerant genotypes adapt to N deficiency by producing greater root mass for efficient uptake of nutrients. In tolerant genotypes, higher abundance of transcripts related to high affinity nitrate transporters (NRT2.2, NRT2.3, NRT2.5, and NRT2.6) and lysine histidine transporter 1 (LHT1), may suggest an improved uptake efficiency of inorganic and organic forms of nitrogen. Higher abundance of SEC14 cytosolic factor family protein transcript in tolerant genotypes could lead to increased membrane stability and tolerance to N-stress.

### Conclusions

Comparison of transcriptomes between N-stress tolerant and sensitive genotypes revealed several common DEG transcripts. Some of these DEGs were evaluated further by comparing the transcriptomes of genotypes grown under full N. The DEG transcripts showed higher expression in tolerant genotypes could be used for transgenic over-expression in sensitive genotypes of sorghum and related crops for increased tolerance to N-stress, which results in increased nitrogen use efficiency for sustainable agriculture.

2. Malleswari Gelli1, Sharon E. Mitchell4, Thomas E. Clemente1,3, Donald P. Weeks2,3, David R. Holding1,3, Ismail M. Dweikat1*. Mapping of QTLs and identification of associated candidate

genes for nitrogen use efficiency traits in sorghum under contrasting nitrogen levels. In review. Molecular Breeding.

**Abstract**

Understanding the genetic variation of sorghum response to limited nitrogen supply is important for elucidating the underlying genetic mechanisms of nitrogen use efficiency (NUE). In this study, two F7 recombinant inbred line populations were developed and used to detect QTLs for multiple agronomic traits. The populations were analyzed under normal N (100 kg.ha-1 fertilizer) and low N (0 kg.ha-1 fertilizer) conditions for two years with two replications each. The linkage maps, spanning a length of 1584 cM and 1527 cM were developed using single nucleotide polymorphisms (SNPs) generated from genotyping-by-sequencing (GBS) technology. Composite interval mapping analysis detected several QTLs for 11 traits tested across two populations. The phenotypic variation explained by individual QTL ranged from 6.2% to 50.8%. Five QTLs were detected consistently across two populations. Co-localized regions affecting multiple traits were detected on chromosomes 1, 3, 6, 7 and 9. These pleiotropic regions were coincident with the genomic regions of cloned QTLs, including Ma3 for flowering time on chromosome 1 and Dw2 for plant height on chromosome 6. In the co-localized regions, Illumina RNA sequencing detected differentially expressed genes related to nitrogen metabolism (ferridoxin-nitrate reductase), glycolysis (phosphofructokinase 2), seed storage proteins, genes involved in plant hormone metabolism and several membrane transporters. Genes underlying the QTL regions could be potential targets for improving sorghum performance under limited N fertilizer through marker assisted selection.

3. Malleswari Gelli1, Sharon E. Mitchell4, Thomas E. Clemente1,3, Donald P. Weeks2,3, David R. Holding1,3, Ismail M. Dweikat1*. Identification, validation of QTLs and associated candidate genes for nitrogen use efficiency traits under different nitrogen conditions in a recombinant inbred line population of Sorghum. Submitted to Genome.

**Abstract**

Sorghum, a cereal of economic importance ensures food and fodder security for millions of rural families in the semi-arid tropics. Despite being a C4 photosynthetic crop, it depends heavily on nitrogen fertilizers for achieving commercial yields. Optimized NUE application in major crops is essential for long-term sustainability of agriculture production. The objective of the present study was to identify and validate quantitative trait loci (QTLs) for NUE traits using a mapping population of 208 recombinant inbred lines (RILs). The mapping population was phenotyped under two different nitrogen regimes with two replications each for two years. A genetic linkage map spaning a length of 1527 cM was developed using 842 single nucleotide polymorphisms (SNPs) generated from genotyping-by-sequencing. The QTL analysis for 11 traits following composite interval mapping identified 32 QTL with 2 to 5 QTL for each trait. QTL detected in the population individually explained phenotypic variation ranged from 6% to 18% for a given trait. Five co-localized regions with QTL effect on multiple traits were detected on chromosomes 1, 3, 8, 9, and 10. We also detected four genomic regions containing QTLs in consistent with China17 population. These genomic regions containing cloned QTLs for dwarfing genes (Dw2 and Dw3) and maturity genes (Ma1). In these consistent co-localized regions Illumina RNA-seq detected

differentially expressed genes (DEGs) related to nitrogen metabolism, ammonia assimilation, plant hormone metabolism and membrane transport. These QTL-linked DEG transcripts may assist in fine mapping, and also for improving NUE of sorghum through marker-assisted breeding.