Project Title: Genomic dissection of anthracnose resistance response in sorghum [*Sorghum bicolor* (L.) Moench]

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Executive Summary

Sorghum [*Sorghum bicolor* (L.) Moench] is the fifth most important grain crop behind maize, wheat, rice, and barley. Today, it is of interest as a source of fermentable sugars for the production of renewable fuels and chemicals, and as a source of biomass for co-firing. The productivity and profitability of sorghum are limited by several biotic constraints, most notably anthracnose caused by the fungal pathogen *Colletotrichum sublineolum*. The most cost-effective and environmentally benign strategy to control anthracnose is through the incorporation of resistance genes. Over the last three years, our research efforts have been directed to identify new sources of resistance in temperate adapted and tropical germplasm, and to delimited genomic regions associated with the observe anthracnose resistant response.

Three biparental mapping populations derived from the resistant lines SC112-14, QL3 and IS18760 were evaluated for anthracnose resistance response in Texas, Georgia, Florida and Puerto Rico. In parallel, three high density recombination maps were constructed and used to identify resistant loci. Anthracnose resistant response in line SC112-14 is controlled by a major locus on chromosome 5. Segregation analysis of 1,500 progenies delimited the resistance locus on chromosome 5 to a 23-kb region harboring three candidate genes, including Sobic.005G17230 identified by GWAS of the sorghum association panel (SAP). The latter gene belongs to a family of genes encoding *F-box* proteins indicating that this resistance response involved in signaling cascades and transcriptional reprograming, rather than recognition of pathotype-associated molecular patterns. In contrast, anthracnose resistant response in lines QL3 and IS18760 is controlled by multiple small-effect genes. Greenhouse evaluation of a representative subset of the three mapping populations against nine pathotypes found that lines susceptible in the field could be resistant to a single pathotype in the greenhouse. Thus, the activation of a resistance response system by a single pathotype could not provide a broader resistance response against multiple pathotypes.

The screening of 1,801 sweet sorghum accessions from the National Plant Germplasm System identified 654 accessions with Brix value larger than 10, which in turn was used to select a subset of 233 accessions for evaluation of anthracnose resistant response. Even though most of the accessions were not completely infected by anthracnose, 28 accessions were completely resistant against pathotypes from Texas, Georgia, Florida and Puerto Rico. Genotyping-by-sequencing analysis of this subset identified 157,843 single nucleotide polymorphisms. Population structure analysis of the subset based on a subset of 2,345 unlinked SNPs found that the genetic diversity could be divided into four populations. The genetic relatedness among accessions within populations suggests most of the resistant germplasm may contain few different resistance sources. These resistance sources present in sweet sorghum germplasm could expedite the development of new resistant sweet sorghum cultivars and hybrids by avoiding time-consuming introgression breeding approaches with non-sweet sorghums serving as donor of the resistance alleles

ACCOMPLISHMENTS:

I - Major goals of the project

The objective of this proposal is to use a genomics-based approach to identify anthracnose (*Colletotrichum sublineolum*) resistance loci from three diverse sorghum germplasm from Ethiopia (SC112-14), Sudan (IS18760), and India (QL3) to establish against which particular pathotypes these loci protect, map their resistance genes and determine the disease resistance mechanism of at least one of these genes. The ultimate goal is to provide plant breeders with the required knowledge to maximize levels of resistance in the intended area of production.

In this regard, three sets of recombinant inbred lines derived from the cross of these three resistance sources (SC112-14, QL3 and IS18760) to a common highly susceptible line PI609251 have already been developed and will be used to identify multiple resistance loci against multiple pathotypes. Likewise, a sweet sorghum diversity panel will be established to identify new sources of anthracnose resistance in this particular germplasm. In fact, this proposal has four particular main objectives:

Objective 1- Genomic dissection of anthracnose resistance response of SC112-14, QL3 and IS18760

Objective 2- Dissection of the anthracnose resistant response into its multiple gene components

Objective 3- Isolation of gene and molecular mechanism of anthracnose resistance response in Bk7 to establish a genetic model for comparative analysis

Objective 4- Identification of novel sources of anthracnose resistance in sweet sorghum germplasm, and SNPs associated with resistance response

II - Accomplished under these goals

Objective 1- Genomic dissection of anthracnose resistance response of SC112-14, QL3 and IS18760
Objective 2- Dissection of the anthracnose resistant response into its multiple gene components

SC112-14

Field evaluation: The RIL's derived from SC112-14 were evaluated for anthracnose resistance response in Florida, Georgia, Texas and Puerto Rico during Summer-Fall 2016 (**Table 1**). A total of eighteen and fifty-one RIL's were resistant and susceptible to anthracnose, respectively, across location. While forty-five RIL's showed variable resistance response across locations.

Linkage map and bin map: A high-density linkage map was constructed for the RIL populations using 9,499 single nucleotide polymorphisms (**Table 2**). The linkage map expanded 1,089.08 cM and included 1,335 recombination events. A representative subset of 34 RILs were selected based on recombination events information (i.e. bin map) and evaluated in the greenhouse against 9 pathotypes. This RIL's subset included 930 recombination events and its linkage map expanded 1,110 cM.

Genome mapping: Genome wide-association analysis of anthracnose resistant response based on the single marker analysis of 9,499 SNPs and 94 RILs identified three loci in chromosome 4, 5 and 9 (**Figure 1**). The locus on chromosome 5 was detected at the four locations and explained up to 70% of the observed variation. This locus expanded a genomic region of ~800 kb (**Table 3**).

Bin mapping: The evaluation of 34 RILs in greenhouse against 9 pathotypes from Texas, Arkansas and Puerto Rico confirmed the anthracnose resistant locus in chromosome 5 (**Table 3**). However, the resistant response observed against two pathotypes from Georgia and Arkansas were not associated with anthracnose resistant locus in chromosome 5 indicating the presence of other loci not been detecting by the bin mapping approach.

Delimitation of locus in chromosome 5. The delimitation of locus on chromosome 5 was based on the analysis of 1,500 segregating progenies and four molecular markers that divided the locus into 3 genomic segments of 386, 23 and 446 kb, respectively. The analysis resulted in the identification of seven recombinants that based on their genotype and anthracnose response suggested the resistance gene is located within or in proximity to the 23 kb genomic interval (**Table 4**). A genome-wide association analysis based on the analysis of 335 accessions from the Sorghum Association Panel evaluated in Puerto Rico and Georgia pinpoint a candidate gene within the 23 kb genomic interval. The candidate gene is characterized by the presence of F-box and Ser-Thr kinase domain.

Table 1 Anthracnose resistance response of recombinant inbred lines (RILs) derived from the cross of SC112-14 and PI609251 evaluated at four locations in 2016.

	Live Oak, FL		Tifton, GA		College Station, TX		Isabela, PR		Overall	
	RILs	χ^2	RILs	χ^2	RILs	χ^2	RILs	χ^2	RILs	χ^2
Resistant[1]	41	0.02	39	0.01	45	0.08	33	<0.001	18	<0.001
Susceptible[1]	65		67		63		78		96	
	X ± S.D.[2]		X ± S.D.		X ± S.D.		X ± S.D.		X ± S.D.	
SC112-14	2.0 ± 0.0		2.0 ± 0.0		2.0 ± 0.0		2.0 ± 0.0		2.0 ± 0.0	
PI 609251	4.0 ± 0.0		4.0 ± 0.0		3.1 ± 0.3		4.8 ± 0.5		3.7 ± 0.7	
RILs	3.1 ± 1.1		3.2 ± 1.1		2.7 ± 0.7		3.5 ± 1.2		3.1 ± 0.9	

[1] Anthracnose resistance response based on a scale of 1 to 5 (Erpelding and Prom, 2004; Prom et al. 2009), where 1 and 2 are resistant, and 3-5 susceptible.
Chi square test against the expected segregation ratio for a single resistant gene in RILs population (1:1)
[2] Means and standard deviation

Table 2 High density linkage map for recombinant inbred line population derived from anthracnose resistant line SC112-14 and susceptible line PI609251.

		RILs (n = 94)		RILs (n = 34)	
Chromosome	SNPs	Recombination	Length (cM)	Recombination	Length (cM)
1	1,617	194	142.68	103	152.36
2	1,026	135	118.68	72	111.56
3	1,054	165	136.92	133	142.15
4	1,145	189	108.08	100	86.41
5	808	136	96.16	157	115.03
6	1,050	125	97.24	87	105.06
7	631	88	98.29	62	98.48
8	614	81	99.48	83	118.11
9	786	107	100.27	69	90.9
10	768	115	91.28	64	89.94
Total	9,499	1,335	1,089.08	930	1,110.00

Figure 1 Genome-wide association analysis for anthracnose resistant response based on the single marker analysis of 9,499 SNPs

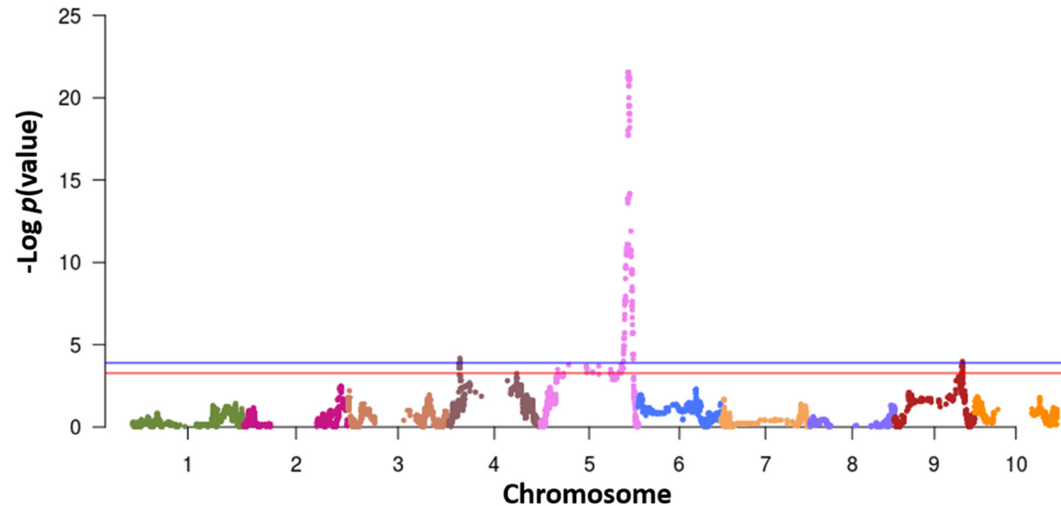


Table 3 Genomic regions associated with anthracnose resistant response based on the analysis of recombinant inbred lines derived from resistant and susceptible lines SC112-14 and PI609251, respectively.

RIL population (n=94)					BIN Mapping (n=34)					
Location	Chr.	Region (Mb)	-Log p (value)	R^2	Isolated	Origin	Chr.	Region (Mb)	-Log p (value)	R^2
Texas	5	64.82 - 65.26	16.91	0.66	AMP46	Arkansas	5	64.77 - 65.52	9.43	0.80
Georgia	5	64.82 - 65.26	14.6	0.61	AMP50	Arkansas	5	64.77 - 65.84	6.57	0.65
	6	42.65 - 42.83	4.51	0.26	PATH20	Texas	5	64.92 - 65.19	4.61	0.53
	9	49.71 - 50.06	3.72	0.22	PATH26	Texas	5	64.77 - 65.26	5.18	0.60
Florida	5	64.82 - 65.26	12.03	0.54	PATH31	Texas	5	64.77 - 65.26	5.77	0.61
Puerto Rico	4	87.21 - 89.30	4.1	0.22	PATH32	Puerto Rico	5	64.77 - 65.65	7.29	0.71
	5	64.82 - 65.26	15.83	0.62	PATH36	Puerto Rico	5	64.45 - 65.26	5.8	0.64
Overall	4	87.21 - 91.57	4.17	0.22	PATH35	Georgia	*n.a.*	*n.a.*	*n.a.*	*n.a.*
	5	64.82 - 65.26	21.28	0.71	AMP48	Arkansas	*n.a.*	*n.a.*	*n.a.*	*n.a.*
	9	49.71 - 50.06	3.97	0.22						

Table 4. Delimitation of anthracnose resistant locus based on the genotype and anthracnose resistant response of seven recombinants identified by the analysis of 1,500 segregating progenies.

	SNPs positions				Anthracnose[1]	
Recombinant	Sobic.05G64784452	Sobic.05G65171076	Sobic.05G65194327	Sobic.05G65640661	Classification	Score
# 467	B[2]	H[2]	H	H	Resistant	2.0
# 649	H	H	H	B	Resistant	2.0
# 176	H	H	H	B	Resistant	2.0
# 105	H	H	H	B	Susceptible	3.0
# 375	H	B	B	B	Susceptible	3.5
# 667	B	B	B	H	Susceptible	3.0
# 1040	B	B	B	H	Susceptible	3.0

[1] Anthracnose resistance response based on a scale of 1 to 5 (Erpelding and Prom, 2004; Prom et al. 2009), where 1 and 2 are resistant, and 3-5 susceptible.
[2] Marker genotype where B and H refers to homozygous for susceptible parent and heterozygous.

QL3 and IS18760

Field evaluation: The RILs derived from QL3 and IS18767 were evaluated for anthracnose resistance response in Florida, Georgia, Texas and Puerto Rico during Summer-Fall 2016. Nevertheless, the RIL's shows segregation for anthracnose resistant response against pathotypes from Texas and Puerto Rico. Therefore, both RIL's population were evaluated for anthracnose resistant response for two additional years in Texas and Puerto Rico (**Table 5**).

Linkage map and bin map: Two linkage maps were constructed for both RILs populations using 1,355 (IS18760) and 1,008 (QL3) single nucleotide polymorphisms (**Table 6**). The linkage map of IS18760 expanded 2,629.30 cM with an average distance of 2.1 cM per single nucleotide polymorphisms. The linkage map of QL3 expanded 3,404.60 cM with an average distance of 3.6 cM per single nucleotide polymorphisms. A representative subset of 25 RILs from each population were selected based on recombination events (i.e. bin map) and evaluated in the greenhouse against 8 pathotypes.

Genome mapping: Composite interval mapping of anthracnose resistant response identified nine quantitative trait loci. A major locus in chromosome 4 was identified in both populations and associated with the anthracnose resistant response in Texas and Puerto Rico (**Figure 2**). This locus explained up to 25% of the observed variation.

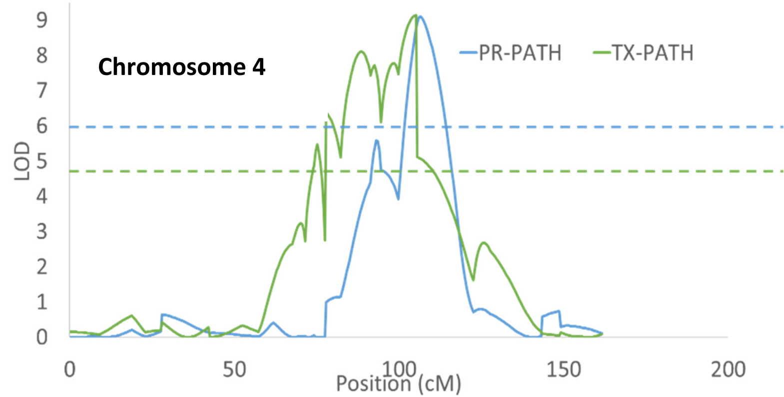


Figure 2. Anthracnose resistance locus on sorghum chromosome 4 identified by CIM analysis of RIL derived from the resistant line IS18760 and QL3

Bin mapping: The resistance response of RILs against particular pathotypes were different than the observed in field evaluation (**Table 7**). Lines that were susceptible in the field showed resistant against single pathotypes. The results indicated the pathogen population diversity in the field or across years determined the observed resistance response. Certainly, the greenhouse evaluation provide valuable genetic information against a particular pathotype that could be used to enhanced field resistance response.

Table 5 Means and standard deviation of anthracnose resistant response of two RILs populations derived from the moderately resistant lines QL3 and IS18760 with the susceptible line PI609251.

	Anthracnose resistant response[1]		
RIL's	Texas[2]	Puerto Rico[2]	Overall
QL3	3.2 ± 0.7	4.0 ± 0.7	3.6 ± 0.6
IS18760	3.0 ± 0.5	3.8 ± 0.7	3.4 ± 0.6

[1] Anthracnose resistance response based on a scale of 1 to 5 (Erpelding and Prom, 2004; Prom et al. 2009), where 1 and 2 are resistant, and 3-5 susceptible.
[2] RILs population evaluate in Texas and Puerto for three consecutive years (2016, 2017 and 2018).

Table 6 High-density linkage maps description for two RILs populations derived from the cross of anthracnose resistant lines IS18760 and QL3 with susceptible line PI609251.

	IS18760			QL3		
Chr.[1]	SNP	cM[2]	Avg.[3]	SNP	Length	Avg.
1	142	327.06	2.32	84	329.14	3.97
2	213	327.31	1.54	116	358.87	3.12
3	233	376.41	1.62	97	370.85	3.86
4	154	309.40	2.02	110	332.12	3.05
5	147	208.89	1.43	137	429.39	3.16
6	111	270.59	2.46	91	389.23	4.32
7	57	131.09	2.34	40	219.01	3.31
8	74	223.26	3.06	117	294.09	2.54
9	122	218.58	1.81	90	347.48	3.90
10	101	236.67	2.34	126	334.42	2.68
	1,355	2,629.30	2.10	1,008	3,404.60	3.60

[1] Chr. refers to chromosome, [2] cM refers to the total recombination distance in centiMorgan according to Kosambi, [3] Avg. refers to the average distance between SNPs

Table 7 Anthracnose resistance response of recombinant inbred lines (RILs) derived from resistant lines QL3 and IS18760 (P_1) with susceptible line PI609251 (P_2) and evaluated against 8 different pathotypes of *Colletotrichum sublineola*, causal agent of sorghum anthracnose.

QL3								
RIL	Pathotype 20	Pathotype 26	Pathotype 29	Pathotype 31	Pathotype 32	Pathotype 36	AMP 48	AMP 50
# 5	S	R	R	R	*	R	R	R
# 8	R	R	*	R	R	R	R	R
# 13	R	S	R	R	S	S	R	S
# 18	S	R	R	R	*	R	R	S
# 33	R	R	R	R	R	R	R	R
# 34	S	S	R	R	S	*	R	S
# 35	*	R	*	R	R	*	R	R
# 38	R	R	R	R	R	R	R	R
# 39	R	R	R	S	R	R	*	R
# 40	R	R	R	R	R	R	R	R
# 41	R	R	R	R	R	R	R	R
# 45	R	R	R	R	R	R	R	R
# 46	R	R	R	R	R	R	R	R
# 51	R	R	R	R	R	S	*	R
# 56	R	R	R	R	*	R	R	R
# 62	*	S	R	R	*	*	R	S
# 70	R	R	R	R	R	R	R	R
# 73	-	R	R	S	-	R	R	R
# 75	R	R	R	R	R	R	R	R
# 84	-	R	R	-	-	-	-	-
# 86	R	R	*	R	R	R	R	R
# 91	R	R	R	R	R	R	R	R
# 95	R	R	R	R	-	R	R	R
# 109	R	R	R	R	R	R	S	R
P_1	R	R	R	R	R	R	R	R
P_2	S	S	S	S	S	S	S	S

IS18760								
RIL	Pathotype 20	Pathotype 26	Pathotype 29	Pathotype 31	Pathotype 32	Pathotype 36	AMP 48	AMP 50
# 5	R	R	R	R	R	R	R	R
# 7	R	R	S	R	*	R	R	R
# 34	S	S	S	R	S	R	S	S
# 36	S	S	R	R	R	R	R	S
# 37	S	S	R	R	*	R	R	S
# 39	R	*	R	R	R	R	R	*
# 44	R	R	R	R	-	-	-	-
# 45	R	R	R	R	R	*	R	R
# 48	R	R	R	R	R	R	R	R
# 58	R	*	*	*	R	*	R	R
# 59	R	R	*	R	R	R	R	R
# 60	R	R	S	R	R	S	S	R
# 65	R	R	R	R	R	R	R	R
# 68	*	R	R	R	R	R	R	R
# 71	R	*	*	S	R	R	R	R
# 73	S	S	R	*	*	R	R	R
# 79	R	R	*	R	R	R	R	S
# 91	R	R	S	R	R	S	R	S
# 96	S	S	*	*	*	R	R	S
# 105	R	S	R	R	R	R	R	R
# 117	-	R	R	R	R	R	R	-
# 118	R	S	R	R	S	R	S	S
# 123	R	S	R	R	*	R	R	S
# 125	R	R	R	R	R	R	R	R
P_1	R	R	R	R	R	R	R	R
P_2	S	S	S	S	S	S	S	S

The pathotypes were based on the pathotype classification described by Prom et al. (2012). The codes and locations of the isolates used in this study are as follow: AMP 1= Pathotype 20 from Texas; AMP 234 =Pathotype 26 from Texas; AMP 172 = Pathotype 29 from Texas; AMP 157=Pathotype 31 from Texas; AMP 205=Pathotype 32 from Puerto Rico; AMP 207= Pathotype 32 from Puerto Rico; AMP 48 and AMP 50 from Arkansas, not yet classified. Disease ratings were based on a scale of 1 to 5 (Erpelding and Prom, 2004; Prom et al. 2009), where 1 and 2 are resistant, and 3-5 susceptible. – no plants due to low germination. * = segregating response i.e., resistance/susceptible.

Objective 3- Isolation of gene and molecular mechanism of anthracnose resistance response in Bk7 to establish a genetic model for comparative analysis

Validation of candidate resistance genes in a QTL on chromosome 9.

At the start of the project we had identified two genomic locations associated with anthracnose resistance from sorghum cultivar 'Bk7', on chromosomes 7 and 9, respectively. The quantitative trait locus on chromosome 7 was very large (40 Mb), with 70 candidate resistance genes, whereas the locus on chromosome 9 was 3.2 Mb, containing 40 candidate genes. This was published by Felderhoff et al. (2016) (see publication list). During the project period we fine-mapped the QTL on chromosome 9 to a 1.5 Mb-region with only 12 candidate genes using a population of sorghum plants obtained from backcrossing a resistant plant to the susceptible parent. This was published by Felderhoff et al. (2017) (see publication list). We determined that all 12 candidate genes are expressed in sorghum leaf tissue exposed to the pathogen, requiring all 12 genes to be evaluated for their role in anthracnose resistance. We used a virus-induced gene silencing protocol with Brome Mosaic Virus, a virus with a genome consisting of three RNA molecules. We cloned and sequenced 200-300 bp fragments of each of the 12 candidate genes, making sure they were specific for these specific genes and not any of their paralogs, and introduced them into a binary vector containing the cDNA encoding RNA3 of Brome Mosaic Virus. Each of the 12 binary vectors were introduced individually in *Agrobacterium tumefaciens* along with two control vectors containing either a fragment of sorghum *phytoene desaturase* (*PDS*) cDNA or a fragment of sorghum *ubiquitin* cDNA. Silencing of the *PDS* and *ubiquitin* genes results in visible changes in the leaves: yellowing and brown spots, respectively, as a way to monitor the efficacy of the inoculation procedure. The individual recombinant *Agrobacterium* strains were then used to co-infiltrate tobacco leaves with an *Agrobacterium* strain containing a plasmid encoding the other two RNA molecules that make up the BMV genome. The leaf infiltration resulted in the production of recombinant virions containing the sorghum cDNA fragments. Tobacco leaf extracts containing virions (verified by PCR) were used to inoculate sorghum leaves with the intent to silence the sorghum genes matching the sorghum cDNA inserts in the virions.

The challenge with this inoculation procedure, which is based on published protocols from Benavente *et al.* (http://www.maydica.org/.../57_206.pdf) at the Noble Foundation in Ardmore, OK, was that results obtained with virions containing the *PDS* cDNA were inconsistent (considerable variation in silencing symptoms among replicate sorghum plants). Since the silencing of candidate resistance genes does not result in a visible phenotype, it is critical that the silencing efficiency is high and consistent, before the silenced plants are challenged with *C. sublineolum*. This lack of consistency turned out to be a common issue observed by others, as described in a publication by Singh *et al.* (doi.org/10.1186/s12870-018-1344-z) in June 2018. These authors made several changed to the VIGS protocol to reduce the level of variation among inoculations. A remaining challenge was that the success of the procedure appears to be dependent on the genotype of the sorghum. Results obtained with the optimized protocol and our resistant recombinant inbred lines indicate a silencing efficiency of 80% in this line (**Figure 3**), but PI 533, a line of interest provided by Dr. Cuevas, had a silencing efficiency of only 28%.

The inefficient VIGS protocol is the reason this part of Objective 3 was incomplete by the end of the project. Experiments with the optimized protocol are, however, in progress, and we expect to have this part of the objective complete by the middle of 2019.

Validation of candidate resistance genes in a QTL on chromosome 7.

In order to identify resistance genes in the QTL on chromosome 7, we used a transcriptome profiling approach, whereby the gene expression was compared between leaves from mock-inoculated controls and plants infected with *C. sublineolum*, using the susceptible genotype 'Early Hegari-Sart' and two resistant recombinant inbred lines containing different segments of the QTL from chromosome 7(these lines did not contain the QTL on chromosome 9). A total of 24 RNA samples were prepared (3 genotypes × 2 treatments (mock vs. *C. sublineolum*) × 4 replicates) and shipped to Novogene, a company that performs transcriptome profiling. We anticipate receiving the data in December.

As part of a complementary experiment, we examined via RT-PCR the expression of three genes encoding germin-like proteins that are located in this QTL. The expression of one of these genes, Sb07g65800, increases in response to inoculation with *C. sublineolum* in a resistant line, while in the susceptible lines expression is low, regardless of whether the plants are challenged with the pathogen (**Figure 4**). Germin-like proteins have been shown to play a role in defense against pathogens in other grass species, as reviewed by Breen and Bellgard (2010; DOI 10.1007/s10142-010-0184-1), because of their ability to produce hydrogen peroxide as an antifungal compound. We plan to follow up on these genes if their change in expression is confirmed in the transcriptome profiling experiment. These experiments of Objective 3 were delayed as a result of Hurricane Irma that destroyed our sorghum field in 2017, requiring more time to generate seed for the expression profiling experiment. We anticipate having completed this experiment in the first quarter of 2019.

Figure 3 Symptoms of virus-induced gene silencing of phytoene desaturase (PDS) in sorghum seedlings is evident from the yellow stripes, whereas leaves mock-inoculated with MES buffer remain green. **A.** Inbred BTx623, mock-inoculated, **B.** Inbred BTx623 inoculated with BMV containing a fragment of the *PDS* cDNA, **C.** Inbred line 2143, mock-inoculated, **D.** Inbred 2143 inoculated with BMV containing a fragment of the *PDS* cDNA.

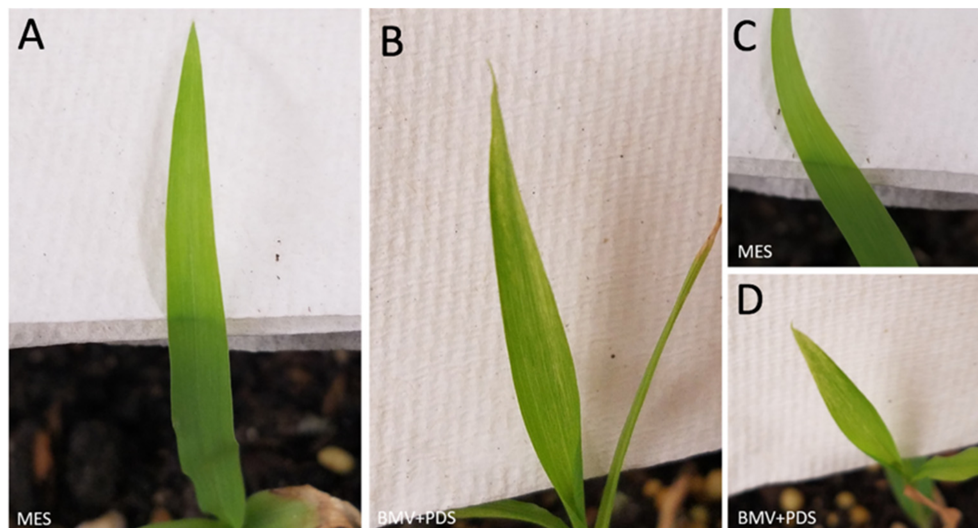
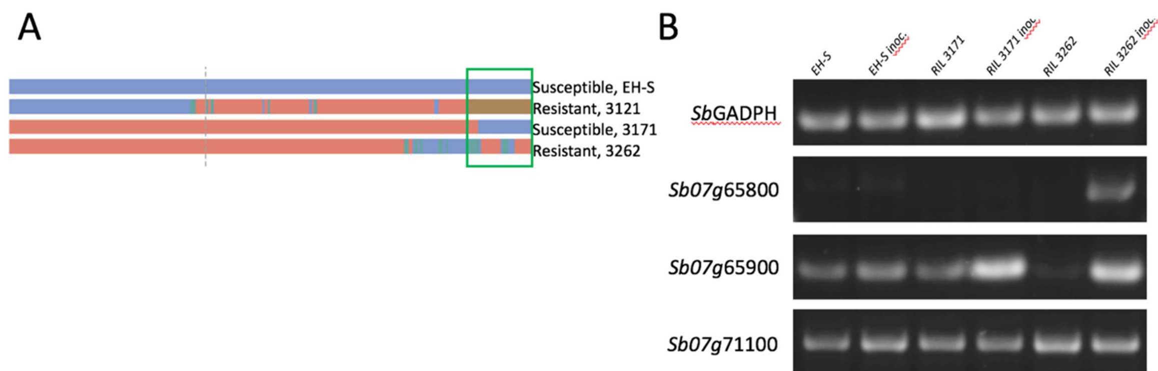


Figure 4 A comparison of the expression of three genes encoding germin-like proteins located in the anthracnose resistance QTL on chromosome 7 among the susceptible genotype Early Hegari-Sart (EH-S), the susceptible recombinant inbred line 3171 and the resistant recombinant inbred line 3262. **A.** The latter two genotypes differ in the alleles at the proximal end of the QTL (blue color represents EH-S alleles, red color Bk7 alleles). **B.** RT-PCR products representing the three genes encoding germin-like proteins and the gene enncoding glyceraldehyde phosphate dehydrogenase (GAPDH), used as control, were separated on an agarose gel. The amount of DNA is representative of the gene expression level. There is an especially large difference in expression of gene *Sb07g65800*, which is expressed highly in response to exposure to *C. sublineolum* in the resistant line 3262, but which is expressed at low levels in the susceptible lines, even in the presence of the pathogen.



Objective 4- Identification of novel sources of anthracnose resistance in sweet sorghum germplasm, and SNPs associated with resistance response

<u>*Screening of NPGS sweet sorghum collection*</u>: A total of 1,801 sweet sorghum accessions from the National Plant Germplasm System were evaluated in single replicated trial in Puerto Rico during 2015 and 2016. The analysis identified 654 accessions with Brix value >10.0 of which 171 accessions had values >15.0 (**Figure 5**).

<u>*Sweet sorghum diversity panel:*</u> Based on Brix values and origin a subset of 233 sweet sorghum accessions were selected for further anthracnose resistance evaluation in Texas, Georgia, Florida and Puerto Rico (**Table 8**). This diversity panel is composed by accessions from 19 countries and advanced breeding materials and represents ~10-15% of the NPGS sweet sorghum collection.

<u>*Genetic diversity and population structure of sweet sorghum diversity panel*</u>: Genotype-by-sequence analysis of the diversity panel identified 157,843 SNPs. Population structure analysis based on 2,345 unlinked SNPs separated most of the accessions (~70%) of the panel into 4 populations (**Figure 6**). Remarkably, most of the advanced germplasm is highly genetically related and are distributed among 3 populations.

Anthracnose resistant response of sweet sorghum diversity panel: Anthracnose resistance response of sweet sorghum diversity panel identified 28 accessions resistant against pathotypes from Texas, Georgia, Florida and Puerto Rico (**Table 9**). The average of anthracnose resistant response was less than 3.0 in the four populations indicating most of the accessions were not completely infected.

Figure 5 Brix values distribution of 1,801 sweet sorghum accessions from the National Plant Germplasm System evaluated in single replicated trial in Puerto Rico

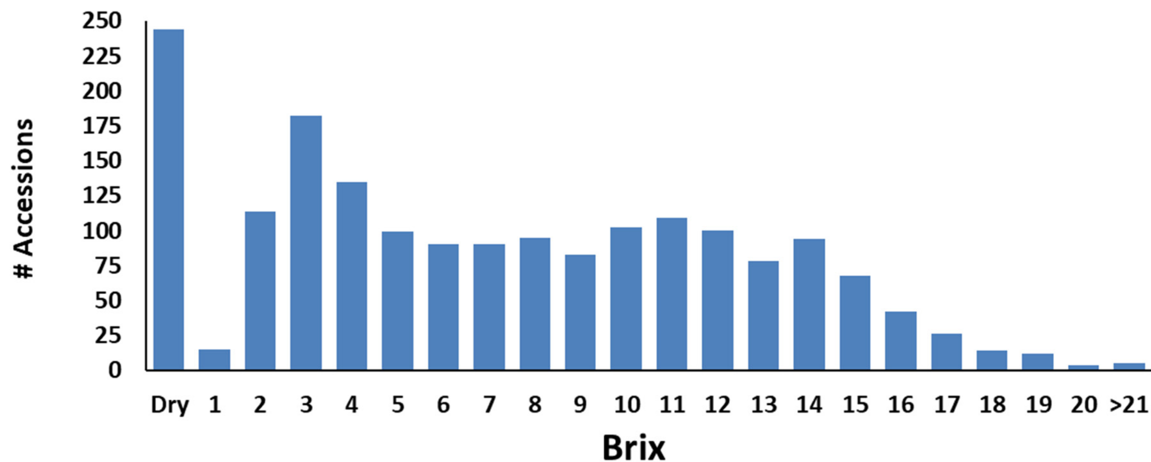


Table 8 Sweet sorghum diversity panel to be evaluated for anthracnose resistance response in Texas, Georgia, Florida and Puerto Rico.

Origin	Num. accessions	Origin	Num. accessions
Advanced breeding	105	Somalia	1
Algeria	1	South Africa	1
Eritrea	1	Sudan	25
Ethiopia	21	Tanzania	8
India	6	Turkey	4
Kenya	6	Uganda	5
Malawi	5	Zaire	12
Mali	1	Zambia	16
Mexico	1	Zimbabwe	9
Nigeria	1		
Portugal	1		

Figure 6 Population structure analysis of 272 sweet sorghum accessions from the National Plant Germplasm System based on 2,345 unlinked single nucleotide polymorphisms.

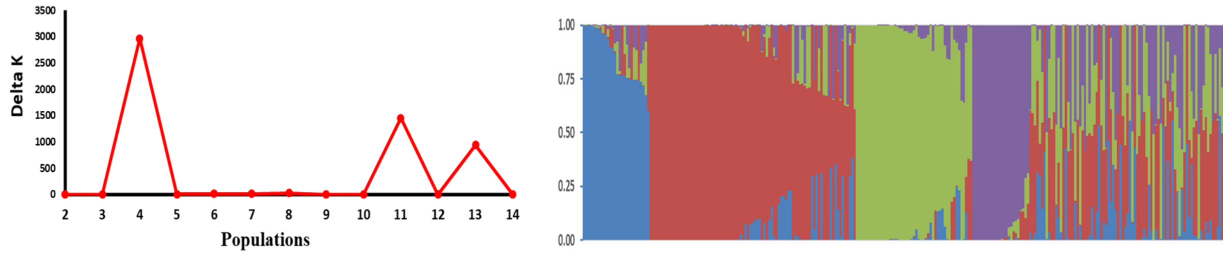


Table 9 Anthracnose resistance response of 224 sweet sorghum accessions from the National Plant Germplasm System collection evaluated in 2017 and 2018. Population structure based on the analysis of 2,633 unlinked single nucleotide polymorphism

	Quantitative[1]						Binary[1]		
	Florida	Georgia	Texas	P.R.[2]	Means		Res.[3]	Susc.[3]	X^2
Pop1	2.3 ± 0.4	2.3 ± 0.4	2.3 ± 0.3	2.1 ± 0.2	2.2 ± 0.2		8	19	0.01
Pop2	3.6 ± 0.9	3.2 ± 0.8	2.6 ± 0.9	2.6 ± 1.0	3.0 ± 0.7		4	69	0.07
Pop3	2.2 ± 0.4	2.4 ± 0.6	2.5 ± 0.6	2.7 ± 0.9	2.4 ± 0.5		5	30	0.75
Pop4	2.8 ± 0.7	2.9 ± 0.8	3.0 ± 0.9	2.6 ± 0.8	2.8 ± 0.6		3	15	0.59
ADX	2.7 ± 0.8	2.8 ± 0.9	2.7 ± 0.8	2.7 ± 1.0	2.7 ± 0.7		8	63	0.75
Means	2.9 ± 0.9	2.8 ± 0.8	2.6 ± 0.8	2.6 ± 0.9		Total	28	196	

[1]Anthracnose resistance based on a scale of 1 to 5 (Erpelding and Prom, 2004; Prom et al. 2009), where 1 and 2 are resistant, and 3-5 susceptible. [2] Average based on 2017 evaluation. [3] Refers to resistant and susceptible to anthracnose.

III - Opportunities for training and professional development

Puerto Rico

1- The project has enable the first professional work opportunity for female technician Giseiry Rosa-Valentin through a 2 years temporary position.

2- Three male field technicians (Israel Beniquez, Roberto Machado and Robert McPhail) were temporary hired (~1,000 hours per year) through funds from this grant to assist in the establishment, maintaining and harvesting of sorghum during the field experiment.

3- One undergraduate student from the Interamerican University of Puerto Rico (Fabiola Lopez) was temporary hired through funds from this grant to assist in laboratory work

4- The project provided educational training opportunity to one female graduate student (Clara Cruet-Burgos master student from the University of Puerto Rico-Mayaguez Campus), who joined my research group in August 2015 and completed her M.S. thesis by December 2017. She is currently a PhD candidate in sorghum genomics with Dr. Rhode at Kansas State University.

Georgia

1- Three undergraduate students (Kelly Perry, Steven Hughes and Christopher Dudley) from the Abraham Baldwin Agriculture College were hired through the length of the project to assist in field related works.

Florida

1- Graduate student trainees: *Dr. Terry Felderhoff* performed the fine-mapping of resistance loci on sorghum chromosome 9. He is currently a post-doc in sorghum genomics with Dr. Geoffrey Morris at Kansas State University. *Lauren Stutts* is a PhD candidate in the Plant Molecular & Cellular Biology program and her dissertation research, supported by the project, is focused on anthracnose resistance in sorghum. *Michael Riley II* is a PhD candidate in the Plant Molecular & Cellular Biology program who assisted with field-related activities for the project. Michael is a minority student. *Nathaniel Ellis* is a PhD candidate in the Plant Molecular & Cellular Biology program who assisted in the project during a 3-month lab rotation in his first year of graduate school.

2- Undergraduate student trainees: *Nicole Gryczuk*, University of Florida, Microbiology & Cell Science; Summer 2017 through Spring 2018. *Thomas Morse*, University of Florida, Microbiology & Cell Science; Summer 2018 *Dominick Padilla*, minority student, Valencia College, Orlando. Summer 2017**.** These students assisted with all aspects of the field experiments. Nicole was paid on project funds for her efforts; Dominick and Thomas had summer research assistantships from the USDA.

3- Visiting scientist: *Dr. Srinivasa P. Rao* from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT, Hyderabad, India) visited during the period October 2015-December 2016 and assisted with field experiments and with the preparation of viral vectors for

gene silencing experiments. He was not paid from project funds. He was offered a position at the USDA-ARS in Stoneville, MS.

Texas

1- In collaboration with Texas A&M University, the project hired two part-time female workers to assist with greenhouse and field evaluations of the sorghum germplasm and RILs against the anthracnose pathogen.

IV – Dissemination of results to scientific community

The results and analysis are still being compiled for peer reviewed publications but have been disseminated to scientific community through conferences and meeting.

Conferences

Cuevas HE, Cruet-Burgos CM, Prom LK, Knoll JE, Stutts L, Vermerris W (2018) Genomic dissection of anthracnose resistance response in *Sorghum bicolor* (L.) Moench. Conference *Sorghum in the 21st Century*, Cape Town, South Africa. *Poster presentation*

Cuevas HE, Cruet-Burgos CM, Prom LK, Knoll JE, Stutts L, Vermerris W (2018) Genomic dissection of anthracnose resistance response in *Sorghum bicolor* (L.) Moench. 2018 Genomic Science PI Meeting Conference, Tysons, VA. *Oral presentation*.

Cuevas HE, Prom LK, Knoll JE, Vermerris W (2018) Mining sorghum genetic diversity to genomic dissect anthracnose resistant response. 11th International Mycological Congress. San Juan, Puerto Rico. *Oral presentation*

Stutts L, Felderhoff TJ, Vermerris W (May 11, 2018) Evaluating candidate genes for anthracnose resistance in sorghum with virus-induced gene silencing. UF Plant Molecular & Cellular Biology Annual Research Symposium. Daytona Beach, FL [oral presentation]

Vermerris W, Silva TN, Stutts L, Riley II MK, Abril A, Cuevas HE (April 30, 2018) Next-generation sweet sorghums for successful cultivation in the southeastern United States. 40th Symposium on Biotechnology for Fuels and Chemicals. Clearwater, FL [poster presentation]

Cuevas HE, Prom LK, Knoll HE, Vermerris W (2017) Genomic dissection of anthracnose resistant response in sorghum [*Sorghum bicolor* (L.)]. 2017 Genomic Science PI Meeting. Washington D.C. *Poster presentation*

Cuevas HE, Prom LK, Cooper EA, Knoll JE, Ni X (2017) Genome-wide association mapping of anthracnose (*Collectotrichum sublineolun*) resistance in U.S. sorghum association panel. *Sorghum Improvement Conferences of North America (SICNA)*. St. Louis, MO. *Poster presentation*

Vermerris W (July 31, 2017) Integrated bioprocessing of sorghum for the sustainable production of renewable fuels and chemicals. Renewable Energy Systems and Sustainability Conference. Lakeland, FL. [invited oral presentation]

Stutts L, Felderhoff TJ, Vermerris W (May 6, 2017) Evaluating candidate resistance genes for anthracnose resistance in sorghum with virus-induced gene silencing. UF Plant Molecular & Cellular Biology Annual Research Symposium. Daytona Beach, FL [oral presentation]

Stutts L, Felderhoff TJ, Vermerris W (April 9, 2017) Evaluating candidate resistance genes for anthracnose resistance in sorghum with virus-induced gene silencing. Southern Section of the American Society of Plant Biologists, Orlando. [poster presentation]

Cruet-Burgos C, Cuevas HE (2016) Genome mapping of anthracnose resistant loci from Ethiopia, India and Sudan population. XXIV Plant and Animal Genome Conference. San Diego, CA. *Poster presentation*

Stutts L, Rao SP, Felderhoff TJ, Vermerris W (November 30, 2016) Visualization of *Colletotrichum sublineolum* growth in *Sorghum bicolor*. Florida Genetics Symposium 2016. University of Florida, Gainesville FL. [poster presentation]

Vermerris W (November 17, 2016) Sorghum genetics and genomics resources. Department of Plant Breeding, Wageningen University, the Netherlands [workshop presentation]

Vermerris W (November 14, 2016) Genetic improvement of sorghum for the production of fuels and chemicals. Department of Plant Breeding, Wageningen University, the Netherlands [departmental seminar]

Vermerris W (July 14, 2016) Genetic improvement of sorghum for the sustainable production of fuels and chemicals. The Allied Genetics Conference. Orlando, FL. [contributed oral presentation]

Felderhoff TJ, McIntyre LM, Saballos A, Olmstead JW, Vermerris W (July 13-17, 2016) A comparison of PCR-based and GBS-based methodologies to fine-map anthracnose resistance loci in sorghum. The Allied Genetics Conference. Orlando, FL. [poster presentation]

Rao SP, Felderhoff TJ, Stutts L, Vermerris W (July 13-17, 2016) Validation of candidate anthracnose resistance genes in sorghum via Brome Mosaic Virus-mediated gene silencing. 13-17 July 2016. The Allied Genetics Conference. Orlando, FL. [poster presentation]

Vermerris W (April 26, 2016) Improving bioenergy sorghums for the sustainable production on low-productivity land. 38th Symposium on Biotechnology for Fuels and Chemicals. Baltimore, MD. [contributed oral presentation].

Vermerris W (March 2, 2016) Sustainable production of fuels and chemicals from sorghum. Indian Institute for Millet Research (IIMR), Hyderabad, India. [seminar]

Vermerris W (March 1, 2016) Sustainable production of fuels and chemicals from sorghum. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad, India. [seminar]

Vermerris W (February 17, 2016) Next-generation sweet sorghums for the sustainable production of fuels and chemicals. 17 February 2016. Dale Smith Memorial Seminar, University of Wisconsin, Madison, WI. [invited seminar].

Vermerris W (January 29, 2016) The prospects of producing fuels and chemicals from sweet sorghum in the Southeastern U.S. Sweet Sorghum Association. Orlando, FL. [invited oral presentation].

Vermerris W (November 18, 2015) Next-generation sweet sorghums for the sustainable production of fuels and chemicals. Department of Crop Production Ecology, Swedish Agricultural University (SLU), Uppsala, Sweden. [seminar]

PhD Dissertation

Felderhoff TJ (August 2016) "Genome-enabled improvement of anthracnose resistance and sugar yield in sweet sorghum". PhD Dissertation. University of Florida.

M.S. Thesis

Cruet-Burgos CM (December 2017) "Genome mapping of anthracnose resistance in sorghum germplasm". M.S. Thesis. University of Puerto Rico-Mayaguez Campus, Mayaguez, Puerto Rico.

Peer reviewed publications (related activities)

Cuevas HE, Prom LK, Cooper EA, Knoll JE, Ni X (2018) Genome-wide association mapping of anthracnose (*Colletotrichum sublineolum*) resistance in the U.S. sorghum association panel Plant Genome 11:170099 doi:10.3835/plantgenome2017.11.009

Cuevas HE, Prom LK, Rosa-Valentin G (2018) Population structure of the NPGS Senegalese sorghum collection and its evaluation to identify new disease resistant genes. Plos One 13: e0191877

Cuevas HE, Prom LK, Cruet-Burgos CM (2018) Genome-wide association mapping of anthracnose (*Colletotrichum sublineolum*) resistance in NPGS Ethiopian sorghum germplasm. G3 (*under revision*)

Felderhoff TJ Olmstead JW, Vermerris W (2017) A cost-benefit analysis to select the most effective method for positional cloning: genotyping by sequencing or allele-specific PCR. Euphytica. 213: 286 doi.org/10.1007/s10681-017-2068-0

Felderhoff TJ, Saballos A, McIntyre LM, Vermerris W (2016). Using genotyping by sequencing to map two novel anthracnose resistance loci in sorghum. G3 [Genes Genomes Genetics] 6: 1935-1946. doi: 10.1534/g3.116.030510