ORIGINAL ARTICLE

GWAS analysis of sorghum association panel lines identifies SNPs associated with disease response to Texas isolates of *Colletotrichum sublineola*

Louis K. Prom[1] · Ezekiel Ahn[2] · Thomas Isakeit[2] · Clint Magill[2]

Abstract

Key message **SNPs identify prospective genes related to response to *Colletotrichum sublineola* (anthracnose) in the sorghum association panel lines.**

Abstract Sorghum association panel (SAP) lines were scored over several years for response to *Colletotrichum sublineola*, the causal agent of the disease anthracnose. Known resistant and susceptible lines were included each year to verify successful inoculation. Over 79,000 single-nucleotide polymorphic (SNP) loci from a publicly available genotype by sequencing dataset available for the SAP lines were used with TASSEL association mapping software to identify chromosomal locations associated with differences in disease response. When the top-scoring SNPs were mapped to the published sorghum genome, in each case, the nearest annotated gene has precedence for a role in host defense.

Introduction

The disease anthracnose is considered to be a global problem in sorghum production. It can infect leaves, stalks, panicles and seeds while exhibiting extreme variability in terms of pathogenicity to host cultivars (Tesso et al. 2012). Although many sorghum cultivars have shown resistance to one or more isolates, that resistance is often fleeting due to variability in the pathogen, *Colletotrichum sublineola* (also often written *C. sublineolum*). Sutton first proposed (Sutton 1968) that appressorium shape for anthracnose isolates from sorghum justified designation as a separate species (*C. sublineola*) from that in maize (*Colletotrichum graminicola*), but it was not until ribosomal ITS DNA sequence data (Sherriff

✉ Louis K. Prom
louis.prom@ars.usda.gov

[1] USDA-ARS, Southern Plains Agricultural Research Center, College Station, TX 77845, USA

[2] Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843, USA

et al. 1995) became available that the separate species designations were generally accepted. As would be expected, the two genomes are overall quite similar, but significant differences have been revealed (Buiate et al. 2017).

Because PCR amplification identified portions of both MAT1 and MAT2 sequences in some *C. sublineola* isolates (neither was found in others) (Zanette et al. 2009), it can be assumed that there is an ascomycetous perfect stage (*Glomerella* sp.) as is the case for other anthracnose-causing pathogens, including *C. graminicola* (Politis 1975). However, as is also the case of the maize pathogen, no reports of crosses leading to ascospore production in natural situations appear in the literature. Even so, there is a considerable variation found among isolates as can be detected based on the use of host differentials that are susceptible to some pathotypes but resistant to others. Examples include identification of eight different response patterns among 12 isolates from Texas, Georgia and Puerto Rico using eight host cultivars (Cardwell 1989), 13 pathotypes among 87 isolates from Arkansas using the same eight cultivars (Moore et al. 2008), while Prom et al. (2012) recorded nine pathotypes among isolates from the USA and Puerto Rico using the same eight cultivars, but adding six additional hosts showed that all 18 isolates differed in pathogenicity factors (Prom et al. 2012). A study from Brazil, with 10 hosts, including the same eight used in prior studies revealed 22 races among 37 isolates (Valèrio et al. 2005). A later study identified 68

unique pathotypes among 480 isolates, with greater variation in virulence detected from isolates taken from plots where three-way hybrids were grown than from plots with pure lines (Costa et al. 2015). DNA sequence variation is also readily detected, as has been documented with increasingly sophisticated technology. Examples include the use of PCR with single RAPD primers (Guthrie et al. 1992; Valèrio et al. 2005), RFLPs (Rosewich et al. 1998) and AFLPs (Chala et al. 2011; Prom et al. 2012).

The sorghum association panel was developed as a community resource by including 277 lines converted for short stature and day-neutral flowering while retaining 90% of the genome of the original exotic cultivar along with 149 other breeding lines. Lines included were selected to include diversity with respect to geographic origin, morphological and genotypic/phenotypic characters that have proven useful in breeding (Casa et al. 2008). A further resource was created when the SAP lines were included in a genotyping by sequencing project that revealed locations of more than 260,000 single-nucleotide polymorphisms that are well spread throughout all ten chromosomes of sorghum (Morris et al. 2013a). The panel is being widely used for genome-wide association studies in order to locate tags for traits useful in breeding. Traits examined using the SAP SNP data for a genome-wide association study (GWAS) include traits such as plant height and inflorescence branch length (Morris et al. 2013b), flavonoid pigment traits (Morris et al. 2013b), grain polyphenol concentrations (Rhodes et al. 2014), seed size (Zhang et al. 2015), stalk rots (Adeyanju et al. 2015) and thermal stress in seedlings (Chopra et al. 2017). A GWAS study based on the 242 lines in the sorghum mini-core collection identified eight genes potentially related to anthracnose resistance in the vicinity of SNP alleles (Upadhyaya et al. 2013). Most recently, another GWAS study also examined anthracnose resistance as measured in Puerto Rico in the SAP accessions using a different analysis procedure. That study was able to identify three candidate resistance genes and showed that resistant accessions came from all four major races of sorghum (Cuevas et al. 2018). Because differences were found in the disease response of a number of the same cultivars when tested in Texas, another GWAS analysis was made using TASSEL (Bradbury et al. 2007). Here, we report finding additional genes that appear to be associated with relative resistance or susceptibility to anthracnose as it occurs in Texas.

Materials and methods

Disease evaluations

In this study, 359 lines from the sorghum association panel (SAP) with SC748 as resistant check and BTx623, RTx430, Tx2911, TAM428, ATx623, Sureno, Dorado as susceptible checks were evaluated for resistance against *C. sublineola*. Seed from the SAP lines were obtained from the USDA-ARS, Plant Genetic Resources Conservation Unit, Griffin, Georgia. The anthracnose evaluations were conducted during the 2010, 2013, 2014 and 2015 growing seasons at the Texas AgriLife Experiment Station, near College Station, Texas. Each line was included in at least two evaluation periods. In the trials, lines were planted in a randomized complete block design, with each line replicated three times. Seeds were planted in 6 m rows at 0.31 m spacing between rows. Standard field preparation which included fall plowing and incorporation of the compound fertilizer NPK was employed during the evaluation years. To control weeds and seedling insects, a pre-emergent insecticide and herbicide were applied before planting and supplemented with hand weeding during the growing season.

The inoculation technique and disease assessment method used were previously described (Prom et al. 2009). Briefly, inoculum was prepared by inoculating autoclaved sorghum grain with a spore suspension from one of eight local isolates of *C. sublineola* (FSP2, FSP5, FSP7, FSP35 and FSP36, from Sorghum line BTx635, FSP46 and FSP50 from RTx 2536, and FSP53 from BSBC) that were harvested after growth on half-strength potato dextrose agar. Each isolate was grown separately on the autoclaved seeds in large, open plastic bags, with daily mixing to facilitate complete colonization. After 3–4 days of incubation, the isolates were mixed to give a uniform inoculum for the plants. Sorghum plants were inoculated 30 days after planting by placing 8–10 *C. sublineola*-colonized grains into the leaf whorls. Disease assessments were conducted 30 days after inoculating the plants and thereafter on a weekly basis for 3 weeks. Plants were rated on a scale of 1–5, where 1 = no symptoms or chlorotic flecks on leaves; 2 = hypersensitive reaction (reddening or red spots) on inoculated leaves, but no acervuli formation and symptoms observed on other leaves; 3 = lesions on inoculated and bottom leaves with acervuli in the center; 4 = necrotic lesions with acervuli observed on inoculated and bottom leaves with infection spreading to middle leaves; and 5 = most leaves are dead due to infection with infection on the flag leaf containing abundant acervuli. The symptom types were then categorized into two reaction classes, resistant = rated as 1 or 2 and susceptible = rated as 3, 4 or 5.

GWAS and SNP mapping

TASSEL (Bradbury et al. 2007) version 5.2.48 was used to conduct a mixed linear model (MLM) association analysis based on mean scores for disease ratings. The 2018 updated version of genome-wide SNP data for the SAP originally made available for public use (Morris et al. 2013a) (link

https://www.morrislab.org/data) was used in this analysis. In order to reduce potential false associations, SNPs with greater than 20% unknown alleles were removed as were those with minor allele frequency (MAF) below 5%. Principal component analysis was used to calculate the population structure which was followed by calculation of kinship data based on the TASSEL 'centered IBS' method. Optimum compression level and P3D variance component estimation were used as MLM options. SNPs with high probability of contribution to anthracnose scores were tracked to the specific chromosome location based on the sorghum genome sequence, version 3.1.1 available at the JGI Phytozome 12.5.1 web site, updated in 2018 (McCormick et al. 2018). For each of the prospective genes included in Table 1, the mean disease rating score for all SAP lines with either of the two prevalent bases was determined and verified to differ significantly ($P < 0.05$) using JMP statistical software from SAS®, version 14.

Results

Due to the size of the collection data for anthracnose response of 359 of the 377 lines in the SAP collection, in most cases, each line was tested in replicates for at least 2 years, with some cultivars included each year to serve as controls. All of the cultivars showed evidence for active defense soon after inoculation in the form of reddish purple pigment (3-deoxyanthocyanidin) typically made by sorghum (Nicholson et al. 1987). Thus, all cultivars were scored between 2 and 5 based on the subsequent level of disease development. Year to year consistency on scoring of the controls rendered further adjustments unnecessary. Consequently, the average disease rating for each cultivar, calculated for between 2 (11 lines) and 14 replications, was used directly for identification of potential QTLs. One cultivar with only two replicates was not included based on extreme differences in scoring of the plants in years, suggesting segregation or seed contamination. Summary of anthracnose scoring data is provided in Supplementary Table 1.

Although the genotyping data provided by Morris et al. identified 267,487 SNPs across the association panel (Morris et al. 2013a), many of the sequencing reads include a number of unidentified bases (*N*). Borrowing from the protocol described for stalk rots (Adeyanju et al. 2015), we eliminated all cases where there were greater than or equal to 20% ambiguous sites, leaving 79,034 SNP markers to use for anthracnose response association analysis. Eliminating minority alleles present in less than 5% of the SAP lines also means that any single gene resistance locus present in the SAP lines below that threshold would also be missed. The Manhattan plot shown in Fig. 1 based on 358 members of the SAP shows strong indication that quantitative trait loci

on eight chromosomes have a role related to resistance to *Colletotrichum sublineola*.

A list of SNP markers that identify 18 chromosomal locations associated with disease response starting with the least likely one to represent chance association is shown in Table 1. These correspond to the TASSEL-based Manhattan plot values below 5.2×10^{-4}. When mapped back to the published genome, all 18 of these SNPs are nearest to genes that have previously been implicated in various resistance responses or identified in other disease association studies. Table 1 also shows the distance in base pairs to the nearest gene, the bases that create the SNP and the fraction of the population with each SNP allele.

Discussion

With an overall genome size estimated at 732 Mb (Paterson 2008) and an overall length in terms of recombination of around 1600 cM (Mace et al. 2009), detection of 79,304 SNPs among the SAP lines suggests an average of one SNP per approximate 10 kb of sequence and 0.02 cM in map distance. However, the use of the restriction endonuclease *ApeK*1 that has partial DNA methylation sensitivity in the genotyping by sequencing technique helps to favor cuts and sequencing from genic regions over transposon-related repeat sequences (Elshire et al. 2011). SNP gene association was made using the latest version (3.1.1) of the sorghum genome available at the Phytozome Web site (v12.1.5) which offers greatly improved gene annotation over prior versions. The latest version took advantage of sequences now available from other cultivars and for transcriptome data from an array of tissues and conditions (McCormick et al. 2018).

On tracking the location of the SNP variants showing significant differences to the annotated genome, all of the genes nearest to the point of the SNP variant have logical roles in host defense. The highest probability of association was a series of 4 SNPs on chromosome 3 between 72,520,274 and 72,520,325 which are near a gene for a pentatricopeptide repeat (PPR). While members of this large gene family with RNA binding capacity are usually associated with organelle development, a PPR has been shown to be involved with defense against necrotrophic fungi in *Arabidopsis* (Laluk et al. 2011). Likewise, PPR protein-encoding sequences have also been identified as candidate QTLs for resistance to *Fusarium graminearum* in soybean (Cheng et al. 2017) and to *Puccinia triticina*, the leaf rust pathogen in wheat (Juliana et al. 2018). The top SNP tagged gene on chromosome 8 has a leucine-rich repeat motif, a feature found in the vast majority of known disease resistance genes (Sekhwal et al. 2015). The gene identified by two SNPs separated by 41,396 base pairs on chromosome 2 encodes 9-*cis*-epoxycarotenoid dioxygenase, an enzyme involved in ABA biosynthesis. While

Table 1 Genes nearest to the most significant SNPs with allele disease score comparisons

Chr.	Location	Nearest gene and function	Base pairs away	SNP base %	TASSEL *P* value	Average score/SNP
3	72,520,274 and 3 more within 50 bp	Sobic.003G420000 (PPR) Pentatricopeptide repeat	809	G: 74% A: 26%	1.5353×10^{-5}	G: 3.263 A: 3.706
8	644,157	Sobic.008G008400 PF13516-leucine-rich repeat (LRR6)	7741	A: 95% G: 5%	1.3698×10^{-4}	A: 3.352 G: 2.709
2	53,144,307 53,102,911	Sobic.002G168700 9-*cis*-epoxycarotenoid dioxygenase	26,951 and 14,445	C: 87% A: 13%	1.3817×10^{-4}	C: 3.499 A: 2.550
2	53,383,079	Sobic.010G246700PF13947 Calcium-binding EGF domain (EGF_CA)// Protein tyrosine kinase (Pkinase_Tyr)// Wall-associated receptor kinase galacturonan-binding (GUB_WAK_bind)	0	C: 88% T: 12%	2.1907×10^{-4}	C: 3.426 T: 2.536
5	1,734,854	Sobic.005G019100 Similar to TRAF-type zinc finger family protein	365	C: 94% T: 6%	2.3012×10^{-4}	C: 3.345 T: 2.998
10	58,670,065	Sobic.010G246700 Xyloglucan:xyloglucosyl transferase	9087	G: 94% A: 6%	2.6589×10^{-4}	G: 3.305 A: 4.194
8	635,089 629,950	Sobic.008G008200 Serine and Sphingolipid biosynthesis protein	2634 and 229	T: 95% C: 5%	2.7336×10^{-4}	T: 3.341 C: 2.730
5	2,438,868 2,438,903 2,438,909	Sobic.005G027400 Similar to Os12g0136200 protein; Heterogeneous nuclear ribonucleoprotein R (RRM superfamily)	0	C: 54% G: 46%	3.4974×10^{-4}	C: 3.571 G: 3.178
6	54,145,482	Sobic.006G186300 PTHR32382:SF5-Fasciclin-like Arabinogalactan Protein	2428	G: 58% A: 42%	3.6124×10^{-4}	G: 3.225 A: 3.495
3	58,345,905	Sobic.003G244200 Similar to vacuolar ATP synthase subunit E Diacylglycerol O-Acyltransferase, The transport of protons against an electrochemical gradient	0	C: 64% A: 36%	3.7465×10^{-4}	C: 3.136 A: 3.695
6	54,390,750	Sobic.006G189700 Similar to OSJNBa0064M23.12 protein, RING-H2 finger protein ATL60	0	T: 90% C: 10%	3.8711×10^{-4}	T: 3.238 C: 4.020
2	61,613,441 61,613,455	Sobic.002G224400 Similar to Putative uncharacterized protein, protein kinase domain, D-mannose-binding lectin	16,661 and 16,675	G: 81% C: 19%	4.1787×10^{-4}	G: 3.418 C: 3.023
2	60,288,158	Sobic.002G210100 Similar to AAA ATPase, central region (50.1 kD)-like protein	1140	A: 87% G: 13%	4.2892×10^{-4}	A: 3.469 G: 2.587
2	61,435,604	Sobic.002G222800 Similar to Putative Avr9 elicitor response protein	782	A: 82% C: 18%	4.3563×10^{-4}	A: 3.449 C: 3.051
2	52,938,340	Sobic.002G168600 Similar to Putative uncharacterized protein, Xanthine/uracil/vitamin C permease family	55,379	G: 87% A: 13%	4.6379×10^{-4}	G: 3.511 A: 2.491
1	67,460,226	Sobic.001G387300 Similar to secondary cell wall-related glycosyltransferase family 47	276	G: 79% T: 21%	4.5839×10^{-4}	G: 3.329 T: 3.594
8	51,178,873	Sobic.008G110000 Weakly similar to Putative dsRNA-binding protein ODB1	27,486	A: 55% C: 45%	5.065×10^{-4}	A: 3.081 C: 3.589

Table 1 (continued)

Chr.	Location	Nearest gene and function	Base pairs away	SNP base %	TASSEL *P* value	Average score/SNP
7	10,842,226	Sobic.007G085600 Similar to putative xyloglucan endo-trans-glycosylase Glycosyl hydrolases family 16, Xyloglucan endo-transglycosylase (XET) C-terminus	12,270	C: 75% G: 25%	5.1907×10^{-4}	C: 3.492 G: 2.733

The top candidate locations of SNP-detected QTLs and nearby genes associated with response to anthracnose are elucidated
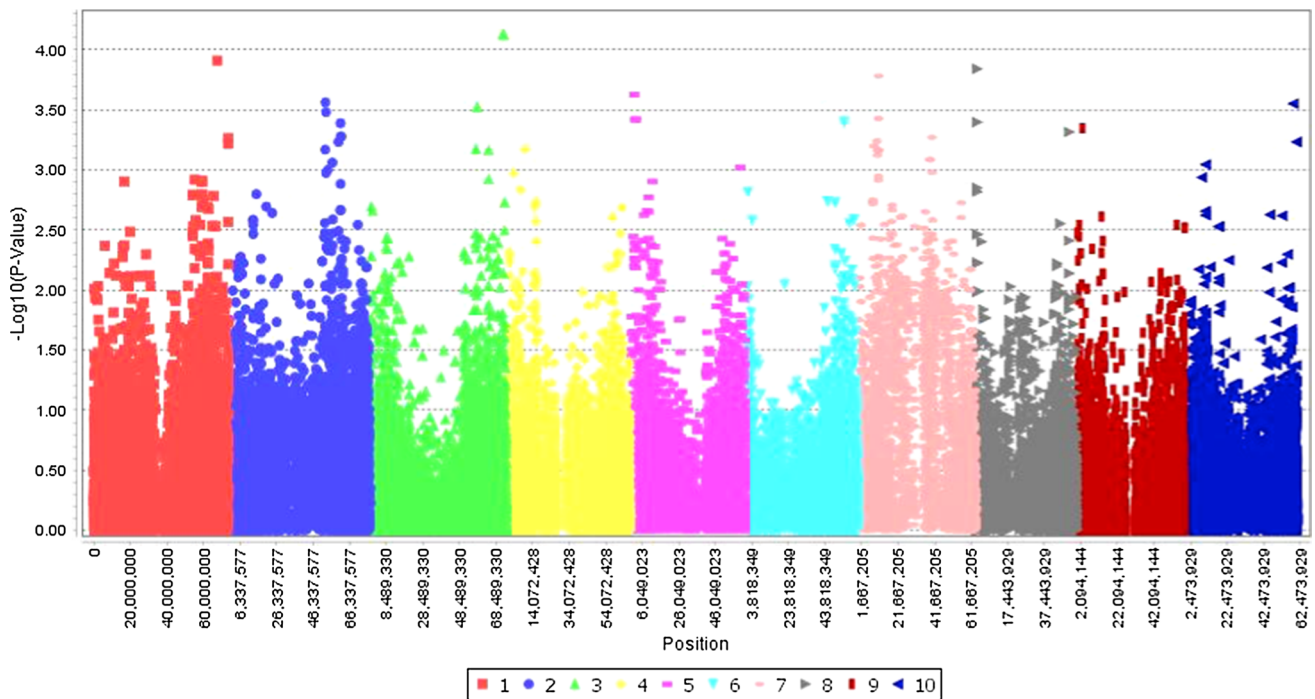

Fig. 1 Manhattan plot showing locations of SNP-detected QTLs associated with response to anthracnose on the ten chromosomes of *Sorghum bicolor*. All SNPs above −Log10 3.00 were examined for potential roles in disease response

known primarily for a role in abiotic stress responses, ABA has been shown to increase the amount of disease for some pathogens and to reduce disease in others (Ton et al. 2009; Liao et al. 2018).

Several of the SNP-identified genes have obvious potential for involvement in disease defense, including genes involved in cell wall synthesis and strengthening. Examples include SNPs near a xyloglucan endo-transglycosylase on chromosome 10 and another on chromosome 7. Levels of mRNA encoding this protein fell in Arabidopsis following inoculation with *Pseudomonas syringae* (Mohr and Cahill 2007). Other cell wall components, including a galacturon-binding receptor kinase located on chromosome 2, the ara-binogalactan protein on chromosome 6 and a glycosyl trans-ferase on chromosome 1, have previously been implicated in disease resistance in plants (Vorwerk et al. 2004; Krattinger et al. 2009).

RING finger proteins such as that identified by a SNP on chromosome 6 have been shown to be involved during infection of rice by *Magnaporthe oryzae* (Li et al. 2014).

It would make sense that energy metabolism would have an effect on host–pathogen interactions, and in fact, other subunits of vacuolar ATP synthase have long been impli-cated (Vera-Estrella et al. 1994) based on elicitor responses in tomato. Thus, differences in the ATP synthase subu-nit E identified on chromosome 3 may also affect disease responses as seen here. Similarly, a gene nearest to another SNP on chromosome 8 identifies a serine-domain gene involved in sphingolipid synthesis that was also identified as a cotton gene associated with Verticillium resistance (Li et al. 2017). Plant mannose-binding lectins (MBLs) are crucial for pepper plant defense signaling during pathogen attack by recognizing specific carbohydrates on pathogen surfaces (Hwang and Hwang 2011); two SNPs related to

MBLs were found on chromosome 2. Also, a marker on chromosome 2 identifies an AAA ATPase, an enzyme known to be involved in immunity in both mammals and plants, and also potentially involved in sorghum immunity as well (Zhu et al. 2016). The first fungal *Avr* gene (*Avr9* gene) was reported in 1991 (Shen et al. 2017); here, a SNP was detected near an *Avr9* elicitor response protein. Interestingly, a member of the xanthine/uracil/vitamin C permease family was spotted on chromosome 2 in this study. When *C. elegans* was infected by *S. aureus*, xanthine/uracil/vitamin C permease was 6.9-fold induced 8 h postinoculation (Irazoqui et al. 2010). While *C. elegans* is not a plant, many similarities in active disease defense in plants and animals have been documented (Nürnberger et al. 2004).

RNA-binding proteins are known to take roles in plant immune responses against microbial pathogens (Woloshen et al. 2011), and on chromosome 8, a SNP near dsRNA-binding protein was detected. Finally, RNA recognition motif (RRM) gene expression has been shown to be highly induced in rice by inoculation with *Xanthomonas oryzae* pv. *oryzicola* (Zhou et al. 2010). In fact, a number of prospects further down the list than those included in Table 1 such as another leucine-rich repeat component on chromosome 8 and a peroxidase on chromosome 4 were also identified. Peroxidases are universally recognized as an early response in host defense (Hammerschmidt 1999).

Essentially, the same population was recently subjected to GWAS analysis by Cuevas et al. (2018) based on response to *C. sublineola* isolates from Puerto Rico. Because the *C. sublineola* isolates differ in the two locations, as do the climatic conditions (Prom et al. 2012), it was considered useful to explore SNP linkage to disease response to local isolates in the Texas environment. Cuevas et al. initially used an enriched mixed linear model (ECMLM) procedure with 268,289 SNP sites, but found no SNPS highly associated with resistance. A follow-up logistic regression analysis that is limited to detecting major genes revealed 3 SNPS on chromosome 5. However, when using TASSEL, we elected to eliminate all SNPs in the database that were ambiguous for 20% or more of the accessions and thus ended up with only 79,034 useful SNPS (Cuevas et al. 2018). Even though some or all of the high-scoring SNPs identified in this study could have occurred by chance, we chose to determine whether or not high-scoring SNPs in the Manhattan plot are associated with genes related to host defense. As documented, that turned out to be the case. Even though we also described two markers on chromosome 5, they were not at the same location as those described by Cuevas et al. Most of the genes described here would be expected to contribute to various aspects of host defense and thus not register as major genes for resistance. Population structure can be important in GWAS studies; however, in both studies, the observation that resistant accessions were found in each of

the five races of sorghum suggested that population structure was not a factor.

We realize that other nearby genes may be even more critical, than those listed here, but the fact that each of the highest scoring SNPs can be associated with genes already known to play a role in host defense strongly suggests their potential importance for use in breeding, as well as for future tests to verify a potential role for these genes in active host defense.

Compliance with ethical standards

Ethical standards All research has been conducted to satisfy TAMU System BSL-1 Biological Safety Standards.

References

Adeyanju A, Little C, Yu J, Tesso T (2015) Genome-wide association study on resistance to stalk rot diseases in grain sorghum. G3 Genes Genom Genet 5:1165. https://doi.org/10.1534/g3.114.016394

Bradbury PJ, Zhang Z, Kroon DE, Casstevens TM, Ramdoss Y, Buckler ES (2007) Tassel: software for association mapping of complex traits in diverse samples. Bioinformatics 23:2633–2635

Buiate EAS, Xavier KV, Moore N, Torres MF, Farman ML, Schardl CL, Vaillancourt LJ (2017) A comparative genomic analysis of putative pathogenicity genes in the host-specific sibling species *colletotrichum graminicola* and *Colletotrichum sublineola*. BMC Genom 18:67. https://doi.org/10.1186/s12864-016-3457-9

Cardwell KF (1989) Pathotypes of *Colletotrichum graminicola* and seed transmission of sorghum anthracnose. Plant Dis 73:255–257

Casa AM, Pressoir G, Brown PJ, Mitchell SE, Rooney WL, Tuinstra MR, Franks CD, Kresovich S (2008) Community resources and strategies for association mapping in sorghum. Crop Sci 48:30–40

Chala A, Tronsmo AM, Brurberg MB (2011) Genetic differentiation and gene flow in *Colletotrichum sublineolum* in Ethiopia, the centre of origin and diversity of sorghum, as revealed by AFLP analysis. Plant Pathol J 60:474–482

Cheng P, Gedling CR, Patil G, Vuong TD, Shannon JG, Dorrance AE, Nguyen HT (2017) Genetic mapping and haplotype analysis of a locus for quantitative resistance to *Fusarium graminearum* in soybean accession pi 567516c. Theroret Appl Genet 130:999–1010

Chopra R, Burow G, Burke JJ, Gladman N, Xin Z (2017) Genome-wide association analysis of seedling traits in diverse sorghum germplasm under thermal stress. BMC Plant Biol 17:12

Costa RV, Zambolim L, Cota LV, Silva DD, Parreira DF, Lanza FE, Souza AGC (2015) Pathotypes of *Colletotrichum sublineolum* in response to sorghum populations with different levels of genetic diversity in Sete Lagoas-MG. J Phytopathol 163:543–553

Cuevas HE, Prom LK, Cooper EA, Knoll JE, Ni X (2018) Genome-wide association mapping of anthracnose (*Colletotrichum sublineoloum*) resistance in the US sorghum association panel. Plant Genome 11:1. https://doi.org/10.3835/plantgenome2017.11.0099

Elshire RJ, Glaubitz JC, Sun Q, Poland JA, Kawamoto K, Buckler ES, Mitchell SE (2011) A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. PLoS ONE 6:e19379

Guthrie PAI, Magill CW, Frederiksen RA (1992) Random amplified polymorphic DNA markers: a system for identifying and differentiating isolates of *Colletotrichum graminicola*. Phytopathology 82:832–835

Hammerschmidt R (1999) Induced disease resistance: how do induced plants stop pathogens? Physiol Mol Plant Pathol 55:77–84

Hwang IS, Hwang BK (2011) The pepper mannose-binding lectin Gene *CaMBL1* is required to regulate cell death and defense responses to microbial pathogens. Plant Physiol 155(1):447–463

Irazoqui JE, Troemel ER, Feinbaum RL, Luhachack LG, Cezairliyan BO, Ausubel FM (2010) Distinct pathogenesis and host responses during infection of *C. elegans* by *P. aeruginosa* and *S. aureus*. PLoS Pathogens 6(7):e1000982. https://doi.org/10.1371/journal.ppat.1000982

Juliana P, Singh RP, Singh PK, Poland JA, Bergstrom GC, Huerta-Espino J, Bhavani S, Crossa J, Sorrells ME (2018) Genome-wide association mapping for resistance to leaf rust, stripe rust and tan spot in wheat reveals potential candidate genes. Theor Appl Genet. https://doi.org/10.1007/s00122-018-3086-6

Krattinger SG, Lagudah ES, Spielmeyer W, Singh RP, Huerta-Espino J, McFadden H, Bossolini E, Selter LL, Keller B (2009) A putative ABC transporter confers durable resistance to multiple fungal pathogens in wheat. Science 323:1360–1363

Laluk K, AbuQamar S, Mengiste T (2011) The arabidopsis mitochondria-localized pentatricopeptide repeat protein PGN functions in defense against necrotrophic fungi and abiotic stress tolerance. Plant Physiol 156:2053–2068

Li W-T, Chen W-L, Yang C, Wang J, Yang L, He M, Wang J-C, Qin P, Wang Y-P, Ma B-T, Li S-G, Chen X-W (2014) Identification and network construction of zinc finger protein (ZFP) genes involved in the rice-'*Magnaporthe oryzae*' interaction [online]. Plant Omics 7:540–548

Li T, Ma X, Li N, Zhou L, Liu Z, Han H, Gui Y, Bao Y, Chen J, Dai X (2017) Genome-wide association study discovered candidate genes of verticillium wilt resistance in upland cotton (*Gossypium hirsutum* L.). Plant Biotechnol J 15:1520–1532

Liao Y, Bai Q, Xu P, Wu T, Guo D, Peng Y, Zhang H, Deng X, Chen X, Luo M, Ali A, Wang W, Wu X (2018) Mutation in rice *abscisic acid2* results in cell death, enhanced disease-resistance, altered seed dormancy and development. Front Plant Sci 9:405. https://doi.org/10.3389/fpls.2018.00405

Mace ES, Rami J-F, Bouchet S, Klein PE, Klein RR, Kilian A, Wenzl P, Xia L, Halloran K, Jordan DR (2009) A consensus genetic map of sorghum that integrates multiple component maps and high-throughput diversity array technology (DaRT) markers. BMC Plant Biol 9:13. https://doi.org/10.1186/1471-2229-9-13

McCormick RF, Truong SK, Sreedasyam A, Jenkins J, Shu S, Sims D, Kennedy M, Amirebrahimi M, Weers Brock D, McKinley B, Mattison A, Morishige Daryl T, Grimwood J, Schmutz J, Mullet JE (2018) The *Sorghum bicolor r*eference genome: improved assembly, gene annotations, a transcriptome atlas, and signatures of genome organization. Plant J 93:338–354

Mohr PG, Cahill DM (2007) Suppression by aba of salicylic acid and lignin accumulation and the expression of multiple genes, in arabidopsis infected with *Pseudomonas syringae* pv. Tomato. Funct Integr Genom 7:181–191

Moore JW, Ditmore M, TeBeest DO (2008) Pathotypes of *Colletotrichum sublineolum* in Arkansas. Plant Dis 92:1415–1420

Morris GP, Ramu P, Deshpande SP, Hash CT, Shah T, Upadhyaya HD, Riera-Lizarazu O, Brown PJ, Acharya CB, Mitchell SE, Harriman J, Glaubitz JC, Buckler ES, Kresovich S (2013a) Population genomic and genome-wide association studies of agroclimatic traits in sorghum. Proc Natl Acad Sci USA 110:453–458

Morris GP, Rhodes DH, Brenton Z, Ramu P, Thayil VM, Deshpande S, Hash CT, Acharya C, Mitchell SE, Buckler ES, Yu J, Kresovich S (2013b) Dissecting genome-wide association signals for loss-of-function phenotypes in sorghum flavonoid pigmentation traits. G3 Genes Genom Genet 3:1. https://doi.org/10.1534/g3.113.008417

Nicholson RL, Kollipara SS, Vincent JR, Lyons PC, Cadena-Gomez G (1987) Phytoalexin synthesis by the sorghum mesocotyl in response to infection by pathogenic and nonpathogenic fungi. Proc Natl Acad Sci USA 84:5520–5524

Nürnberger T, Brunner F, Kemmerling B, Piater L (2004) Innate immunity in plants and animals: striking similarities and obvious differences. Immunol Rev 198:249–266

Paterson AH (2008) Genomics of sorghum. Int J Plant Genom 2008:1. https://doi.org/10.1155/2008/362451

Politis DJ (1975) The identity and perfect state of *Colletotrichum graminicola*. Mycologia 67:56–62

Prom LK, Perumal R, Erpelding J, Isakeit T, Montes-Garcia N, Magill CW (2009) A pictorial technique for mass screening of sorghum germplasm for anthracnose (*Colletotrichum sublineolum*) resistance. Open Agric J. https://doi.org/10.2174/1874331500903010020

Prom LK, Perumal R, Erattaimuthu SR, Little CR, No EG, Erpelding JE, Rooney WL, Odvody GN, Magill CW (2012) Genetic diversity and pathotype determination of *Colletotrichum sublineolum* isolates causing anthracnose in sorghum. Eur J Plant Pathol 133:671–685

Rhodes DH, Hoffmann L, Rooney WL, Ramu P, Morris GP, Kresovich S (2014) Genome-wide association study of grain polyphenol concentrations in global sorghum [S*orghum bicolor* (L.) *moench*] germplasm. J Agric Food Chem 62:10916–10927

Rosewich UL, Pettway RE, McDonald BA, Duncan RR, Frederiksen RA (1998) Genetic structure and emporal dynamics of a *Colletotrichum graminicola* population in a sorghum disease nursery. Phytopathology 88:1087–1093

Sekhwal KM, Li P, Lam I, Wang X, Cloutier S, You MF (2015) Disease resistance gene analogs (RGAs) in plants. Int J Mol Sci 16:19248–19290

Shen Y, Liu N, Li C, Wang X, Xu X, Chen W, Xing G, Zheng W (2017) The early response during the interaction of fungal phytopathogen and host plant. Open Biol 7(5):170057. https://doi.org/10.1098/rsob.170057

Sherriff C, Whelan MJ, Arnold GM, Bailey JA (1995) RDNA sequence analysis confirms the distinction between *Colletotrichum graminicola* and *C. sublineolum*. Mycol Res 99:475–478

Sutton BC (1968) The appressoria of *Colletotrichum graminicola* and *C. falcatum*. Can J Bot 46:873–876

Tesso TRP, Little CR, Adeyanju A, Radwan GL, Prom LK, Magill CW (2012) Sorghum pathology and biotechnology—a fungal disease perspective: part II. Anthracnose, stalk rot, and downy mildew. Eur J Plant Sci Biotechnol 6:33–44

Ton J, Flors V, Mauch-Mani B (2009) The multifaceted role of ABA in disease resistance. Trends Plant Sci 14:310–317

Upadhyaya HD, Wang Y, Sharma R, Sharma S (2013) Identification of genetic markers linked to anthracnose resistance in sorghum using association analysis. Theor Appl Genet 126:649–657. https://doi.org/10.1007/s00122-013-2081-1

Valèrio H, Rèsende M, Weikert-Oliveira R, Casela C (2005) Virulence and molecular diversity in *Colletotrichum graminicola f*rom Brazil. Mycopathologia 159:449–459

Vera-Estrella R, Barkla BJ, Higgins VJ, Blumwald E (1994) Plant defense response to fungal pathogens (activation of host-plasma

membrane H$^+$-atpase by elicitor-induced enzyme dephosphorylation). Plant Physiol 104:1. https://doi.org/10.1104/pp.104.1.209

Vorwerk S, Somerville S, Somerville C (2004) The role of plant cell wall polysaccharide composition in disease resistance. Trends Plant Sci 9:203–209

Woloshen V, Huang S, Li X (2011) RNA-binding proteins in plant immunity. J Pathog 2011:278697. https://doi.org/10.4061/2011/278697

Zanette GF, Nóbrega GMA, Meirelles LDP (2009) Morphogenetic characterization of *Colletotrichum sublineolum* strains, causal agent of anthracnose of sorghum. Trop Plant Pathol 34:146–151

Zhang D, Li J, Compton RO, Robertson J, Goff VH, Epps E, Kong W, Kim C, Paterson AH (2015) Comparative genetics of seed size traits in divergent cereal lineages represented by sorghum *Panicoidae*) and rice (*Oryzoidae)*. G3 Genes Genom Genet 5:1. https://doi.org/10.1534/g3.115.017590

Zhou Y-L, Xu M-R, Zhao M-F, Xie X-W, Zhu L-H, Fu B-Y, Li Z-K (2010) Genome-wide gene responses in a transgenic rice line carrying the maize resistance gene Rxo1 to the rice bacterial streak pathogen, xanthomonas oryzae pv. Oryzicola. BMC Genom 11:1. https://doi.org/10.1186/1471-2164-11-78

Zhu X, Yin J, Liang S, Liang R, Zhou X, Chen Z (2016) The multivesicular bodies (MVBs)-localized AAA ATPase LRD6-6 inhibits immunity and cell death likely through regulating MVBs-mediated vesicular trafficking in rice. PLoS Genet 12(9):e1006311. https://doi.org/10.1371/journal.pgen.1006311