

## Genomic dissection of anthracnose resistant response in sorghum [*Sorghum bicolor* (L.) Moench]

Hugo E. Cuevas<sup>1, \*</sup> ([hugo.cuevas@ars.usda.gov](mailto:hugo.cuevas@ars.usda.gov)), Clara M. Cruet<sup>2</sup>, Louis K. Prom<sup>3</sup>, Joseph E. Knoll<sup>4</sup>, Lauren Stutts<sup>5</sup>, Wilfred Vermerris<sup>6</sup>

<sup>1</sup>USDA-ARS Tropical Agriculture Research Station, Mayaguez, PR; <sup>2</sup> University of Puerto Rico; Mayaguez Campus, Department of Biology. Mayaguez, P.R. 00680; <sup>3</sup>USDA-ARS Crop Germplasm Research Unit, College Station, TX; <sup>4</sup>USDA-ARS Crop Genetics and Breeding Research Unit, Tifton, GA; <sup>5</sup>University of Florida, Program in Plant Molecular & Cellular Biology and <sup>6</sup>Department of Microbiology & Cell Science, Gainesville, FL

### Project Goals:

**The goal of this project is to use a genomics-based approach to identify anthracnose resistance loci from diverse sorghum germplasm, to establish against which pathotypes these loci protect, and to determine the disease resistance mechanism of at least one of these genes. This information will provide plant breeders a tool kit that can be used to maximize levels of resistance in different areas of production.**

The productivity and profitability of sorghum [*Sorghum bicolor* (L.) Moench] is reduced by susceptibility to fungal diseases, such as anthracnose, caused by *Colletotrichum sublineolum*. The identification of anthracnose resistance loci from different sorghum accessions is imperative to develop new varieties with broader resistant response and to increase its durability. We evaluated the anthracnose resistant response of 335 accessions from a sorghum association panel (SAP) (Cuevas *et al.* 2018, Plant Genome), 300 exotic sorghum accessions from NPGS Ethiopian germplasm collection, and two sets of recombinant inbred lines derived from anthracnose-resistant sources SC112-14 (Ethiopia) and QL3 (India). In parallel, two novel QTL associated with the resistant response in sorghum line Bk7 (chromosomes 7 and 9; Felderhoff *et al.* 2016,G3) are now being analyzed in more detail to identify the underlying resistance genes.

The evaluation of SAP identified 75 accessions resistant to anthracnose (Cuevas *et al.*, 2018, Plant Genome). A phylogenetic analysis of these accessions showed high genetic diversity and multiple resistance sources. Genome-wide association scans (GWAS) using 268,289 single-nucleotide polymorphisms (SNPs; Morris *et al.* 2013) and logistic regressions for binary measures of resistance responses identified three loci within a region on chromosome 5 that have been previously associated with three sources of anthracnose resistance. Candidate genes within these loci were related to R-gene families, signaling cascades and transcriptional reprogramming, suggesting that the resistance response is controlled by multiple defense mechanisms. The strategic integration of exotic resistant germplasm into the SAP is needed to identify additional rare resistance alleles via GWAS.

The evaluation of 297 exotic sorghum accessions from NPGS Ethiopian germplasm collection identified 143 resistant accessions. Genetic characterization of this germplasm (Cuevas *et al.* 2017; BMC Genomics) and its anthracnose resistant response were merged with phenotypic and genetic characterization of the SAP (Morris *et al.* 2013) for a large GWAS comprising 592 accessions and 219,037 SNPs. Logistic regressions for binary measures of resistance responses identified the previously associated locus on chromosome 5 and an additional locus on chromosome 3, while mixed linear model using quantitative resistant response identified a locus

on chromosome 9. Candidate genes within loci on chromosome 5 and 3 include a resistance gene belonging to a family of genes encoding *F-box* proteins, while a candidate resistance gene on chromosome 9 is a gene with leucine-rich repeat and NACHT domain (i.e. R-gene family). Resistant alleles for loci on chromosomes 3 and 9 are present in the SAP at low frequency, thus, the integration of NPGS Ethiopian germplasm increased its frequency and power of detection.

Two sets of recombinant inbred lines (RILs) derived from the anthracnose-resistant sources SC112-14 (Ethiopia) and QL3 (India) were evaluated for anthracnose resistant response in Puerto Rico, Florida, Georgia and Texas for two consecutive years to identify broad resistance against multiple isolates of the pathogen. In parallel, a subset of RILs were evaluated in the greenhouse against eight anthracnose pathotypes to identify particular resistance loci. Composite interval mapping using two high-density genetic maps constructed using genotyping by sequencing revealed that the resistance in QL3 is controlled by multiple loci, while the resistance in SC112-14 is controlled by a single locus on chromosome 5. Segregation analysis of 1,500 individuals derived from SC112-14 delimited this locus to a 22 kb region and confirmed one of the GWAS loci on chromosome 5. Greenhouse evaluation validated this locus for the eight pathotypes. In contrast, greenhouse evaluation for QL3 identified two loci for four pathotypes that were not detected based on field evaluations.

Visual observation of the resistant line Bk7 and the susceptible line Early Hegari Sart in response to infection with *C. sublineolum* shows that rates of germination and appressorium formation were reduced in Bk7, and that this line also produces more deoxyanthocyanidins, visible as orange pigments. Mycelial growth is being monitored with a transgenic strain of *C. sublineolum* expressing the green fluorescent protein. We also determined that the resistant locus on chromosome 9 present in Bk7 included 12 candidate genes (Felderhoff et al., 2017) that were validated to be expressed in leaves. Each of the 12 candidate genes from Bk7 is now being targeted for down-regulation using virus-induced gene silencing (VIGS) with a modified bromo mosaic virus. Down-regulation of the gene(s) responsible for the anthracnose resistance is expected to result in successful infection by the pathogen. The locus on chromosome 7 contains too many candidate genes for VIGS, and expression profiling is being pursued to identify differentially expressed genes as candidates.

## References

- Cuevas et al. (2018). Genome-wide association mapping of anthracnose (*Colletotrichum sublineolum*) resistance in the U.S. sorghum association panel. *Plant Genome* (accepted)
- Cuevas et al. (2017). Genomic characterization of a core set of the USDA-NPGS Ethiopian sorghum germplasm collection: implications for germplasm conservation, evaluation, and utilization in crop improvement. *BMC Genomics* **18**, 108.
- Felderhoff et al. (2016). Using genotyping by sequencing to map two novel anthracnose resistance loci in *Sorghum bicolor*. *G3-Genes Genomes Genetics* **6**, 1935-1946.
- Felderhoff et al. (2017) A cost-benefit analysis to select the most effective method for positional cloning: genotyping by sequencing versus allele-specific PCR. *Euphytica* 213: 286
- Morris et al. (2013). Population genomic and genome-wide association studies of agroclimatic traits in sorghum. *Proceedings of the National Academy of Sciences of the United States of America* **110**, 453-458.

## Funding statement.

This research was supported by the DOE Office of Science, Office of Biological and Environmental Research (BER), grant no. DE-SC0014171.