GENETIC STUDY OF RESISTANCE TO CHARCOAL ROT AND FUSARIUM STALK ROT DISEASES OF SORGHUM

by

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B. Agric., Ahmadu Bello University, 2000
M.Sc., Ahmadu Bello University, 2006

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Abstract

Fusarium stalk rot and charcoal rot caused by *Fusarium thapsinum* and *Macrophomina phaseolina* respectively are devastating global diseases in sorghum that lead to severe quality and yield loss each year. In this study, three sets of interrelated experiments were conducted that will potentially lead to the development of resistance based control option to these diseases.

The first experiment was aimed at identifying sources of resistance to infection by *M. phaseolina* and *F. thapsinum* in a diverse panel of 300 sorghum genotypes. The genotypes were evaluated in three environments following artificial inoculation. Out of a total of 300 genotypes evaluated, 95 genotypes were found to have resistance to *M. phaseolina* and 77 to *F. thapsinum* of which 53 genotypes were resistant to both pathogens.

In the second experiment, a set of 79,132 single nucleotide polymorphisms (SNPs) markers were used in an association study to identify genomic regions underlying stalk rot resistance using a multi-locus mixed model association mapping approach. We identified 14 loci associated with stalk rot and a set of candidate genes that appear to be involved in connected functions controlling plant defense response to stalk rot resistance. The associated SNPs accounted for 19-30% of phenotypic variation observed within and across environments. An analysis of associated allele frequencies within the major sorghum subpopulations revealed enrichment for resistant alleles in the durra and caudatum subpopulations compared with other subpopulations. The findings suggest a complicated molecular mechanism of resistance to stalk rots.

The objective of the third experiment was to determine the functional relationship between stay-green trait, leaf dhurrin and soluble sugar levels and resistance to stalk rot diseases. Fourteen genotypic groups derived from a Tx642 × Tx7000 RIL population carrying combinations of stay-green quantitative trait loci were evaluated under three environments in four replications. The *stg* QTL had variable effects on stalk rot disease. Genotypes carrying *stg*1, *stg*3, *stg*1,3 and *stg*1,2,3,4 expressed good levels of resistance to *M. phaseolina* but the combination of *stg*1 and *stg*3 was required to express the same level of resistance to *F. thapsinum*. Other *stg* QTL blocks such as *stg*2 and *stg*4 did not

have any impact on stalk rot resistance caused by both pathogens. There were no significant correlations between leaf dhurrin, soluble sugar concentration, and resistance to any of the pathogens.

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Chapter 1 - Review of Literature

Overview

Stalk rots are caused by various species of fungal organisms. Two or more causal pathogens usually occur together, making identification of the primary pathogen difficult. The most important stalk rotting organisms, however, are *Macrophomina phaseolina* (Tassi) Goidanich, the causal organism of charcoal rot, and a number of *Fusarium* spp, the pathogens responsible for Fusarium stalk rot (Edmunds and Zummo, 1975; Bramel-Cox et al., 1988 Wildermutch et al., 1997). Fusarium stalk rot can usually be distinguished from, charcoal rot because of the less pronounced pigmentation and disintegration of pith tissues and slower rot rate of *Fusarium*. The fungi, ubiquitous on plant debris of many crops, are not aggressive pathogens capable of attacking vigorous plant tissue, but are able to overcome senescing tissue. Each of these stalk rots is associated with environmental stresses. Fusarium stalk rot is generally less virulent than charcoal rot. The incidence of both diseases is generally associated with high temperature, drought stresses, and senescence (Rosenow et al., 1977). The stress is a predisposing factor to invasion of the fungi. Bramel-Cox et al. (1988) reported a significant general combining ability effect for resistance to both *Macrophomina phaseolina* (Tassi) Goid and *Fusarium moniliforme* Sheldon (sensu lato) under both dryland and normal conditions. However, resistance was dependent on environment especially for *F. moniliforme* (sensu lato).

Apart from root and stalk disintegration, both charcoal rot and Fusarium stalk rot under severe infestation can lead to crop lodging, which may further undermine the grain filling process and eventually reduce grain yield. Under severe conditions complete lodging and a grain yield loss of about 64% has been reported for charcoal rot (Mughogho and Pande, 1983). The disease can also result in significant reduction in fodder quality in infected stalks (Manici et al., 1995; Mayek-Perez et al., 2001).

Host plant resistance has been considered as the most practical way to manage these diseases. However, sorghum breeding efforts have not yielded any cultivar with a satisfactory level of field resistance to charcoal rot. The polygenic nature of charcoal rot

resistance, and its interaction with moisture stress and temperature has complicated the breeding efforts for resistance (Rajkumar et al., 2007).

Resistance to maturity-related stalk rots has complex inheritance patterns linked to environmental and physiological interactions in plants. Consequently, selection of genotypes that will reliably maintain healthy stalks and have high yields over many environments is difficult to identify. A better understanding and elucidation of the nature of the interactions is a prerequisite to select genotypes and recommend cultural practices for more stable crop performance. These interactions are explained by the photosynthetic stress translocation balance concept of predisposition to root and stalk rots (Dodd, 1977). According to this theory, root and stalk rot predisposition begins with senescence of root tissue due to an insufficient supply of carbohydrates for normal metabolic functions. The senescing cell, apparently unable to produce normal resistance metabolites, are invaded by microorganisms that are only weakly pathogenic on vigorous cells. As more root tissue is destroyed, the ability of the plant to obtain water from the soil is reduced. The plant eventually reaches the point where transpiration rates exceed water uptake and consequently, permanent wilting occurs. Several microorganisms now invade and digest the remaining stalk structure, which results in lodging.

In a study involving sorghum families derived from diallel crosses between two nonsenescent, resistant inbreds and two nonsenescent susceptible inbreds, Tenkouano et al. (1993) reported that charcoal rot resistance was regulated by dominant and recessive epistatic interactions between two non-senescence inducing loci and a third locus with modifying effect.

Economic importance

Macrophomina phaseolina infects more than 500 plant hosts in many areas of the world (Ali and Dennis, 1992). Hosts may include major food crops (maize, sorghum; Su et al., 2001), pulse crops (common bean; Mayek-Perez et al., 2001), green gram (Raguchander et al., 1997), fiber crops like jute (De and Chattopadhyay, 1992), cotton (Aly et al., 2007), and oil crops such as soybean (Ali and Dennis, 1992), sunflower (Khan, 2007), and sesame (Dinakaran and Mohammed, 2001). Softwood and other forest trees such as *Abies*, *Pinus*, *Pseudotsuga*, *Cassia* (Lodha et al., 1986; McCain and

Scharpf, 1989), fruit trees (*Citrus* spp., *Cocos nucifera*, *Coffea* spp., *Ziziphus mauritiana*, *Leucaena* spp.), medicinal plants and weed species (Lodha et al., 1986; Songa and Hillocks, 1996) are also hosts. Recently, increased incidence of the pathogen on diverse crop species has been reported worldwide (Aviles et al., 2008; Khangura and Aberra, 2009; Mahmoud and Budak, 2011; Sharifi and Mahdavi, 2012) highlighting the importance of this disease to crop production in drought-prone regions. The fungus has a wide geographical distribution, and is especially found in tropical and subtropical countries with arid to semi-arid climates in Africa, Asia, Europe, and North and South America (Diourte et al., 1995; Gray et al., 1990; Wrather et al., 2001; Wrather et al., 2003). The pathogen can result in severe crop losses. For example, charcoal rot is a serious problem of soybean, which accounted for a total yield loss of $173.80 million in the United States during the 2002 crop season (Wrather et al., 2003). In Bangladesh, the fiber yield of jute is reduced by 30% due to this pathogen.

The genus *Fusarium* represents the most important group of fungal pathogens, causing various diseases on nearly every economical plant species. Several *Fusarium* spp. are potentially pathogenic to sorghum (Leslie et al., 2005). Stalks or root infection can reduce yield and grain quality, while lodging can increase difficulty in harvesting (Tesso et al., 2005). Panicle infection can result in grain of reduced quality and usability (Menkir et al., 1996). Mycotoxins are produced by many *Fusarium* spp. (Leslie et al., 2005; Nelson et al., 1992). Accumulation of these metabolites occurs at various stages particularly in stored grains and ensiled stover (Amigot et al., 2006; da Silva et al., 2000). Deterioration by grain molding fungi and contamination by mycotoxins is common in traditional small holder systems such as those used in developing countries and may cause serious problems as infected grains are the source of health concerns when used as food or feed or for making various types of beverages (Dejene et al., 2004; Sashidhar et al., 1992). Long-term exposure to mycotoxins can result in life-threatening diseases to animals and possibly to humans (Voss et al., 2007).

Charcoal rot is generally more destructive, it can destroy a field of sorghum in 2 to 3 days compared to Fusarium stalk rot which may require 2 to 3 weeks to do so. Fusarium stalk rots can be more important in certain regions. Jardine and Leslie (1992) indicated that the average yield loss caused by this disease in Kansas is about 4%, while

the loss at specific locations can reach as much 50%. Recent reports from South Central Kansas experiment stations support this estimate, indicating that stalk-rot-induced lodging of up to 60% is common among high yielding hybrids and the incidence can be even higher under stressful conditions. When converted to monetary terms, the annual economic loss associated with this disease in Kansas surpasses $15 million per year. Significant yield losses due to stalk rot have been reported in Africa (Frowd, 1980; Omar et al., 1985; Hulluka and Esele, 1992), India (Khune et al., 1984; Seetharama et al., 1987), and Australia (Trimboli, 1982).

Causal organisms

Several bacterial and fungal pathogens have been reported as causing stalk rots in sorghum and these include: *Macrophomina phaseolina* (charcoal rot), various *Fusarium spp* (Fusarium stalk rot), and *Colletotrichum graminicola* (anthracnose stalk rot). Stalk rot diseases of lesser importance or with a more limited distribution include Pokkah boeng (*F. proliferatum*, *F. subglutinans*, and *F. thapsinum*), bacterial stalk rot (*Erwinia* spp.), and *Acremonium* wilt (*Acremonium strictum*). *M. phaseolina* and several *Fusarium* spp are the most common and important. *F. thapsinum* and *F. andiyazi* have been reported as the most dominant species affecting sorghum (Funnell-Harris and Pedersen, 2008; Leslie et al., 1990; Tesso et al., 2010). In Kansas, where over 40% of the sorghum in the USA is produced, *Fusarium* spp are the predominant causal agents of stalk rot disease (Reed et al., 1983; Jardine and Leslie, 1992)

Biology and epidemiology of the major causal agents (*M. phaseolina* and *F. thapsinum*)

The fungus, *M. phaseolina* is an anamorphic fungus in the ascomycete family Botryosphaeriaceae (Slippers et al., 2013). Growth of the fungus in soil is fueled by nutrients stored in the microsclerotia, so growth continues even after the soil nutrient levels become insufficient for fungal competitors to grow. On account of this characteristic, the pathogen competes well with other soil pathogens when soil nutrient levels are low and the temperature is above 30ºC. Microsclerotia germinate from a few cells at a time on the surface, or in close proximity of the roots. Root exudates induce

germination of microsclerotia, and when in proximity to or on the roots, microsclerotia germinate from a few cells at a time. Numerous germ tubes are formed, which give rise to appresoria on the anticlinal walls of epidermal cells. The appresoria penetrate the epidermal cell walls by mechanical pressure and enzymatic digestion or via wounds and natural openings (Gupta et al., 2012). Ammon et al. (1975) observed that within 3 days of inoculation, appresoria are produced on the root surface at the tip of the primary hyphae. During the initial stages of pathogenesis, mycelium penetrates the root epidermis and is primarily restricted to the intercellular spaces of the cortex of the primary roots. Consequently, adjacent cells collapse and heavily infected plants may die prematurely probably due to the production of fungal toxins, e.g., phaseolinone or botryodiplodin (Ramezani et al., 2007) and plugging host vessels. After plant death, colonization by mycelia and formation of microsclerotia in host tissue continue until the tissues have dried. After decay of root and plant debris, microsclerotia are released into the soil and the cycle continues.

Similar to *M. phaseolina*, infection by *Fusarium* spp. usually occurs when the host is weakened by predisposing factors (Dodd, 1980). The pathogens persist in soil, on crop residue and on weed hosts as mycelium or as conidiomata, such as sporodochia. The infection starts on the cortical tissues of the roots and eventually invades the vascular tissues as the pathogen progresses towards the stalk (Zummo, 1980). *Fusarium* spp. do not produce microsclerotia. But upon sudden occurrence of dry conditions, the pathogen appears to form sclerotia-like structures for its survival (Khune et al., 1984). *Fusarium thapsinum* is characterized by the production of yellow pigments on lab media. However, pigmentation is variable in *F. thapsinum* strains. The teleomorph, *G. thapsina*, can be formed under laboratory conditions by crossing strains of opposite mating types on carrot agar (Klittich et al., 1997). Symptoms of the disease are prevalent in tissues that are injured or damaged by insects. The infected plant parts contain large areas of reddish pith and the upper internodes have discolored vascular bundles. Premature plant death, poor grain development and crop lodging are some of the characteristic symptoms of Fusarium stalk rot.

The *Fusarium* species that are responsible for stalk rot can survive between crops in infected stubble for up to 3 years and perhaps on roots of weed grasses. The dominant

Fusarium spp that attack grain sorghum are known to infect winter cereals such as wheat and barley. Zero tominimum tillage tends to favor their survival in stubble, and successive sorghum crops will gradually build up the levels of these pathogens.

Relationship between stalk rots and the stay-green trait

Stay-green is the general term given to a variant of annual crop species in which normal senescence is delayed compared with a standard reference genotype. Stay-green can be viewed as a consequence of the balance between N demand by the grain and N supply during the grain filling stage. Rapid premature leaf death generally occurs in sorghum when water is limiting during the grain-filling period (Tuinstra et al., 1997). During post-anthesis drought, genotypes possessing the stay-green trait maintain more photosynthetically active leaves than genotypes not possessing this trait (Borrell and Hammer, 2000). Expression of stay-green has been reported in other cereals including maize (Wang et al., 2012; Zheng et al., 2009), rice (You et al., 2007), wheat, (Kumari et al., 2013), and oat (Helsel and Frey, 1978).

Stay-green phenotypes have been classified into five types on the basis of time and duration of senescence (Thomas and Howarth, 2000). In Type A stay-green, there is a delayed initiation of yellowing. Senescence is initiated late but then proceeds at a normal rate. Type B stay-greens initiate senescence on schedule, but thereafter senesce comparatively slowly. In Type C stay-green phenotype, specific defects in chlorophyll degradation pathway are involved. Chlorophyll may be retained more or less indefinitely. Stay-green of the frozen spinach or herbarium specimen kind are referred to as Type D. In type E stay-green, chlorophyll content remains constant but enzyme activity is reduced (Thomas and Smart, 1993). Classifying stay-greens has been useful in understanding the kinds of modified gene or physiological process underlying the phenotype, but, in practice, particular stay-greens can be combinations of two or more different functional types. Retention of green leaf area at maturity (GLAM), which is a visual stay-green rating, has been used as an indicator of post-anthesis drought resistance in sorghum (Borrell et al., 2000). Field-based screening methods for stay-green drought tolerance are difficult to manage and highly constrained due to the need for near perfect moisture and drought stress conditions across multiple locations to acquire a meaningful assessment of

the stay-green trait. A quantitative assay that would allow the trait to be measured year-round under controlled and easily reproducible conditions and is potentially high throughput has been reported by (Burke et al., 2013).

Four major QTLs (*stg*1,*stg*2,*stg*3,*stg*4) conferring stay-green in grain sorghum have been reported (Crasta et al., 1999; Harris et al., 2007; Kebede et al., 2001; Tao et al., 2000; Tuinstra et al., 1996; Xu et al., 2000). Stay-green has also been reported in other cereals like rice (You et al., 2007); wheat (Vijayalakshmi et al., 2010) and maize (Wang et al., 2012, Zheng et al., 2009). Several breeding programs are using marker-assisted selection and/or marker-assisted backcrossing to incorporate stay-green in advanced breeding lines (Galyuon et al., 2005).

Stay-green in sorghum is considered an important trait; it improves genotype adaptation to post-flowering drought stress, particularly in environments in which the plant depends largely on stored soil moisture to fill grain and mature (Rosenow et al., 1977). Specifically, stay-green has been associated with reduced lodging (Mughogho and Pande, 1984), lower susceptibility to charcoal rot (Mughogho and Pande, 1984), higher levels of stem carbohydrates both during and after grain filling (McBee, 1984), and improved grain filling and better grain yield under stress conditions (Rosenow and Clark, 1981). Because of these benefits, selection for enhanced stay-green has been an important component of breeding for improved drought tolerance and improved grain yield in breeding programs in the United States (Rosenow et al., 1983) and Australia (Henzell et al., 1992) for many years.

Drought stress and the stay-green gene have been reported to have a great impact on stalk rot disease development. Symptoms of post-flowering drought stress susceptibility include premature plant (leaf and stem) death or plant senescence, stalk collapse and lodging, charcoal rot infection, and a significant reduction in seed size, particularly at the base of the panicle. Tolerance is indicated when plants remain green and fill grain normally. Such green stalks also have good resistance to stalk lodging and charcoal rot. Stay-green is as an important post-flowering drought resistance trait. Genotypes possessing stay-green are able to maintain a greater green leaf area under post-flowering drought than their senescent counterparts (Rosenow et al., 1997). Recent studies have shown that leaves stay-green not only because of small sink demand but also

due to higher leaf nitrogen status and transpiration efficiency, resulting in maintenance of photosynthetic capacity and, ultimately, higher grain yield and lodging resistance (Borrell and Douglas, 1997; Borrell et al., 1999, 2000b; Borrell and Hammer, 2000). It is unsure whether or not the trait itself provides resistance or just tolerance to drought conditions. However, reports indicate that there is an interaction between irrigation, stay-green, and the occurrence of charcoal rot.

Dhurrin and stay-green in sorghum

Sorghum exhibits genetic variability for the stay-green trait, but the expression of the trait can also be affected by the nitrogen status of the plant. Dhurrin is a cyanogenic glucoside found in sorghum. Dhurrin is reported to act as a nitrogen storage compound. When tissues containing dhurrin are crushed, hydrogen cyanide (HCN) is released as part of dhurrin decomposition. HCN is toxic to humans and livestock. Recent studies have shown a relationship between higher dhurrin content in seedling leaves and improved stay-green at grain fill.

The content of the cyanogenic glucoside dhurrin in sorghum varies depending on plant age and growth conditions. The cyanide potential is highest shortly after onset of germination. At this stage, nitrogen application has no effect on dhurrin content, whereas in older plants, nitrogen application induces an increase. At all growth stages, the content of dhurrin correlates well with the activity of the two biosynthetic enzyme CYP79A1 and CYP71E1 and with the protein and mRNA levels of the two enzymes. During development, the activity of CYP79A1 is lower than the activity of CYP71E1, suggesting that CYP79A1 catalyzes the rate-limiting step in dhurrin synthesis as has been shown using etiolated seedlings. The site of dhurrin synthesis shifts from leaves to stem during plant development (Busk and Moller, 2002). The toxicity of hydrogen cyanide renders it obvious to assume that cyanogenic glucosides repel herbivores (Jones, 1998). However, many trials do not support this hypothesis (Hruska, 1988) and effectiveness may be strongly influenced by the feeding strategy of the animals (Compton and Jones, 1985). With regard to the interaction between plants and microorganisms, the release of hydrogen cyanide from cyanogenic glucosides may be more damaging to the plant than to the microorganism because of inhibition of phytoalexin production (Lieberei et al.,

1989). In accordance, highly cyanogenic plants are preferred by some fungi and insects compared to plants with lower cyanogenic potential (Nahrstedt, 1996; Møller and Seigler, 1999). Aglycones released from cyanogenic glucosides formed from Phenylalanine or Tyrosine may give rise to the formation of compounds with antifungal activities (Siebert et al., 1996). Other possible roles of cyanogenic glucosides are as nitrogen storage compounds or as osmoprotectants (Forslund and Jonsson, 1997).

Control of stalk rots

Coincident with increased stalk rot disease incidence are several cultural practices suspected of contributing to the disease. These include: soil nitrogen fertility, excessive plant populations, irrigation during dry periods after heading so as to eliminate predisposing water stress conditions, and crop rotation. However, the most practical way to control charcoal rot is to grow varieties that are resistant to charcoal rot and/or tolerant to predisposing stress factors. Efforts have also been made to manage the disease using biological methods by using fluorescent pseudomanads associated with the rhizophere (Das et al., 2008). However this bio-control measure was meant to be a useful component of integrated management the disease. The constitutive overexpression of rice chitinase in sorghum plants seems to offer protection against the stalk rot disease. Transgenic sorghum expressing high levels of chitinase have been reported to exhibit less stalk rot development when exposed to *F. thapsinum* conidia (Waniska et al., 2001).

Pathogenesis-related (PR) proteins such as chitinase are inducible plant defenses that restrict the spread of the pathogen in incompatible interactions and allow for systemic acquired resistance.

The effect on the charcoal rot fungus by common herbicides used in sorghum such as atrazine, alachlor, and metolachlor on growth and colonization of grain sorghum roots by *Macrophomina phaseolina* showed that all three herbicides reduced fungal colony diameter and increased production of microsclerotia both in laboratory and green house conditions (Russin et al., 1995).

A good understanding of all the processes involved between the sorghum plant, microbiota found in the soil, and cultural practices that can assist or hinder these interactions is required for managing stalk rots. Certain agricultural practices can alter the

soil environment, indirectly or directly, thereby creating conditions that are less favorable for pathogens to survive and attack plants or by just moving them about and in the process reduce the incidence of disease.

Genomic mapping in sorghum

The recently completed sorghum genome sequence of a leading inbred BTx623 by Paterson et al. (2009) has provided a foundation for invigorating progress toward relating sorghum genes to their functions. Sorghum is a diverse genus consisting of both cultivated and wild species, many of which are inter-fertile. *Sorghum bicolor* (2n=20) is the most important taxon that includes the cultivated races. Sorghum is usually self-pollinated and is a functional diploid possessing considerable genetic and morphological diversity. Sorghum is an important target for plant genomics due to its adaptation to harsh environments, wide genetic diversity, and relatively small genome size (Menz et al., 2002). The sorghum genome contains 750 Mb of DNA, which is somewhat larger than that of rice (430 Mb) but 3- to 4-fold smaller than that of maize (2400 Mb) (Arumuganathan and Earle, 1991). Maize and sorghum diverged from a common ancestor 15 to 20 million years ago (Doebley et al., 1990) and are more closely related than either is to rice. Sorghum genome mapping based on DNA markers began in the early 1990s and several genetic maps of moderate marker density have been constructed. The first complete sorghum maps with 10 linkage groups developed based on DNA probes previously mapped in maize were reported by Pereira et al. (1994), and Chittenden et al. (1994). Genomic mapping strategies are increasingly being adopted to develop genetic linkage maps and to identify genomic regions influencing traits of economic importance in sorghum, e.g, stay-green, fertility restoration, pest and disease resistance, photoperiod sensitivity, grain quality and bioenergy traits (Sivasukurman et al., 2012; Harris et al., 2007; Klein et al., 2005; Parh et al., 2006; Tao et al., 2003; Chantereau et al., 2001 and Crasta et al., 1999).

Sorghum is fitting for association mapping methods because of its medium-range patterns of linkage disequilibrium (Hamblin et al. 2005) and its self-pollinating mating system. Extensive ex-situ sorghum germplasm collections exist within the U.S. National Plant Germplasm System and ICRISAT. Early characterization of complementary

association genetics panels developed by a group of US scientists (Kresovich et al., 2005), and by Subprogram 1 of the Generation Challenge Program, is in progress. At present, more than 750 SSR alleles and 1402 SNP alleles discovered in 3.3Mb of sequence (Hamblin et al., 2005, Hamblin et al., 2005, Casa et al., 2005; Schloss et al., 2002) are freely available from the Comparative Grass Genomics Center relational database (Paterson et al., 2008). Extensive studies of sequence variation in sorghum show that haplotype diversity is low, even when nucleotide diversity is high. For regions of average length 671bp surveyed in 17 genotypes, the median number of haplotypes was three and the mode was two (Hamblin et al., 2005). Common sequence variation can therefore be captured in a small sample of genotypes.

Genetic variation consists of sequence variation and structure alteration. Sequence variation normally is manifested by SNPs, short sequence insertions and deletions (indels), microsatellites or simple sequence repeats, and transposable elements. Numerous marker systems designed to capture those variations have been used in genetic studies of sorghum. Restriction fragment length polymorphisms (RFLP), random amplified polymorphic DNA (RAPD) markers, amplified fragment polymorphisms (AFLP), simple sequence repeat (SSR) and inter sequence repeat (ISSR), markers have been widely used in sorghum. More recently, single nucleotide polymorphisms (SNPs) have become markers of choice primarily due to their ubiquitousness. SNPs are very useful for high resolution genetic mapping, marker discovery by association analysis, and investigation of evolutionary history of populations (Morris et al., 2013). Development of the Illumina BeadArray platform and GoldenGate SNP assay have made high-throughput SNP discovery feasible in sorghum. SNP development using genotype-by-sequencing (GBS) is now possible because of the advancement of next generation sequencing technology. This is a very simple, cost effective, and a rapid approach that is very specific, highly reproducible, and may reach important regions of the genome that are inaccessible to other sequence capture approaches (Elshire et al., 2011). The library development for GBS is also very simple, requires minute quantities of DNA, avoids random shearing and can be completed in only two steps (Poland and Rife, 2012).

References

Ali, S.M., and Dennis, J. (1992). Host range and physiological specialization of *Macrophomina phaseolina* isolated from field peas in South-Australia. Australian Journal of Experimental Agriculture 32, 1121-1125.

Aly, A.A., Abdel-Sattar, M.A., Omar, M.R., and Abd-Elsalam, K.A. (2007). Differential antagonism of Trichoderma sp against *Macrophomina phaseolina*. Journal of Plant Protection Research 47, 91-102.

Amigot, S.L., Fulgueira, C.L., Bottai, H., and Basilico, J.C. (2006). New parameters to evaluate forage quality. Postharvest Biology and Technology 41, 215-224.

Ammon, V., Wyllie, T.D., and Brown, M.F. (1975). Investigation of infection process of *Macrophomina phaseolina* on surface of soybean roots using scanning electron-microscopy. Mycopathologia 55, 77-81.

Andrew H.P. (2008). Genomics of sorghum. International Journal of Plant Genomics. doi:10.1155/2008/362451

Arumuganathan, K. and Earle, E.D. (1991). Nuclear DNA content of some important plant species. Plant Molecular Biology Reporter 9: 208-218.

Aviles, M., Castillo, S., Bascon, J., Zea-Bonilla, T., Martin-Sanchez, P.M., and Perez-Jimenez, R. M. (2008). First report of *Macrophomina phaseolina* causing crown and root rot of strawberry in Spain. Plant Pathology 57, 382-382.

Borrell, A.K., Hammer, G.L., and Henzell, R.G. (2000). Does maintaining green leaf area in sorghum improve yield under drought II. Dry matter production and yield. Crop Science 40, 1037-1048.

Borrell, A.K. and Douglas, A.C.L. (1997). Maintaining green leaf area in grain sorghum increased nitrogen uptake under post-anthesis drought. International Sorghum and Millets Newsletter 38: 89-91.

Borrell, A.K. and Hammar, G.L. (2000). Nitrogen dynamics and the physiological basis of stay-green in sorghum. Crop Science 40: 1295-1307.

Borrell, A.K., Hammar, G.L., and Henzell, R.O. (2000b). Does maintaining green leaf area in sorghum improve yield under drought. II. Dry matter production and yield. Crop Science 40: 1037-1048.

Bramel-cox, P.J., Stein, I.S., Rodgers, D.M., and L.E. Claflin (1988) Inheritance of resistance to *Macrophomina phaseolina* (Tassi) Goid and *Fusarium moniliforme* Sheldon in sorghum. Crop science 28:37-40.

Burke, J.J., Chen, J.P., Burow, G., Mechref, Y., Rosenow, D., Payton, P., Xin, Z.G., and Hayes, C.M. (2013). Leaf dhurrin content is a quantitative measure of the level of

pre- and postflowering drought tolerance in sorghum. Crop Science 53, 1056-1065.

Busk, P.K., and Moller, B.L. (2002). Dhurrin synthesis in sorghum is regulated at the transcriptional level and induced by nitrogen fertilization in older plants. Plant Physiology 129, 1222-1231.

Casa, A.M., Mitchell, S.E., Hamblin, M.T., Sun, H., Bowers, J.E., Paterson, A.H., Aquadro, C.F. and Kresovich, S. (2005). Diversity and selection in sorghum: simultaneous analyses using simple sequence repeats. Theoretical and Applied Genetics, 111(1), 23-30.

Chantereau, J., Trouche, G., Rami, J.F., Deu, M., Barro, C., and Grivet, L. (2001). RFLP mapping of QTLs for photoperiod response in tropical sorghum. Euphytica 120, 183-194.

Chittenden, L.M., Schertz, K.F., Lin, Y-R., Wing, R.A. and Paterson, A.H. (1994). A detailed RFLP map of *Sorghum bicolor* × *S. propinquum*, suitable for high-density mapping, suggests ancestral duplication of Sorghum chromosomes or chromosomal segments. Theoretical and Applied Genetics 87, 925-933.

Compton, S.G., Jones, D.A. (1985). An investigation of the responses of herbivores to cyanogenesis in *Lotus corniculatus*. Biological Journal Linnean Society 26, 21-38.

Crasta, O.R., Xu, W.W., Rosenow, D.T., Mullet, J., and Nguyen, H.T. (1999). Mapping of post-flowering drought resistance traits in grain sorghum: association between QTLs influencing premature senescence and maturity. Molecular and General Genetics 262, 579-588.

Da Silva, J.B., Pozzi, C.R., Mallozzi, M.A.B., Ortega, E.M., and Correa, B. (2000). Mycoflora and occurrence of aflatoxin B-1 and fumonisin B-1 during storage of Brazilian sorghum. Journal of Agricultural and Food Chemistry 48, 4352-4356.

Das, I.K., Indira, S., Annapurna, A., Prabhakar, and Seetharama, N.(2008). Biocontrol of charcoal rot in sorghum by fluorescent P*seudomonas* associated with the rhizosphere. Crop protection 27, 1407-1414.

De, B.K., and Chattopadhyay, S.B. (1992). Effect of potash on stem rot disease of jute caused by *Macrophomina phaseolina*. Journal of Mycopathological Research 30, 51-55.

Dejene, M., Yuen, J., and Sigvald, R. (2004). The impact of storage methods on storage environment and sorghum grain quality. Seed Science and Technology 32, 511-529.

Dinakaran, D., and Mohammed, S.E.N. (2001). Identification of resistant sources to root rot of sesame caused by *Macrophomina phaseolina* (Tassi.) Goid. Sesame and Safflower Newsletter 16, 68-71.

Diourte, M., Starr, J.L., Jeger, M.J., Stack, J.P., and Rosenow, D.T. (1995). Charcoal rot (*Macrophomina phaseolina*) resistance and the effects of water-stress on disease development in sorghum. Plant Pathology 44, 196-202.

Dodd, J.L. (1977). A photosynthetic stress-translocation balance concept of corn stalk rot. Proceedings, 32nd Annual corn and sorghum research conference 32, 122-130.

Dodd, J.L. (1980). The photosynthetic stress translocation balance concept of sorghum stalk rot. In: Proceedings of International Workshop of Sorghum Diseases, Texas A&M University/ICRISAT Press, 11-15 December 1978, Hyderabad, India, pp 300-305.

Doebley, J., Durbin, M., Golenberg, E.M., Clegg, M.T. and Mam D.P. (1990). Evolutionary analysis of the large subunit of carboxylase (rbcL) nucleotide sequence among the grasses (Gramineae). Evolution 44, 1097-1108

Edmunds, L.K., and Zummo, N. (1975). Sorghum diseases in the United States and their control. USDA Handout No. 468. U.S. Gov. Print Office, Washington, DC.

Elshire, R.J., Glaubitz, J.C., Sun, Q., Poland, J.A., Kawamoto, K., Buckler, E.S., and Mitchell, S.E. (2011). A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. Plos One 6(5): e19379. doi:10.1371/journal.pone.0019379

Forslund, K., Jonsson. L. (1997). Cyanogenic glucosides and their metabolic enzymes in barley, in relation to nitrogen levels. Physiologia Plantarum. 101, 367-372.

Frowd, J.A. (1980). Sorghum stalk rots in West Africa. p. 322-324. In Proc. of the Int. Workshop of Sorghum Diseases, Hyderabad, India. 11-15 Dec. 1978. Texas A&M University, College Station, and ICRISAT, Patancheru, India.

Funnell-Harris, D.L., and Pedersen, J.F. (2008). Inoculation strategies to assess biological interactions between Fusarium and Alternaria species infecting sorghum. Canadian Journal of Plant Pathology 30, 404-413.

Galyuon, I.K.A., Hash, C.T., Bidinger, F.R., Mahalakshmi, V., Folkertsma, R.T., and Howarth, C.J. (2005). Functioning under drought stress of stay-green sorghum developed by marker-assisted selection. Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology 141, S314-S314.

Gray, F.A., Kolp, B.J., and Mohamed, M.A. (1990). A disease survey of crops grown in the Bay Region of Somalia, East Africa. FAO Plant Protection Bulletin 38, 39-47.

Gupta, G.K., Sharma, S.K., and Ramteke, R. (2012). Biology, epidemiology and management of the pathogenic Fungus *Macrophomina phaseolina* (Tassi) Goid with special reference to charcoal rot of soybean (Glycine max (L.) Merrill). Journal of Phytopathology 160, 167-180.

Hamblin, M.T., Fernandez, M.G.S., Casa, A.M., Mitchell, S.E., Paterson, A.H., and Kresovich, S. (2005). Equilibrium processes cannot explain high levels of short- and medium-range linkage disequilibrium in the domesticated grass *Sorghum bicolor*. Genetics, 171, 1247-1256.

Hamblin, M.T., Mitchell, S.E., White, G.M., Gallego, J., Kukatla, R., Wing, R.A., and Kresovich, S. (2004). Comparative population genetics of the panicoid grasses: sequence polymorphism, linkage disequilibrium and selection in a diverse sample of *Sorghum bicolor*. Genetics 167, 471-483.

Harris, K., Subudhi, P.K., Borrell, A., Jordan, D., Rosenow, D., Nguyen, H., Klein, P., Klein, R., and Mullet, J. (2007). Sorghum stay-green QTL individually reduce post-flowering drought-induced leaf senescence. Journal of Experimental Botany 58, 327-338.

Helsel, D.B., and Frey, K.J. (1978). Grain yield variations in oats associated with differences in leaf area duration among oat lines. Crop Science 18, 765-769.

Henzell, R.G., R.L. Brengman, D.S. Fletcher, and McCosker, A.N. (1992). Relationships between yield and non-senescence (stay-green) in some grain sorghum hybrids grown under terminal drought stress. p. 355-358. In M.A. Foale et al. (ed.) Proc. of the Second Australian Sorghum Conference. Gatton, Australia. 4-6 Feb. 1992. Occasional Publication no. 68. Australian Inst. of Agric. Sci., Melbourne, Australia.

Hruska, A.J. (1998). Cyanogenic glucosides as defense compounds: a review of the evidence. Journal of Chemical Ecology 14, 2213-2217.

Hulluka, M., and Esele, J.P.E. (1992). Sorghum diseases in East Africa. p. 21-24. In W.A.J. Milliano et al. (ed.) Sorghum and millet diseases: A second world review. ICRISAT, Patancheru, AP, India.

Jardine, D.J., and Leslie, J.F. (1992). Aggressiveness of Gibberella fujikuroi (*Fusarium moniliforme*) isolates to grain sorghum under greenhouse conditions. Plant Diseases 76, 897-900.

Jenae, L.S., and Mitchell R.T. (2013). Relationship between dhurrin and stay-green in *Sorghum bicolor* (L.) Moench. Poster session presented at the annual meeting of the ASA, CSSA, and SSSA, Nov. 3-6, 2013, in Tampa, FL.

Jones, D.A. (1998). Why are so many food plants cyanogenic. Phytochemistry 47, 155-162.

Kebede, H., Subudhi, P.K., Rosenow, D.T., and Nguyen, H.T. (2001). Quantitative trait loci influencing drought tolerance in grain sorghum (*Sorghum bicolor* L. Moench). Theoretical and Applied Genetics 103, 266-276.

Khan, S.N. (2007). *Macrophomina phaseolina* as causal agent for charcoal rot of sunflower. Mycopathologia 5 111-118.

Khangura, R., and Aberra, M. (2009). First Report of Charcoal Rot on Canola Caused by *Macrophomina phaseolina* in Western Australia. Plant Disease 93, 666-667.

Khune, N.N., D.E. Kurhekar, J.G. Raut, and Wangikar, P.D. (1984). Stalk rot of sorghum caused by *Fusarium moniliforme*. Indian Phytopathology 37, 316-319.

Klein, R.R., Klein, P.E., Mullet, J., Minx, P., Rooney, W.L., and Schertz, K.F. (2005). Fertility restorer locus Rf 1 of sorghum (*Sorghum bicolor* L.) encodes a pentatricopeptide repeat protein not present in the colinear region of rice chromosome 12. Theoretical and Applied Genetics 111, 994-1012

Klittich, C.J.R., Leslie, J.F., Nelson, P.E., and Marasas, W.F.O. (1997). *Fusarium thapsinum* (*Gibberella thapsina*): A new species in section Liseola from sorghum. Mycologia 89, 643-652.

Kresovich, S., Barbazuk, B., Bedell, J.A., Borrell, A., Buell, C.R., Burke, J., and Warek, U. (2005). Toward sequencing the sorghum genome: a US National Science Foundation-sponsored workshop report. Plant Physiology, 138, 1898-1902

Kumari, M., Pudake, R.N., Singh, V.P., and Joshi, A.K (2013). Association of stay-green trait with canopy temperature depression and yield traits under terminal heat stress in wheat (*Triticum aestivum* L.). Euphytica 190, 87-97.

Leslie, J.F., Pearson, C.A.S., Nelson, P.E., and Toussoun, T.A. (1990). *Fusarium* spp from corn, sorghum, and soybean fields in the central and eastern United States. Phytopathology 80, 343-350.

Leslie, J.F., Zeller, K.A., Lamprecht, S.C., Rheeder, J.P., and Marasas, W.F.O. (2005). Toxicity, pathogenicity, and genetic differentiation of five species of *Fusarium* from sorghum and millet. Phytopathology 95, 275-283.

Lieberei R, Biehl B, Giesemann A, Junqueira N.T.V. (1989). Cyanogenesis inhibits active defense reactions in plants. Plant Physiol. 90, 33-36.

Lodha, S., Gupta, G.K., and Singh, S. (1986). Crop disease situation and some new records in Indian arid zone. Annals of Arid Zone 25, 311-320.

Mace, E.S., Rami, J.F., Bouchet, S., Klein, P.E., Klein, R.R., Kilian, A. (2009). A consensus genetic map of sorghum that integrates multiple component maps and high-throughput Diversity Array Technology (DArT) markers. BMC Plant Biology 9, 13.

Mahmoud, A., and Budak, H. (2011). First report of charcoal rot caused by *Macrophomina phaseolina* in sunflower in Turkey. Plant Disease 95, 223-223.

Manici, L.M., Cerato, C. and Caputo, F. (1995). Pathogenic and biologic variability of *Macrophomina phaseolina* (Tassi) Goid. isolates in different areas of sunflower cultivation in Italy. Proceedings of sunflower conference Pisa, Italy.

Mayek-perez, N., Lopez-caataneda, C., Gonzalez-Chavira, M., Garch-Espinosa, R., Acosta-Gallegos, J., Vega, O. M., and Simpson, J. (2001). Variability of Mexican isolates of *Macrophomina phaseolina* based on pathogenesis and AFLP genotype. Physiological and Molecular Plant Pathology 59, 257-264.

Mayek-Perez, N., Lopez-Castaneda, C., Lopez-Salinas, E., Cumpian-Gutierrez, J., and Acosta-Gallegos, J. A. (2001). Resistance to *Macrophomina phaseolina* (Tassi) Goid. in common bean under field conditions in Mexico. Agrociencia (Montecillo) 35, 649-661.

McBee, G.G. (1984). Relation of senescence, nonsenescence, and kernel maturity to carbohydrate metabolism in sorghum. p. 119-129. In L.K. Mughogho (ed.) Sorghum root and stalk rots: A critical review. Proc. Consultative Group Discussion of Research Needs and Strategies for Control of Sorghum Root and Stalk Rot diseases, Bellagio, Italy. 27 Nov.-2 Dec. 1983. ICRISAT, Patancheru, A.P., India.

McCain, A.H., and Scharpf, R.F. (1989). Effect of inoculum density of *Macrophomina-phaseolina* on seedling susceptibility of 6 conifer species. European Journal of Forest Pathology 19, 119-123.

Menkir, A., Ejeta, G., Butler, L.G., Melakeberhan, A., and Warren, H.L. (1996). Fungal invasion of kernels and grain mold damage assessment in diverse sorghum germ plasm. Plant Disease 80, 1399-1402.

Menz M.A., Klein R.R., Mullet J.E., Obert J.A., Unruh N.C., Klein P.E. (2002) A high-density genetic map of *Sorghum bicolor* (L.) Moench based on 2926 AFLP, RFLP and SSR markers. Plant Molecular Biology 48, 483-499.

Møller, B.L., Seigler, D.S. (1999). Biosynthesis of cyanogenic glycosides, cyanolipids, and related compounds. In: Singh BK, editor. Plant Amino Acids. Biochemistry and Biotechnology. New York: Marcel Dekker; pp. 563-609.

Morris, G.P., Ramu, P., Deshpande, S.P., Hash, C.T., Shah, T., Upadhyaya, H.D., Riera-Lizarazu, O., Brown, P.J., Acharya, C.B., Mitchell, S.E., Harriman, J., Glaubitz, J. C., Buckler, E.S., and Kresovich, S. (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.

Mughogho, L.K. and Pande, S. (1983). Charcoal rot of sorghum. In: Sorghum Root and Stalk Rots, A critical Review: Proceedings of the Consultative Group Discussion

of Research Needs and Strategies for Control of Sorghum Root and Stalk Rot Diseases,Bellagio, Italy.

Nahrstedt A. Relationships between the defense systems of plants and insects: the cyanogenic system of the moth *Zygaena trifolii*. In: Romeo JT, editor. Recent Advances in Phytochemistry. Vol. 33. New York: Plenum Press; 1996. pp. 217-230.

Nelson, P.E., Plattner, R.D., Shackelford, D.D., and Desjardins, A.E. (1992). Fumonisin-b1 production by *Fusarium* species other than *F. moniliforme* in section Liseola and by some related species. Applied and Environmental Microbiology 58, 984-989.

Omar, M.E., Frederiksen, R.A., and Rosenow, D.T. (1985). Collaborative sorghum disease studies in Sudan. Sorghum Newsletter 28:93.

Parh, D.K, Jordan, D.R., Aitken, E.A.B., Gogel, B.J., McIntyre, C.L., Godwin, I.D. (2006). Genetic Components of Variance and the Role of Pollen Traits in Sorghum Ergot Resistance. Crop Science 46, 2387-2395

Paterson, A.H., Bowers, J.E., Bruggmann, R., Dubchak, I., Grimwood, J., et al., (2009). The *Sorghum bicolor* genome and the diversification of grasses. Nature 457, 551-556.

Pereira, M.G., Lee, M., Bramel-Cox, P., Woodman, W., Doebley, J. and Whitkus, R. (1994). Construction of an RFLP map in sorghum and comparative mapping in maize. Genome 37: 236-243.

Poland, J.A., and Rife, T.W. (2012). Genotyping-by-sequencing for plant breeding and genetics. Plant Genome 5, 92-102.

Raguchander, T., Rajappan, K., and Samiappan, R. (1997). Evaluating methods of application of biocontrol agent in the control of mungbean root rot. Indian Phytopathology 50, 229-234.

Rajkumar, Fakrudin Bashasab and Mahaling S Kuruvinashetti. (2007). Genetic variability of sorghum charcoal rot pathogen (*Macrophomina phaseolina*) assessed by random DNA markers. Plant Pathology Journal 23, 45-50.

Ramezani, M., Shier, W.T., Abbas, H.K., Tonos, J.L., Baird, R.E., and Sciumbato, G.L. (2007). Soybean charcoal rot disease fungus *Macrophomina phaseolina* in Mississippi produces the phytotoxin (-)-botryodiplodin but no detectable phaseolinone. Journal of Natural Products 70, 128-129.

Rosenow, D.T., Johnson, J.W., Frederiksen, R.A., and Miller, F.R. (1977). Relationship of nonsenescence to lodging and charcoal rot in sorghum. Agronomy Abstracts, 69-69.

Rosenow, D.T., and Clark, L.E. (1981). Drought tolerance in sorghum. p. 18-31. In H.D. Loden and D. Wilkinson (ed.) Proc. 36th Annu. Corn and Sorghum Industry Res. Conf. Chicago, IL. 9-11 Dec. 1981. Am. Seed Trade Assoc., Washington, DC.

Rosenow, D.T., Ejeta, G., Clark, L.E., Grilbert, M.L., Henzell, R.G., Borrell, A.K., and Muchow, R.C. (1997). Breeding for pre- and post-flowering drought stress resistance in sorghum. In International Conference on Genetic Improvement of Sorghum and Pearl Millet, Lubbock, TX, September 22-27, 1996, pp. 400-411.

Russin, J.S., Carter, C.H., and Griffin, J.L. (1995). Effects of grain-sorghum (*Sorghum-bicolor*) herbicides on charcoal rot fungus. Weed Technology 9, 343-351.

Sashidhar, R.B., Ramakrishna, Y., and Bhat, R.V. (1992). Molds and mycotoxins in sorghum stored in traditional containers in India. Journal of Stored Products Research 28, 257-260.

Schloss, S., Mitchell, S., White, G., Kukatla, R., Bowers, J., Paterson, A., and Kresovich, S. (2002). Characterization of RFLP probe sequences for gene discovery and SSR development in *Sorghum bicolor* (L.) Moench. Theoretical and Applied Genetics, 105, 912-920.

Seetharama, N.R., Sachan, C., Huda, A.K., Gill, K.S., Rao, K.N., and Bidinger, F.R. (1991). Effect of pattern and severity of moisture deficit stress on stalk rot incidence in sorghum. II. Effect of source/ sink relationships. Field Crops Research. 26, 355-374.

Sharifi, K., and Mahdavi, M. (2012). First report of strawberry crown and root rot caused by *Macrophomina phaseolina* in Iran. Iranian Journal of Plant Pathology 47, Pe479-En161.

Siebert, M., Sommer, S., Li, S., Wang, Z., Severin, K., and Heide, L. (1996). Genetic engineering of plant secondary metabolism. Accumulation of 4-hydroxybenzoate glucosides as a result of the expression of the bacterial ubiC gene in tobacco. Plant Physiology. 112, 811-819.

Slippers, B., E. Boissin, A.J.L. Phillips, J.Z. Groenewald, L. Lombard, M.J. Wingfield, et al. (2013). Phylogenetic lineages in the Botryosphaeriales: a systematic and evolutionary framework. Studies in Mycology: 31-49.

Songa, W., and Hillocks, R.J. (1996). Legume hosts of *Macrophomina phaseolina* in Kenya and effect of crop species on soil inoculum levels. Journal of Phytopathology 144, 387-391.

Su, G., Suh, S.O., Schneider, R.W., and Russin, J.S. (2001). Host specialization in the charcoal rot fungus, *Macrophomina phaseolina*. Phytopathology 91, 120-126.

Sukumaran, S., Xiang, W., Bean, S., Pedersen, J.F., Tuinstra, M.R., Tesso, T.T., Hamblin, M.T. and Yu, J. (2012). Association mapping for grain quality in a diverse sorghum collection. Plant Genome. 5, 126-135.

Tao, Y.Z., Hardy, A., Drenth, J., Henzell, R.G., Franzmann, B.A., Jordan, D.R., Butler, D.G., McIntyre, C.L. (2003). Identifications of two different mechanisms for sorghum midge resistance through QTL mapping. Theoretical and Applied Genetics, 107, 116-122.

Tao, Y.Z., Henzell, R.G., Jordan, D.R., Butler, D.G., Kelly, A.M., and McIntyre, C.L. (2000). Identification of genomic regions associated with stay-green in sorghum by testing RILs in multiple environments. Theoretical and Applied Genetics 100, 1225-1232.

Tenkouano, .A., Miller, F.R., Frederiksen, R.A., and D.T. Rosenow. (1993). Genetics of nonsenesence and charcoal rot resistance in sorghum. Theoretical and Applied Genetics 85, 644-648.

Tesso, T.T., Claflin, L.E., and Tuinstra, M.R. (2005). Analysis of stalk rot resistance and genetic diversity among drought tolerant sorghum genotypes. Crop Science 45, 645-652.

Tesso, T.T., Ochanda, N., Little, C.R., Claflin, L., and Tuinstra, M.R. (2010). Analysis of host plant resistance to multiple *Fusarium* spp associated with stalk rot disease in sorghum *Sorghum bicolor* (L.) Moench. Field Crops Research 118, 177-182.

Thomas, H., and Howarth, C.J. (2000). Five ways to stay-green. Journal of Experimental Botany 51, 329-337.

Thomas, H., and Smart, C.M. (1993). Crops that Stay-green. Annals of Applied Biology 123, 193-219.

Trimboli, D.S., and Burgess, L.W. (1982). The fungi associated with stalk and root rot of grain sorghum in New South Wales. Newsletter 25:105.

Tuinstra, M.R., Grote, E.M., Goldsbrough, P.B., and Ejeta, G. (1996). Identification of quantitative trait loci associated with pre-flowering drought tolerance in sorghum. Crop Science 36, 1337-1344.

Tuinstra, M.R., Grote, E.M., Goldsbrough, P.B., and Ejeta, G. (1997). Genetic analysis of post-flowering drought tolerance and components of grain development in *Sorghum bicolor* (L.) Moench. Molecular Breeding 3, 439-448.

Vijayalakshmi, K., Fritz, A.K., Paulsen, G.M., Bai, G., Pandravada, S., and Gill, B.S. (2010). Modeling and mapping QTL for senescence-related traits in winter wheat under high temperature. Molecular Breeding 26, 163-175.

Voss, K.A., Smith, G.W., and Haschek, W.M. (2007). Fumonisins: Toxicokinetics, mechanism of action and toxicity. Animal Feed Science and Technology 137, 299-325.

Wang, A.-y., Li, Y., and Zhang, C.-q. (2012). QTL mapping for stay-green in maize (*Zea mays)*. Canadian Journal of Plant Science 92, 249-256.

Waniska, R.D., Venkatesha, R.T., Chandrashekar, A., Krishnaveni, S., Bejosano, F.P., Jeoung, J., Jayaraj, J., Muthukrishnan, S., and Liang, G.H. (2001). Antifungal proteins and other mechanisms in the control of sorghum stalk rot and grain mold. Journal of Agricultural and Food Chemistry 49, 4732-4742.

Wildermutch, G.B., Thompson, J.P., and Robertson, L.N. (1997). Biological change: Disease, insects and beneficial organisms. In Sustainable crop production in the sub tropics-An Australian perspective. Queensland Dep. of Primary Industries, Brisbane, QLD, Australia.

Wrather, J.A., Anderson, T.R., Arsyad, D.M., Tan, Y., Ploper, L.D., Porta-Puglia, A., Ram, H.H., and Yorinori, J.T. (2001). Soybean disease loss estimates for the top ten soybean-producing countries in 1998. Canadian Journal of Plant Pathology 23, 115-121.

Wrather, J.A., Koenning, S.R., and Anderson, T.R. (2003). Effect of diseases on soybean yields in the United States and Ontario (1999 to 2002). Plant Health Progress, 0-16.

Xu, W.W., Subudhi, P.K., Crasta, O.R., Rosenow, D.T., Mullet, J.E., and Nguyen, H.T. (2000). Molecular mapping of QTLs conferring stay-green in grain sorghum (*Sorghum bicolor* L. Moench). Genome 43, 461-469.

You, S.-C., Cho, S.H., Zhang, H., Paik, H.C., Lee, C.-H., Li, J., Yoo, J.H., Lee, B.W., Koh, H.J., Seo, H. S., and Paek, N.C. (2007). Quantitative trait loci associated with functional stay-green SNU-SG1 in rice. Molecules and Cells 24, 83-94.

Zheng, H.J., Wu, A.Z., Zheng, C.C., Wang, Y.F., Cai, R., Shen, X.F., Xu, R.R., Liu, P., Kong, L.J., and Dong, S.T. (2009). QTL mapping of maize (*Zea mays*) stay-green traits and their relationship to yield. Plant Breeding 128, 54-62.

Zummo, N. (1980). *Fusarium* disease complex of sorghum in West Africa. In: Bengtson G.D. (ed.), Sorghum Diseases: a World Review. ICRISAT, India, pp 297-299.

Chapter 2 - Resistance to Stalk-rots

in a sorghum Diversity panel

Abstract

Fusarium stalk rot and charcoal rot are the primary stalk rot diseases that cause significant yield loss in sorghum. Fusarium stalk rot caused by *Fusarium* spp. including *F. thapsinum, F. proliferatum*, and *F. andiyazi*) are important when high temperatures and drought stress occur during grain filling followed by exposure to cooler and wetter conditions. However, charcoal rot (caused by *Macrophomina phaseolina*) occurs during prolonged post-flowering drought stress and does not require a period of cooler temperatures or higher moisture in order to manifest the disease. The diseases are important biotic constraints to sorghum production worldwide and are especially critical under mechanized production. The objective of the study was to identify sources of resistance to stalk rot diseases. A sorghum diversity panel consisting of 300 genotypes was evaluated for resistance to both *M. phaseolina* and *F. thapsinum* in three environments (Manhattan, KS 2011, Manhattan, KS 2012, and Ottawa, KS 2012) following artificial inoculation. A randomized complete block design with two replications was used. The panel displayed large genetic variability for stalk rot related traits. Response to infection by pathogens varied significantly and was not affected by botanical race and geographic origin of genotypes. Out of a total of 300 genotypes evaluated, 95 genotypes were found to have resistance to *M. phaseolina* and 77 to *F. thapsinum* of which, 53 genotypes were resistant to both pathogens. These should serve as useful sources for stalk rot resistance breeding efforts in future.

Key words: Sorghum, *Fusarium thapsinum*, *Macrophomina phaseolina*, stalk rots, diversity panel

Introduction

Sorghum (*Sorghum bicolor* L. Moench), a grass belonging to the family Andropogonaceae, is a resilient species able to grow under a variety of conditions. Together with maize, barley, wheat, rice and sugarcane, it forms a significant part of the world's major crops cultivated both for animal and human consumption (Jwa et al., 2006). Sorghum is particularly important under small-scale subsistence production in arid and infertile soils due to its tolerance to drought and waterlogging, adaptation to marginal soils and its more prudent use of soil nitrogen fertilizer than other cereal crops (Sasaki and Antonio, 2009). According to the Food and Agriculture Organization crop production statistics, sorghum is the fifth most important grain crop in the world. Its yearly production has been stabilized at 60 million tons per year with an average area harvested ~44 million hectares annually. However, in addition to abiotic stresses, sporadic occurrences of various disease and insect pests have become major impediments to realizing high yield potential. Developing cultivars resistant to these stresses is the key to improving sorghum productivity in farmers' fields.

Sorghum stalk rots are caused by a complex of soil-borne fungal pathogens. Fusarium stalk rot and charcoal rot are the primary stalk rot diseases that cause significant yield loss in sorghum. Fusarium stalk rots caused by *Fusarium spp*. including *F. thapsinum, F. proliferatum*, and *F. andiyazi* are important when high temperatures and drought stress occur during grain filling followed by exposure to cooler and wetter conditions. However, charcoal rot caused by *Macrophomina phaseolina* occurs during prolonged post-flowering drought stress and does not require a period of cooler temperatures or higher moisture in order to manifest itself. The diseases are important biotic constraints to sorghum production worldwide and are best managed through host plant resistance. Both diseases reduce crop yield through direct interference with the grain filling process (degraded vascular tissues reduce transportation of water, minerals and assimilates that cause further reduction in yield and quality of grains), inducing lodging and increasing harvesting problems. Stalk rot pathogens can be distributed through rain, agricultural equipment, wind and animals and survive in plants, soil or plant debris either as spores, hyphae, or resting structures (Waniska et al., 2002). Germination of resting structures, mycelium or spores is stimulated by root and seed

exudates (Idris et al., 2008) and the pathogens gain access to the roots through natural root wounds or injuries caused by machinery, insects or other causes (Claflin, 2000). Primary infection starts in the cortex tissues and spreads towards the vascular tissues of the root (Zummo, 1984). Studies conducted by Jardine and Leslie (1992) estimated the average yield loss caused by this disease in Kansas at 4%, while the loss at specific locations can reach high as 50%. Recent reports from South Central Kansas experiment stations support this estimate, indicating that stalk rot induced lodging of up to 60% is common among high yielding hybrids and the incidence can be even higher under stressful conditions. When converted to monetary terms, the annual economic loss associated with these diseases in Kansas surpasses $15 million per year. Significant yield losses due to stalk rot have been reported in other regions of the world where the crop is cultivated including Africa (Frowd, 1980; Omar et al., 1985; Hulluka and Esele, 1992), India (Khune et al., 1984; Seetharama et al., 1987), and Australia (Trimboli, 1982).

Availability of the desirable genetic sources is a prerequisite for genetic improvement of any trait. The ever increasing population and the growing challenge of food security require that productivity be increased at a faster rate than ever before. Much of the anticipated increase in productivity will rely on exploiting the existing sources of genetic variability. Thus, genetic resources will be the main drivers of future progress in developing new cultivars (Grenier et al., 2001; Bhattacharjee et al., 200; Upadhyaya et al., 2009). Large amounts of plant genetic materials have been collected and preserved in gene banks throughout the world. Given the large number of collections and the level of variability present among them, finding ways of harnessing these resources for crop improvement has been one of the issues scientists struggle with. The use of core collections was proposed to overcome this problem (Frankel et al., 1984) and has been used to systematically capture the existing variability in a sub-sample of the collections that is small enough for use in experimentation.

To this end, the U.S. sorghum research community has created a panel of 378 photoperiod insensitive grain sorghum genotypes from diverse geographic and climatic regions including 230 converted African landraces, U.S. adapted breeding lines and cultivars (Stephens et al., 1967), and 148 elite grain or forage sorghum cultivars and other genotypes of genetic and historical importance. The population has been evaluated for

several key plant characteristics including drought and heat stress tolerance (Mutava et al., 2011), grain quality (Sukumaran et al., 2012), protein content and digestibility (Bean, unpublished). Based on these data, the K-State sorghum breeding program has utilized selected sources of large seed size and grain quality in its breeding program. The current study is based on further exploiting the potential value of this resource for improving stalk rot resistance in grain sorghum.

The objective of this study was thus to identify sources of resistance to infection by *M. phaseolina* and *F. thapsinum*.

Materials and methods

Genetic materials

Three hundred lines from the sorghum diversity panel, including 251 converted germplasm, and 49 breeding lines or releases from the U.S public breeding programs and other sources of both tropical and temperate origin and U.S adapted historical cultivars were included in the study. The materials represent wide geographical origin, from all parts of Africa, Asia, and the Americas. They also represent diverse plant morphotypes, including plant and seed color, panicle forms, plant height, maturity range, seed size, and botanical races. All races were adequately represented in the panel. The materials also vary for agronomic potential and response to numerous biotic and abiotic stresses other than those targeted in this study. The test panel also contained known sources of resistance to *M. phaseolina* and *F. thapsinum* and known susceptible sources to both pathogens.

Experimental design and management

The materials were planted in 5 m long single row plot spaced 0.75m apart. The design was randomized complete block with 2 replications in three environments. The test environments were Manhattan, KS during the 2011 and 2012 seasons and Ottawa, KS during the 2012 season. Both of the 2011 and 2012 Manhattan tests were conducted at the Ashland Bottoms KSU Agronomy research farm near Manhattan, KS on well-drained Smolan Silty Clay Loam soils prone to moisture stress. The Ottawa 2012 test was conducted at the KSU North East Agricultural Experiment Station near Ottawa, KS. The

soil at Ottawa is Harney Silt Loam. The test was conducted under uniquely dry and hot environments both in 2011 and 2012 seasons that were not typical for the areas. How much effect this may have had on the development of the disease is not clear. All fields received optimal fertilization and pre-planting weed control practices commonly used for sorghum. Post-emergence weeds were removed by hand as soon as they appeared. At flowering, six uniform plants in a plot were tagged with two distinct color tapes, three plants for each color to use for pathogen inoculation.

Inoculum preparation and inoculation

Two fungal pathogens were selected for this study: *Fusarium thapsinum and Macrophomina phaseolina*. The choice of pathogens was based on previous reports that these two pathogens are the most virulent organisms causing stalk rot diseases in sorghum (Tesso et al., 2010). Pure cultures of the pathogens were provided by Dr. Christopher Little at Kansas State University.

For *F. thapsinum,* liquid inoculum suspensions were initiated from pure cultures of the pathogen using potato dextrose broth (DIFCO, Detroit, MI). The suspensions were maintained at room temperature on a rotary shaker until conidia were produced and then strained through four layers of cheese cloth to separate the conidia from the mycelial mass. The concentration of the suspension was determined by counting the number of conidia under a microscope using a hemacytometer. The concentration was then adjusted to 5 x 10^4 conidia ml^{-1} by diluting using 10 m*M* (pH 7.2) phosphate-buffered saline. The suspension was kept on ice until inoculation. An Idico filler-plug gun (Forestry Suppliers, Inc., Jackson MS) equipped with a stainless steel needle similar to that described by Toman and White (1993) was used to deliver approximately 1 ml of suspension into the pith of three of the tagged plants in each plot at 14 d after flowering.

For *M. phaseolina*, the pathogen was sub-cultured into several fresh potato dextrose agar plates, into which sterile toothpicks were inserted. The plates were incubated at 30°C for two weeks. At 14 d after flowering, the remaining three tagged plants from each plot were inoculated with an infested toothpick inserted perpendicularly into each stalk. For both pathogens, inoculations were made on the basal stalk about 10 cm above the soil surface

Data collection

Twenty eight days after inoculation, the entire stalk of the inoculated plants were harvested and scored for disease severity. The scoring was done by longitudinally splitting the stalks and measuring the length of the visible lesion and by counting the number of diseased nodes crossed within the lesion.

Total lesion length (cm) was measured as the entire length of the visible lesion in the pith of the stalk and major lesion length (cm) as the length of uninterrupted lesion measured on both sides of the inoculation site. Plant height was recorded as the length of the plant from the soil surface to the tip of the panicle. We also determined relative lesion length as the ratio of lesion length to plant height. Days to flowering was recorded as the number of days from planting to when 50% of plants in a plot have reached 50% anthesis.

Statistical analysis

The analysis of variance was conducted for 8 disease related traits using the R software package. Broad sense heritability was estimated using the method outlined by Holland et al. (2003). Pearson correlation coefficients between traits were estimated using means pooled over the three environments.

Using the means for all traits, hierarchical clustering was performed with a software package Pvclust (Ryota and Hidetoshi, 2011) in R (version 3.0.1, 64 bit). This procedure was used to partition the 300 genotypes into different disease response groups; resistant, moderately resistant, moderately susceptible, and susceptible. Using a Dunnett's test, the mean disease score of each group were compared to the means of the known resistant checks; SC599 for *F. thapsinum*, and Tx642 for *M. phaseolina* and a susceptible check Tx7000 for both pathogens.

Results

The analysis of variance for 8 traits, their broad-sense heritability, and descriptive statistics are presented in Table 1. The difference among the genotypes was highly significant ($P \leq 0.0001$) for all disease parameters and phonological traits. The combined genotypic mean for total lesion length for *M. phaseolina* (TLM) ranged from 1.83 to 16.2

cm with a mean of 5.38 ± 2.78. The value for major lesion length (MLM) was similar with the range being 1.83 to 12.92 cm and a mean of 4.65 ± 2.16. The range of values for the disease parameters indicates the breadth of genetic variability for the trait and the prospect for genetic improvement. The result for *F. thapsinum* was similar. The genotype mean for total lesion length for *F. thapsinum* (TLF) ranged from 2.28 to 17.02 cm and for major lesion length (MLF) from 2.19 to 13.67 cm; the overall mean for TLF and MLF was 5.83 ± 2.35 and 5.02 ± 1.93, respectively. The score for both *M. phaseolina* and *F. thapsinum* was comparable although the former is generally considered more aggressive.

The coefficient of variation was high for all traits except days to flowering, but given the quantitative nature of the disease traits and the scoring methods used, the coefficient of variation observed is not out of range. Broad-sense heritability estimates were moderate to low for all disease traits ranging from 21 to 39%. As expected, broad-sense heritability was high for plant height (53%) and days to flowering (49%). Pearson correlation coefficients between all the traits are presented in Table 2.2. There were highly significant positive correlations between all disease related traits both within and between pathogens indicating that the pattern of reaction of genotypes to both pathogens was similar. There is also a significant correlation between plant height and all of the disease related traits for both pathogens. However, there was no correlation between relative lesion length for both total and major lesion lengths and plant height, which shows that the relative lesion length was not affected by plant height. Days to flowering was significantly (r = -0.24) correlated with total lesion length for *M. phaseolina* and was not significant with all other parameters.

Fig 2.1 shows the summary of disease reaction of the sorghum diversity panel. Using a hierarchical clustering procedure and a Dunnet's test to test differences between group means, resistant and susceptible checks, and individual disease scores, the genotypes were classified into four disease reaction groups: resistant, moderately resistant, moderately susceptible and susceptible. According to this grouping, 95 genotypes were resistant to *M. phaseolina* while 77 genotypes were resistant to *F. thapsinum*. Another 72 genotypes were moderately resistant to *M. phaseolina* while 80 were moderately resistant to *F. thapsinum*. Likewise another 81 and 49 genotypes were grouped as moderately susceptible and susceptible to *M. phaseolina* respectively, while

83 and 60 genotypes were moderately susceptible and susceptible to *F. thapsinum*. A total of 53 genotypes were resistant to both *M. phaseolina* and *F. thapsinum.*

Relationship between stalk-rot disease reaction and racial distribution

The racial distribution of the 300 sorghum genotypes in the diversity panel is shown in Fig 2.2 Twenty-five percent of the genotypes belonged to the caudatum race, 10% to durra, 8% to guinea, 6% to kafir, 5% to bicolor, 4.3% were breeding lines, 16% were cultivars of unknown race. The remaining 27% were representatives of mixed races like caudatum-bicolor, durra-bicolor, durra-caudatum, guinea-bicolor, guinea-caudatum, guinea-durra, guinea-kafir, kafir-bicolor, kafir-caudatum and kafir-durra. The caudatum race had the highest representation in the diversity panel followed by durra, guinea, kafir and bicolor, respectively. The caudatum race, mainly from central Africa, has been widely used in breeding programs and all modern hybrids in the U.S. are caudatums or contain substantial pedigree of caudatum. Because of its large panicle structure, caudatum seems to offer higher yield potential though there is significant variation within races for yield as well as other traits like seed color and grain quality. The race bicolor is predominant in Africa and found in almost all parts of the continent with no clear geographical distribution or ecological adaptation. Guineas are predominantly from West Africa and have hard seeds that are resistant to insects and mold damage under wet conditions. They are grown in the high rainfall areas of the region. The race kafir was evolved in southern Africa and also commonly grown in India. Durra is a drought-tolerant race and is present in Eastern Africa and India. It is adapted to wide agro ecologies, including wide moisture and temperature regimes.

Racial distribution of genotypes with respect to reaction to *Fusarium* stalk rot resistance is shown in Fig 2.3 Sources of resistance to both Fusarium stalk rot and charcoal rot are not limited to just one or a few races. The 95 genotypes identified as resistant to *F. thapsinum* represented all races of sorghum (Fig 2.3 A). However, there was a larger representation of the caudatum race within the resistant group. Similar racial representations were observed for the moderately resistant (Fig 2.3 B), moderately susceptible (Fig 2.3 C), and susceptible (Fig 2.3 D) group. The distribution of the genotypes in different disease reaction groups for *M. phaseolina* was similar to that of *F.*

thapsinum (Fig 2.4). The five basic races were represented in each group; however, the proportion of the caudatum and durra races in the resistant group was higher than the other races.

Relationship between stalk rot disease reaction and geographical distribution

The geographical distribution of members of the diversity panel is presented in Fig 2.5. Sudan, Ethiopia, USA, India, Nigeria, and Uganda were the highest contributors to the panel at 18%, 15%, 12%, 10%, 9.3% and 6.7%, respectively. Materials from Sudan and Ethiopia made up 33% of the entire panel; these makes sense since the two countries harbor the largest genetic variability for the crop.

Unlike the botanical race, geographic origin of genotypes seems to have some degree of influence on the response of genotypes to the diseases. As shown in Fig 2.6, 38% of genotypes resistant to Fusarium stalk rot are of Ethiopian origin followed by Sudan (17%). Though Sudan has the largest representation in the panel, the proportion of Sudanese materials resistant to Fusarium stalk rot were less than half of those from Ethiopia indicating some influence of geographical adaptation. However, a higher frequency of genotypes originating from Sudan was grouped in the susceptible category (Fig 2.6 D). The distribution of genotypes with respect to charcoal rot resistance (Fig 2.7 A) had the same trend as Fusarium stalk rot with 14 and 16 genotypes originating from Ethiopia and Sudan, respectively, making up the greatest proportion of the resistant group. However, Sudanese materials also make up the highest proportion of genotypes susceptible to the disease (Fig 2.7 D). It was also observed that genotypes originating from Japan, Kenya, Western Ghana and Gambia were predominantly resistant to charcoal rot though the representation from these countries is generally very low.

Table 2.3 shows the list of top ten genotypes identified as resistant to stalk rot diseases caused by *F. thapsinum* and *M. phaseolina*. The elite breeding line RTx430 bred at Texas A&M University was found to be resistant to infection by *M. phaseolina*. This line has been included in previous stalk rot resistance studies conducted in Texas and Kansas and was not considered resistant. However, this study shows a completely different result and this may be due to either environmental effect or differences in the pathogen isolates used in the studies. SC15 is another resistant line of Sudanese origin. It

belongs to a mixed race of guinea-bicolor and was noted to have the best resistance to infection by *F. thapsinum*. RTx2737 was also observed among the top ten genotypes to have dual resistance to *F. thapsinum* and *M. phaseolina* infection. The origin of the resistant genotypes is largely from the east African countries of Ethiopia, Sudan, and Uganda with few genotypes from Chad, Nigeria, Congo, and China also among the top 10. No evidence of racial effect on stalk rot resistance was observed.

Discussion

The incidence of stalk rot diseases is sporadic; whenever it happens, it can cause serious damage to yield and quality of sorghum. Apart from the likely loss caused as a result of damage to roots and stalks, the disease predisposes infected plants to lodging that can cause even more damage. The disease is very complex in that not only numerous pathogens are involved but also the environmental conditions that trigger the diseases are poorly understood. Generally, prolonged drought after flowering period is believed to trigger infection by *M. phaseolina* whereas *F. thapsinum* requires a wet and cool period that follows hot and dry condition in order to cause disease. The year 2013 was sufficiently moist in much of the Midwestern U.S. and sorghum enjoyed ample moisture and ideal temperature throughout the season. Though such an environment may be perceived as less ideal for disease development, heavy stalk rot infection occurred later in the season at an epidemic scale covering large areas from Texas to Kansas. Our sorghum breeding nursery was affected by the disease with entire root systems in some of the genotypes, being completely disintegrated leaving the plants flat; whereas, other genotypes were healthy and intact and there was wide variability between genotypes in the nursery.

Previous studies have suggested the use of resistant cultivars as the most effective and environmentally sound approach for addressing stalk rot problems. Our observation in 2013, where varietal differences were vividly evident in breeding nurseries agrees with this recommendation. The objective of the present study was to identify stable sources of resistance to infection by the two major stalk rot organisms, *M. phaseolina* and *F. thapsinum*. Although stalk rot is caused by a complex of pathogens, these two species have been reported to be the major causal organisms. *M. phaseolina* has been known to

occur worldwide and is much more aggressive than other pathogens (Zummo, 1984). While almost all *Fusarium* spp. do cause stalk rot in sorghum, *F. thapsinum* was shown to be the most aggressive species (Tesso et al., 2010).

The present study evaluated the largest set of genotypes (300) ever studied under artificial inoculation and revealed significant genetic variation for resistance to both diseases. Genotypes with a range of disease responses were present among the population evaluated, which were grouped into four response categories, resistant, moderately resistant, moderately susceptible and susceptible based on hierarchical clustering performed on individual genotype response data (Table 2.1). Of the 300 genotypes evaluated, 95 and 77 were found to be resistant to *M. phaseolina* and *F. thapsinum*, respectively with 53 genotypes resistant to both pathogens. The remaining genotypes were distributed among the different resistance groups. There seems to be more genotypes resistant to *Macrophomina* than to *Fusarium* despite that the former was more aggressive.

The environments were not considered ideal for development of *Macrophomina* and this may have obscured the expected aggressiveness. Nevertheless, given the number and level of genetic variability present within the diversity panel (Casa et al., 2008) and the results of similar studies conducted earlier on smaller set of genotypes (Rosenow et al., 1983; Bramel-Cox et al., 1988; Tesso et al., 2005), the extent of genetic variation observed for resistance to both pathogens in the present study was not unexpected. Levels of resistance seem to be randomly distributed across the diversity continuum and were not specific to any genetically differentiated subgroup or specific racial group. However, geographical origin seemed to have a slight impact in that many of the resistance sources came from east African. This agrees with numerous previous reports where east Africa is considered a source for post-flowering drought tolerance genotypes, a character closely related to stalk rot resistance. However, the significant number of genotypes that are resistant to both pathogens was interesting. The pathogens belong to completely different genera and have distinct biology. However, though there are specific preferences, the pathogens seem to have significant ecological overlap in that they both over winter in infected plant debris and the soil, and their infection triggered by similar types of environmental conditions during crop maturation. Thus, although certain genotypes of

sorghum produce antifungal proteins that provide resistance to fungal colonization (Darnetty et al., 2006), which may be the case in the current study, part of the dual pathogen resistance observed may be the result of overlapping ecological preferences of the two species. In other words, genotypes that are tolerant to abiotic stress conditions that trigger these diseases even though they carry different alleles for resistance may tend to show less infection by the pathogens. Previous studies have also reported similar results where several sorghum genotypes were found to have enhanced resistance to *Macrophomina* as well as several species of *Fusarium* (Tesso et al., 2005). However, these authors reported consistently higher levels of infection among plants inoculated with *M. phaseolina* than those treated with any of the *Fusarium* spp. The current study differs in that though *M. phaseolina* was always considered more virulent and aggressive, disease severity was not higher than that of *F. thapsinum*. This may be the result of the difference in inoculum doses of *Macrophomina* and *Fusarium* pathogens or due to specific environmental preference of the pathogens although they all are commonly triggered by the same kind of environmental conditions. Unlike the study by Tesso et al. (2005) where *Macrophomina* and *Fusarium* inoculum were delivered as calibrated liquid suspensions the current study utilized the toothpick inoculation procedure for *M. phaseolina* that does not account for specific inoculum concentration. Therefore, it is likely that the amount of *Macrophomina* inoculum delivered by the colonized toothpick to the plants may be low resulting in disease severity that is not higher than *F. thapsinum* as expected. Also, the current test was carried out in an environment presumed to be less ideal for *M. phaseolina* infection. Previous studies have shown that *Macrophomina* prefers a more arid environment such as those found in Texas and New Mexico (Rosenow et al., 1977), while *Fusarium* becomes more damaging in more temperate type environments, including the Central Great Plains of the U S (Jardine and Leslie, 1992). Hence, besides the inoculum dose, the fact that Kansas is a less ideal environment for *Macrophomina*, or the isolate used in the study may have compromised virulence, or both may have contributed to *M. phaseolina* not expressing the typical aggressiveness.

Another important observation from this study is that new genotypes were found to be more resistant than the known checks (Table 2.3). This indicates that wider scale germplasm screening would yield new genotypes immune to these diseases. The

difficulty with that is the lack of high throughput screening method. The present inoculation and screening procedures are crude and labor intensive. Thus only a relatively small set of genotypes can be accurately phenotyped in a reasonable amount of time. While the stalk and root rot problems in sorghum are real, any effort to address them through deployment of resistant germplasm should first focus on optimizing and simplifying screening protocols to add ease and accuracy to the procedure. On the other hand, some of the genotypes that were grouped among the top resistance sources to both *M. phaseolina* and *F. thapsinum* were not considered so in previous studies. The appearance of genotypes such as Tx430 and Tx2737 as resistant to both pathogens, and more resistant than the resistant checks, was difficult to explain. Although they were not in the susceptible groups when evaluated as inbreds per se, they never were on the top or even close to the resistant checks such as Tx642 and SC599. The only two reasons we can speculate in relation to this result may be the kind of pathogen isolates used in the studies and the possible genotype by environment interaction effects. Much of the earlier studies conducted on *Macrophomina* over the last fifteen years was based on the isolate collected in Texas by Dr. Gary Odvody. Under those tests genotypes like Tx430 and Tx2737 never performed well and their hybrids were susceptible to the disease. Similarly, although many of the *Fusarium* studies previously conducted were based on various strains of the former *F. monliforme*, more recent studies after the reclassification of the *F. moniliforme* species complex (Leslie, 1995) was based on specific species of *F. thapsinum*, *F. andiazi*, *F. proliferatum,* etc. Strains used in the current study were isolated from infected sorghum plants but there is no evidence of their pathogenicity.

Conclusion

Like for many other traits, sorghum seems to harbor significant genetic variability for resistance to stalk rot diseases. However, research for improving stalk rot resistance in sorghum is difficult and complex primarily due to the complexity of the disease itself and due to lack of high throughput screening methods that can be used in breeding programs. Enhancing resistance to the disease should involve large scale screening to identify more stable sources with resistance to multiple causal pathogens, mapping the key genes involved and incorporating them into breeding programs if the immense potential of the

crop are to be harnessed. Moreover, there are significant genotype × environment × isolate interaction effects on the development of the diseases, which bolsters the need to establish the most robust and virulent isolate for both pathogens and selection of suitable locations for use in germplasm screening and hybrid evaluation. The new sources identified as resistant to both *F. thapsinum* and *M. phaseolina* are significant additions to the resistance pool and broaden the germplasm base to offer alternative sources for programs seeking to develop stalk rot resistant cultivars/hybrids.

References

Bhattacharjee, R., Khairwal, I.S., Bramel, P.J., and Reddy, K.N. (2007). Establishment of a pearl millet *Pennisetum glaucum* (L.) R. Br. core collection based on geographical distribution and quantitative traits. Euphytica 155, 35-45.

Bramel-Cox, P.J., Stein, I.S., Rodgers, D.M., and Claflin, L.E. (1988). Inheritance of Resistance to *Macrophomina phaseolina* (Tassi) Goid. and *Fusarium moniliforme* Sheldom in Sorghum. Crop science 28, 37-40.

Casa, A.M., G. Pressoir, P.J. Brown, S.E. Mitchell, W.L. Rooney, M.R. Tuinstra, et al. (2008). Community resources and strategies for association mapping in sorghum. Crop Science 48, 30-40.

Choudhary, S., Sinclair, T.R., and Prasad, P.V. (2013). Hydraulic conductance of intact plants of two contrasting sorghum lines, SC15 and SC1205. Functional Plant Biology 40, 730-738.

Claflin, L.E. (2000). Fusarium root and stalk rot. p 28-30. In: Frederikson, R.A. (Ed). Compendium of Sorghum Diseases. 2nd Edition. APS Press, St Paul, MN.

Darnetty, J.F.L., Muthukrishnan, S., Swegle, M., Vigers, A.J., and Selitrennikoff, C.P. (1993). Variability in antifungal proteins in the grains of maize, sorghum and wheat. Physiologia Plantarum 88, 339-349.

Frankel, O.H., and Brown, A.H.D. (1984). Plant genetic resources today: a critical appraisal. Pages 249-268 in: Crop Genetic Resources: Conservation and Evaluation. J.H.W. Holden and J.T. Williams, eds. Allen and Unwin, Winchester, MA.

Frowd, J.A. (1980). Sorghum stalk rots in West Africa. p. 322–324. *In* Proc. of the Int. Workshop of Sorghum Diseases, Hyderabad, India.11–15 Dec. 1978. Texas A&M University, College Station, and ICRISAT, Patancheru, India.

Grenier, C., Hamon, P., and Bramel-Cox, P.J. (2001). Core collection of sorghum: II. Comparison of three random sampling strategies. Crop Science 41, 241-246.

Hulluka, M., and Esele, J.P.E. (1992). Sorghum diseases in East Africa. p. 21–24. *In* W.A.J. Milliano et al. (ed.) Sorghum and millet diseases: A second world review. ICRISAT, Patancheru, AP, India.

Idris, H. A., Labuschagne, N., and Korsten, L. (2008). Suppression of *Pythium ultimum* root rot of sorghum by rhizobacterial isolates from Ethiopia and South Africa. Biological Control 45, 72-84.

Jardine, D.J., and Leslie, J.F. (1992). Aggressiveness of Gibberella-Fujikuroi (*Fusarium moniliforme*) isolates to grain-sorghum under greenhouse conditions. Plant Disease 76, 897-900.

Jwa, N-S., Agrawal, G.K., Tamogami, S., Yonekura, M., Han, O., Iwahashi, H. and Rakwal, R. (2006). Role of defense/stress-related marker genes, proteins and secondary metabolites in defining rice self-defense mechanisms. Plant Physiology and Biochemistry 44, 261-273

Khune, N.N., Kurhekar, D.E., Raut, J.G., and Wangikar, P.D. (1984). Stalk rot of sorghum caused by *Fusarium moniliforme.* Indian Phytopathology 37, 316–319.

Leslie, J.F. (1995). Gibberella-Fujikuroi - Available populations and variable traits. Canadian Journal of Botany 73, S282-S291.

Miller, F.R. (1984). Registration of RTx430 sorghum parental line. *Crop Science* 24, 1224-1224. Chicago.

Mutava, R.N., Prasad, P.V.V., Tuinstra, M.R., Kofoid, M.D., and Yu, J. (2011). Characterization of sorghum genotypes for traits related to drought tolerance. Field Crops Research 123, 10-18.

Omar, M.E., Frederiksen, R.A., and Rosenow, D.T. (1985). Collaborative sorghum disease studies in Sudan. Sorghum Newsletter 28:93.

R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Rosenow, D.T., Johnson, J.W., Frederiksen, R.A., and Miller, F.R. (1977). Relationship of nonsenescence to lodging and charcoal rot in sorghum. Agronomy Abstracts 69-69.

Rosenow, D.T., Quisenberry, J.E., Wendt, C.W., and Clark, L.E. (1983). Drought tolerant sorghum and cotton germplasm. Agricultural Water Management, 7, 207-222.

Ryota, S. and Hidetoshi, S. (2011). pvclust: Hierarchical Clustering with P-Values via Multiscale Bootstrap Resampling. R package version 1.2-2. http://CRAN.R-project.org/package=pvclust

Sasaki, T., and Antonio, B.A. (2009). Sorghum in sequence. Nature 457, 547-548.

Seetharama, N., R.C. Sachan, A.K. Huda, K.S. Gill, K.N. Rao, and Bidinger, F.R. (1991). Effect of pattern and severity of moisture deficit stress on stalk rot incidence in sorghum. II. Effect of source/ sink relationships. Field Crops Research. 26, 355–374.

Stephens, J.C., Miller, F.R., and Rosenow, D.T. (1967). Conversion of alien sorghums to early combine genotypes. Crop Science 7, 396.

Sukumaran, S., Xiang, W.W., Bean, S.R., Pedersen, J.F., Kresovich, S., Tuinstra, M.R., Tesso, T.T., Hamblin, M.T., and Yu, J.M. (2012). Association mapping for grain quality in a diverse sorghum collection. Plant Genome 5, 126-135.

Tesso, T.T., Ochanda, N., Little, C.R., Claflin, L., and Tuinstra, M.R. (2010). Analysis of host plant resistance to multiple *Fusarium* spp associated with stalk rot disease in sorghum *Sorghum bicolor* (L.) Moench. Field Crops Research 118, 177-182.

Toman, J., and White, D.G. (1993). Inheritance of resistance to anthracnose stalk rot of corn. Phytopathology 83, 981-986.

Trimboli, D.S., and Burgess, L.W. (1982). The fungi associated with stalk and root rot of grain sorghum in New South Wales. Newsletter. 25:105.

Upadhyaya, H.D., Pundir, R.P.S., Dwivedi, S.L., Gowda, C.L.L., Reddy, V.G., and Singh, S. (2009). Developing a mini core collection of sorghum for diversified utilization of germplasm. Crop Science 49, 1769-1780.

Waniska, R.D., Venkatesha, R.T., Chandrashekar, A., Krishnaveni, S., Bejosano, F.P., Jeoung, J-M., Jayaraman, J., Muthukrishnan, S. and Liang, G.H. (2002). Antifungal proteins and other mechanisms in the control of sorghum stalk rot and grain mold. p 287-297. In: Leslie, J.F. (Ed). Sorghum and millets diseases. Iowa State Press, Ames

Zummo, N. (1984). Fusarium root and stalk disease complex. p 25-29. In: Mughogho, L.K. (Ed). Sorghum root and stalk rots, a critical review: Proceedings of the consultative group discussion on research needs and strategies for control of sorghum root and stalk diseases, 27 Nov - 2 Dec 1983, Bellagio, Italy. Patancheru, A. P. 502 324, India, ICRISAT.

Tables and Figures

Table 2.1. Combined analysis of variance, descriptive statistics and broad-sense heritability for eight traits related to stalk rot disease measured in the sorghum diversity panel (N = 300).

Trait	Mean ± SD[a]	Coefficient of Variation	Range	*P* value (genotype)	Heritability (%)
TLM	5.38 ± 2.78	51.65	1.83 - 16.72	<0.0001	38.7
MLM	4.65 ± 2.16	46.56	1.83 - 12.92	<0.0001	38.1
RMLM	0.04 ± 0.02	38.91	0.021 – 0.126	<0.0001	21.7
TLF	5.83 ± 2.35	40.20	2.28 - 17.03	<0.0001	37.1
MLF	5.02 ± 1.93	38.55	2.19 - 13.67	<0.0001	40.7
RMLF	0.05 ± 0.02	33.79	0.021 – 0.119	<0.0001	31.3
PH (cm)	103.99 ± 23.87	22.96	59.97 - 192.9	<0.0001	52.6
DF	65.62 ± 6.54	9.97	41.5 - 80.5	<0.0001	49.0

TLM = Total lesion length *Macrophomina,* MLM = Major lesion length *Macrophomina,* TLF = Total lesion length *Fusarium*, MLF= Major Lesion length *Fusarium*, RMLM = Relative major lesion length *Macrophomina,* RMLF = Relative major lesion length *Fusarium*, PH= Plant height, DF= days to flowering.
[a] standard deviation

Table 2.2. Pearson correlation coefficients between crop phenology and disease parameters for both *Fusarium thapsinum* and *Macrophomina phaseolina* (N=300).

	Correlation (*r*)						
Trait	TLM	MLM	RMLM	TLF	MLF	RMLF	PH
MLM	0.89**	-					
RMLM	0.73**	0.87**	-				
TLF	0.82**	0.76**	0.62**	-			
MLF	0.75**	0.8**	0.67**	0.9**	-		
RMLF	0.58**	0.65**	0.76**	0.78**	0.88**	-	
PH	0.41**	0.43**	-0.04	0.36*	0.38*	-0.05	-
DF	-0.24*	-0.08	-0.05	-0.09	0.01	0.03	-0.01

**Significant at $P<0.01$, *Significant at $P<0.05$

TLM = Total lesion length *Macrophomina,* MLM = Major lesion length *Macrophomina,* TLF = Total lesion length *Fusarium*, MLF= Major lesion length *Fusarium*, RMLM= Relative major lesion length *Macrophomina,* RMLF= Relative major lesion length *Fusarium*, PH= Plant height, DF= days to flowering

Table 2.3. The list of top ten genotypes identified as resistant to stalk rot diseases caused by *Macrophomina phaseolina* and *Fusarium thapsinum*.

Macrophomina phaseolina			*Fusarium thapsinum*		
Genotypes	origin	Race	Genotypes	origin	Race
*RTx430	Ethiopia	Breeding line	SC15	Sudan	Guinea-bicolor
*SC323	China	Caudatum	SC299	Nigeria	Guinea
*SC115	Chad	Caudatum-bicolor	*RTx430	Ethiopia	Breeding line
SC325	Congo	Caudatum	RTx437	Ethiopia	Breeding line
SC58	Ethiopia	Caudatum	*RTx2737	Ethiopia	Breeding line
RTx436	Ethiopia	Inbred	*SC115	Chad	Caudatum-bicolor
*RTx2737	Ethiopia	Breeding line	*SC323	China	Caudatum
SC17	Sudan	Bicolor	SC13	Sudan	Durra-bicolor
SC348	Nigeria	Caudatum	SC805	Uganda	Caudatum
SC574	Uganda	Caudatum	SC348	Nigeria	Caudatum

*Genotypes resistant to both *Fusarium thapsinum* and *Macrophomina phaseolina* inducing stalk rot

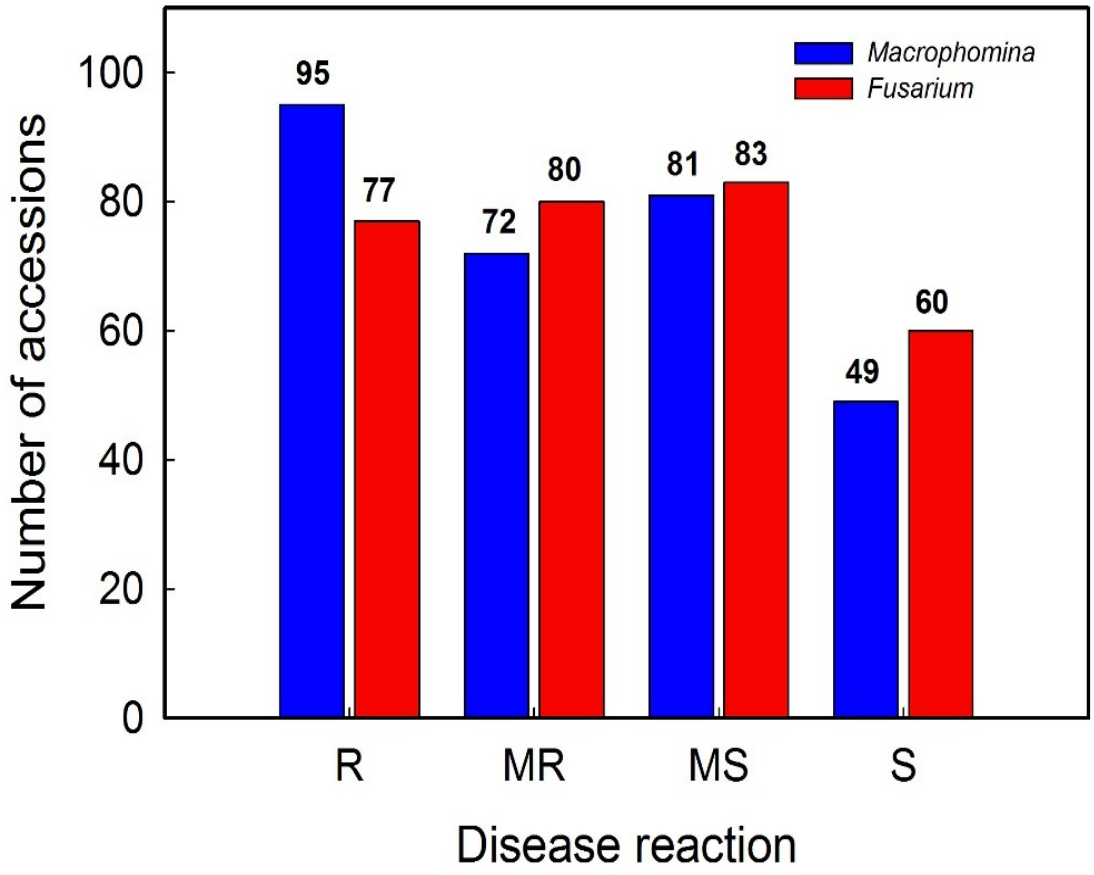

Figure 2.1. Summary of disease reaction of the sorghum diversity panel. R= resistant, MR = moderately resistant, MS = moderately susceptible, and S = susceptible.

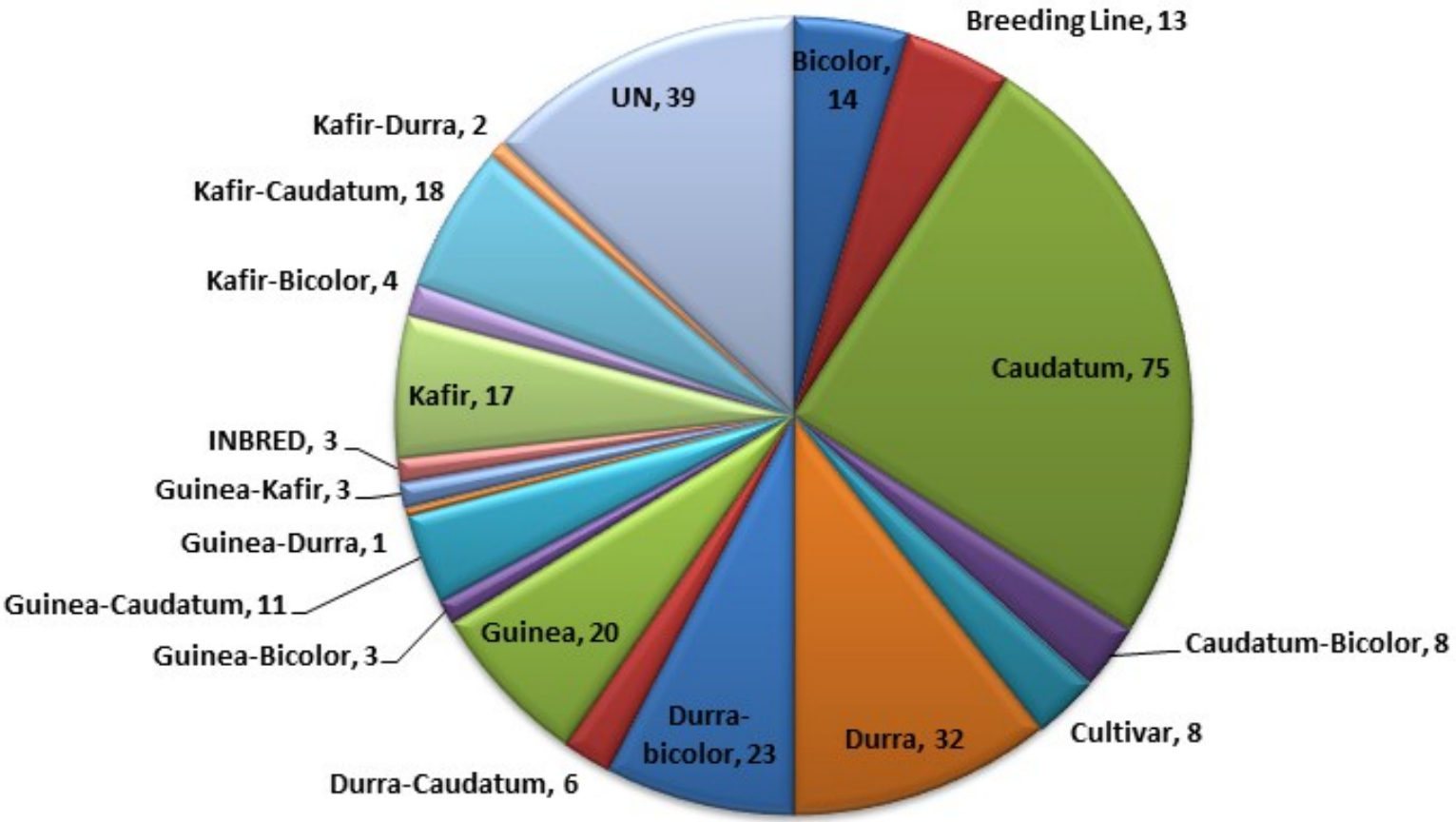

Figure 2.2. Racial distribution of the sorghum diversity panel used in the study.

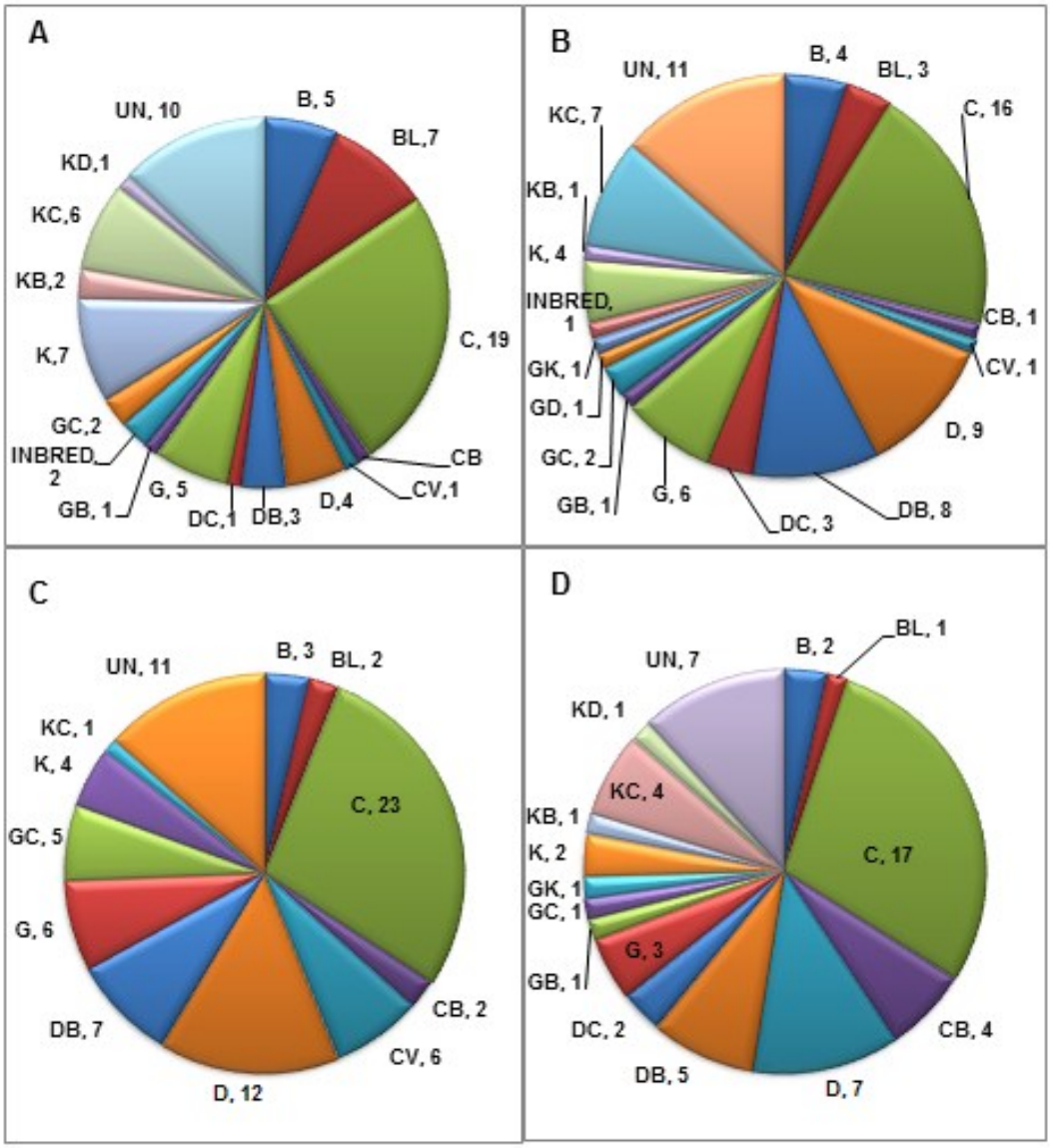

Figure 2.3. Racial distribution of study genotypes with respect to the *Fusarium* stalk-rot disease reaction groups (A) Resistant (B) Moderately-resistant (C) Moderately-susceptible (D) Susceptible; B= Bicolor, BL= Breeding Line, C= Caudatum, CB= Caudatum-Bicolor, CV= Cultivar, D= Durra, DB= Durra-bicolor, DC= Durra-Caudatum, G= Guinea, GB= Guinea-Bicolor, GC= Guinea- Caudatum, GD= Guinea-Durra, GK= Guinea-Kafir, K= Kafir, KB= Kafir-Bicolor, KC= Kafir-Caudatum, KD= Kafir-Durra, UN = Unknown.

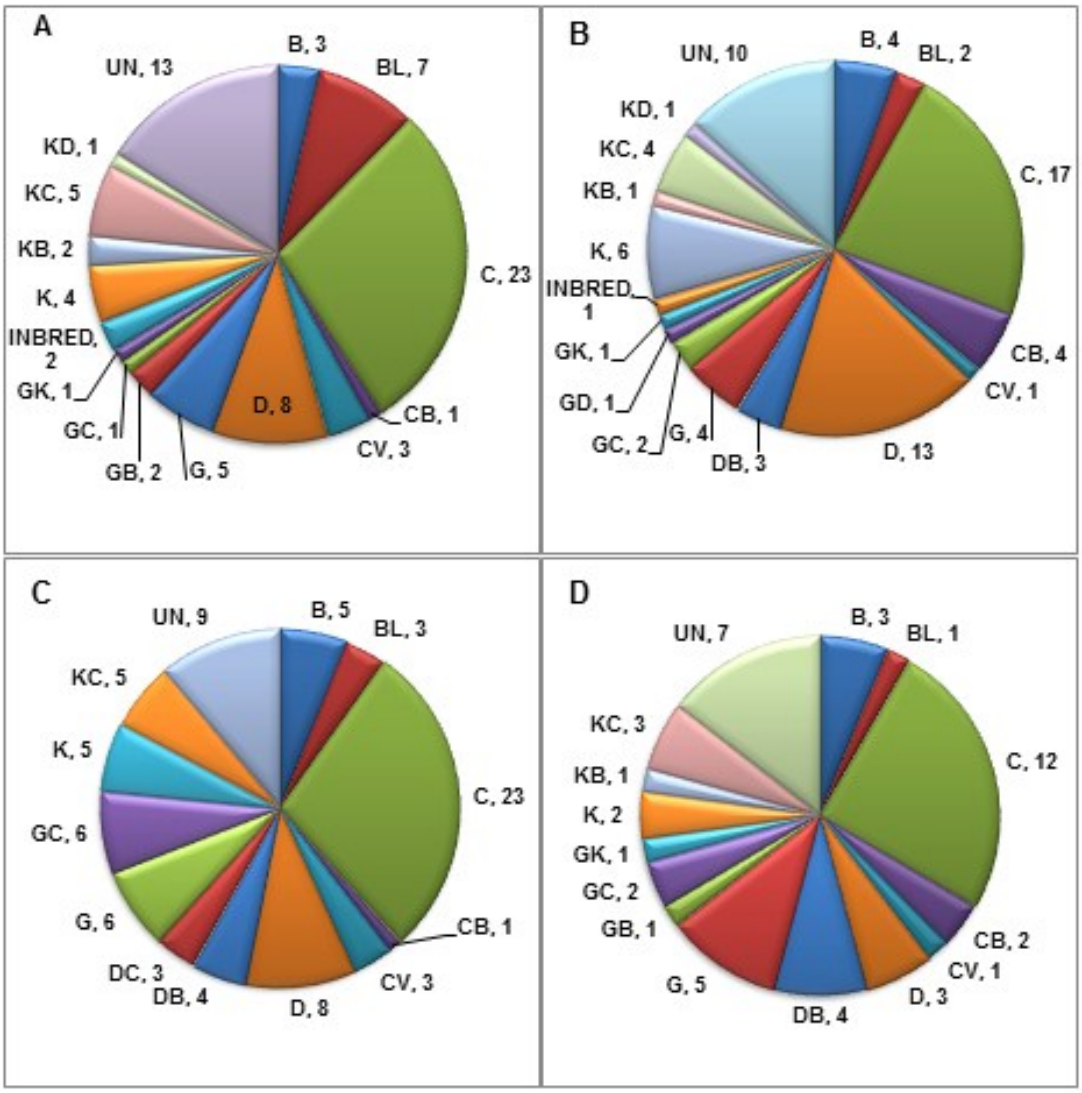

Figure 2.4. Racial distribution of study genotypes with respect to the *Macrophomina* disease reaction groups (A) Resistant (B) Moderately-resistant (C) Moderately-susceptible (D) Susceptible; B= Bicolor, BL= Breeding Line, C= Caudatum, CB= Caudatum-Bicolor, CV= Cultivar, D= Durra, DB= Durra-bicolor, DC= Durra-Caudatum, G= Guinea, GB= Guinea-Bicolor, GC= Guinea- Caudatum, GD= Guinea-Durra, GK= Guinea-Kafir, K= Kafir, KB= Kafir-Bicolor, KC= Kafir-Caudatum, KD= Kafir-Durra, UN = Unknown.

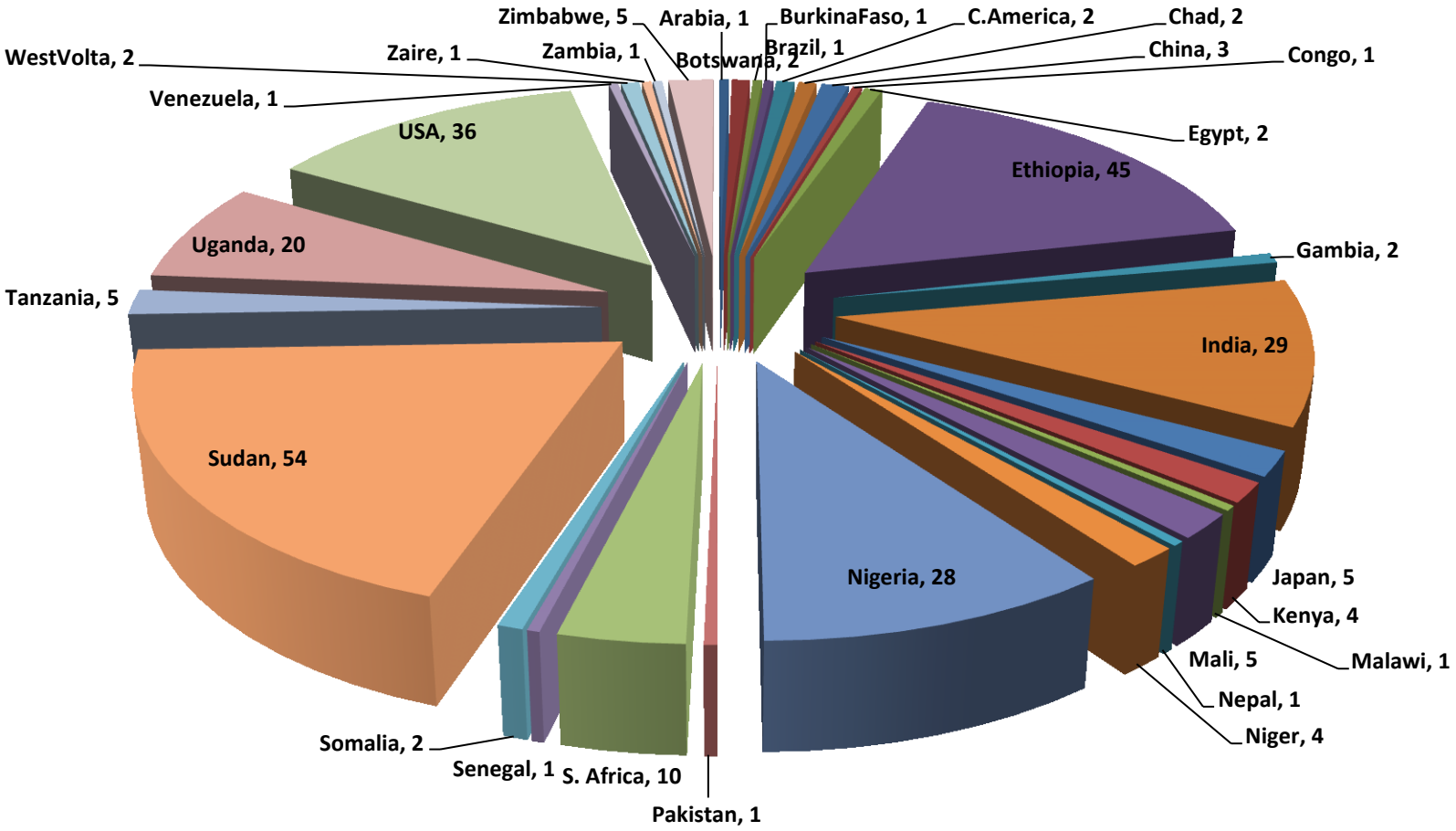

Figure 2.5. Geographical distribution of the sorghum diversity panel used in the study.

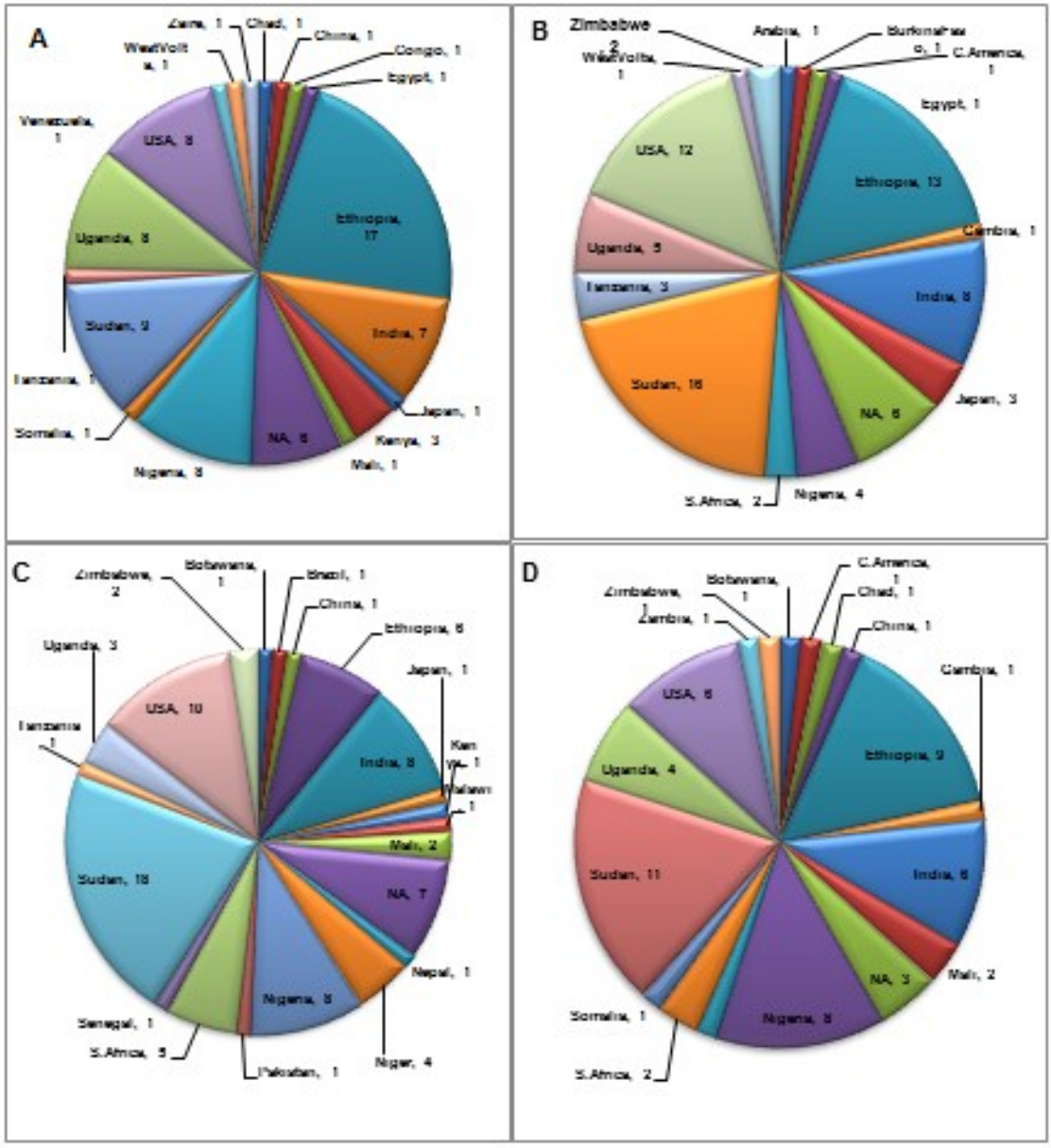

Figure 2.6. Geographical distribution with respect to the Fusarium stalk rot disease reaction groups. (A) Resistance (B) Moderately resistant (C) Moderately-susceptible (D) Susceptible. B= Bicolor, BL= Breeding Line, C= Caudatum, CB= Caudatum-Bicolor, CV= Cultivar, D= Durra, DB= Durra-bicolor, DC= Durra-Caudatum, G= Guinea, GB= Guinea-Bicolor, GC= Guinea- Caudatum, GD= Guinea-Durra, GK= Guinea-Kafir, K= Kafir, KB= Kafir-Bicolor, KC= Kafir-Caudatum, KD= Kafir-Durra, UN = Unknown

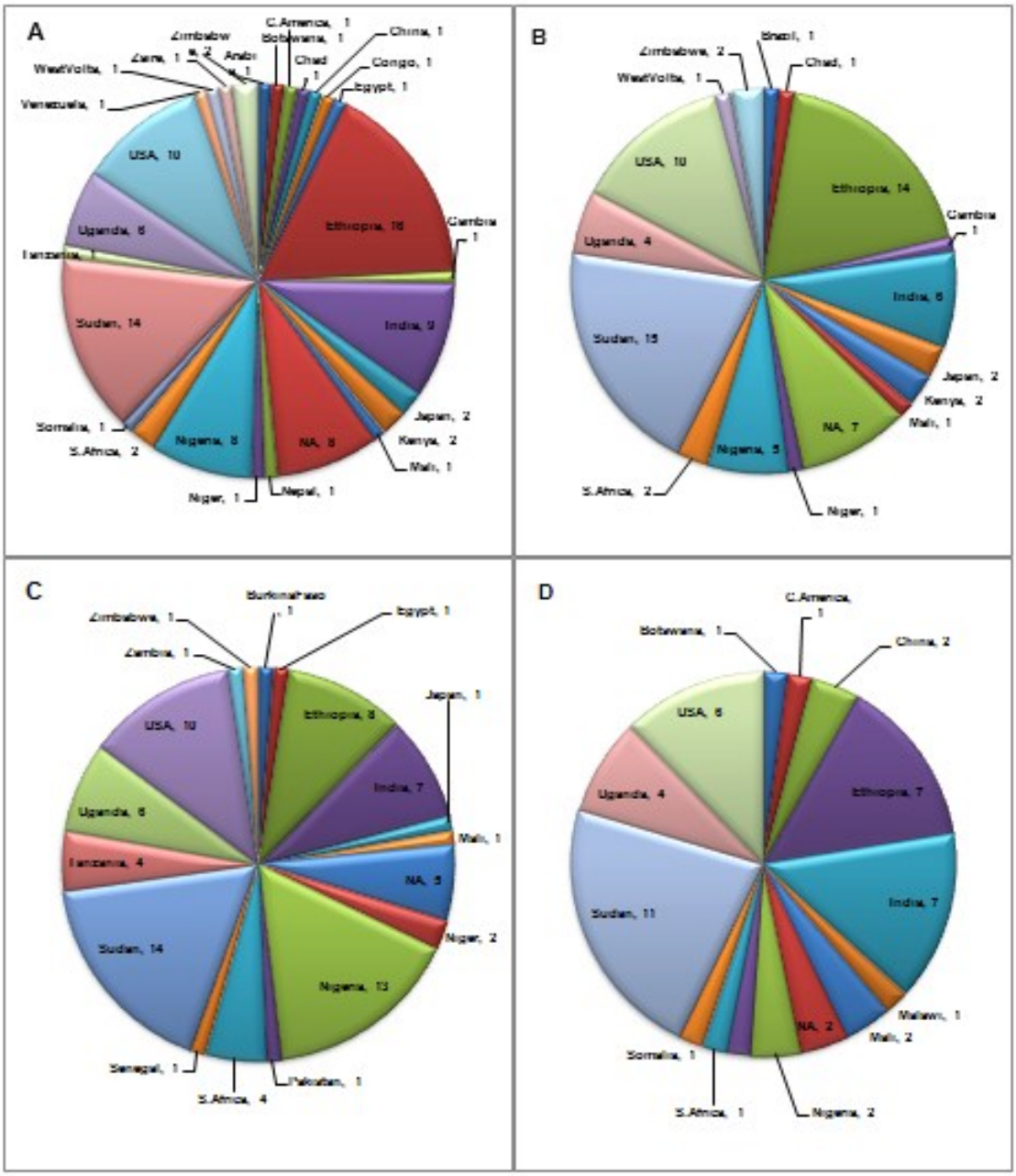

Figure 2.7. Geographical distributions with respect to the charcoal rot disease reaction groups. (A) Resistance (B) Moderately resistant (C) Moderately susceptible, (D) Susceptible; B= Bicolor, BL= Breeding Line, C= Caudatum, CB= Caudatum-Bicolor, CV= Cultivar, D= Durra, DB= Durra-bicolor, DC= Durra-Caudatum, G= Guinea, GB= Guinea-Bicolor, GC= Guinea- Caudatum, GD= Guinea-Durra, GK= Guinea-Kafir, K= Kafir, KB= Kafir-Bicolor, KC= Kafir-Caudatum, KD= Kafir-Durra, UN = Unknown.

Chapter 3 - Genome wide association study on resistance to stalk rots in sorghum

Abstract

Fusarium stalk rot and charcoal rot are the primary stalk rot diseases that cause significant yield loss in sorghum. They are important biotic constraints to sorghum production worldwide and host plant resistance has been considered as the most feasible approach for controlling the disease. The high environment dependence of the disease and its sporadic nature has led to slow progress in breeding efforts. A set of 79,132 single nucleotide polymorphisms (SNPs) markers were used in an association test to identify genomic regions underlying stalk rot resistance using a multi-locus mixed model association mapping approach that accounted for background genomic relationships. The genotypes were evaluated in three environments (Manhattan, KS 2011, Manhattan, KS 2012, and Ottawa, KS 2012) using artificial field inoculations. Adequate genetic variation for stalk rot resistance traits was observed in the study. We identified fourteen associated loci and a set of candidate genes that appear to be involved in connected functions controlling plant defense response to stalk rot resistance. Each associated SNP had a relatively small effect on disease resistance. The associated SNPs accounted for between 19-30% of phenotypic variation observed within and across environments. Linkage disequilibrium analyses of the significant SNPs suggested that they are genetically independent. An analysis of associated allele frequencies within the major sorghum subpopulations revealed enrichment for resistant alleles in the durra and caudatum subpopulations compared with other subpopulations. The findings suggested a complicated molecular mechanism of sorghum resistance against stalk rots caused by the fungus *Macrophomina phaseolina* and *Fusarium thapsinum*.

Key words: Sorghum, *Fusarium thapsinum*, *Macrophomina phaseolina*, stalk rots, association mapping, diversity panel

Introduction

Sorghum is the world's fifth most important cereal crop. It is a staple food for more than half a billion people in the world, 60 percent of whom are living in the African continent. However, diseases and pests, in addition to abiotic stresses, are major impediments to realizing the high yield potential that sorghum ought to offer as a C4 cereal. Developing cultivars resistant to these biotic and abiotic stresses is paramount to improving sorghum productivity in farmers' fields.

Stalk-rots are an important disease of sorghum worldwide. Recently, increased incidence of stalk rot have been reported (Mahmoud and Budak 2011; Khangura and Aberra 2009) highlighting the importance of the disease especially in regions that are already prone to drought. Charcoal rot, a soil borne necrotrophic fungal pathogen is the most aggressive stalk rot disease of sorghum (Crasta et al. 1999). The disease is caused by the fungus *Macrophomina phaseolina* (Tassi) Goid. *M. phaseolina* infects sorghum through roots and continues to the base of the plant, resulting in disintegration of the pith cells, premature leaf and plant senescence, and crop lodging. *Fusarium* (*Fusarium* spp.) stalk-rot is another important disease of grain sorghum. *Fusarium thapsinum* is the most common fungus associated with sorghum stalk rots in more temperate environments including much of the central great plains of the United States (Bramel-cox et al. 1988; Klittich et al. 1997; Marasas et al. 2001; Leslie 1995; Leslie et al. 1990). Although *Fusarium* is often regarded as a weak pathogen or a secondary invader, *Fusarium* infections become more aggressive under appropriate environmental condition. Both *F thapsinum* and *M phaseolina* have been reported as the most aggressive pathogens causing stalk-rot in Kansas (Tesso et al. 2010). Significant yield losses due to stalk rot have been reported throughout the world including Africa (Frowd, 1980; Omar et al., 1985; Hulluka and Esele, 1992), India (Khune et al., 1984; Seetharama et al., 1987), Australia (Trimboli and Burgess, 1982), and the USA (Jardine and Leslie 1992; Reed et al. 1983). Current disease control depends on the combination of field management practices and the use of resistant cultivars (Waniska et al. 2001).

Conventional breeding for sorghum resistance to stalk rots has mainly focused on indirect selection for the stay-green trait. This may be as a result of the difficulty of field phenotyping for stalk-rot disease related traits. Genetic engineering is also not a feasible

approach both from the wide gap in the knowledge of molecular processes mediating host-pathogen interactions as well as from the regulatory standpoint as sorghum GM is not widely deployed. Also, there are conflicting reports on the nature of genetic factors that condition resistance to charcoal. (Pecina-Quintero et al. 1999; Sahib et al. 1990). Understanding the genetic control will facilitate cultivar improvement for disease and secure global food production.

Genome wide association mapping has arisen as a powerful tool for high-resolution mapping of loci underlying quantitative traits, because it takes advantage of accumulated historic recombination events in natural populations and it holds promise for identifying causative polymorphism of complex traits (Stich et al. 2008). Although genome wide association studies (GWAS) have the potential to pinpoint genetic polymorphisms underlying human diseases and agriculturally important traits, false discoveries are a major concern (Abiola et al. 2003) and can be partially attributed to spurious associations caused by population structure and unequal relatedness among individuals in a given cohort. Population stratification was initially addressed using general linear model (GLM)-based methods such as structured association (Pritchard et al. 2000), genomic control (Devlin and Roeder 1999) and family-based tests of association (Abecasis et al. 2000). Association analysis uses preexisting germplasm such as landraces, modern cultivars, and advanced breeding lines to detect potential associations between molecular markers and traits of interest (Zhu et al., 2008). Association analysis has been successfully applied to identify marker–trait associations in different crops (Beattie et al. 2010; Kump et al. 2011; Roy et al. 2010; Wang et al. 2012a; Yu et al. 2006; Zhu et al. 2008; Gupta et al. 2005). In sorghum, an association mapping approach has been used to map agronomic and quality traits such as grain quality (Sukumaran et al. 2012; Figueiredo et al. 2010), height and maturity (Upadhyaya et al. 2012; Brown et al. 2008), agro-climatic traits (Morris et al. 2013), biomass, grain, and saccharification yield traits (Yi-Hong Wang, 2012), days to heading, flowering time, culm length, number of tillers, number of panicles and panicle length (Shehzad et al. 2009). Associating of causal polymorphism with complex phenotypes can provide a better understanding of the mechanisms underlying developmental and biochemical constraints, thus enabling accelerated crop improvement.

In the present study, 300 genotypes from the sorghum association panel (SAP) characterized at 265,487 SNPs was used in association analysis. Our objective was to identify marker-trait associations for stalk rot disease resistance in sorghum.

Materials and methods

Genetic materials

Three hundred genotypes from the sorghum association panel, including 251 Sorghum Conversion Program (SCP) lines, and 49 breeding lines and their progenitors from the U.S. served as the genetic material for this study. The sorghum genotypes were planted in a randomized complete block design with two replications. The experiment was conducted in three environments; Manhattan 2011 (39°N, 96°W), Manhattan 2012 (39°N, 96°W) and Ottawa 2012 (38°N, 95°W), KS. Plots were 6 m long single rows spaced 0.75 m apart with 0.6m alley at the end of each plot. Three grams each of the entries were drilled into the plots after treatment with herbicide safener (Maxim 4FS, Apron XL, Concept III, and colorant). Three weeks after emergence the seedlings were thinned to approximately 40 plants per plot. Fertilizer phosphorus (di-ammonium phosphate, DAP) and nitrogen (urea) were applied at the rate of 34 kg P_2O_5 ha^{-1} and 90 kg N ha^{-1}, respectively, at Manhattan and 25 kg P_2O_5 ha^{-1} and 90 kg N ha^{-1}, respectively, at Ottawa. Pre-emergence weeds were controlled with a pre-plant application of 0.24 L ha^{-1} Dual plus 0.68 kg ha^{-1} Atrazine. Post emergence weeds were removed manually to ensure normal growth. Fields trials near Manhattan were conducted on Smolan Silty Clay Loam soils and trials in Ottawa were conducted on Harney Silt Loam soils.

Inoculum preparation and inoculation

Two fungal pathogens were selected for this study; *Fusarium thapsinum and Macrophomina phaseolina*. The selection of these two pathogens was based on previous reports that these two pathogens are the most virulent stalk rot causing pathogens of sorghum (Tesso et al. 2010). Pure fungal cultures were provided by Dr. Chris Little's lab at Kansas State University. Liquid inoculum suspensions from pure cultures of the *Fusarium thapsinum* pathogen were initiated in potato dextrose broth (DIFCO, Detroit, MI). The suspensions were initiated on a shaker at room temperature until conidia were produced and then strained through four layers of cheese cloth to separate the conidia from the mycelia mass. Concentration of the suspension was determined by counting the number of conidia under a microscope using a hemacytometer. The concentration was then adjusted to 5 x 10^4 conidia ml^{-1} by diluting using 10 m*M* (pH 7.2) Phosphate-buffered saline. The suspension was kept on ice until inoculation. An Idico filler-plug gun (Forestry suppliers, Inc., Jackson MS) equipped with a stainless steel needle similar to that described by (Toman and White 1993) was used to deliver approximately 1 ml of suspension into the pith of the sorghum plants. For *M. phaseolina*, the pathogen strain was sub-cultured into several fresh potato dextrose agar plates, into which sterile toothpicks were inserted and incubated at 30°C for two weeks.

Field inoculation

For *Macrophomina phaseolina,* at 14 d after flowering, three randomly tagged plants from each plot were inoculated with an infested toothpick inserted perpendicularly into each stalk about 10 cm above the soil line (internodal region) in holes made with a sterilized needle. For *Fusarium thapsinum*, inoculations were performed with an Idico filler-plug gun (Forestry Suppliers, Inc., Jackson, MS) equipped with a stainless steel needle similar to that described by Toman and White (1993). At 14 d after flowering, three randomly tagged plants from each plot were artificially inoculated with approximately 5×10^4 conidia in the pith of the stalk approximately 10 cm above the soil surface.

Disease evaluation and scoring

Twenty eight days after field inoculation, plants were cut at the base and split longitudinally to score disease severity. Disease scores were obtained by measuring the length of the visible lesion in the pith of the stalk as a measure of disease progression in the sorghum stalks.

Data was collected on the following traits:

1. Total lesion Length (cm) : length of the visible necrotic lesion in the pith of the stalk
2. Major lesion length (cm): length of the major necrotic lesion in the pith of the stalk
3. Relative lesion length: Ratio of lesion length to plant height
4. Plant height (cm): Distance from the soil surface to the top of the panicle
5. Flowering time (FT): Days from sowing to when 50% of plants in a plot have reached 50% anthesis

Genotypic data

The genotypic data was a community resource of 265,487 SNPs (Morris et al., 2013). To avoid potential false findings, we selected only 79,132 SNP markers that mapped to defined single locations in the sorghum genome and that had <20% missing data and MAF>0.05 were used in the association analysis.

Statistical analysis

Estimation of least-square means and repeatability:

To obtain inbred line means adjusted for environmental effects, a similar method used by (Zila et al. 2013) was implemented. The Manhattan and Ottawa experiments were analyzed individually first and then combined in a second multi-environment analysis. The mixed linear model for the Manhattan experiment across years included line as a fixed effect, flowering time as a fixed linear covariate, and year, line × year interaction and replication within year as random effects. In the combined experiment analysis, each combination of location and year was considered an environment. The combined analysis model included a fixed line effect, flowering time as a fixed covariate nested within environment, a random line × environment interaction effect, and nested random

experimental design effects (replication within environment). All analyses were weighted by the number of stalks scored within each plot and utilized a heterogeneous error variance structure. In both experiments, larger predicted stalk rot trait values were associated with larger residuals, so a natural logarithmic transformation of raw stalk rot scores (which largely eliminated the relationship between residual variance and predicted values) was used for all analyses. Least square means were estimated for inbred lines within each environment and across environments using ASReml version 3 (Gilmour et al. 2009). A second analysis was performed treating inbred lines as random effects for the purposes of estimating repeatability for stalk rot resistance traits in the diversity panel. The same models as above were used except lines were treated as random effects to obtain estimates of genetic variance.

Line mean-basis repeatability was estimated as

$$\widehat{H}_c = 1 - \frac{\sigma^2_{PPE}}{2\hat{\sigma}^2_G}$$

Where σ^2_{PPE} is the average prediction error variance for all pairwise comparisons of lines and $\hat{\sigma}^2_G$ the estimated genetic variance (Cullis et al. 2006). We estimated line mean-basis repeatability for each environment individually, across years for the Manhattan environments and for the combined data set across all environments. The model used to estimate line mean-basis repeatability in the combined data set was further modified by nesting the random line effect within environment and modeling the genotype-environment effect (G) matrix as unstructured, thereby allowing estimation of unique genetic variance within each environment and a unique genetic correlation between each pair of environments. The same models used to compute stalk rot resistance trait repeatability were used to estimate flowering time and plant height repeatability, but flowering time was treated as the dependent variable instead of as a fixed linear covariate.

Population structure and kinship analysis

Population structure was investigated in order to define suitable covariates for association genetic models. Population structure was analyzed using a Bayesian model

based clustering method implemented in the STRUCTURE software (Pritchard et al. 2000) to detect population structure and assign individuals to subpopulations. This analysis requires selectively neutral polymorphic unlinked markers. Unlinked markers were selected for this analysis using the PLINK software to generate a pruned subset of SNPs that are in approximate linkage equilibrium with each other. The final SNP dataset used to analyze the population structure thus contained 25,190 SNP markers from 10 LGs. An initial cluster 2 through 10 were tested with ten replications for each value of putative *k* clusters and the log probability of data was estimated using the admixture model with correlated allele frequency. The initial 10^5 steps were discarded as burn-in to allow the run parameters to attain convergence, after which data were collected for an additional 5 x 10^5 steps. Using stability of the rate of change of log likelihood across grouping patterns within the ten runs, the optimal (*k*) was observed between *k*=4 and 5. The optimal k was determined based on: 1, - likelihood plot of models; 2, - stability of grouping patterns across 20 runs; and 3, - germplasm information. Subsequently, we tested clusters 4 and 5 using an initial burn-in of 150,000 after which data was collected for an additional 350,000 steps with 20 replications for each cluster. Further, the membership coefficients for the most optimal number of clusters based on this analysis were permuted using the Greedy algorithm to match the various replicates for that value of *k* as closely as possible. This was done using the CLUMPP software (Jakobsson and Rosenberg 2007). Finally, the membership assignments of individuals in various clusters were visualized using a plotting function in the DISTRUCT software (Rosenberg 2004). Probabilities of subpopulation membership coefficients (*Qk*) were used for assigning lines to subpopulations. Lines with highest membership probability *Qk* less than 0.8 for all *k* were considered to result from admixture, hence, classified as "mixed". Kinship (K) was estimated using the software SPAGeDi 1.4 (Hardy and Vekemans 2002) using the method of (Ritland 1996).

Genotypic correlation analysis

We estimated genetic correlations among stalk rot traits using a multivariate model using the ASReml version 3 (Gilmour et al. 2009). Least square means estimated from the

combined analysis were used to estimate genotypic correlations between the diseases related traits.

Linkage disequilibrium analysis

Linkage disequilibrium analyses of the significantly associated SNPs were performed with HAPLOVIEW v.4.2 (Barrett et al. 2005).

Association analysis

The least square means for the inbred lines was used as the input phenotype. We conducted a GWAS with 79,132 genome wide SNPs using a univariate unified mixed linear model (Yu et al. 2006) that eliminated the need to recompute variance components. The optimum compression mixed linear model and P3D options (*i.e.*, population parameters previously determined), which increases computational speed and statistical power, were implemented by clustering individuals in groups (Zhang et al. 2010) in the Genome Association and Prediction Integrated Tool package (GAPIT) (Lipka et al. 2012). To control for population structure and familial relatedness, the mixed model included principal components (Price et al. 2006) and a kinship (coancestry) matrix (Ritland 1996). An R^2 statistic was used to assess the amount of phenotypic variation explained by the model. The (Benjamini and Hochberg 1995) procedure was used to control for the multiple testing problem at false-discovery rates (FDRs) of 5%.

In addition to the unified mixed linear model, a multi-locus mixed model (MLMM) (Segura et al. 2012) was used to dissect complex association signals that involved major effect loci. The MLMM employs stepwise mixed-model regression with forward inclusion and backward elimination, thus allowing for a more exhaustive exploration of the model space. In contrast to the unified mixed model with P3D, the MLMM re-estimates the genetic and error variance components at each step of the regression. Specifically, all SNPs on a chromosome (*i.e.*, chromosome-wide) with a major effect locus were considered for inclusion into the final model. The optimal model was selected using the extended Bayesian information criterion (Chen and Chen 2008). We then reanalyzed GWAS for each trait with MLMM-identified SNPs included as covariates in the unified mixed linear model for better control of major effect loci.

Allele frequency analysis

Lines were grouped into one of five major sorghum subpopulations (caudatum, durra, guinea, bicolor and kafir) based on the population structure analysis. Lines of mixed ancestry (the result of admixture among the subpopulations) were dropped from the analysis. Based on the results of the GWAS analyses, the frequencies of alleles that reduced disease severity at significant SNPs were estimated within each subpopulation using the FREQ procedure in R software version 3.0.2 (R CORE TEAM 2013). At each SNP locus, a Fisher's exact test in R software version 3.0.2 (R CORE TEAM 2013) was used to test the null hypothesis that frequency of the allele conferring increased disease resistance was the same across all four subpopulations.

Candidate genes

The genes located within or adjacent to associated SNPs were identified using the sorghum genome browser (Goodstein et al. 2012).

Results

Phenotypic variation

The extent of phenotypic variation for stalk rot resistance to two fungal pathogens; *F thapsinum* and *M phaseolina* were assessed with the use of a sorghum diversity panel consisting of 300 sorghum genotypes. Significant phenotypic variation for *Fusarium* and *Macrophomina* stalk rot resistance was observed in both the Manhattan and Ottawa experiments. For the Fusarium pathogen, the mean ranges for total and major lesion length observed among inbred lines of the association panel were higher in the Manhattan (1.33 - 34.67cm) compared with the Ottawa environment (1.67 -14.34 cm). Similar range for the mean relative total and major lesion length with an overall mean of 0.05 and 0.04 respectively were also observed at both environments (Table 3.1). Similar findings were also observed for the *Macrophomina* pathogen at both environments.

Environmental correlations

In the combined analysis, significant line × environment interactions were observed for all stalk rot disease related traits. Estimates of unique genotypic covariance from the mixed model analysis for each trait and for each pair of environments showed that the two Manhattan environments had a much stronger genotypic correlation for the eight stalk rot traits (Table 3.2) than did any other pair of environments. Thus, there was little genotype-by-environment (G×E) interaction between the two Manhattan environments compared with other environments. In contrast, pair-wise genotypic correlations were much lower between Manhattan and Ottawa environments for all traits (Table 3.2), causing much of the observed G×E interaction in the combined analysis. Therefore, we conducted separate association analyses on three different sets of phenotypic mean values for the eight stalk rot traits: (1) means from two Manhattan environments, (2) means from one Ottawa environments, and (3) means from the combined analysis of all three environments.

Genotypic correlations

Genetic correlations were estimated between the eight stalk rot traits including plant height (PH) and flowering time (FT) (Table 3.3). The traits: major lesion length *Macrophomina* (MLM), total lesion length *Macrophomina* (TLM), major lesion length *Fusarium* (MLF), and total lesion length *Fusarium* (TLF) were highly genetically correlated with each other($|\rho g| > 0.94$). Similarly, the traits: relative major lesion length *Macrophomina* (RMLM), relative total lesion length *Macrophomina* (RTLM), relative major lesion length *Fusarium* (RMLF), and relative total lesion length *Fusarium* (RTLF) had high genetic correlation between each other($|\rho g| > 0.82$). However, genetic correlations between RTLF and TLM, RTLF and MLF, RTLM and TLF were low compared to other genetic correlations. Generally, genetic correlations were lower between traits (MLM, TLM, MLF, and TLF) and (RMLM, RTLM, RMLF, and RTLF). For PH, genetic correlations were low for all stalk rot traits with a range of (-0.04 to 0.69). Negative genetic correlations were observed between the eight stalk rot traits and FT.

Assessment of population structure

Previous studies, (Sukumaran et al. 2012; Casa et al. 2008; Brown et al. 2011) have assigned lines to groups based on a probability of membership $P \geq 0.5$. Compared with previous studies, some lines were reassigned from the five well established groups [Bicolor (BIC) Caudatum (CAU), Durra (DUR), Guinea (GUI) and Kafir (KAF) to the admixed group (containing lines with the probability of membership in each of the five major germplasm groups <0.8). We were left with four major groups after the reassignment. The BIC group was reassigned to the admixed group. A large majority of the lines that were reassigned from one of the population groups to the mixed group in the current analysis had high probability of membership (P = 0.6-0.79) in their previously-assigned group (Table 3.4; Fig 3.13); i.e., close to the arbitrary threshold used for group classification.

Genome-wide analysis

We examined the genetic basis of natural variation for stalk rot resistance in sorghum by using an association panel of diverse inbred lines with a unified mixed linear model that controls for population structure and familial relatedness. A total of 3 SNPs were significant for at least one trait at a genome-wide bonferroni corrected threshold estimated based on effective number of independent tests (Table 3.5 and Figure 3.1-3.11a). Given the complex nature of inheritance of stalk rot resistance in sorghum and also given that most of the significant SNPs in the unified mixed model were located within a 2Mb region on chromosome 9 (Table 3.5), we used the MLMM of (Segura et al. 2012) on a chromosome-wide scale to further dissect the complex association signals which we observed for stalk rot resistance (Table 3.6 and Figure 3.1-3.11b).

Based on the MLMM we identified 14 major effect loci associated with stalk rot resistance from the combined analysis, MN all years and Ottawa analysis. These results indicate that the association panel provides sufficient statistical power to detect causative alleles. In the combined analysis, the optimal model contained one significant SNP for MLF (S9_57816733) (Figure 3.1b). This same SNP was associated with TLF (Figure 3.3b). The SNP S9_57222599 was significant from the optimal model for MLM and TLM (Figure 3.2b and 3.4b). These SNPs had the strongest significant association signals

in the unified mixed model as well (Table 3.5). The individual SNPs accounted for between 11-16% of the total phenotypic variation explained by the traits. The two MLMM-identified SNPs in the combined analysis were essentially in linkage equilibrium (r^2=0.45) with each other (Figure 3.12).

In the MN all years analysis, one significant SNP (S9_57272115) was included in the MLMM obtained optimal model for MLM, TLF, and TLM (Figure 3.5b, 3.8b and 3.9b). This SNP accounted for about 14% of the phenotypic variation observed for each trait. This SNP also had the strongest association signal for the 3 traits in the unified mixed model (Table 3.5). For RMLF, two SNPs (S2_64623580 and S7_55702883) were included in the optimal model obtained with the forward-backward stepwise approach of MLMM (Figure 3.6b). The two SNPs accounted for a 24% of the total phenotypic variation for RMLF. For RMLM, four SNPs (S2_27851064, S2_60129082, S7_58532122 and S9_57231207) were included in the optimal model obtained with the forward-backward stepwise approach of MLMM (Figure 3.7b). The two SNPs accounted for a 33% of the total phenotypic variation for RMLM.

In the Ottawa analysis, four SNPs (S3_8738404, S4_65815798, S8_51279094 and S9_56646280) were included in the MLMM obtained optimal model for RTLM (Figure 3.10b). These SNPs accounted for 34% of the total phenotypic variation observed for the trait. For TLM, one SNP (S8_39230116) was included in the optimal model obtained with the forward-backward stepwise approach of MLMM. The SNP accounted for a 13% of the total phenotypic variation for TLF (Figure 3.11b). Clearly, stalk rot resistance is subject to Genotype × Environment, which might be related to the severity of the stress. Such effects will be reflected in QTL × Environment interaction leading to SNPs being significant at one environment but not the other.

Linkage disequilibrium (LD)

Classic LD parameters (D' and r^2) as implemented by HAPLOVIEW v.4.2 (Barrett et al. 2005) were used to test whether the significant SNPs were in strong LD with each other (Figure 3.12). The presence of very strong LD would raise concerns about a high rate of false positives present in our results whilst complete absence of LD between the significant SNPs would provide evidence of complete independence between QTLs. We

only observed significant LD between SNPs S9_57222599 and S9_5722115 (r^2= 0.52), both located on chromosome 9 at 57222599 and 5722115 Mb, respectively, suggesting their effects are not independent. For the rest of the SNPs we did not observe signs of strong inter-QTL LD resulting from population substructure and admixture within our panel even for closely linked significant SNPs observed in the combined analysis such as S9_57222599 and S9_57816733 both mapping on chromosome 9 with (r^2= 0.20). With an average r^2 value of 0.06 for all 91 possible combinations between the 14 significantly associated SNPs, most observed r^2 values were <0.30. Only four, which were amongst the six possible combinations between SNPs S9_57222599, S9_57231207, S9_57272115, and S9_57816733, were greater than 0.3 and none exceeded 0.5 (Figure 3.12). Such r^2 values are not strong enough to suggest non-independence between QTL regions but they might add some evidence of co-selection, a reasonable situation when measuring traits of high agronomic/economic importance are subjected to a long history of breeding and selection.

Allele distribution at significant SNPs

Allele frequency at the 14 SNPs significantly associated with stalk rot resistance in four major sorghum sub-populations: CAU, DUR, GUI, and KAF were estimated (Table 3.7). In the combined analysis, the allele that reduced disease severity on the chromosome 9 SNP locus S9_57816733 was over-represented in the CAU and DUR groups compared to GUI and KAF ($P = 3.01 \times 10^{-4}$). The second SNP, S9_57222599 is represented in all four subpopulations but the allele frequencies are not high enough to be considered different between the subpopulations ($P = 0.13$). In the MN all year's analysis, the allele that reduced disease severity linked to SNP S9_57272115 had high frequencies in all the subpopulations. The allele on the second SNP S9_57231207 was only represented in the CAU and GUI sub-populations. On chromosome 7, the allele on the SNP S7_55702883 is significantly over-represented in the CAU compared to the other sub-populations ($P = 2.02 \times 10^{-16}$). The allele reducing disease severity linked to SNP S7_58532122 is significantly over-represented in the DUR sub-population ($P = 0.0013$). On chromosome 2 in the MN all years analysis, the allele on SNP S2_64623580 which reduced disease severity is significantly ($P = 1.41 \times 10^{-7}$) over represented in CAU and DUR compared to

GUI ad KAF sub-population. For the SNP S2_60129082, the allele reducing disease severity was significantly ($P = 4 \times 10^{-6}$) over-represented in the GUI and KAF compared to the CAU and DUR subpopulations. The allele reducing disease severity for SNP S2_27851064 was significantly ($P = 2 \times 10^{-16}$) over represented in the DUR and GUI compared to the CAU and KAF sub-populations. In the Ottawa analysis on chromosome 3, the allele reducing disease severity on SNP S3_8738404 was only present in CAU, DUR and KAF subpopulations. On chromosome 4 the allele reducing disease severity was significantly over-represented in the DUR subpopulation. On SNP S8_39230116, the allele reducing disease severity was only present in the DUR and significantly ($P = 5.14 \times 10^{-8}$) over represented. Also on chromosome 8, the allele on SNP S8_51279094 reducing disease severity was significantly ($P = 5.14 \times 10^{-8}$) over represented in the DUR and GUI. On chromosome 9, from the Ottawa analysis, the allele on SNP S9_57231207 reducing disease severity present only in the CAU and GUI and significantly ($P = 14 \times 10^{-8}$) over represented compared to the other two subpopulations (Table 3.7).

Candidate genes colocalized with associated SNPs

Using the sorghum genome browser (Goodstein et al. 2012), we identified the genes that contained SNPs that showed statistical significance with the stalk rot resistance. Two genes identified in the combined analysis (Table 3.6), have reported predicted gene functions related to immune response pathways including; a ring finger domain containing protein, tyrosine kinase protein, chalcone and stilbene synthases (Dao et al. 2011). In the MN all year analysis, genes with predicted functions included; an AP2 domain containing protein, a homobox domain containing protein, a protein kinase domain, an auxin-induced protein, a UDP-glucuronosyltransferase gene and a pentatricopeptide repeat-containing protein. In the Ottawa analysis, the SNP S3_8738404 is located in an intronic region of hAT family dimerisation domain with similarity to the zinc finger protein. SNP S4_65815798 is located in the intronic region of an acetylglucosaminyltransferase family gene (Table 3.6). SNP S8_51279094 is located in a sterol regulatory element-binding (SERP) domain containing protein.

Discussion

In the present study we describe the application of genome wide association mapping in a panel of diverse sorghum genotypes for resistance to two predominant pathogens causing sorghum stalk rot disease; *Macrophomina phaseolina* and *Fusarium thapsinum*. Sorghum germplasm used in this study represent an excellent reservoir of genetic variation for the application of GWAS. It is assumed that these genotypes possess a diversity of alleles. Such diversity can be examined and exploited for sorghum genetic improvement by identifying useful alleles for genetic improvement. GWAS offers one approach of achieving the objective.

Means and repeatability

GWAS are strongly influenced by the quality of the phenotypic data (Rafalski 2010). Plant maturity differences can confound with stalk rot response; we used FT as a fixed linear covariate to try to account for this. In the present study repeatability on a line means basis were moderate to high with a range of 0.62-0.97 for all traits indicating that, QTL are predominantly responsible for the broad phenotypic variation reflected in the panel. The efficiency of artificial selection could be enhanced through connecting phenotypic information to molecular markers associated with QTL alleles that enhance stalk rot resistance. One of the major constraints in breeding for stalk rot resistance is identifying a phenotype that underlies the genetics of stalk rot infection. Here we have used a novel additional phenotype "relative lesion length" which is a ratio of length of infection to plant height to account for the distribution of plant height in the panel which was assumed to be correlated with lesion length. The repeatability of these derived traits (RMLM, RMLF, RTLM and RTLF) on a line mean basis was lower than the corresponding (MLM, MLF, TLM and TLF) traits (Table 3.1). The possible reason for this is the lower variation for the derived traits in the panel having accounted for by plant height. This repeatability were obtained by modeling each line as a random sample from the reference population, modeled by a genotypic variance-covariance structure equal to the genotypic variance multiplied by an identity matrix.

Genotypic correlations

Strong genotypic correlations between resistance to the two fungal diseases; charcoal rot and Fusarium stalk were obtained, implying that the genetic mechanisms controlling these traits; (TLM, MLM, RTLM and RMLM for *Macrophomina*) and (TLM, MLM, RTLM and RMLM for *Fusarium*) were partially shared (Table 3.3). Previous reports have suggested that some genotypes with charcoal rot resistance could also be resistant to *Fusarium* stalk rot (Tesso et al. 2010; Bramelcox et al. 1988). The genotypic correlations between plant height and the derived traits RMLM, RMLF, RTLM and RTLF were low and not significant after accounting for plant height. In contrast, the traits (MLM, MLF, TLM and TLF) were moderately correlated with plant height indicating that the derived traits may be beneficial to account for the confounding nature of plant height on stalk rot resistance in a diverse panel.

Association analysis results

Two SNPs were significantly associated with stalk rot resistance in the combined analysis; S9_5816733 (TLM and MLM), S9_57222599 (TLF and MLF), and all localized to the same chromosome. None of these SNPs were identified in the MN al year and Ottawa analysis. However, the SNPs that were significantly associated with stalk rot resistance on chromosome 9 in the other analysis *vis* combined analysis (S9_5816733 and S9_57222599), MN all years (S9_57272115 and S9_57231207), and Ottawa (S9_56646280) were located within a 1 Mb region on chromosome 9 (which we would here call SNPs 1, 2, 3, 4, and 5 respectively). There appeared to be a relatively high LD between SNPs 2 and 1 (r^2 = 0.52) and between SNPs 2 and 3 (r^2 = 0.45). Thus it is possible that these SNPs are associated with the same underlying causal variation. However, SNPs 4 and 5 are in low LD with the other SNPs, suggesting that they are distinct from the causal polymorphisms with which the other SNPs are associated. The chromosome 9 SNPs colocalized with previously described plant height (57272115 Mb) locus dw1/SbHt9.1 (Brown et al. 2008). This locus is 29 kb away from a GA2 oxidase, a catabolic enzyme in the gibberellin pathway which is proposed as the gene underlying plant height QTL SbHt9.1/Dw1(Wang et al. 2012b). The derived traits RMLM and RTLM which had no genotypic correlation with plant height and accounted for plant

height in the diversity panel also mapped to this region. Therefore, the stalk rot resistance causal variations observed on chromosome 9 are not causal variations due to plant height. Furthermore, the SNPs identified with significant associations to stalk rot resistance are located within predicted genes with functions related to the immune response pathway. While we may not rule out a pleiotropic effect of the plant height locus on stalk rot resistance, this is a subject of further research. The fact that we identified precisely different QTLs with the same data set across environments indicates the diversity of the environments used in the present study. However, the chromosome 9 SNPs mapped to the same genomic region. Although we might expect such effects to be minor, we have sampled a limited number of environments yet highlighted the potential importance of QTL × Environment interactions. Future work should be directed towards suitably augmenting the genetic diversity of the panel and increasing the environmental diversity to which it is exposed in order to obtain a complete survey of stalk rot resistance genetics. The directions of the allelic effects were consistent for all the significant SNPs. Although, the SNPs explained a relatively large portion of the total variation in line means, each SNP had a relatively small additive effect, with a range of 0.84 - 1.50. In every case, an increase in disease resistance was associated with the rare allele at each locus. Alleles enhancing stalk rot resistance were overrepresented in the caudatum germplasm group relative to other groups for the combined analysis while they were overrepresented in the durra germplasm group relative to other groups for the MN all years, and Ottawa analysis. Durra sorghums are predominantly found in the warm semi-arid or warm desert climates of the Horn of Africa, Sahel, Arabian Peninsula, and west and central India (Morris et al. 2013). These results are congruent with the fact that stalk rot resistance is related to drought tolerance and staygreen under terminal moisture stress conditions. Several reports have related staygreen and drought tolerance to stalk rot resistance (Crasta et al. 1999; Rosenow et al. 1977; Rosenow et al. 1983).

Candidate genes

We used the BTx623 sorghum genome reference sequence to identify genes that either included or were nearby SNPs significantly associated with stalk rot resistance. One of the chromosome 9 genes (Sb09g029260) in the combined analysis belongs to the

Chalcone and stilbene synthase protein family. Chalcone synthase is a key enzyme of the flavonoid/isoflavonoid biosynthesis pathway. Besides being part of the plant developmental program the CHS gene expression is induced in plants under stress conditions such as UV light, bacterial or fungal infection. CHS expression causes accumulation of flavonoid and isoflavonoid phytoalexins and is involved in the salicylic acid defense pathway (Dao et al. 2011). The second gene (Sb09g028280.1) belongs to the family of ROP GTPase protein an important signaling molecule. ROP GTPase signaling regulates hormone responses and disease resistance (Tao et al. 2002; Ono et al. 2001; Kawasaki et al. 1999). In the MN all years analysis, the gene on chromosome 9 (Sobic.009G233100.1) belongs to the AP2 transcription factor family found only in plants that encodes proteins involved in regulation of disease resistance pathways (Gutterson and Reuber 2004). On chromosome 7, the gene Sb07g021660 encodes a protein kinases known to be involved in disease response, gene (Sb02g029630, Sb07g023700) are predicted genes of unknown function. Gene (Sb02g025370) belongs to a class of proteins coding for a PPR repeat (pentatricopeptide repeat-containing protein). PPR genes show features in common with disease resistance genes (Foxe and Wright 2009). In Arabidopsis, PPR proteins have been shown to function in defense against neurotropic fungi and abiotic stress tolerance (Laluk et al. 2011). On chromosome 9, the gene (Sb09g028320.1) belongs to the UDP-glucuronosyltransferase family of proteins. Altering glucosinolate profiles in plants have been shown to modulate disease resistance in plants (Brader et al. 2006).

In the Ottawa analysis, the SNPs on chromosome 8 (sobic.008G098200.1) and 9 (Sb09g027770) were located within genes of unknown function. The SNP on chromosome 3 is located downstream of a gene (Sb03g008300) coding for a BED zinc finger domain protein which are major components of R proteins (Aravind 2000; Tuskan et al. 2006). The SNP on chromosome 4 is located upstream of a gene (Sb04g036090) coding for acetylglucosaminyltransferase protein a major component of signal transduction pathways leading to systemic acquired resistance in plants. Infection of plants by necrotizing pathogens may lead to the induction of a complex set of defense responses resulting in a restriction of pathogen growth and spread. The infected leaves develop a hypersensitive response (HR), i.e., rapid, localized cell death occurring at the

sites of pathogen entry (Heath 2000). Concomitant with the HR is the accumulation of salicylic acid and several classes of pathogenesis-related (PR) proteins, many of which exhibiting antimicrobial activity (Ward et al. 1991). Subsequently, an enhancement of the plant defensive capacity against a broad spectrum of pathogens is observed. This resistance is expressed locally as well as in distal, uninfected tissues and can last for several weeks to months. It is known as Systemic Acquired Resistance or SAR (Klessig and Malamy 1994). The SNP on chromosome 8 is located downstream of a gene (Sb08g020320) coding for a sterol regulatory element-binding protein.

Conclusion

In conclusion, progress in understanding the genetic basis of stalk rot resistance in sorghum is often constrained by difficulty in reproducing results under both field environments and controlled experiments in addition to lack of high throughput phenotyping protocols. The current study is the first attempt to apply GWAS to stalk rot resistance. We have used the MLMM in a sorghum association panel as a powerful way to survey the allelic diversity to discover loci associated with natural variation in resistance to two stalk rot pathogens; *Macrophomina phaseolina* and *Fusarium thapsinum.* We identified fourteen associated loci and a set of candidate genes that appear to be involved in connected functions controlling plant defense response. This research may serve as a basis for resistant genes or QTL cloning to understand further the complicated molecular mechanism of resistance to stalk rots in sorghum against *Macrophomina phaseolina* and *Fusarium thapsinum.* To validate the significant association signals, further research is required. Further studies may focus on, deep association work (i.e., resequencing the rest of the gene from the panel), QTL mapping/fine-mapping, characterization of near-isogenic lines, gene knock-outs, complementation studies, larger sample sizes, association analysis in more diverse populations, and validation of findings in other sorghum lines and environments to identify other genetic factors contributing to phenotypic variations.

References

Abecasis GR, Cardon LR, Cookson WOC (2000) A general test of association for quantitative traits in nuclear families. American Journal of Human Genetics 66 (1):279-292. doi:10.1086/302698

Abiola O, Angel JM, Avner P, Bachmanov AA, Belknap JK, Bennett B, Blankenhorn EP, Blizard DA, Bolivar V, Brockmann GA, Buck KJ, Bureau JF, Casley WL, Chesler EJ, Cheverud JM, Churchill GA, Cook M, Crabbe JC, Crusio WE, Darvasi A, de Haan G, Demant P, Doerge RW, Elliott RW, Farber CR, Flaherty L, Flint J, Gershenfeld H, Gu J, Gu WK, Himmelbauer H, Hitzemann R, Hsu HC, Hunter K, Iraqi FA, Jansen RC, Johnson TE, Jones BC, Kempermann G, Lammert F, Lu L, Manly KF, Matthews DB, Medrano JF, Mehrabian M, Mittleman G, Mock BA, Mogil JS, Montagutelli X, Morahan G, Mountz JD, Nagase H, Nowakowski RS, O'Hara BR, Osadchuk AV, Paigen B, Palmer AA, Peirce JL, Pomp D, Rosemann M, Rosen GD, Schalkwyk LC, Seltzer Z, Settle S, Shimomura K, Shou SM, Sikela JM, Siracusa LD, Spearow JL, Teuscher C, Threadgill DW, Toth LA, Toye AA, Vadasz C, Van Zant G, Wakeland E, Williams RW, Zhang HG, Zou F, Complex Trait C (2003) The nature and identification of quantitative trait loci: a community's view. Nature Reviews Genetics 4 (11):911-916. doi:10.1038/nrg1206

Aravind L (2000) The BED finger, a novel DNA-binding domain in chromatin-boundary-element-binding proteins and transposases. Trends in Biochemical Sciences 25 (9):421-423. doi:10.1016/s0968-0004(00)01620-0

Barrett JC, Fry B, Maller J, Daly MJ (2005) Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 21 (2):263-265. doi:10.1093/bioinformatics/bth457

Beattie AD, Edney MJ, Scoles GJ, Rossnagel BC (2010) Association Mapping of Malting Quality Data from Western Canadian Two-row Barley Cooperative Trials. Crop Science 50 (5):1649-1663. doi:10.2135/cropsci2009.06.0334

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate - a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society Series B-Methodological 57 (1):289-300

Brader G, Mikkelsen MD, Halkier BA, Palva ET (2006) Altering glucosinolate profiles modulates disease resistance in plants. Plant Journal 46 (5):758-767. doi:10.1111/j.1365-313X.2006.02743.x

Bramel-cox PJ, Stein IS, Rodgers DM, Claflin LE (1988) Inheritance of Resistance to Macrophomina-phaseolina (Tassi) Goid And *Fusarium moniliforme* Sheldon In Sorghum. Crop Science 28 (1):37-40

Brown PJ, Myles S, Kresovich S (2011) Genetic Support for Phenotype-based Racial Classification in Sorghum. Crop Science 51 (1):224-230. doi:10.2135/cropsci2010.03.0179

Brown PJ, Rooney WL, Franks C, Kresovich S (2008) Efficient mapping of plant height quantitative trait loci in a sorghum association population with introgressed dwarfing genes. Genetics 180 (1):629-637. doi:10.1534/genetics.108.092239

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 Science 48 (1):30-40. doi:10.2135/cropsci2007.02.0080

Chen JH, Chen ZH (2008) Extended Bayesian information criteria for model selection with large model spaces. Biometrika 95 (3):759-771. doi:10.1093/biomet/asn034

Crasta OR, Xu WW, Rosenow DT, Mullet J, Nguyen HT (1999) Mapping of post-flowering drought resistance traits in grain sorghum: association between QTLs influencing premature senescence and maturity. Molecular and General Genetics 262 (3):579-588

Cullis BR, Smith AB, Coombes NE (2006) On the design of early generation variety trials with correlated data. Journal of Agricultural Biological and Environmental Statistics 11 (4):381-393. doi:10.1198/108571106x154443

Dao TTH, Linthorst HJM, Verpoorte R (2011) Chalcone synthase and its functions in plant resistance. Phytochemistry Reviews 10 (3):397-412. doi:10.1007/s11101-011-9211-7

Devlin B, Roeder K (1999) Genomic control for association studies. Biometrics 55 (4):997-1004. doi:10.1111/j.0006-341X.1999.00997.x

Figueiredo LFD, Sine B, Chantereau J, Mestres C, Fliedel G, Rami JF, Glaszmann JC, Deu M, Courtois B (2010) Variability of grain quality in sorghum: association with polymorphism in Sh2, Bt2, SssI, Ae1, Wx and O2. Theoretical and Applied Genetics 121 (6):1171-1185. doi:10.1007/s00122-010-1380-z

Foxe JP, Wright SI (2009) Signature of Diversifying Selection on Members of the Pentatricopeptide Repeat Protein Family in Arabidopsis lyrata. Genetics 183 (2):663-672. doi:10.1534/genetics.109.104778

Frowd JA, (1980) Sorghum stalk rots in West Africa. p. 322–324. *In* Proc. of the Int. Workshop of Sorghum Diseases, Hyderabad, India. 11–15 Dec. 1978. Texas A&M University, College Station, and ICRISAT, Patancheru, India.

Gao X, Becker LC, Becker DM, Starmer JD, Province MA (2010) Avoiding the high Bonferroni penalty in genome-wide association studies. Genetic Epidemiology 34 (1):100-105. doi:10.1002/gepi.20430

Gebrekidan B, Kebede Y (1979) Highlights of sorghum improvement in Ethiopia. Sorghum Newsl. 22:2-5.

Gilmour AR, Gogel BJ, Cullis BR, Thompson R (2009) *ASReml User Guide Release 3.0*. VSN 558 International Ltd, Hemel Hempstead, HP1 1ES, UK.

Goodstein DM, Shu SQ, Howson R, Neupane R, Hayes RD, Fazo J, Mitros T, Dirks W, Hellsten U, Putnam N, Rokhsar DS (2012) Phytozome: a comparative platform for green plant genomics. Nucleic Acids Research 40 (D1):D1178-D1186. doi:10.1093/nar/gkr944

Gupta PK, Rustgi S, Kulwal PL (2005) Linkage disequilibrium and association studies in higher plants: Present status and future prospects. Plant Molecular Biology 57 (4):461-485. doi:10.1007/s11103-005-0257-z

Gutterson N, Reuber TL (2004) Regulation of disease resistance pathways by AP2/ERF transcription factors. Current Opinion in Plant Biology 7 (4):465-471. doi:10.1016/j.pbi.2004.04.007

Hardy OJ, Vekemans X (2002) SPAGEDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. Molecular Ecology Notes 2 (4):618-620. doi:10.1046/j.1471-8286.2002.00305.x

Hulluka M, Esele JPE (1992) Sorghum diseases in East Africa. p. 21–24. In W.A.J. Milliano et al. (ed.) Sorghum and millet diseases: A second world review. ICRISAT, Patancheru, AP, India.

Heath MC (2000) Hypersensitive response-related death. Plant Molecular Biology 44 (3):321-334. doi:10.1023/a:1026592509060

Jakobsson M, Rosenberg NA (2007) CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics 23 (14):1801-1806. doi:10.1093/bioinformatics/btm233

Jardine DJ, Leslie JF (1992) Aggressiveness of Gibberella-Fujikuroi (*Fusarium-moniliforme*) Isolates to Grain-Sorghum under Greenhouse Conditions. Plant Disease 76 (9):897-900

Kawasaki T, Henmi K, Ono E, Hatakeyama S, Iwano M, Satoh H, Shimamoto K (1999) The small GTP-binding protein Rac is a regulator of cell death in plants. Proceedings of the National Academy of Sciences of the United States of America 96 (19):10922-10926. doi:10.1073/pnas.96.19.10922

Khangura R, Aberra M (2009) First Report of Charcoal Rot on Canola Caused by Macrophomina phaseolina in Western Australia. Plant Disease 93 (6):666-667. doi:10.1094/pdis-93-6-0666c

Khune NN, (1984) Stalk rot of sorghum caused by *Fusarium moniliforme*. Indian Phytopathology. 37:316–319.

Klessig DF, Malamy J (1994) The Salicylic-Acid Signal in Plants. Plant Molecular Biology 26 (5):1439-1458. doi:10.1007/bf00016484

Klittich CJR, Leslie JF, Nelson PE, Marasas WFO (1997) *Fusarium thapsinum* (*Gibberella thapsina*): A new species in section Liseola from sorghum. Mycologia 89 (4):643-652. doi:10.2307/3761002

Kump KL, Bradbury PJ, Wisser RJ, Buckler ES, Belcher AR, Oropeza-Rosas MA, Zwonitzer JC, Kresovich S, McMullen MD, Ware D, Balint-Kurti PJ, Holland JB (2011) Genome-wide association study of quantitative resistance to southern leaf blight in the maize nested association mapping population. Nature Genetics 43 (2):163-U120. doi:10.1038/ng.747

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 Physiology 156 (4):2053-2068. doi:10.1104/pp.111.177501

Leslie JF (1995) Gibberella-Fujikuroi - Available Populations and Variable Traits. Canadian Journal of Botany 73:S282-S291

Leslie JF, Pearson CAS, Nelson PE, Toussoun TA (1990) Fusarium Spp from Corn, Sorghum, and Soybean Fields in the Central and Eastern United-States. Phytopathology 80 (4):343-350. doi:10.1094/Phyto-80-343

Lipka AE, Tian F, Wang QS, Peiffer J, Li M, Bradbury PJ, Gore MA, Buckler ES, Zhang ZW (2012) GAPIT: genome association and prediction integrated tool. Bioinformatics 28 (18):2397-2399. doi:10.1093/bioinformatics/bts444

Mahmoud A, Budak H (2011) First Report of Charcoal Rot Caused by Macrophomina phaseolina in Sunflower in Turkey. Plant Disease 95 (2):223-223. doi:10.1094/pdis-09-10-0631

Marasas WFO, Rheeder JP, Lamprecht SC, Zeller KA, Leslie JF (2001) Fusarium andiyazi sp nov., a new species from sorghum. Mycologia 93 (6):1203-1210. doi:10.2307/3761681

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 (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 (2):453-458. doi:10.1073/pnas.1215985110

Omar M, (1985) Collaborative sorghum disease studies in Sudan: Sorghum Newsletter. 28:93.

Ono E, Wong HL, Kawasaki T, Hasegawa M, Kodama O, Shimamoto K (2001) Essential role of the small GTPase Rac in disease resistance of rice. Proceedings of the National Academy of Sciences of the United States of America 98 (2):759-764. doi:10.1073/pnas.021273498

Pecina-Quintero V, Williams-Alanis H, Vandemark GJ (1999) Diallel analysis of resistance to Macrophomina phaseolina in sorghum. Cereal Research Communications 27 (1-2):99-106

Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D (2006) Principal components analysis corrects for stratification in genome-wide association studies. Nature Genetics 38 (8):904-909. doi:10.1038/ng1847

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155 (2):945-959

R CORE TEAM, 2014 *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical 594 Computing, Vienna, Austria.

Rafalski JA (2010) Association genetics in crop improvement. Current Opinion in Plant Biology 13 (2):174-180. doi:10.1016/j.pbi.2009.12.004

Reed JE, Partridge JE, Nordquist PT (1983) Fungal Colonization of Stalks and Roots of Grain-Sorghum during the Growing-Season. Plant Disease 67 (4):417-420. doi:10.1094/pd-67-417

Ritland K (1996) Estimators for pairwise relatedness and individual inbreeding coefficients. Genetical Research 67 (2):175-185

Rosenberg NA (2004) DISTRUCT: a program for the graphical display of population structure. Molecular Ecology Notes 4 (1):137-138. doi:10.1046/j.1471-8286.2003.00566.x

Rosenow DT, Johnson JW, Frederiksen RA, Miller FR (1977) Relationship of nonsenescence to lodging and charcoal rot in sorghum. Agronomy Abstracts:69-69

Rosenow DT, Quisenberry JE, Wendt CW, Clark LE (1983) Drought Tolerant Sorghum and Cotton Germplasm. Agricultural Water Management 7 (1-3):207-222. doi:10.1016/0378-3774(83)90084-7

Roy JK, Smith KP, Muehlbauer GJ, Chao SM, Close TJ, Steffenson BJ (2010) Association mapping of spot blotch resistance in wild barley. Molecular Breeding 26 (2):243-256. doi:10.1007/s11032-010-9402-8

Sahib KH, Reddy BB, Ali SM (1990) Genetics of Charcoal Rot Resistance In Rabi Sorghum. Indian Journal of Genetics and Plant Breeding 50 (3):263-267

Segura V, Vilhjalmsson BJ, Platt A, Korte A, Seren U, Long Q, Nordborg M (2012) An efficient multi-locus mixed-model approach for genome-wide association studies in structured populations. Nature Genetics 44 (7):825-U144. doi:10.1038/ng.2314

Seetharama N (1987) Effect of pattern and severity of moisture deficit stress on stalk rot incidence in sorghum: I. Use of line source irrigation technique, and the effect of time of inoculation. Field Crops Research. 15:289–308.

Shehzad T, Iwata H, Okuno K (2009) Genome-wide association mapping of quantitative traits in sorghum (*Sorghum bicolor* (L.) Moench) by using multiple models. Breeding Science 59 (3):217-227

Stich B, Mohring J, Piepho HP, Heckenberger M, Buckler ES, Melchinger AE (2008) Comparison of mixed-model approaches for association mapping. Genetics 178 (3):1745-1754. doi:10.1534/genetics.107.079707

Sukumaran S, Xiang WW, Bean SR, Pedersen JF, Kresovich S, Tuinstra MR, Tesso TT, Hamblin MT, Yu JM (2012) Association Mapping for Grain Quality in a Diverse Sorghum Collection. Plant Genome 5 (3):126-135. doi:10.3835/plantgenome2012.07.0016

Tao LZ, Cheung AY, Wu HM (2002) Plant Rac-like GTPases are activated by auxin and mediate auxin-responsive gene expression. Plant Cell 14 (11):2745-2760. doi:10.1105/tpc.006320

Tesso TT, Ochanda N, Little CR, Claflin L, Tuinstra MR (2010) Analysis of host plant resistance to multiple Fusarium species associated with stalk rot disease in sorghum *Sorghum bicolor* (L.) Moench. Field Crops Research 118 (2):177-182. doi:10.1016/j.fcr.2010.05.010

Toman J, White DG (1993) Inheritance of resistance to anthracnose stalk rot of corn. Phytopathology 83 (9):981-986

Trimboli DS (1982) The fungi associated with stalk and root rot of grain sorghum in New South Wales. Sorghum Newsletter 25:105.

Tuskan GA, DiFazio S, Jansson S, Bohlmann J, Grigoriev I, Hellsten U, Putnam N, Ralph S, Rombauts S, Salamov A, Schein J, Sterck L, Aerts A, Bhalerao RR, Bhalerao RP, Blaudez D, Boerjan W, Brun A, Brunner A, Busov V, Campbell M, Carlson J, Chalot M, Chapman J, Chen GL, Cooper D, Coutinho PM, Couturier J, Covert S, Cronk Q, Cunningham R, Davis J, Degroeve S, Dejardin A, dePamphilis C, Detter J, Dirks B, Dubchak I, Duplessis S, Ehlting J, Ellis B, Gendler K, Goodstein D, Gribskov M, Grimwood J, Groover A, Gunter L, Hamberger B, Heinze B, Helariutta Y, Henrissat B, Holligan D, Holt R, Huang W, Islam-Faridi N, Jones S, Jones-Rhoades M, Jorgensen R, Joshi C, Kangasjarvi J, Karlsson J, Kelleher C, Kirkpatrick R, Kirst M, Kohler A, Kalluri U, Larimer F, Leebens-Mack J, Leple JC, Locascio P, Lou Y, Lucas S, Martin F, Montanini B, Napoli C, Nelson DR, Nelson C, Nieminen K, Nilsson O, Pereda V, Peter G,

Philippe R, Pilate G, Poliakov A, Razumovskaya J, Richardson P, Rinaldi C, Ritland K, Rouze P, Ryaboy D, Schmutz J, Schrader J, Segerman B, Shin H, Siddiqui A, Sterky F, Terry A, Tsai CJ, Uberbacher E, Unneberg P, Vahala J, Wall K, Wessler S, Yang G, Yin T, Douglas C, Marra M, Sandberg G, Van de Peer Y, Rokhsar D (2006) The genome of black cottonwood, *Populus trichocarpa* (Torr. & Gray). Science 313 (5793):1596-1604. doi:10.1126/science.1128691

Upadhyaya HD, Wang YH, Sharma S, Singh S (2012) Association mapping of height and maturity across five environments using the sorghum mini core collection. Genome 55 (6):471-479. doi:10.1139/g2012-034

Wang A-y, Li Y, Zhang C-q (2012a) QTL mapping for stay-green in maize (*Zea mays*). Canadian Journal of Plant Science 92 (2):249-256. doi:10.4141/cjps2011-108

Wang Y-H, Bible P, Loganantharaj R, Upadhyaya HD (2012b) Identification of SSR markers associated with height using pool-based genome-wide association mapping in sorghum. Molecular Breeding 30 (1):281-292. doi:10.1007/s11032-011-9617-3

Waniska RD, Venkatesha RT, Chandrashekar A, Krishnaveni S, Bejosano FP, Jeoung J, Jayaraj J, Muthukrishnan S, Liang GH (2001) Antifungal proteins and other mechanisms in the control of sorghum stalk rot and grain mold. Journal of Agricultural and Food Chemistry 49 (10):4732-4742. doi:10.1021/jf010007f

Ward ER, Uknes SJ, Williams SC, Dincher SS, Wiederhold DL, Alexander DC, Ahlgoy P, Metraux JP, Ryals JA (1991) Coordinate Gene Activity in Response to agents that Induce Systemic Acquired-Resistance. Plant Cell 3 (10):1085-1094

Yi-Hong W, 2012. Association Mapping of Sorghum Biomass, Grain, and Saccharification Yield Traits Using Simple Sequence Repeat Markers. Poster presentation. International plant and animal genome conference. January 12-14 2012 San Diego, Califonia.

Yu JM, Pressoir G, Briggs WH, Bi IV, Yamasaki M, Doebley JF, McMullen MD, Gaut BS, Nielsen DM, Holland JB, Kresovich S, Buckler ES (2006) A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. Nature Genetics 38 (2):203-208. doi:10.1038/ng1702

Zhang ZW, Ersoz E, Lai CQ, Todhunter RJ, Tiwari HK, Gore MA, Bradbury PJ, Yu JM, Arnett DK, Ordovas JM, Buckler ES (2010) Mixed linear model approach adapted for genome-wide association studies. Nature Genetics 42 (4):355-U118. doi:10.1038/ng.546

Zhu CS, Gore M, Buckler ES, Yu JM (2008) Status and Prospects of Association Mapping in Plants. Plant Genome 1 (1):5-20. doi:10.3835/plantgenome2008.02.0089

Zila CT, Samayoa LF, Santiago R, Butron A, Holland JB (2013) A genome-wide association study reveals genes associated with Fusarium ear rot resistance in a maize core diversity panel. G3-Genes Genomes Genetics 3 (11):2095-2104. doi:10.1534/g3.113.007328

Tables and Figures

Table 3.1. Means and repeatability estimates for stalk rot traits. Estimates are reported for each environment individually, across years within the Manhattan environments and combined across environments.

Environment	MN 2011		MN 2012		OT 2012		MN, all years		Combined	
Traits[a]	Mean[a]	$\widehat{H}_c$	Mean	$\widehat{H}_c$	Mean	$\widehat{H}_c$	Mean	$\widehat{H}_c$	Mean	$\widehat{H}_c$
MLF	4.22	0.72	5.40	0.67	3.74	0.82	4.77	0.96	4.43	0.75
MLM	3.79	0.81	4.38	0.68	3.85	0.93	4.07	0.94	4.00	0.80
TLF	5.24	0.70	5.89	0.65	4.09	0.79	5.56	0.95	5.06	0.69
TLM	4.61	0.73	4.63	0.70	4.19	0.93	4.62	0.93	4.49	0.78
RTLF	0.05	0.64	0.05	0.67	0.05	0.71	0.05	0.97	0.05	0.64
RTLM	0.04	0.66	0.04	0.65	0.05	0.89	0.04	0.96	0.04	0.71
RMLF	0.04	0.62	0.05	0.66	0.05	0.73	0.04	0.97	0.04	0.66
RMLM	0.04	0.69	0.04	0.62	0.05	0.87	0.04	0.97	0.04	0.73
PH	107.2	0.81	119.8	0.83	84.9	0.75	113.5	0.87	104	0.78
FT	63.3	0.88	63.4	0.47	70.1	0.90	63.3	0.68	65.4	0.53

[a]MLF ,major lesion length Fusarium; MLM, major lesion length Macrophomina; TLF, total lesion length Fusarium; TLM, total lesion length Macrophomina; RTLF, relative total lesion length Fusarium; RTLM, relative total lesion length Macrophomina; RMLF, relative major lesion length Fusarium; RMLM, relative major lesion length Macrophomina; FT, flowering time; PH, plant height

[b]Means are reported as the average of the line least square means calculated within and across environments.

Table 3.2. Genotypic covariance/variance/correlation matrix for stalk rots traits from the combined analysis of a sorghum diversity panel evaluated in three environments. The diagonal (bold) is an estimate of genetic variance ($\sigma\hat{G}^2$) plus the genotype by environment interaction ($\sigma\hat{G}E^2$) within each environment. Estimates of covariance between pairs of environments are shown below the diagonal, and genetic correlations between inbred lines in each pair of environments are shown above the diagonal.

	Environment				Environment		
Traits	MN 2011	MN 2012	OT 2012	Traits	MN 2011	MN 2012	OT 2012
	Major lesion length *Fusarium*				Major lesion length *Macrophomina*		
MN 2011	**0.14**	0.83	0.66		**0.17**	0.89	0.51
MN 2012	0.11	**0.12**	0.72		0.14	**0.15**	0.66
OT 2012	0.09	0.09	**0.12**		0.10	0.13	**0.24**
	Total lesion length *Fusarium*				Total lesion length *Macrophomina*		
MN 2011	**0.14**	0.75	0.48		**0.18**	0.89	0.57
MN 2012	0.10	**0.13**	0.66		0.16	**0.17**	0.68
OT 2012	0.07	0.09	**0.14**		0.13	0.15	**0.29**
	Relative total lesion length *Fusarium*				Relative total lesion length *Macrophomina*		
MN 2011	**0.11**	0.70	0.36		**0.12**	0.84	0.44
MN 2012	0.08	**0.13**	0.52		0.10	**0.12**	0.47
OT 2012	0.04	0.06	**0.11**		0.07	0.08	**0.22**
	Relative major lesion length *Fusarium*				Relative major lesion length *Macrophomina*		
MN 2011	**0.10**	0.77	0.56		**0.11**	0.85	0.35
MN 2012	0.08	**0.11**	0.56		0.09	**0.10**	0.44
OT 2012	0.05	0.05	**0.09**		0.05	0.06	**0.18**
	Plant height				Flowering time		
MN 2011	**587.70**	0.79	0.88		**0.33**	0.76	0.82
MN 2012	613.80	**1029.00**	0.99		0.20	**0.21**	0.76
OT 2012	318.00	473.10	**223.30**		0.30	0.22	**0.39**

Table 3.3. Genotypic correlations from the combined analysis of a sorghum diversity panel evaluated in three environments. The diagonal (bold) is an estimate of genetic variance ($\hat{\sigma}G^2$) for each trait. Estimates of covariance between pairs of traits) are shown below the diagonal, and genetic correlations between traits are shown above the diagonal.

Traits[a]	MLF	MLM	TLF	TLM	RTLF	RTLM	RMLF	RMLM	PH	FT
MLF	**0.09**	0.94	0.99	0.9	0.74	0.8	0.78	0.85	0.59	-0.03
MLM	0.1	**0.13**	0.97	0.99	0.63	0.84	0.62	0.85	0.69	-0.22
TLF	0.09	0.1	**0.09**	0.94	0.06	0.86	0.79	0.9	0.57	-0.1
TLM	0.11	0.14	0.11	**0.16**	0.07	0.1	0.6	0.86	0.65	-0.31
RTLF	0.06	0.06	0.06	0.06	**0.06**	0.86	0.99	0.93	-0.09	-0.14
RTLM	0.08	0.09	0.08	0.11	0.07	**0.1**	0.82	0.98	0.22	-0.41
RMLF	0.06	0.06	0.06	0.06	0.06	0.06	**0.06**	0.89	-0.04	-0.06
RMLM	0.07	0.08	0.07	0.09	0.06	0.08	0.06	**0.07**	0.21	-0.31
PH	3.92	5.36	3.65	5.58	-0.47	1.45	-0.19	1.22	**467.4**	0.01
FT	-0.05	-0.36	-0.13	-0.57	-0.16	-0.58	-0.07	-0.38	0.99	**21.04**

[a] MLF ,major lesion length Fusarium; MLM, major lesion length Macrophomina; TLF, total lesion length Fusarium; TLM, total lesion length Macrophomina; RTLF, relative total lesion length Fusarium; RTLM, relative total lesion length Macrophomina; RMLF, relative major lesion length Fusarium; RMLM, relative major lesion length Macrophomina; FT, flowering time; PH, plant height.

Table 3.4. List of sorghum lines in population structure analysis (based on 25,000 SNPs) showing the subpopulations they are assigned to, and their probability values of membership.

Taxa	New assignment	Traditional classification	CAU	BIC	KAF	DUR	GUI	Taxa	New assignment	Traditional classification	CAU	BIC	KAF	DUR	GUI
PI152651	MIXED	cultivar	0.22	0.42	0.18	0.04	0.13	PI576366	MIXED	durra-bicolor	0.23	0.32	0.13	0.32	0.01
PI34911	CAUDATUM	kafir	0.85	0.15	0.00	0.00	0.00	PI576373	MIXED	caudatum-bicolor	0.16	0.26	0.48	0.08	0.03
PI533750	DURRA	durra-bicolor	0.00	0.00	0.00	0.94	0.06	PI576375	DURRA	durra-bicolor	0.00	0.00	0.06	0.85	0.09
PI533752	CAUDATUM	caudatum	0.82	0.07	0.11	0.00	0.00	PI576376	DURRA	durra-bicolor	0.00	0.02	0.00	0.97	0.00
PI533754	MIXED	bicolor	0.03	0.17	0.00	0.53	0.26	PI576380	CAUDATUM	caudatum	0.98	0.00	0.00	0.00	0.02
PI533755	MIXED	caudatum	0.22	0.58	0.00	0.10	0.10	PI576381	DURRA	durra-bicolor	0.00	0.01	0.02	0.98	0.00
PI533757	CAUDATUM	kafir-caudatum	1.00	0.00	0.00	0.00	0.00	PI576385	MIXED	kafir	0.00	0.00	0.52	0.00	0.48
PI533758	CAUDATUM	caudatum	0.99	0.00	0.01	0.00	0.00	PI576386	CAUDATUM	other	0.85	0.02	0.02	0.07	0.04
PI533759	CAUDATUM	caudatum	0.88	0.10	0.00	0.00	0.02	PI576387	CAUDATUM	other	0.91	0.05	0.00	0.03	0.00
PI533761	MIXED	durra	0.55	0.36	0.00	0.01	0.08	PI576390	MIXED	durra	0.00	0.18	0.11	0.62	0.09
PI533762	MIXED	durra-caudatum	0.48	0.36	0.02	0.03	0.12	PI576391	DURRA	bicolor	0.00	0.20	0.01	0.80	0.00
PI533766	GUINEA	guinea	0.00	0.02	0.00	0.00	0.98	PI576393	MIXED	kafir	0.50	0.15	0.12	0.03	0.20
PI533769	MIXED	kafir-caudatum	0.72	0.18	0.00	0.04	0.06	PI576394	KAFIR	caudatum	0.00	0.01	0.96	0.01	0.02
PI533776	GUINEA	caudatum	0.00	0.00	0.00	0.00	1.00	PI576399	MIXED	caudatum	0.26	0.21	0.20	0.00	0.32
PI533785	GUINEA	guinea	0.00	0.00	0.00	0.00	1.00	PI576401	DURRA	durra	0.00	0.19	0.00	0.79	0.02
PI533788	MIXED	durra-caudatum	0.34	0.30	0.00	0.35	0.02	PI576418	GUINEA	guinea	0.00	0.00	0.00	0.00	1.00
PI533789	MIXED	caudatum	0.48	0.39	0.00	0.05	0.08	PI576422	KAFIR	kafir	0.00	0.00	1.00	0.00	0.00
PI533792	CAUDATUM	caudatum	1.00	0.00	0.00	0.00	0.00	PI576425	DURRA	durra	0.00	0.02	0.00	0.96	0.01
PI533794	CAUDATUM	caudatum	1.00	0.00	0.00	0.00	0.00	PI576426	DURRA	durra-bicolor	0.00	0.02	0.04	0.94	0.00
PI533799	CAUDATUM	caudatum	1.00	0.00	0.00	0.00	0.00	PI576428	CAUDATUM	caudatum	0.94	0.00	0.06	0.00	0.00
PI533800	CAUDATUM	caudatum	0.84	0.09	0.00	0.07	0.00	PI576435	MIXED	kafir-bicolor	0.66	0.16	0.11	0.03	0.04
PI533807	MIXED	kafir-caudatum	0.77	0.12	0.00	0.00	0.11	PI576437	MIXED	other	0.34	0.23	0.11	0.13	0.20
PI533810	DURRA	durra	0.00	0.15	0.00	0.85	0.00	PI595699	DURRA	caudatum	0.00	0.14	0.03	0.83	0.00
PI533814	DURRA	durra	0.00	0.15	0.00	0.83	0.02	PI595702	KAFIR	kafir-caudatum	0.00	0.01	0.99	0.00	0.00
PI533821	MIXED	caudatum	0.18	0.29	0.29	0.00	0.24	PI595714	CAUDATUM	caudatum	0.80	0.14	0.00	0.00	0.05
PI533822	MIXED	kafir-caudatum	0.76	0.10	0.06	0.00	0.08	PI595718	MIXED	kafir-caudatum	0.58	0.30	0.01	0.08	0.02
PI533824	MIXED	durra-caudatum	0.10	0.23	0.05	0.42	0.20	PI595720	DURRA	durra-bicolor	0.00	0.00	0.00	1.00	0.00
PI533831	KAFIR	kafir-caudatum	0.00	0.00	1.00	0.00	0.00	PI595739	CAUDATUM	caudatum	0.95	0.00	0.02	0.00	0.03

PI533833	CAUDATUM	caudatum-bicolor	0.88	0.10	0.02	0.00	0.00	PI595740	MIXED	caudatum	0.40	0.11	0.40	0.05	0.04
PI533838	MIXED	caudatum	0.42	0.08	0.13	0.05	0.32	PI595741	MIXED	durra	0.00	0.09	0.37	0.00	0.53
PI533841	MIXED	guinea	0.23	0.06	0.04	0.00	0.67	PI595743	MIXED	guinea-caudatum	0.10	0.00	0.50	0.12	0.28
PI533842	DURRA	durra	0.00	0.15	0.00	0.85	0.00	PI595744	MIXED	kafir-caudatum	0.20	0.36	0.27	0.01	0.15
PI533843	MIXED	guinea	0.12	0.22	0.31	0.23	0.11	PI595745	MIXED	guinea-caudatum	0.75	0.11	0.07	0.06	0.00
PI533845	MIXED	guinea	0.11	0.22	0.48	0.05	0.14	PI597945	MIXED	durra	0.02	0.27	0.04	0.54	0.13
PI533852	DURRA	durra	0.00	0.16	0.08	0.75	0.00	PI597946	MIXED	guinea-bicolor	0.51	0.08	0.39	0.03	0.00
PI533855	MIXED	guinea-bicolor	0.06	0.21	0.46	0.15	0.12	PI597950	MIXED	kafir-bicolor	0.13	0.24	0.31	0.21	0.11
PI533856	DURRA	durra	0.00	0.15	0.00	0.85	0.00	PI597951	GUINEA	guinea	0.00	0.01	0.08	0.00	0.91
PI533866	MIXED	caudatum-bicolor	0.15	0.21	0.02	0.14	0.48	PI597952	MIXED	caudatum	0.61	0.15	0.17	0.04	0.04
PI533869	MIXED	guinea	0.10	0.21	0.49	0.00	0.20	PI597957	MIXED	durra-bicolor	0.00	0.00	0.08	0.83	0.09
PI533871	GUINEA	caudatum	0.10	0.04	0.03	0.00	0.83	PI597960	GUINEA	caudatum-bicolor	0.07	0.04	0.03	0.00	0.86
PI533876	MIXED	caudatum	0.41	0.26	0.10	0.06	0.17	PI597961	CAUDATUM	caudatum	0.89	0.01	0.10	0.00	0.00
PI533877	GUINEA	caudatum	0.00	0.00	0.00	0.00	1.00	PI597964	CAUDATUM	caudatum	0.99	0.00	0.00	0.00	0.01
PI533878	GUINEA	caudatum	0.00	0.00	0.05	0.00	0.95	PI597965	CAUDATUM	caudatum	0.93	0.07	0.00	0.00	0.00
PI533901	MIXED	caudatum	0.45	0.30	0.11	0.03	0.12	PI597966	CAUDATUM	caudatum	0.87	0.08	0.05	0.00	0.00
PI533902	MIXED	durra-bicolor	0.02	0.28	0.05	0.47	0.18	PI597967	CAUDATUM	caudatum	1.00	0.00	0.00	0.00	0.00
PI533910	MIXED	caudatum	0.53	0.26	0.17	0.03	0.01	PI597971	MIXED	caudatum	0.50	0.26	0.11	0.07	0.06
PI533911	MIXED	caudatum	0.55	0.22	0.01	0.06	0.16	PI597972	MIXED	durra-caudatum	0.34	0.25	0.02	0.27	0.11
PI533912	MIXED	caudatum	0.46	0.34	0.17	0.00	0.03	PI597973	MIXED	durra-bicolor	0.12	0.26	0.00	0.43	0.19
PI533913	MIXED	guinea-caudatum	0.43	0.14	0.19	0.03	0.21	PI597976	GUINEA	guinea	0.00	0.01	0.00	0.00	0.99
PI533919	MIXED	durra-bicolor	0.00	0.11	0.00	0.75	0.14	PI597980	MIXED	caudatum	0.73	0.11	0.00	0.00	0.17
PI533927	MIXED	bicolor	0.12	0.27	0.33	0.08	0.20	PI597982	MIXED	caudatum	0.52	0.36	0.07	0.01	0.05
PI533937	KAFIR	kafir	0.00	0.00	1.00	0.00	0.00	PI607931	MIXED	cultivar	0.52	0.07	0.41	0.00	0.00
PI533938	MIXED	caudatum	0.10	0.13	0.42	0.15	0.21	PI613536	MIXED	caudatum-bicolor	0.27	0.21	0.00	0.38	0.14
PI533939	MIXED	durra	0.28	0.29	0.19	0.07	0.17	PI629034	MIXED	breedingline	0.55	0.09	0.00	0.00	0.36
PI533940	MIXED	bicolor	0.00	0.03	0.79	0.14	0.04	PI629040	MIXED	inbredline	0.27	0.09	0.08	0.33	0.24
PI533943	MIXED	durra-bicolor	0.00	0.13	0.30	0.55	0.02	PI641874	MIXED	NA	0.00	0.01	0.35	0.64	0.00
PI533948	KAFIR	guinea-kafir	0.00	0.00	1.00	0.00	0.00	PI655977	CAUDATUM	breedingline	0.85	0.08	0.00	0.07	0.00
PI533955	KAFIR	kafir-caudatum	0.00	0.00	1.00	0.00	0.00	PI655978	MIXED	breedingline	0.00	0.15	0.15	0.06	0.64
PI533956	MIXED	durra-bicolor	0.09	0.12	0.38	0.14	0.28	PI655979	MIXED	inbredline	0.24	0.20	0.04	0.09	0.44
PI533957	MIXED	caudatum	0.19	0.17	0.22	0.12	0.30	PI655986	MIXED	breedingline	0.01	0.11	0.47	0.39	0.03
PI533961	CAUDATUM	caudatum	0.87	0.08	0.05	0.00	0.00	PI655987	KAFIR	NA	0.00	0.00	0.92	0.08	0.00
PI533962	CAUDATUM	caudatum	0.99	0.01	0.00	0.00	0.00	PI655989	MIXED	breedingline	0.00	0.04	0.80	0.14	0.02

PI533964	CAUDATUM	caudatum	1.00	0.00	0.00	0.00	0.00	PI655990	MIXED	kafir	0.01	0.13	0.16	0.66	0.04
PI533965	CAUDATUM	caudatum-bicolor	0.79	0.13	0.00	0.00	0.08	PI655991	KAFIR	kafir	0.00	0.00	1.00	0.00	0.00
PI533967	CAUDATUM	caudatum	0.94	0.06	0.00	0.00	0.00	PI655992	KAFIR	kafir	0.00	0.00	1.00	0.00	0.00
PI533970	CAUDATUM	caudatum	0.90	0.08	0.02	0.00	0.00	PI655993	KAFIR	kafir	0.00	0.00	0.85	0.15	0.00
PI533972	CAUDATUM	caudatum	1.00	0.00	0.00	0.00	0.00	PI655996	MIXED	breedingline	0.14	0.18	0.00	0.00	0.67
PI533976	CAUDATUM	caudatum	0.84	0.06	0.09	0.00	0.00	PI655998	MIXED	NA	0.00	0.00	0.33	0.00	0.67
PI533979	KAFIR	kafir	0.00	0.00	1.00	0.00	0.00	PI656001	MIXED	breedingline	0.64	0.10	0.13	0.14	0.00
PI533985	CAUDATUM	caudatum	0.90	0.10	0.00	0.00	0.00	PI656010	GUINEA	NA	0.00	0.14	0.00	0.00	0.86
PI533986	CAUDATUM	caudatum	0.67	0.29	0.00	0.01	0.02	PI656015	MIXED	bicolor	0.23	0.58	0.00	0.08	0.10
PI533987	MIXED	caudatum	0.59	0.32	0.00	0.02	0.07	PI656018	KAFIR	breedingline	0.00	0.00	0.94	0.06	0.00
PI533989	CAUDATUM	durra	1.00	0.00	0.00	0.00	0.00	PI656019	MIXED	kafir	0.00	0.00	0.69	0.23	0.08
PI533991	MIXED	guinea-caudatum	0.79	0.00	0.15	0.06	0.00	PI656022	KAFIR	breedingline	0.01	0.01	0.99	0.00	0.00
PI533996	MIXED	durra-caudatum	0.41	0.33	0.05	0.09	0.12	PI656023	KAFIR	kafir	0.00	0.00	1.00	0.00	0.00
PI533997	MIXED	guinea	0.08	0.19	0.40	0.07	0.26	PI656025	DURRA	NA	0.00	0.30	0.00	0.66	0.04
PI533998	KAFIR	guinea	0.00	0.04	0.86	0.07	0.04	PI656027	MIXED	cultivar	0.76	0.09	0.01	0.13	0.02
PI534009	MIXED	durra	0.00	0.18	0.06	0.63	0.13	PI656029	DURRA	durra	0.00	0.00	0.11	0.89	0.00
PI534021	DURRA	durra	0.00	0.15	0.00	0.85	0.00	PI656034	CAUDATUM	cultivar	1.00	0.00	0.00	0.00	0.00
PI534028	DURRA	durra	0.00	0.13	0.10	0.77	0.00	PI656035	CAUDATUM	NA	0.73	0.23	0.01	0.00	0.04
PI534037	MIXED	guinea-caudatum	0.05	0.11	0.29	0.04	0.52	PI656048	MIXED	cultivar	0.73	0.00	0.27	0.00	0.00
PI534053	MIXED	caudatum	0.39	0.40	0.08	0.10	0.04	PI656051	CAUDATUM	cultivar	0.91	0.00	0.00	0.00	0.09
PI534054	MIXED	kafir-caudatum	0.06	0.13	0.69	0.00	0.12	PI656058	MIXED	kafir	0.37	0.04	0.58	0.00	0.00
PI534063	GUINEA	guinea-caudatum	0.00	0.00	0.00	0.00	1.00	PI656063	MIXED	NA	0.23	0.00	0.57	0.19	0.00
PI534070	GUINEA	guinea	0.00	0.00	0.00	0.00	1.00	PI656071	CAUDATUM	caudatum	0.78	0.15	0.00	0.04	0.02
PI534075	GUINEA	caudatum	0.00	0.00	0.00	0.00	1.00	PI656072	DURRA	durra	0.00	0.00	0.00	1.00	0.00
PI534079	MIXED	caudatum-bicolor	0.52	0.07	0.00	0.04	0.38	PI656074	MIXED	guinea-caudatum	0.24	0.46	0.07	0.05	0.18
PI534096	MIXED	guinea	0.07	0.27	0.15	0.10	0.41	PI656075	CAUDATUM	guinea	0.91	0.00	0.09	0.00	0.00
PI534097	KAFIR	kafir	0.00	0.02	0.89	0.09	0.00	PI656076	CAUDATUM	caudatum	0.97	0.00	0.03	0.00	0.00
PI534099	CAUDATUM	caudatum	0.90	0.10	0.00	0.00	0.00	PI656077	MIXED	durra-bicolor	0.05	0.12	0.00	0.72	0.11
PI534101	MIXED	caudatum	0.72	0.13	0.09	0.01	0.04	PI656078	MIXED	kafir-durra	0.07	0.28	0.13	0.10	0.42
PI534104	MIXED	kafir-caudatum	0.53	0.38	0.00	0.04	0.05	PI656080	MIXED	guinea	0.09	0.18	0.55	0.00	0.17
PI534105	CAUDATUM	kafir-caudatum	0.89	0.06	0.05	0.00	0.00	PI656081	MIXED	guinea	0.07	0.31	0.18	0.09	0.35
PI534108	CAUDATUM	kafir-caudatum	0.89	0.00	0.11	0.00	0.00	PI656082	DURRA	bicolor	0.00	0.10	0.00	0.86	0.04
PI534112	CAUDATUM	other	0.98	0.00	0.02	0.00	0.00	PI656083	MIXED	caudatum	0.48	0.41	0.01	0.00	0.10
PI534114	CAUDATUM	caudatum	0.92	0.00	0.08	0.00	0.00	PI656085	CAUDATUM	NA	0.89	0.00	0.11	0.00	0.00

PI534116	MIXED	durra-bicolor	0.06	0.17	0.22	0.39	0.16	PI656086	MIXED	durra-bicolor	0.38	0.21	0.00	0.30	0.11
PI534117	MIXED	bicolor	0.07	0.26	0.33	0.03	0.31	PI656088	DURRA	other	0.00	0.04	0.00	0.96	0.00
PI534123	MIXED	durra-bicolor	0.09	0.29	0.07	0.35	0.21	PI656089	DURRA	durra	0.00	0.05	0.00	0.95	0.00
PI534124	MIXED	guinea-bicolor	0.07	0.29	0.06	0.33	0.24	PI656090	MIXED	guinea-caudatum	0.40	0.29	0.13	0.08	0.10
PI534127	DURRA	kafir-bicolor	0.00	0.08	0.02	0.84	0.06	PI656091	DURRA	durra	0.00	0.00	0.00	1.00	0.00
PI534128	DURRA	durra	0.00	0.00	0.04	0.96	0.00	PI656092	DURRA	durra	0.00	0.00	0.05	0.95	0.00
PI534132	DURRA	durra	0.00	0.00	0.07	0.93	0.00	PI656093	GUINEA	guinea	0.00	0.01	0.08	0.06	0.85
PI534133	DURRA	durra	0.00	0.00	0.00	1.00	0.00	PI656094	GUINEA	guinea	0.00	0.01	0.00	0.00	0.99
PI534135	DURRA	durra	0.00	0.00	0.00	1.00	0.00	PI656095	GUINEA	caudatum	0.00	0.07	0.00	0.00	0.93
PI534137	MIXED	caudatum	0.36	0.30	0.05	0.24	0.05	PI656096	GUINEA	caudatum	0.00	0.00	0.00	0.00	1.00
PI534138	MIXED	caudatum	0.24	0.40	0.12	0.01	0.23	PI656097	DURRA	durra	0.00	0.15	0.00	0.85	0.00
PI534139	MIXED	guinea-caudatum	0.06	0.24	0.35	0.11	0.24	PI656099	DURRA	durra	0.00	0.15	0.00	0.85	0.00
PI534144	MIXED	durra-caudatum	0.15	0.35	0.14	0.02	0.34	PI656100	DURRA	durra	0.00	0.15	0.00	0.85	0.00
PI534145	GUINEA	other	0.00	0.02	0.00	0.00	0.98	PI656101	MIXED	guinea	0.28	0.57	0.00	0.06	0.09
PI534148	DURRA	durra-bicolor	0.00	0.03	0.00	0.97	0.00	PI656102	MIXED	caudatum-bicolor	0.29	0.20	0.26	0.00	0.25
PI534155	MIXED	durra-bicolor	0.01	0.10	0.02	0.78	0.08	PI656103	MIXED	bicolor	0.00	0.22	0.00	0.66	0.12
PI534157	CAUDATUM	caudatum	0.97	0.00	0.00	0.00	0.03	PI656104	MIXED	bicolor	0.00	0.15	0.24	0.62	0.00
PI534163	MIXED	caudatum	0.67	0.02	0.31	0.00	0.00	PI656105	MIXED	kafir-caudatum	0.69	0.00	0.28	0.03	0.00
PI534167	MIXED	durra-bicolor	0.00	0.07	0.11	0.72	0.10	PI656106	CAUDATUM	caudatum	0.87	0.00	0.13	0.00	0.00
PI542718	DURRA	NA	0.00	0.30	0.00	0.66	0.04	PI656107	MIXED	caudatum	0.47	0.39	0.00	0.11	0.02
PI561071	MIXED	inbredline	0.20	0.19	0.11	0.17	0.33	PI656108	DURRA	durra	0.00	0.17	0.05	0.78	0.00
PI561472	CAUDATUM	cultivar	0.92	0.04	0.04	0.00	0.00	PI656110	MIXED	durra-bicolor	0.00	0.21	0.01	0.61	0.17
PI576332	KAFIR	bicolor	0.01	0.08	0.86	0.05	0.01	PI656111	MIXED	kafir-durra	0.18	0.16	0.44	0.00	0.23
PI576333	KAFIR	guinea-kafir	0.00	0.00	1.00	0.00	0.00	PI656112	MIXED	guinea-kafir	0.01	0.09	0.22	0.57	0.11
PI576339	KAFIR	kafir-caudatum	0.00	0.00	1.00	0.00	0.00	PI656113	GUINEA	durra	0.00	0.00	0.00	0.00	1.00
PI576340	KAFIR	kafir-caudatum	0.00	0.00	1.00	0.00	0.00	PI656114	MIXED	durra-bicolor	0.21	0.01	0.19	0.43	0.16
PI576345	KAFIR	caudatum	0.00	0.06	0.92	0.00	0.03	PI656115	MIXED	guinea	0.07	0.27	0.19	0.08	0.38
PI576347	MIXED	bicolor	0.19	0.30	0.20	0.17	0.14	PI656117	CAUDATUM	caudatum	1.00	0.00	0.00	0.00	0.00
PI576348	MIXED	bicolor	0.03	0.30	0.22	0.26	0.19	PI656118	CAUDATUM	NA	0.89	0.08	0.00	0.00	0.03
PI576349	MIXED	bicolor	0.05	0.32	0.18	0.29	0.16	PI656119	GUINEA	caudatum	0.00	0.00	0.15	0.00	0.85
PI576350	MIXED	kafir-caudatum	0.08	0.23	0.35	0.16	0.18	PI656120	MIXED	caudatum	0.74	0.00	0.22	0.04	0.00
PI576352	KAFIR	kafir	0.00	0.00	1.00	0.00	0.00	PI656121	MIXED	NA	0.44	0.11	0.05	0.08	0.32
PI576364	KAFIR	caudatum	0.00	0.04	0.94	0.02	0.00								

CAU, caudatum; BIC, bicolor; DUR, durra; GUI, guinea; KAF, kafir.

Table 3.5. Chromosome locations, and other summary statistics for SNPs significantly associated stalk rot resistance in the combined, MN all years and Ottawa analysis from the unified mixed model.

Chromosome	SNP Physical Position, bp	P-Value	MAF[a]	Trait[b]
Combined analysis				
9	57816733	2.87E-06	0.28	MLF
9	57272115	4.18E-06	0.25	MLF
6	60030948	5.65E-06	0.10	MLF
9	57222599	1.48E-07	0.28	MLM
9	57476134	2.28E-07	0.30	MLM
9	57272296	5.58E-07	0.27	MLM
9	56152890	6.56E-07	0.34	MLM
9	57169768	7.50E-07	0.23	MLM
9	57272115	7.72E-07	0.25	MLM
3	60176979	4.97E-05	0.23	RMLF
2	64623580	9.16E-05	0.44	RMLF
7	58280357	1.43E-05	0.35	RMLM
7	56256841	1.95E-05	0.39	RMLM
3	60176979	7.62E-05	0.23	RTLF
7	56152038	1.35E-05	0.47	RTLM
9	56152890	1.87E-05	0.34	RTLM
9	57272115	1.72E-06	0.25	TLF
9	57222599	2.07E-06	0.28	TLF
9	57816733	2.66E-06	0.28	TLF
9	57222599	8.93E-08	0.28	TLM
9	56152890	1.17E-07	0.34	TLM
9	57476134	2.25E-07	0.30	TLM
9	56508161	4.94E-07	0.41	TLM
9	57236778	5.82E-07	0.29	TLM
9	57272296	6.91E-07	0.27	TLM
9	57272115	7.47E-07	0.25	TLM
9	56562984	9.71E-07	0.34	TLM
MN all years				
9	57272115	2.93E-06	0.25	MLF
9	57816733	3.36E-06	0.28	MLF
9	57383556	6.39E-06	0.30	MLF
9	57236791	6.70E-06	0.30	MLF
9	57272115	4.04E-07	0.25	MLM
9	57272296	6.16E-07	0.27	MLM
9	57476134	6.53E-07	0.30	MLM
9	57169768	6.69E-07	0.23	MLM

9	57383556	9.89E-07	0.30	MLM
2	60129082	9.67E-06	0.40	RMLM
6	59739236	1.89E-05	0.46	RTLF
2	64623580	1.61E-05	0.44	RMLF
7	59890439	1.07E-05	0.32	RTLM
9	57272115	2.48E-06	0.25	TLF
9	57816733	5.56E-06	0.28	TLF
9	57236791	6.72E-06	0.30	TLF
9	56152890	2.82E-07	0.34	TLM
9	57272115	4.34E-07	0.25	TLM
9	57383556	4.99E-07	0.30	TLM
9	57222599	5.93E-07	0.28	TLM
9	57476134	9.28E-07	0.30	TLM
Ottawa analysis				
1	11814753	1.70E-05	0.12	MLF
4	55646941	1.78E-05	0.31	MLF
2	69967056	3.73E-05	0.17	MLM
1	42155829	5.19E-05	0.10	MLM
3	60195553	4.48E-05	0.31	RMLF
4	55646941	6.59E-05	0.31	RMLF
1	66836006	1.20E-05	0.08	RMLM
8	51279094	2.55E-05	0.40	RMLM
6	54221186	6.27E-05	0.17	RTLF
10	3137796	8.26E-05	0.17	RTLF
1	61119284	2.68E-05	0.32	RTLM
8	51279094	3.48E-05	0.40	RTLM
10	3137796	2.38E-05	0.17	TLF
9	56802949	2.61E-05	0.18	TLF
1	27040589	6.39E-05	0.32	TLM
1	61119285	6.57E-05	0.32	TLM

[a]MAF, minor allele frequency.

[b]MLF, major lesion length Fusarium; MLM, major lesion length Macrophomina; TLF, total lesion length Fusarium; TLM, total lesion length Macrophomina; RTLF, relative total lesion length Fusarium; RTLM, relative total lesion length Macrophomina; RMLF, relative major lesion length Fusarium; RMLM, relative major lesion length Macrophomina

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Table 3.6. Chromosome locations, allele effect estimates and other summary statistics for SNPs significantly associated stalk rot resistance in the combined, MN all years and Ottawa analysis from the multi-locus mixed model.

Chromosome	SNP Physical Position, bp	P-Value[e]	Trait[d]	Allele	N[a]	Allele[b] Effect	R^{2c}
Combined analysis							
9	57816733	8.87E-07	MLF	A	174	1.14	0.12
				G	61		
9	57222599	7.01E-09	MLM	C	169	1.20	0.16
				G	57		
9	57816733	6.98E-07	TLF	A	174	1.50	0.11
				G	61		
9	57222599	8.60E-09	TLM	C	169	1.30	0.16
				G	57		
MN all years analysis							
9	57272115	1.18E-07	MLM	A	58	0.84	0.13
				G	186		
7	55702883	1.42E-07	RMLF	T	154	0.99	
				G	62		
2	64623580	2.74E-07	RMLF	G	122	0.99	0.24
				A	91		
9	57272115	3.68E-07	TLF	A	58	0.87	0.13
				G	186		
9	57272115	1.23E-07	TLM	A	58	0.83	0.14
				G	186		
2	60129082	3.84E-10	RMLM	C	133	0.99	
				T	84		
7	58532122	8.28E-09	RMLM	G	203	0.99	
				A	30		
9	57231207	5.70E-08	RMLM	T	213	0.99	
				C	13		
2	27851064	5.13E-06	RMLM	A	153	1.00	0.33
				G	76		
Ottawa analysis							
3	8738404	1.63E-08	RTLM	A	200	1.00	
				G	27		
9	56646280	1.06E-07	RTLM	A	84		
				T	149	0.99	
4	65815798	1.20E-06	RTLM	T	211	0.99	
				C	17		
8	51279094	5.23E-06	RTLM	C	129	0.99	0.34
				T	79		
8	39230116	1.18E-06	TLF	G	203	1.21	0.13
				A	21		

[a] N, total number of lines with the specific SNP genotype.

[b] Allele effects are reported back-transformed to the original scale in cm.

[c] R^2, proportion of total line mean variance explained by SNP as computed by GAPIT.

[d] MLF,major lesion length *Fusarium*; MLM, major lesion length *Macrophomina*; TLF, total lesion length *Fusarium*; TLM, total lesion length *Macrophomina;* RTLF, relative total lesion length *Fusarium*; RTLM, relative total lesion length *Macrophomina;* RMLF, relative major lesion length *Fusarium*; R MLM, relative major lesion length *Macrophomina*.

Table 3.7. Allele frequencies of significantly associated SNPs at the combined, MN all years and Ottawa environments in four sorghum major subpopulations

Chromosome	SNP Physical	Allele increasing	Allele Frequency (%)[a]					*N*[b]			
Environment	Position, bp	resistance	CAU	DUR	GUI	KAF	P-value*	CAU	DUR	GUI	KAF
Combined											
9	57816733	G	41.2	27.8	9.5	0.0	3.01E-04	51	36	21	24
9	57222599	G	35.3	16.7	9.5	20.8	1.29E-01	51	36	21	24
MN all years											
9	57272115	G	60.8	97.2	66.7	70.8	8.12E-04	51	36	21	24
7	55702883	G	72.6	8.3	0.0	0.0	2.20E-16	51	36	21	24
2	64623580	A	37.3	66.7	9.5	8.3	1.41E-07	51	36	21	24
2	60129082	T	11.7	36.1	71.4	58.3	4.00E-06	51	36	21	24
7	58532122	A	5.9	22.2	4.8	4.2	4.22E-02	51	36	21	24
9	57231207	C	13.7	0.0	9.5	0.0	1.33E-02	51	36	21	24
2	27851064	G	2.0	69.4	85.7	12.5	2.20E-16	51	36	21	24
Ottawa											
3	8738404	G	9.8	2.8	0.0	8.3	2.55E-04	46	34	21	24
9	56646280	A	39.2	19.9	28.5	37.5	9.99E-03	44	34	21	24
4	65815798	C	0.0	27.8	0.0	0.0	1.53E-06	50	33	21	24
8	51279094	T	27.4	69.4	52.4	4.0	5.41E-08	44	29	21	24
8	39230116	A	0.0	30.6	0.0	0.0	5.41E-08	47	36	21	24

CAU, caudatum; DUR, durra; GUI, guinea; KAF, kafir.

**P*-values after testing the null that the proportions (probability of success) in subpopulations are the same.

[a]Alleles are for homozygous genotype.

[b]*N*, total number of lines included in the analysis.

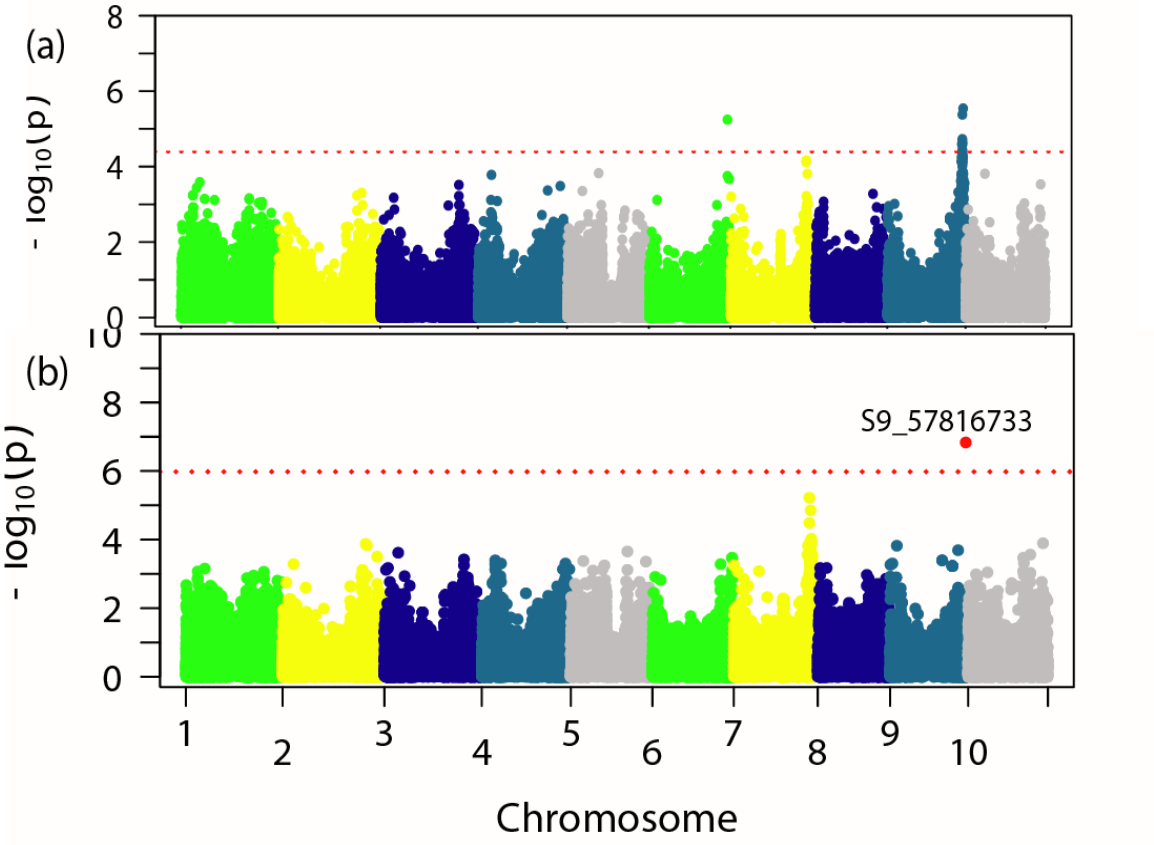

Figure 3.1. Manhattan plots for stalk rot resistance trait: major lesion length Fusarium (MLF) in sorghum from the combined analysis (a). A single-locus mixed model (marked in red; Bonferroni-corrected threshold of 0.05; dashed horizontal line) (b). A multi-locus mixed model (MLMM) identifies one SNP on chromosome 9 (marked in red) from the optimum model. The vertical axis indicates the $-\log_{10}$ of P-value scores, and the horizontal axis indicates chromosomes and physical positions of SNPs.

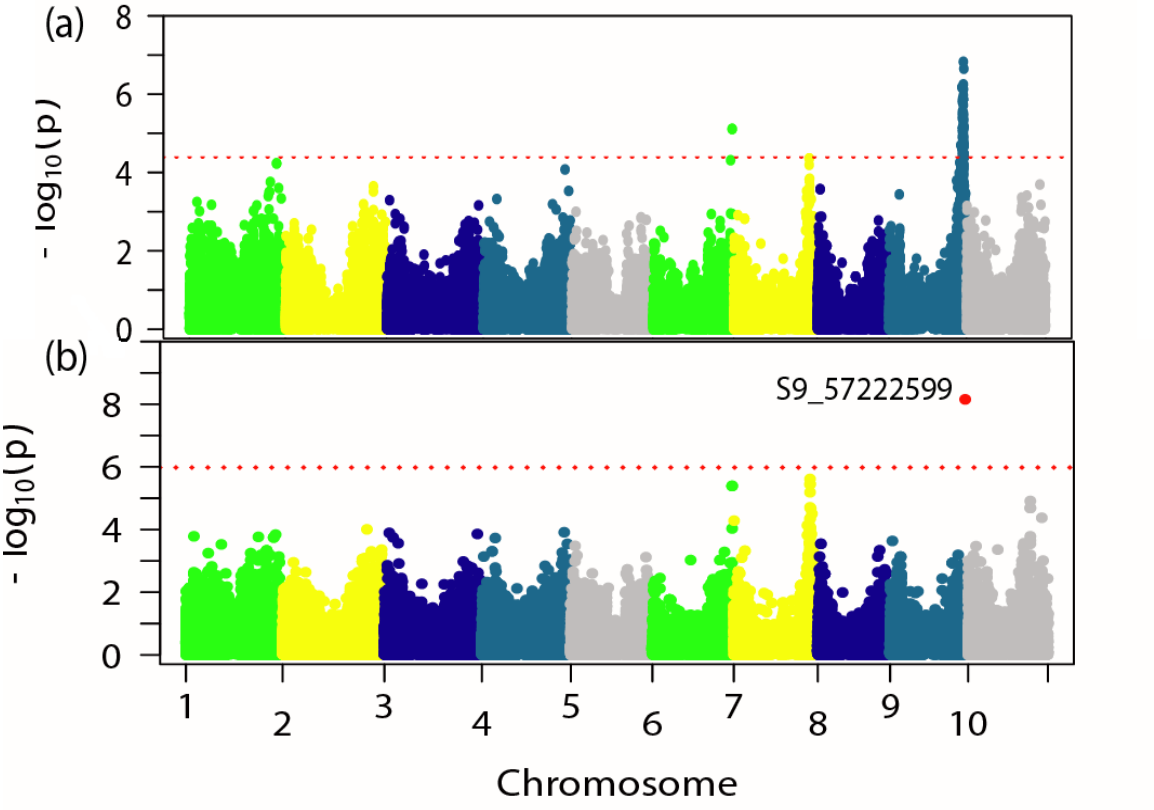

Figure 3.2. Manhattan plots for stalk rot resistance trait: major lesion length Macromophina (MLM) in sorghum from the combined analysis (a). A single-locus mixed model (marked in red; Bonferroni-corrected threshold of 0.05; dashed horizontal line) (b). A multi-locus mixed model (MLMM) identifies one SNP on chromosome 9 (marked in red) from the optimum model. The vertical axis indicates the $-\log_{10}$ of P-value scores, and the horizontal axis indicates chromosomes and physical positions of SNPs

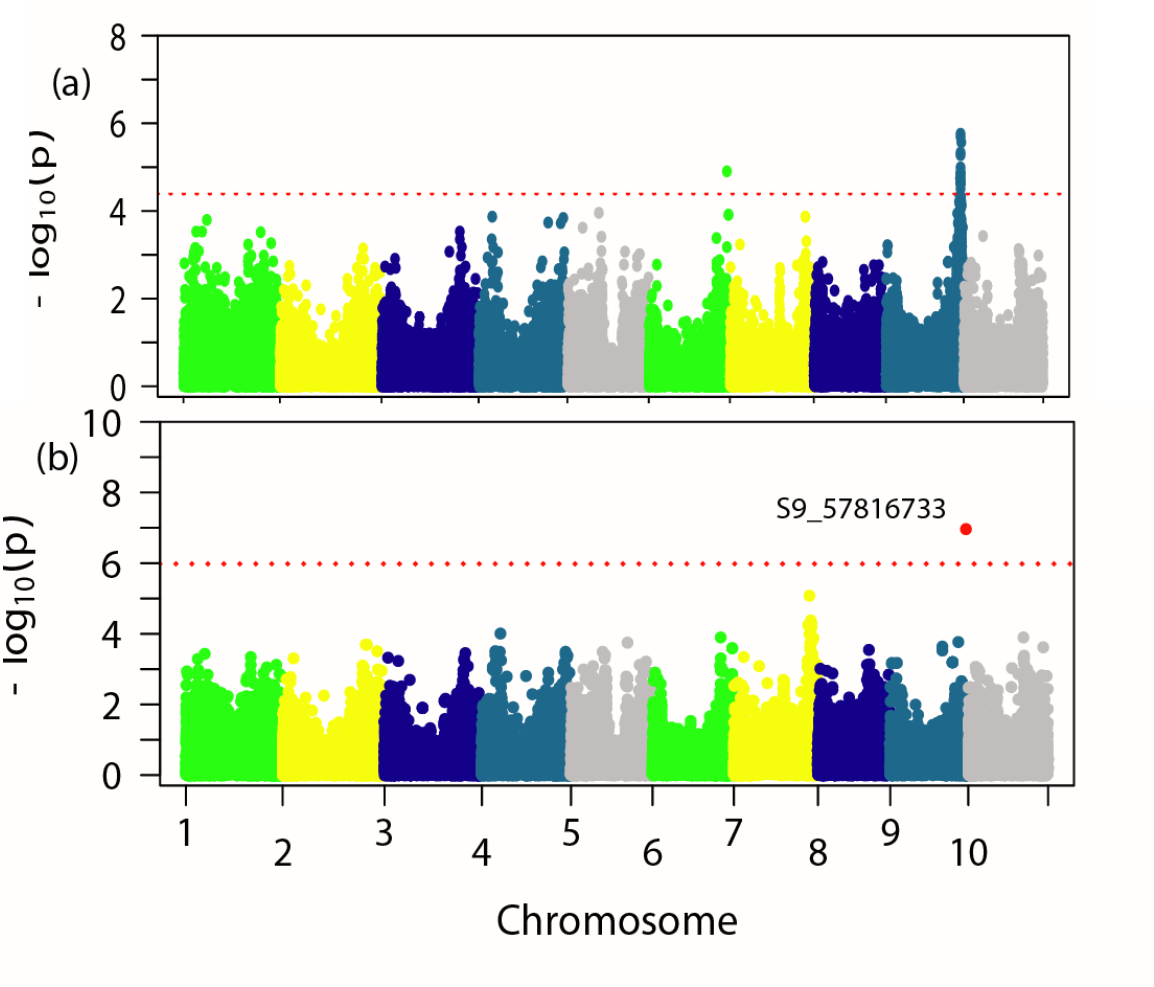

Figure 3.3. Manhattan plots for stalk rot resistance trait: total lesion length Fusarium (TLF) in sorghum from the combined analysis (a). A single-locus mixed model (marked in red; Bonferroni-corrected threshold of 0.05; dashed horizontal line) (b). A multi-locus mixed model (MLMM) identifies one SNP on chromosome 9 (marked in red) from the optimum model. The vertical axis indicates the $-\log_{10}$ of P-value scores, and the horizontal axis indicates chromosomes and physical positions of SNPs.

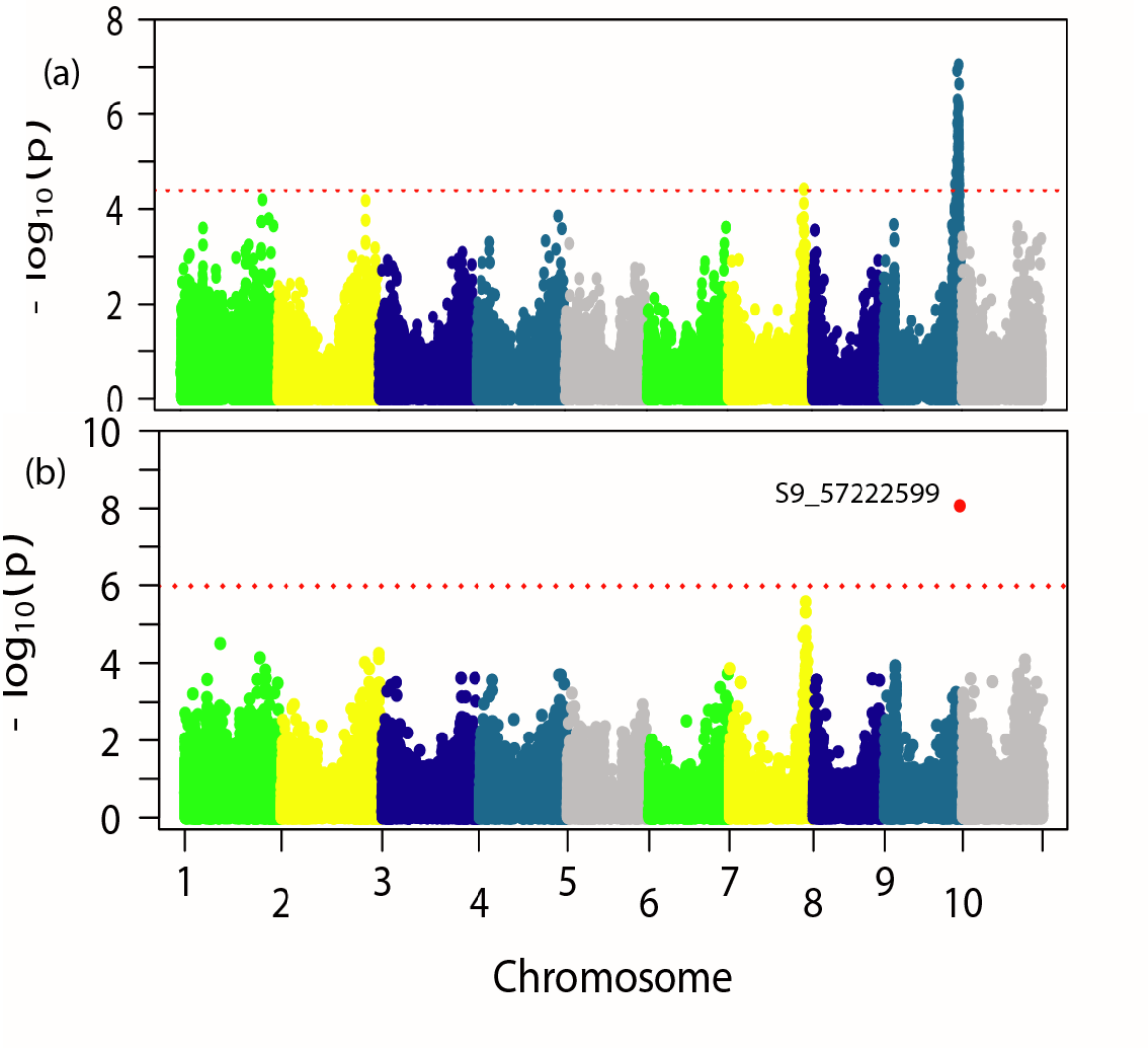

Figure 3.4. Manhattan plots for stalk rot resistance trait: total lesion length Macrophomina (TLM) in sorghum from the combined analysis (a). A single-locus mixed model (marked in red; Bonferroni-corrected threshold of 0.05; dashed horizontal line) (b). A multi-locus mixed model (MLMM) identifies one SNP on chromosome 9 (marked in red) from the optimum model. The vertical axis indicates the $-\log_{10}$ of P-value scores, and the horizontal axis indicates chromosomes and physical positions of SNPs.

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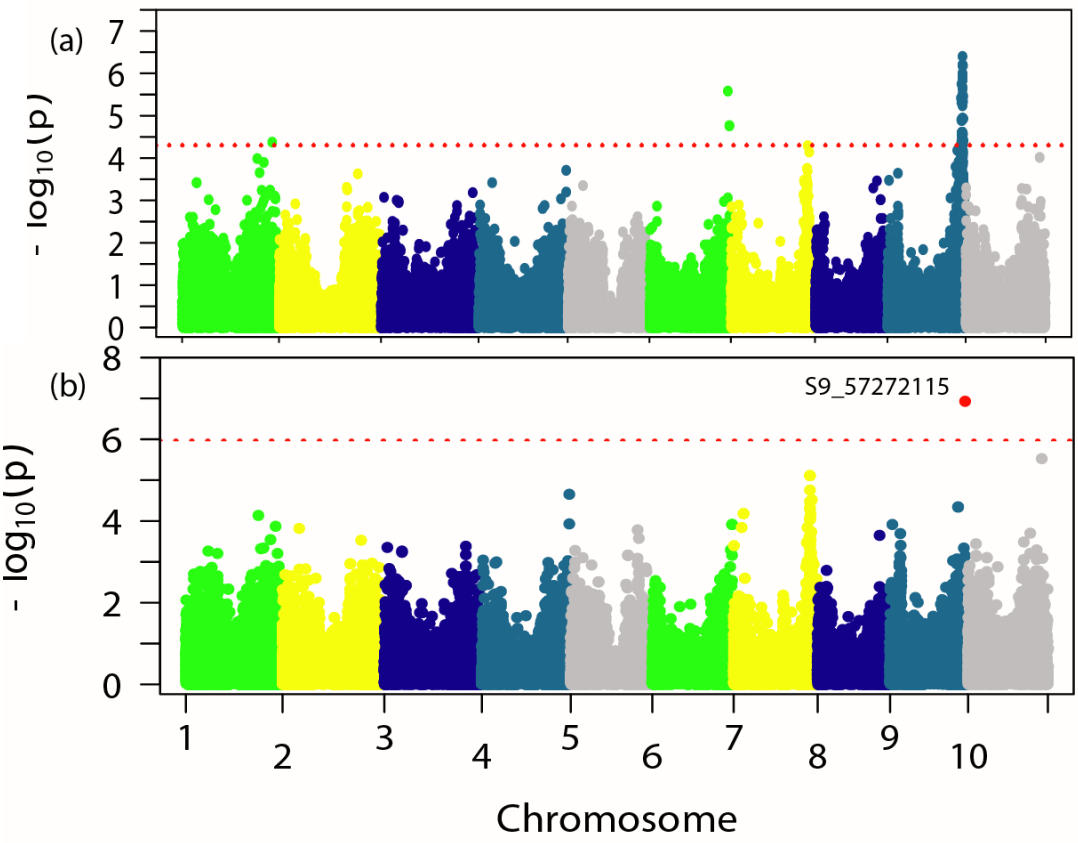

Figure 3.5. Manhattan plots for stalk rot resistance trait: major lesion length Macrophomina (MLM) in sorghum from the Manhattan, all years' analysis (a). A single-locus mixed model (marked in red; Bonferroni-corrected threshold of 0.05; dashed horizontal line) (b). A multi-locus mixed model (MLMM) identifies one SNP on chromosome 9 (marked in red) from the optimum model. The vertical axis indicates the $-\log_{10}$ of P-value scores, and the horizontal axis indicates chromosomes and physical positions of SNPs.

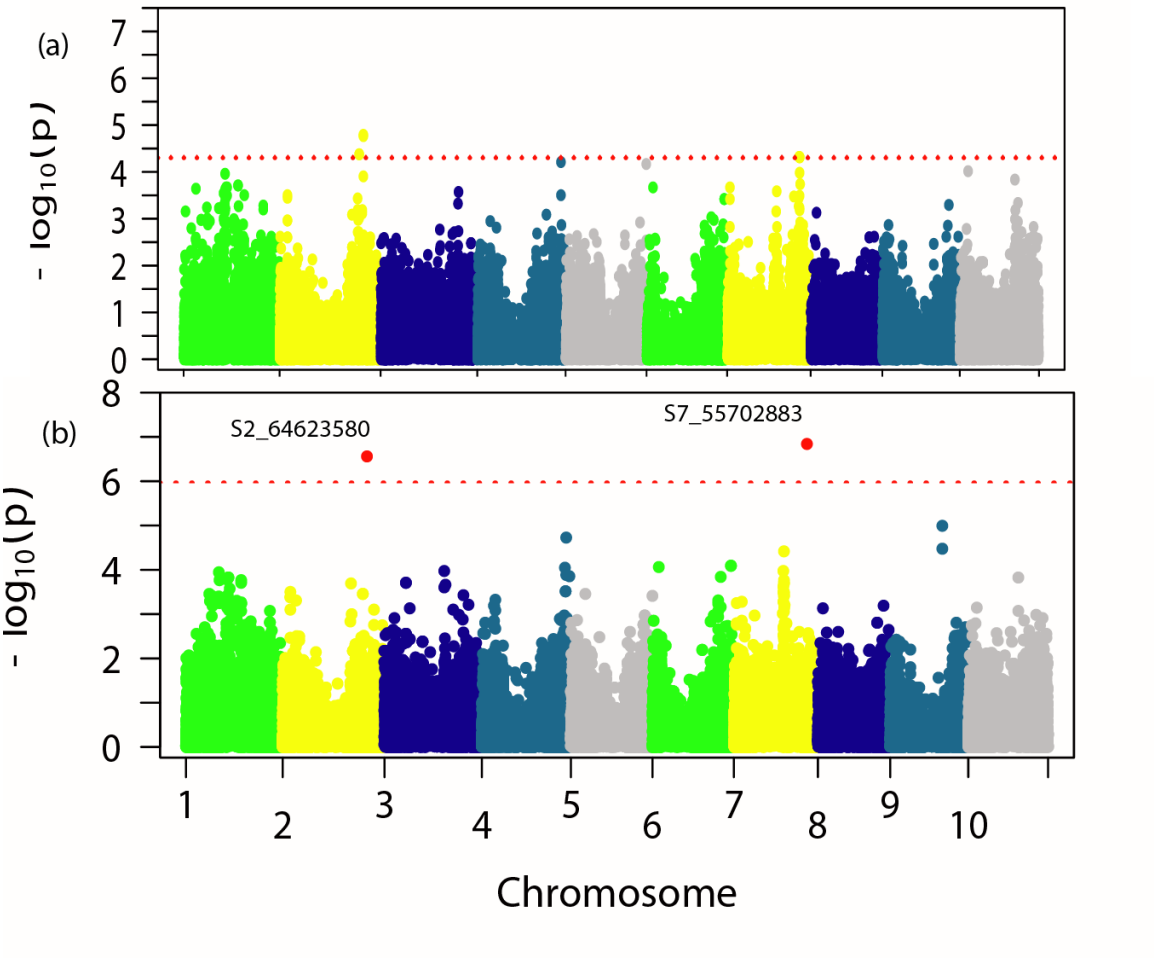

Figure 3.6. Manhattan plots for stalk rot resistance trait: relative major lesion length Fusarium (RMLF) in sorghum from the Manhattan, all years' analysis (a). A single-locus mixed model (marked in red; Bonferroni-corrected threshold of 0.05; dashed horizontal line) (b). A multi-locus mixed model (MLMM) identifies one SNP on chromosome 2 and 7 (marked in red) from the optimum model. The vertical axis indicates the $-\log_{10}$ of P-value scores, and the horizontal axis indicates chromosomes and physical positions of SNPs.

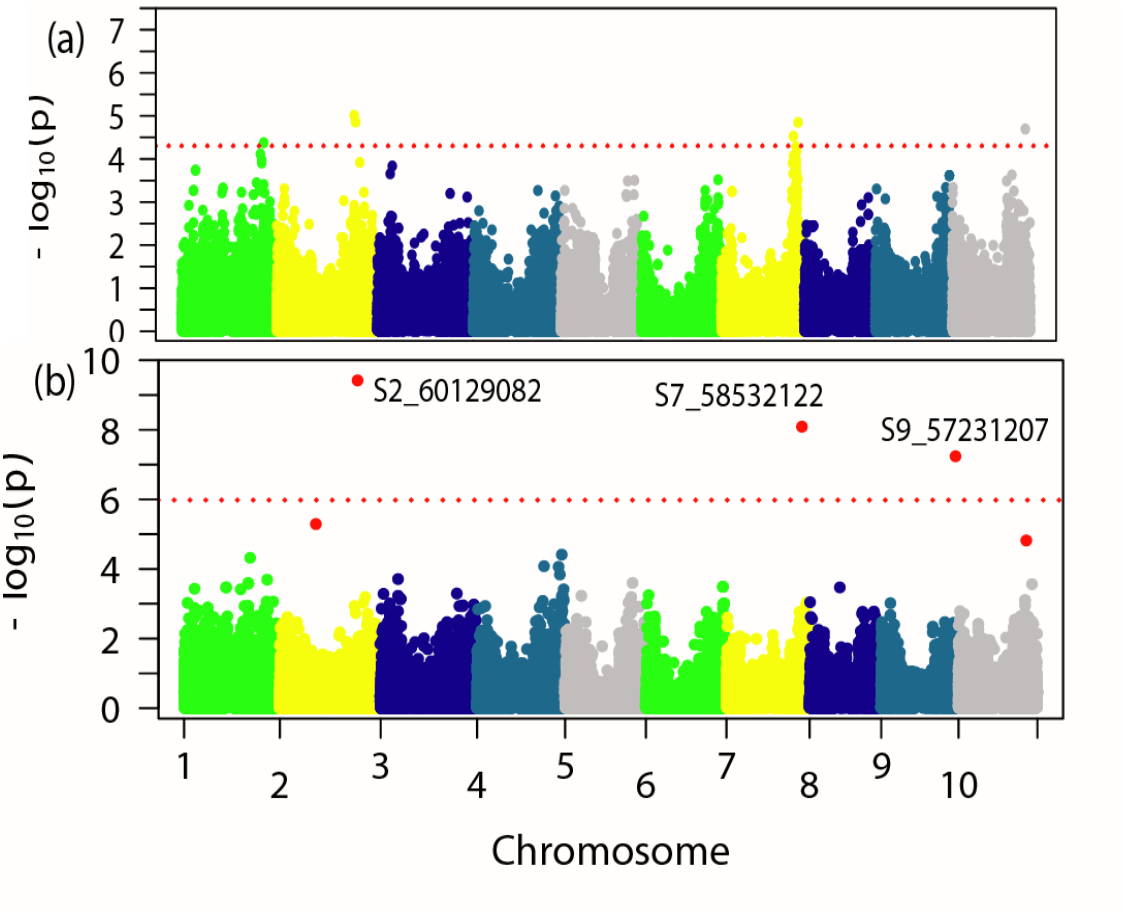

Figure 3.7. Manhattan plots for stalk rot resistance trait: relative major lesion length Macrophomina (RMLM) in sorghum from the Manhattan, all years' analysis (a). A single-locus mixed model (marked in red; Bonferroni-corrected threshold of 0.05; dashed horizontal line) (b). A multi-locus mixed model (MLMM) identifies one SNP on chromosome 2, 7 and 9 (marked in red) from the optimum model. The vertical axis indicates the $-\log_{10}$ of P-value scores, and the horizontal axis indicates chromosomes and physical positions of SNPs.

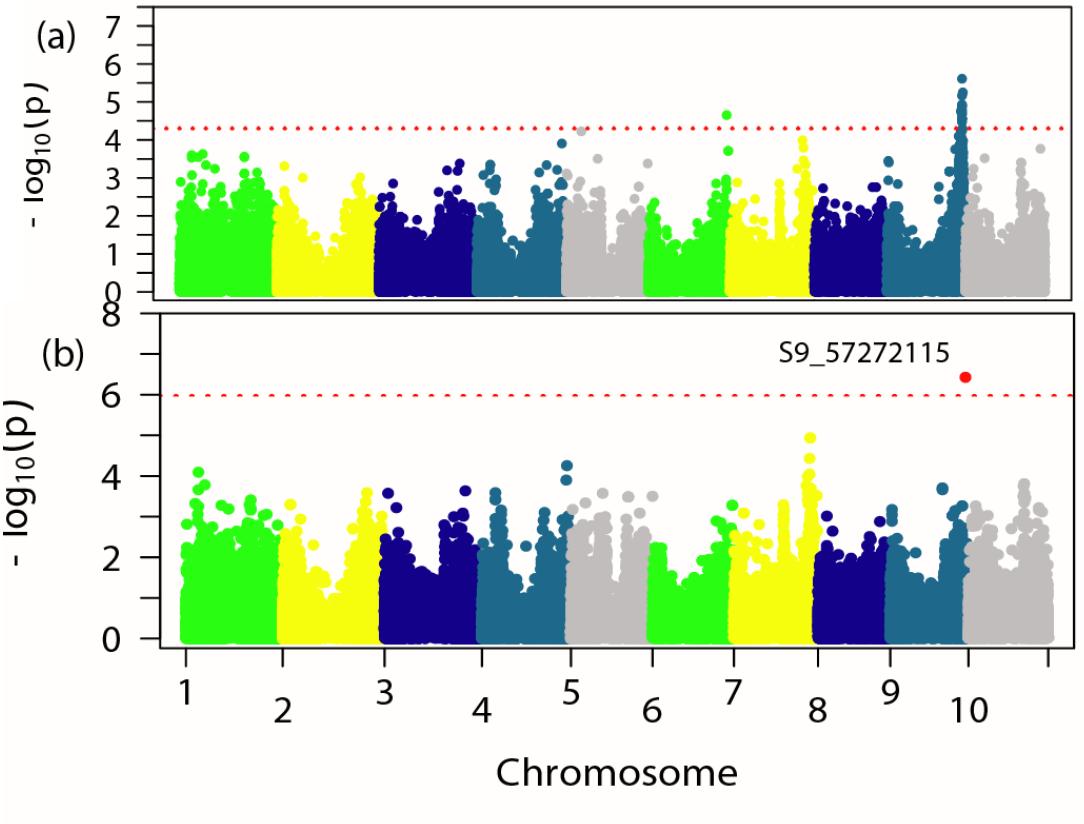

Figure 3.8. Manhattan plots for stalk rot resistance trait: total length Fusarium (TLF) in sorghum from the Manhattan, all years' analysis (a). A single-locus mixed model (marked in red; Bonferroni-corrected threshold of 0.05; dashed horizontal line) (b). A multi-locus mixed model (MLMM) identifies one SNP on chromosome 9 (marked in red) from the optimum model. The vertical axis indicates the $-\log_{10}$ of P-value scores, and the horizontal axis indicates chromosomes and physical positions of SNPs.

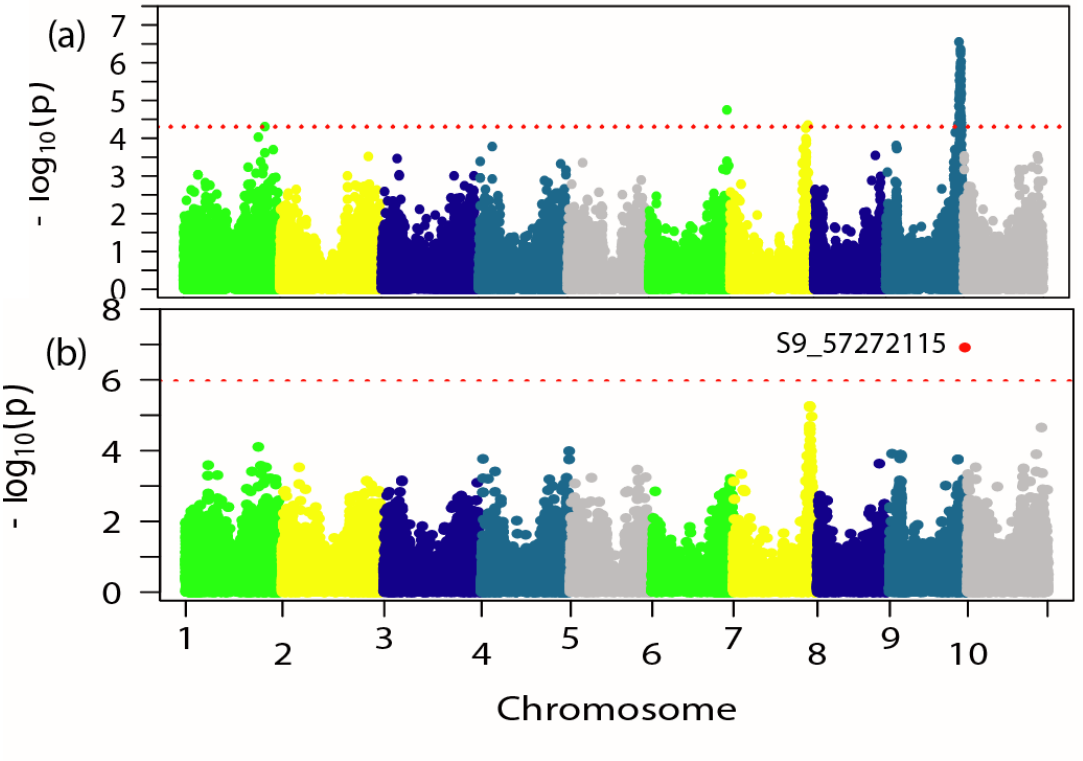

Figure 3.9. Manhattan plots for stalk rot resistance trait: total lesion length Macrophomina (TLM) in sorghum from the Manhattan, all years' analysis (a). A single-locus mixed model (marked in red; Bonferroni-corrected threshold of 0.05; dashed horizontal line) (b). A multi-locus mixed model (MLMM) identifies one SNP on chromosome 9 (marked in red) from the optimum model. The vertical axis indicates the $-\log_{10}$ of P-value scores, and the horizontal axis indicates chromosomes and physical positions of SNPs.

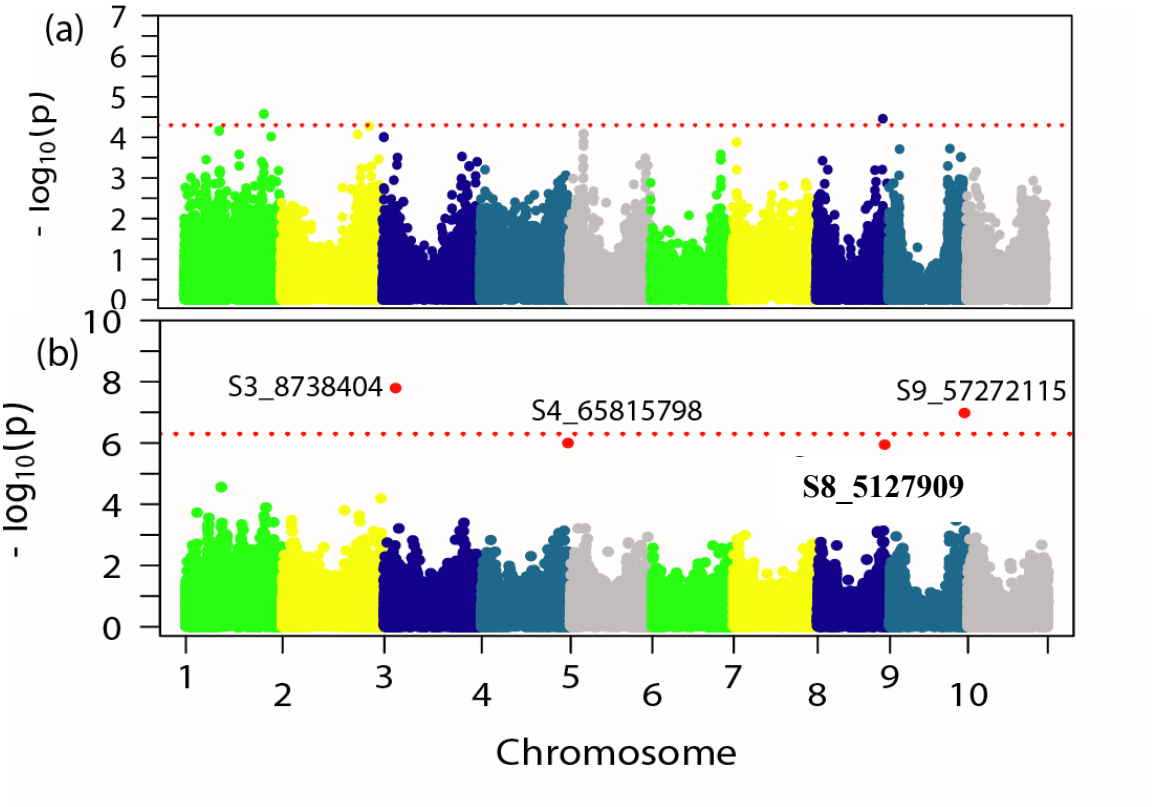

Figure 3.10. Manhattan plots for stalk rot resistance trait: relative total lesion length Macrophomina (RTLM) in sorghum from the Ottawa analysis (a). A single-locus mixed model (marked in red; Bonferroni-corrected threshold of 0.05; dashed horizontal line) (b). A multi-locus mixed model (MLMM) identifies one SNP on chromosome 3, 4, 8 and 9 (marked in red) from the optimum model. The vertical axis indicates the $-\log_{10}$ of P-value scores, and the horizontal axis indicates chromosomes and physical positions of SNPs.

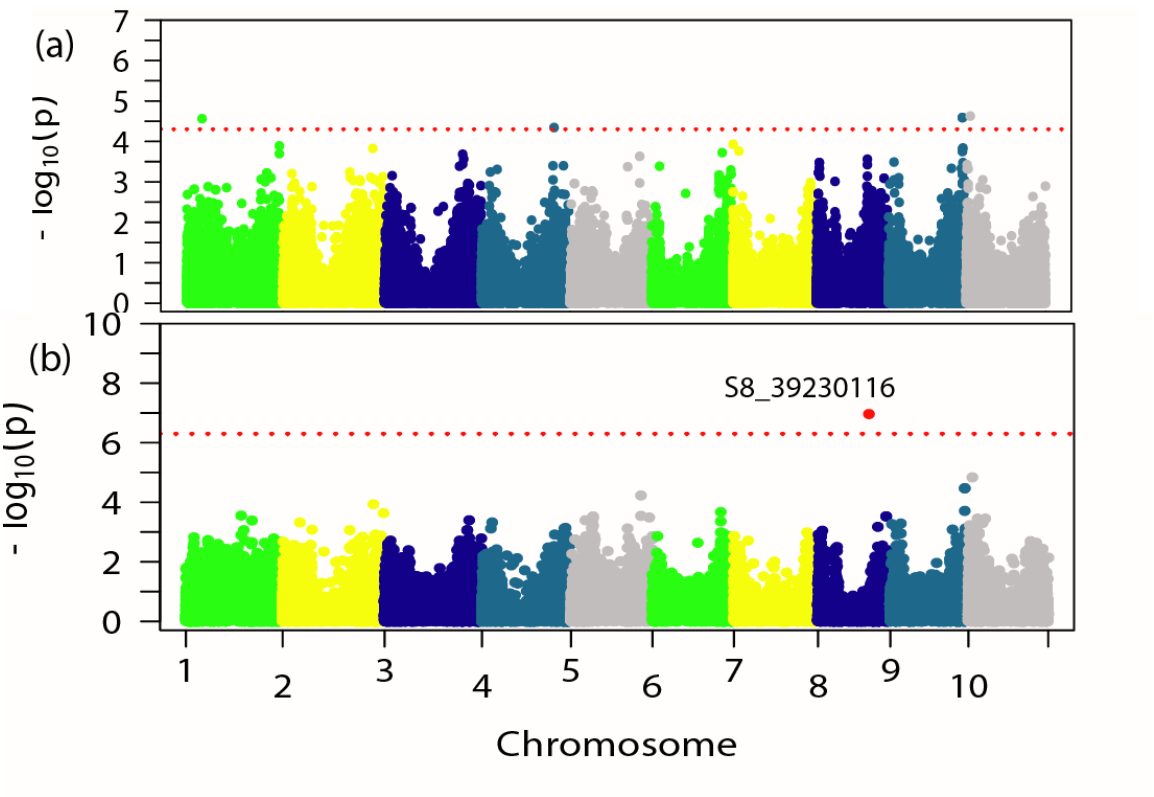

Figure 3.11. Manhattan plots for stalk rot resistance trait: total lesion length Fusarium (TLF) in sorghum from the Ottawa analysis (a). A single-locus mixed model (marked in red; Bonferroni-corrected threshold of 0.05; dashed horizontal line) (b). A multi-locus mixed model (MLMM) identifies one SNP on chromosome 8 (marked in red) from the optimum model. The vertical axis indicates the $-\log_{10}$ of P-value scores, and the horizontal axis indicates chromosomes and physical positions of SNPs.

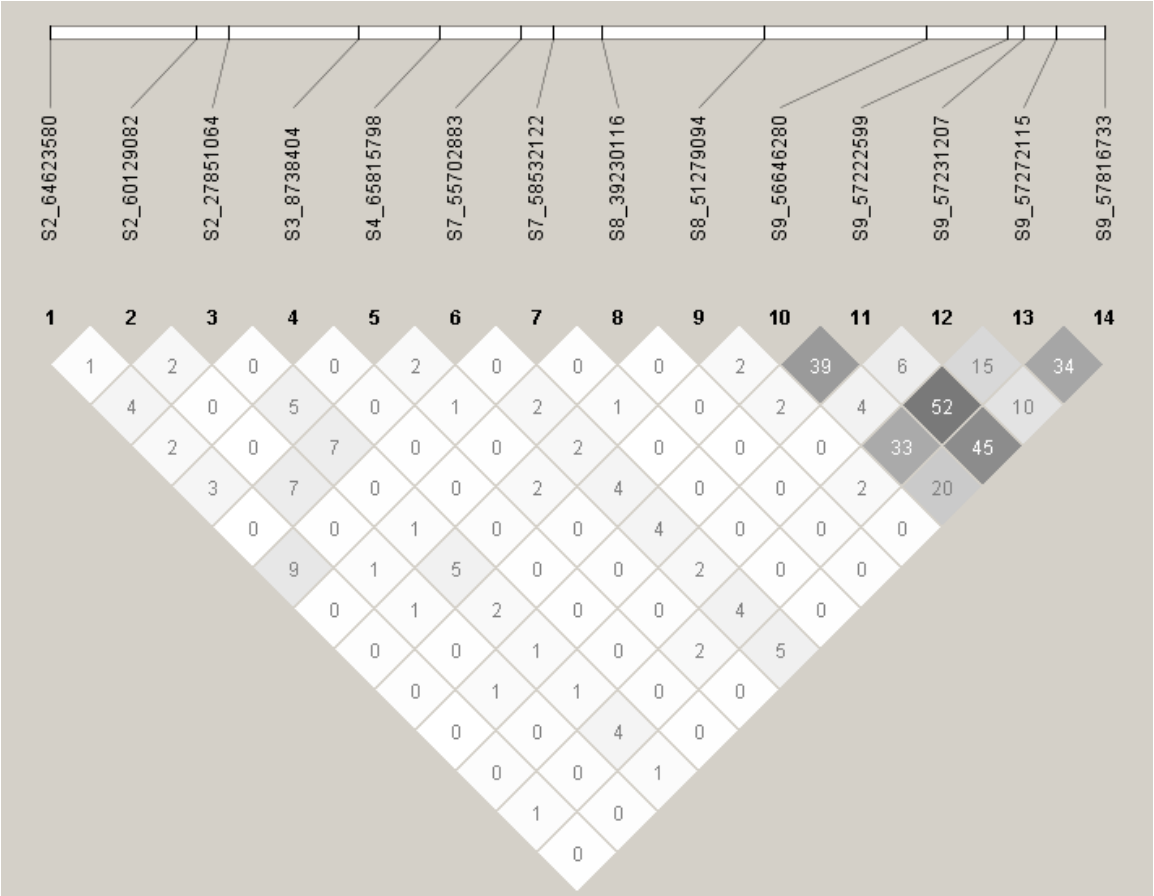

Figure 3.12. Linkage Disequilibrium (LD) among significant SNP markers. HAPLOVIEW v.4.2 (Barrett et al., 2005) pairwise LD values (r2*100) for 14 SNPs based on genotypes of 257 individuals were used to test whether all the SNPs significantly associated with stalk rot resistance tolerance were in strong LD with each other; white, $r^2=0$; shades of gray, $0 < r^2 < 1$.

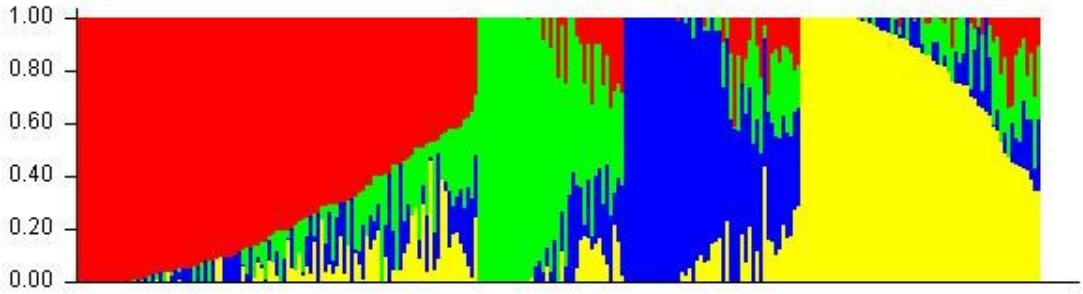

Figure 3.13. Population structure plot for the 257 association population based on 25,000 SNPs. The red, green, blue and yellow bars correspond to the caudatum (CAU), kafir (KAF), guinea (GUI) and Durra (DUR) and, respectively, while vertical bars represent sorghum lines.

Chapter 4 - Study of Recombinant Inbred Lines (RILs) Differing for Stay-green QTL, Dhurrin and Leaf Sugar Concentration for resistance to infection by stalk rot pathogens

Abstract

The association between post-flowering drought tolerance, the stay-green phenotype, and stalk rot resistance has been reported in sorghum [*Sorghum bicolor* (L.) Moench] and used as an indirect selection criteria to improve stalk rot resistance, especially charcoal rot caused by *Macrophomina phaseolina*. However, the mechanisms governing this association between drought tolerance and charcoal rot resistance, apart from the common knowledge that charcoal rot requires dry environment to prevail is not fully known. Understanding the mechanisms relating the stay-green and associated traits such as dhurrin and leaf sugar levels with response to stalk-rot will be important in the development of new stalk-rot resistant cultivars. The objective of this study was thus to determine the functional relationship between stay-green trait and resistance to stalk rot diseases caused by *Macrophomina phaseolina* and *Fusarium thapsinum*. Fourteen genotypic groups derived from Tx642 × Tx7000 RIL population carrying combination for stay-green quantitative trait loci were evaluated under three environments in four replications. The stg QTL had variable effects on stalk rot disease. Genotypes carrying *stg*1, *stg*3, *stg*1, 3 and *stg*1,2,3,4 expressed good levels of resistance to *M. phaseolina* but it required the combination of *stg*1 and *stg*3 to express same level of resistance to *F. thapsinum*. Other *stg* QTL blocks such as *stg*2 and *stg*4 did not have any impact on stalk rot resistance caused by both pathogens. Also there was no correlation between leaf dhurrin, and soluble sugar concentration and resistance to any of the pathogens.

Key words: Sorghum, *Macrophomina phaseolina*, *Fusarium thapsinum*, stay-green, quantitative trait loci (QTL).

Introduction

Genetic variation for stalk rot resistance in sorghum is well-documented (Dodman et al., 1992; Tesso et al., 2005). Not much effort has been made to directly improve stalk rot resistance in sorghum; but much of the progress in addressing this and the associated lodging problems was achieved through selection for post-flowering drought tolerance. The key trait of interest in post-flowering drought tolerance breeding has been the stay-green trait. Stay-green also known as non-senescence is the ability of a plant to maintain active green leaf area to continue to photosynthesize under limited moisture during crop maturation. It is strongly associated with post-flowering drought tolerance and allows plants to avoid premature senescence due to drought stress. Stay-green genotypes have been reported to have the ability to continue to fill grain normally under drought stress (Rosenow and Clark, 1981) and exhibit resistance to charcoal rot (Rosenow, 1984; Borrell et al., 2000b) and lodging (Henzell et al., 1984). Because of these, the trait has attracted significant attention over the last decades and almost all breeding lines released in the recent past and many commercial hybrids have stay-green trait incorporated into them.

Quantitative trait loci (QTL) associated with the stay-green phenotype has been mapped in numerous populations including the famous Tx642 × Tx7000 recombinant inbred population (Crasta et al., 1999; Haussmann et al., 2003; Kebede et al., 2001; Subudhi et al., 2000; Tao et al., 2000; Tuinstra et al., 1997; Xu et al., 2000). Of several stay-green QTL mapped in this population, four of them, *Stg*1-*Stg*4, were the major ones that were consistently expressed under multiple environments and jointly account for 53.5% of the phenotypic variance for the trait (Sanchez et al., 2002). Moreover, these QTL have also been shown to individually reduce leaf senescence induced by post-flowering drought stress (Harris et al., 2007). Genetic studies on populations derived from Tx642 showed that genes conferring the stay-green trait act in various levels of dominance (Walulu et al., 1994) or in an additive fashion (VanOosterom et al., 1996).

Stay-green genotypes were reported to contain higher concentration of basal stem sugars (Duncan, 1984) and cytokinins (McBee, 1984) compared to senescent genotypes, which may reduce the rate of drought-induced senescence (Thomas and Smart, 1993). Increased accumulation of soluble sugars found in stay-green genotypes besides reducing

dependence on stored assimilates for filling grains (McBee et al., 1983) were reported to contribute to reduced incidence of stalk and root rot diseases. Previous studies have indicated that high concentration of sugars in basal stalks contribute to increased resistance to major stalk rot pathogens (Clark and Miller, 1980; Hirematch and Parvatikar, 1985; Rajewiski and Francis, 1991). Thus indirect selection for charcoal rot resistance through stay-green trait has been effective (Rosenow, 1984).

Further research in recent years substantiate the original findings by confirming that stay- green sorghums tend to accumulate high amount of biochemical compounds and sugars (Burke et al., 2013). One of the key compounds consistently found in stay-green genotypes was the cyanogenic glucoside, dhurrin. Cyanogenic glycosides are produced in plants to mediate some general and specialized functions such as to provide chemical defense system, for mediating plant-insect interactions (Zagrobelny et al., 2004), and also to serve as nitrogen storage compounds (Busk and Moller, 2002; Selmar et al., 1988). Dhurrin is found in all vegetative tissues in sorghum (O'Donnell et al., 2013); the site of dhurrin synthesis shifts from leaves to stem during plant development (Busk and Moller, 2002). When tissues containing dhurrin are crushed, hydrogen cyanide (HCN) is released as part of dhurrin decomposition. Sorghum may accumulate high concentration of cyanogenic glycoside dhurrin when subjected to drought stress.

Recent studies have shown the relationship between dhurrin levels and the degree of pre- or post-flowering drought tolerance (Burke et al., 2013). Highly cyanogenic plants have been reported to be preferred by some fungi and insects compared with plants with lower cyanogenic potential (Nahrstedt, 1996; Møller and Seigler, 1999). But Aglycones released from cyanogenic glucosides formed from Phe or Tyr amino acids may give rise to the formation of compounds with antifungal activities (Siebert et al., 1996).

Despite the relative paucity of published evidence highlighting the value of stay-green, there has been little research conducted to understand the genetic mechanism underlying the stay-green and stalk rot resistance complex in sorghum and the relationship between them. There seems to be a direct correlation between senescence and susceptibility to stalk rots (Borrell et al., 2000a) but it is not clear whether there is a cause and effect relationship between the two. It is possible that the stay-green genes may provide tolerance to drought and at the same time confer resistance to stalk rot

(pleiotropic effect) or indirectly contribute to stalk rot resistance through reducing the negative effects of drought.

Therefore, in this project we examined a sub-set of genotypes derived from the popular Tx642 ×Tx7000 RIL population to shed more light on the functional association between stay- green trait and resistance to stalk rot diseases. The objectives of this study were thus: (1) to determine the reaction of recombinant inbred (RI) genotypes with varying stay-green QTLs to stalk rot resistance, and (2) to examine any possible relationship between leaf dhurrin and soluble sugar levels and stalk rot disease severity.

Materials and methods

Genetic materials

An F_{12} RIL (recombinant inbred line) population from the cross Tx642 × Tx7000 was used for this study. The development of this RIL population was earlier described by (Xu et al., 2000). Tx642, a BC_1 derivative of IS12555, durra sorghum from Ethiopia is a B-line and does not restore fertility when crossed with A1 cytoplasm. It responds distinctly to drought stress at pre-flowering and post-flowering stages compared to Tx7000. It is susceptible to pre-flowering drought and highly resistant to post-flowering drought (stay-green trait) with a relatively low yield potential as inbred per se. Tx7000 is an elite high yielding public R-line commonly used for developing sorghum hybrids in the United States and is tolerant to pre-flowering drought but very susceptible to post-flowering drought. Tx642 is also resistant to charcoal rot while Tx7000 has been used as a susceptible check in all charcoal rot related studies.

The entire population of Tx642 × Tx7000 was provided by Dr. Bill Rooney and *stg* QTL profile for each of the genotypes by Dr. Patricia Klein of Texas A&M University. Sub-sample of the population selected based on the number and types of stay-green QTL they carry were used in this study. For the four stay-green QTL (*stg*1-*stg*4), there were a total of 14 combinations of QTL groups. Wherever possible, four genotypes were selected for each group and whatever number of genotypes available was used for those less than that. Accordingly, seven of the 14 QTL groups had 4 genotypes each, two of them had 3, three of them had 2 and another two of them had just 1 genotype. For those QTL groups that have only one or two genotypic representation, multiple rows of

the genotypes were grown to make the number of reading comparable to those that have three or four representation. The parental lines Tx642 and Tx7000 were also included as complete QTL and zero QTL check along with Tx430 as non-*stg* check. Overall, 45 genotypes were included in the study. Summary of the number of genotypes and the number and types of *stg* QTL they carry is presented in Table 4.1.

Experimental design and management

The selected genotypes including the parents were evaluated at three environments using a randomized complete block design with four replications. The test environments were the 2012 and 2013 crop seasons at the Ashland Bottom Kansas state University (KSU) Agronomy research farm near Manhattan KS and the KSU East Central Agricultural Experiment Station near Ottawa KS in 2013. Soils are well-drained Smolan Silty Clay Loam and Harney Silt Loam for Manhattan and Ottawa, respectively. Plots were 6 m long single rows spaced 0.75 m apart with 0.6m alley between blocks. Three grams each of the selected RILs were drilled into the plots after treatment with herbicide safener (Maxim 4FS, Apron XL, Concept III, and colorant). Three weeks after emergence the seedlings were thinned to approximately 0.15m between plants. Fertilizer phosphorous (di-ammonium phosphate, DAP) and nitrogen (urea) were applied at the rate of 34 kg P_2O_5 ha^{-1} and 90 kg N ha^{-1} at Manhattan and 25 kg P_2O_5 ha^{-1} and 90 kg N ha^{-1} at Ottawa. Pre-emergence weeds were controlled with a pre-plant application of 0.24 L ha^{-1} Dual plus 0.68 kg ha^{-1} Atrazine. Post emergence weeds were removed manually to ensure normal growth. The Manhattan 2012 environment was characterized by serious drought and high temperature stress while both Manhattan and Ottawa during the 2013 season had optimal moisture and temperature. At flowering, eight uniform plants in a plot were tagged, four each with two distinct color tapes, to use for pathogen inoculation.

Dhurrin and leaf sugar assay

A one centimeter leaf disc samples were collected midway between the base and the tip of the top third leaf from each genotype prior to flowering using leaf punch from both Manhattan and Ottawa 2013 tests. Samples were collected from five different plants in a row and immediately placed in an ice box and covered to transport to the laboratory. In the laboratory, the samples were transferred into a dry-ice container and shipped to Dr.

John Burke's lab at the USDA Cropping Systems Research Laboratory, Lubbock, TX for biochemical analysis. High performance liquid chromatography analysis for dhurrin and leaf sugars was carried out using the methods described by (Burke et al., 2013). Dhurrin and leaf sugars were extracted from five leaf punches by incubating the samples in 1 mL 80% ethanol at 60°C for 1h followed by a 5-min cool down time at room temperature. The extract was centrifuged at 10,621 × *g* for 10 min and 200 µL supernatant was transferred into a clean Eppendorf tube and dried by vacuum centrifugation in a Savant SpeedVac SVC100 (Savant Instruments Inc.) set to the low drying rate. The extracts were then suspended in 200 µL deionized water and suspension samples equivalent to 50 to 100 µg of fresh weight of leaf tissues were separated on a 4.6 × 250-mm YMC Polyamine II column (Waters Corporation) with a mobile phase of 70% C_2H_3N in water (v/v) at a flow rate of 1.0 mL min^{-1} and dhurrin and leaf sugars were analyzed using a VP Series HPLC (high performance liquid chromatography) system fitted with an evaporative light scattering detector-LT (Shimadzu Scientific Instruments). Presence of dhurrin and leaf sugars in the samples was detected by their retention time in comparison with the corresponding dhurrin and leaf sugar standards. Quantification of dhurrin and leaf sugar concentration was calculated using peak area.

Inoculums preparation and inoculation

Two fungal pathogens were selected for this study: *Fusarium thapsinum and Macrophomina phaseolina*. The selection of these two pathogens was based on previous reports that these two species are the most virulent stalk rot pathogens of sorghum (Tesso et al., 2010). Pure fungal cultures were provided by Dr. Chris Little, Department of plant pathology, Kansas State University. Liquid inoculum suspensions from pure cultures of the *Fusarium thapsinum* pathogen were initiated in potato dextrose broth (DIFCO, Detroit, MI). The suspensions were initiated on a shaker at room temperature until conidia were produced and then strained through four layers of cheese cloth to separate the conidia from the mycelia mass. Concentration of the suspension was determined by counting the number of conidia under a microscope using a hemacytometer. The concentration was then adjusted to 5 x 10^4 conidia ml^{-1} by diluting the suspension using 10 m*M* (pH 7.2) Phosphate-buffered saline. The suspension was kept on ice until

inoculation. An Idico filler-plug gun (Forestry suppliers, Inc., Jackson MS) equipped with a stainless steel needle similar to that described by (Toman and White, 1993) was used to deliver approximately 1 ml of suspension into the pith of three tagged plants per plot at 14 d after flowering. For *M. phaseolina*, the pathogen strain was sub-cultured into several fresh potato dextrose agar plates, into which sterile toothpicks were inserted and incubated at 30°C for two weeks. Then infected toothpicks were used to inoculate the plants on the basal stalk approximately 10 cm above the soil surface. Control plants (one per genotype) in the case of *F. thapsinum* were injected using sterilized water, whereas for *M. phaseolina* with sterilized toothpicks.

Data collection and statistical analysis

Twenty eight days after inoculation, plants were cut at the base and split longitudinally to score disease severity. Disease scores were obtained by measuring the length of the visible lesion in the pith of the stalk as a measure of disease progression in the sorghum stalks. Data were analyzed using the PROC GLM procedure in SAS (version 9.1.3) both for individual environment as well as the combined data. All factors, genotype, block and environment and their interactions were considered random effects. Significant means for all traits were separated using Fischer's protected LSD (SAS, V 9.1.3). Pearson correlation coefficients were estimated from combined environments data (Manhattan 2013 and Ottawa 2013) to determine the relationship between disease scores, dhurrin and leaf sugar concentrations using PROC CORR procedure in SAS (version 9.1.3).

Results

Analysis of variance

The combined analysis of variance for stalk rot parameters, dhurrin and leaf sugar concentration as affected by genotypes and environment is presented in Table 4.2. The environment had a significant effect on mean disease score for both *F. thapsinum* and *M. phaseolina*. However, it did not have significant effect on dhurrin concentration and all components of leaf sugars (Table 4.2). The effect of genotype, however, was highly significant ($P \leq 0.001$) for lesion length score both for *M. phaseolina* and *F. thapsinum*. It was also highly significant ($P \leq 0.01$) for dhurrin and significant ($P \leq 0.05$) for sucrose

content. The genotype did not have significant effect on leaf glucose and fructose concentrations. Similar to the genotype effect, the QTL effect was highly significant for *M. phaseolina* and *F. thapsinum*, dhurrin and sucrose concentrations; but was not significant for glucose and fructose concentrations. The genotype × environment effect was highly significant for dhurrin concentration, significant for *F. thapsinum* and sucrose concentration and was not highly significant for the other parameters. The QTL × environment effect was significant only for dhurrin concentration.

Genotypic response to infection by Fusarium and Macrophomina pathogens

Across environment mean disease rating following inoculation, dhurrin and leaf sugar content as affected by stg QTL blocks presented in Table 3. The environment and genotype effects for disease development of both *M. phaseolina* and *F. thapsinum* were highly significant (Table 4.2). The genotype × environment effect was only significant for *F. thapsinum*, dhurrin and sucrose concentrations. As a result, in addition to the combined analysis, a second analysis was performed for individual environments.

Mean disease score for *F. thapsinum* among genotypes in the combined analysis ranged from 3.3 cm in the stay-green QTL donor parent Tx642 to 7.8 cm in the susceptible parent Tx7000. Most of the stay-green RI genotypes had intermediate values between the parents with mean disease reaction in genotypes combining *stg*1 and *stg*3 QTL being close to that of Tx642 (5.0cm) (Table 4.3). But when the two QTL occur independently, it seems resistance is compromised. Genotypes that carry *stg*2, *stg*4 and those that combine both *stg*2 and *stg*4 QTL were as susceptible as Tx7000. Although genotypes combining *stg*1 and *stg*3 were the most resistant, stacking additional *stg* QTL (*stg*2, *stg*4 or both) with *stg*1*,3* seems to undo the resistance. Accordingly, genotypes carrying *stg*2 or *stg*4 QTL in addition to either *stg*1 or *stg*3 or both tend to lose resistance and the disease score approaches that of the susceptible parent.

For *M. phaseolina*, mean disease score was lowest (3.2cm) in Tx642 and was again highest (7.8cm) in the susceptible parent Tx7000. Unlike *F. thapsinum* where *stg*1 and *stg*3 should occur together for resistance to express, both *stg*1 and *stg*3 seem to contribute to resistance individually as well as in combination with other *stg* QTL. Again *stg*2 and *stg*4 whether they occur together or separately seem to have little or no contribution to

resistance to *M. phaseolina*. Response to infection by both pathogen groups of RI genotypes carrying all the *stg* QTL blocks was markedly different from that of Tx642 which indicates that additional gene blocks may be responsible for conferring resistance to the diseases besides these four QTL blocks.

Because there was no significant genotype × environment interaction effect for *M. phaseolina* and the significant G × E effect for *F. thapsinum* is of a scale and did not involve change in ranking patterns, the performance of the genotypes across environments tend to be consistent (Tables 4.4, 4.5 and 4.6). Accordingly, mean lesion length for both *F. thapsinum* and *M. phaseolina* ranged from 2.6 to 3.9 cm in the parental genotype Tx642 to the highest of 6.8 to 8.7 cm in the other parent Tx7000. The across genotype mean lesion length for both pathogens were 5.7 and 4.3 cm at Manhattan 2012, 6.5 and 6.3 cm at Ottawa 2013 and 6.1 and 4.7 cm at Manhattan 2013.

In the Manhattan 2012 environment (Table 4.4), genotypes with the *stg*3, *stg*1,3, *stg*1,4 and *stg* 1,3,4 had lower lesion lengths of (3.4 to 4.8 cm) for *F. thapsinum* and these scores were not significantly different from that of Tx642 (3.9 cm). The highest score at this environment (7.7cm) was recorded in the susceptible parent Tx7000 and *stg*4 (8.3cm) and *stg*2, 4 (6.9cm) genotypes. For *M. phaseolina*, genotypes carrying *stg*3, *stg*1,3, *stg*2,3, and *stg*1,3,4 had lesion lengths of 3.0 cm and below which is lower than 3.4 cm recorded in the resistant parent Tx642.

At Manhattan 2013, mean lesion length for *F. thapsinum* was lowest (2.6 cm) in Tx642 followed by RI genotypes *stg*1,3, *stg*1,4 and *stg*1,3,4. Genotypes with *stg*2, *stg*2,4 and *stg*4 were comparable to that of the susceptible check Tx7000 while others have intermediate scores. For *M. phaseolina*, genotypes *stg*1, *stg*1,3,4, *stg*1,2,3,4, and *stg*3,4 had men lesion length of 4cm and lower while the resistant and susceptible parents had lesion lengths of 2.7cm and 6.8cm, respectively. As it was the case in Manhattan 2012 for both pathogens and Manhattan 2013 for *F. thapsinum*, genotype *stg*2,4 had the highest mean lesion length for *M. phaseolina* in 2013. This shows that though their contribution to stay-green has been validated, some of the QTLs both individually and in combination with others seem to have no or little relationship with stalk rot resistance. The Ottawa 2013 environment was not any different from the Manhattan 2012 and 2013 environments. Consistent with data for other environments as well as the combined

analysis, the lowest lesion length of 4.0cm for *F. thapsinum* was recorded in *stg*1,3 and this was not significantly different from the 3.7 cm recorded in Tx642 at this environment. The score for other genotypes was similar to the other locations. Likewise, genotype *stg*3 had the best performance for *M. phaseolina* similar to Tx642 at this environment. In the 2013 evaluation, another genotype (Tx430) not known to possess any of these QTL blocks was included for comparison and it consistently expressed high degree of resistance to both pathogens. This indicates that besides the stay-green character there are other genetic/physiological events associated with resistance to stalk rot diseases.

To summarize, some of the stay-green QTL blocks, besides conferring post-flowering drought tolerance either directly or indirectly contribute to stalk rot resistance. For *F. thapsinum*, the effects of the QTL blocks were not so marked such that only few and specific QTL combinations provided resistance comparable to that of Tx642. Only *stg*1 and *stg*3 when combined into one genotype provide the desired level of resistance. When disaggregated or when another *stg* QTL is included, it seems to break up with the positive interaction between the two and the resistance breaking down (Fig 4.1). For *M. phaseolina*, there seems to be more room for combining the different *stg* blocks. QTL blocks *stg*1 and *stg*3 whether they occur together or separately seem to provide the same level of resistance. Also combination of *stg*1,3,4 and *stg*1,2,3,4 also provide acceptable level of resistance (Fig 4.1). The effect of the number of stg QTL blocks on parameters studied was not significant in individual environments. However in the combined analysis, significant effects were observed for disease related traits, dhurrin and sucrose (Appendix 2). These significant effects could be attributed to the parental genotypes. There was no significant difference between genotypes with varying number of QTLs. Data on nodes crossed was not significant for any of the parameters and hence was not presented. Only summary of the genotypic response for the trait is included in the appendix A1.

All of the control genotypes expressed discoloration near the inoculation site either in response to wounds or due to infection by spontaneous pathogens that may have used the open wounds. No effort was made to isolate a pathogen from the control wounded tissue.

Dhurrin and soluble sugars

There was marked difference for dhurrin content between the test genotype groups; but its relationship with stalk rot disease was not clear. Mean dhurrin content ranged from the lowest of 39.45 µg cm^{-2} in RI genotype carrying *stg*1, *stg*3 (genotypes that showed the least infection by both *M. phaseolina* and *F. thapsinum*), to the highest of 90.6 µg cm^{-2} in RI genotypes combining *stg*1, *stg*2 and *stg*3 QTL blocks. Another group of genotypes that combine all *stg*1-*stg*4 QTL blocks and those that combine *stg*2 and *stg*4 also had among the lowest dhurrin content of 47.2 and 47.85µg cm^{-2}, respectively. The drought susceptible parent Tx7000 and the tolerant parent Tx642 had mean dhurrin content of 40.3 and 67.4µg cm^{-2} respectively.

Also there was marked difference between the RI genotypes for soluble sugar concentration except for fructose that ranged from 94.2µg cm^{-2} in *stg*3 genotypes to 122.6 µg cm^{-2} in *stg*1,3 genotypes. Mean fructose content in parents, Tx7000 and Tx642 was, 107.7 and 119.7µg cm^{-2}, respectively. Similar to fructose, mean glucose levels of the RI genotypes was also within the range of readings of the parental genotypes. Only three genotype groups, *stg*3, *stg*4 and *stg*3,4, had mean glucose levels slightly lower than 110.6 µg cm^{-2} recorded in one of the susceptible parents Tx7000 and none of the RI genotypes exceeded the resistant parent Tx642 which had the reading of 124.4 µg cm^{-2}. Variation for sucrose levels was relatively larger ranging from a low of about 11.5 µg cm^{-2} in several RI genotypes including Tx7000 to a high of 30 µg cm^{-2} in *stg*3 genotypes. The resistant parent Tx642 had only 14.2 µg cm^{-2}. Some eight of the fourteen RI genotype groups had mean sucrose content higher than the resistant parent.

Because there was highly significant G × E effect for dhurrin levels, we carried out separate ANOVA for the two locations. For Manhattan 2013 environment, mean dhurrin levels ranged from as low as 48.9 µg cm^{-2} in *stg*3, 4 genotypes to a high of 77.7µg cm^{-2} in *stg*3 genotypes. Only four *stg* genotype groups had mean dhurrin content less than that of the susceptible parent Tx7000 (58.5 µg cm^{-2}) and ten of the fourteen *stg* groups were higher than the other parent Tx642 (61.7 µg cm^{-2}). For Ottawa 2013, the domain of the dhurrin levels was much larger ranging from the low of 22 µg cm^{-2} in Tx7000 to a high of 106 µg cm^{-2} in *stg*1,2,3 with an overall entry mean of 65.3 µg cm^{-2}. Tx642 had a mean dhurrin level of 73.1 µg cm^{-2} exceeded by six of the 14 *stg* groups.

Soluble sugars in the leaves were significantly different among entries. At Ottawa 2013, the highest fructose and glucose levels of 166 and 182µg cm^{-2}, respectively, were observed in the check genotype Tx430. While the parental sources Tx7000 and Tx642 were 96 and 108 µg cm^{-2}, respectively, for fructose; and 97 and 114 µg $cm^{-2}$for glucose with, several genotypes exceeding the high sugar parent. Genotypes *stg*1, *stg*1,2, *stg*1,2,3, *stg*1,2,4, *stg*1,3, *stg*2, and *stg*2,3 were higher than Tx642 both for fructose and glucose levels. Invariably, genotypes with the highest fructose and glucose had the lowest sucrose levels and the vice-versa (Table 4.5). Accordingly, the check genotype Tx430 that had the highest glucose and fructose levels had the lowest sucrose while *stg*3 that was the lowest both in fructose and glucose was highest in sucrose levels. Unlike Ottawa 2013, Tx430 had among the lowest levels both for fructose and glucose while Tx642 being the highest for both. Here the relationship between fructose/glucose and sucrose was again almost direct opposite with Tx430 and *stg*1 that had the least fructose/glucose levels showing the highest sucrose. However, Tx642 that had the highest fructose and glucose had an intermediate sucrose and *stg*1,3,4 that had the lowest fructose and glucose also had among the lowest sucrose levels (Table 4.6). There was no significant correlation between dhurrin, fructose, glucose and sucrose levels and disease score for both pathogens. However there was significant (positive) association between dhurrin and sucrose and significant (negative) correlation between fructose and sucrose and between glucose and sucrose. Correlation between glucose and fructose was positive and highly significant (0.99) (Table 4.7). Also there was significant and positive correlation between disease score for the two pathogens.

Discussion

Previous studies have shown that stalk rot causing pathogens are commonly found in the soil and also in the plant system almost at all stages of growth. However, they are capable of causing disease only when the host condition is weakened either by prolonged drought stress or due to senescence or both. As a result, stalk rots are often observed in plants that are in senescence phase of development that are also subjected to severe drought stress. Thus, plant characteristics that enhance drought tolerance or delay senescence are considered to contribute to reduced stalk rot incidence. The current study investigated the

disease response of sorghum genotypes varying for non-senescence (stay-green) QTL to infection by common pathogens causing Fusarium stalk rot and charcoal rot. Because previous studies found significant association between stay-green phenotype and leaf dhurrin content (Burke et al., 2013), we also evaluated the relationship between the levels of dhurrin and soluble sugars in the leaves and severity of both charcoal rot and Fusarium stalk rot.

Although all of the four major QTL (*stg*1, *stg*2, *stg*3 and *stg*4) contribute to stay-green expression jointly accounting for 53% of the total variation observed for the trait, not all of these QTL blocks have an effect on genotypic response to stalk rot diseases (Tables 3-6, Fig 1). Only certain *stg* blocks either independently or in combination with each other seems to have effect on the development of the disease.

For *M. phaseolina*, *stg*1, *stg*3 and their combination (*stg*1,3) consistently resulted in low infection rate comparable to that in the resistant parent Tx642. Also genotypes carrying *stg*3,4, *stg*1*,3,4* and *stg*1,2,3,4 gave resistant reaction to charcoal rot. The resistance in *stg*1,2,3,4 genotype, however, was not as strong as that of Tx642 though both carry all the major QTL. This indicates the presence of other QTL loci in the Tx642 background that may have positive contribution to charcoal rot resistance but may not necessarily be to stay-green. The resistance reaction of the check genotype Tx430 which is not known to carry any of the four stg QTL but expresses the highest resistance to the disease confirms this hypothesis. However, *stg*2, *stg*4, *stg*2,4 do not seem to have any contribution to charcoal rot resistance. Moreover, they undermine the role of other QTL blocks such as *stg*1 and *stg*3 that were consistently associated with low charcoal rot infection when they occur in combination with them such as in *stg*1,2, *stg*1*,2,3* and *stg*1,2,4 where infection by *M. phaseolina* was apparently higher. This response was consistent across all test environments except only *stg*3 was more important in Manhattan 2012 and *stg*1 in Manhattan 2013 (Fig1-4). The role of the different *stg* QTL blocks seems to be slightly different for *F. thapsinum*. Unlike *M. phaseolina* where either *stg*1 or *stg*3 or their combination *stg*1,3 seem to effectively reduce the development of stalk rot disease, *stg*1 and *stg*3 need to be stacked in order to reduce infection by *F. thapsinum* and this does not seem to be affected by environment. However, it seems additional QTL blocks may be present that contribute to reduced infection both by *Macrophomina* and

Fusarium spp. Previous studies have ranked the contribution of the four QTL to the expression of stay-green trait as *Stg*2 > *Stg*1 > *Stg*3 >*Stg*4 (Xu et al., 2000). The trend for delayed onset of leaf senescence has been reported for *Stg*3 near-isogenic sorghum lines (Harris et al., 2007). With respect to disease resistance, this ranking doesn't hold true given that *stg*2 was apparently not important.

Contrary to the growing expectations, correlation between the disease scores, and dhurrin and leaf sugar levels were not significant. There was a tendency for genotypes with intermediate levels of dhurrin to have lower disease reactions to both pathogens especially for the parental genotypes Tx642 and Tx7000. Dhurrin is a cyanogenic glycoside located in the vacuole of the epidermal cells, whereas the catabolic enzymes (β-glucosidases and α-hydroxynitrilases) are in the mesophyll cells (Conn, 1991). In intact plant tissues, enzymes and substrates are kept separate in cells: only when plant tissues are lesioned or destroyed, as a consequence of biotic or abiotic factors, do enzymes and substrate come into contact, releasing the bioactive compound (hydrogen cyanide) that is involved in limiting plant infection.

In the Ottawa2013 environment, the genotype Tx7000 had the lowest dhurrin level (22 μg cm^{-2}). Tx7000 genotype also showed the highest lesion length for the disease related traits. While the dhurrin levels in the resistant genotypes (Tx642 and Tx430) were not the highest, they were more than double that observed in the genotype Tx7000. However, genotypes with the highest dhurrin levels in the leaves did not necessarily have lower lesion lengths as expected. Earlier studies have shown that, with regard to the interaction between plants and microorganisms, the release of hydrogen cyanide from cyanogenic glycosides may be more damaging to the plant than to the microorganism because of inhibition of phytoalexin production (Lieberei et al., 1989). In accordance, highly cyanogenic plants are likely to be preferred by some fungi and insects compared with plants with lower cyanogenic potential (Busk and Moller, 2002); Møller and Seigler, 1999). We did not observe significant differences between genotypes for dhurrin and leaf sugar levels at Manhattan 2013. This could be attributed to the growing environment. Leaf tissues at the Manhattan 2013 environment were collected after a series of heavy rainfall. The concentrations of dhurrin increases when plants are drought stressed (Gleadow et al., 2012; O'Donnell et al., 2013).

Conclusion

The results of this study confirm the relationship between post-flowering drought tolerance and resistance to stalk rot pathogens. However, not all genomic loci that confer tolerance to post-flowering drought stress are responsible for resistance to infection by stalk rot pathogens. In the current study, out of the four QTL blocks known to confer stay-green, only two of them *stg*1 and *stg*3 seem to have functional relationship with stalk rot resistance. Their mechanism of action seem to be different for the two pathogen groups with both loci needing to occur in one background in order to express resistance to *F. thapsinum* while either of the loci seem to work well to confer resistance against *M. phaseolina*. Moreover, there seems to be additional QTL loci that seem to contribute to resistance to both diseases. Hence future work besides optimizing the phenotyping assay should explore additional sources to discover new alleles that may further boost resistance to these diseases for use in breeding programs.

References

Borrell, A.K., Hammer, G.L., and Douglas, A.C.L. (2000a). Does maintaining green leaf area in sorghum improve yield under drought? I. Leaf growth and senescence. Crop Science 40, 1026-1037.

Borrell, A.K., Hammer, G.L., and Henzell, R.G. (2000b). Does maintaining green leaf area in sorghum improve yield under drought? II. Dry matter production and yield. Crop Science 40, 1037-1048.

Burke, J.J., Chen, J.P., Burow, G., Mechref, Y., Rosenow, D., Payton, P., Xin, Z.G., and Hayes, C.M. (2013). Leaf dhurrin content is a quantitative measure of the level of pre- and postflowering drought tolerance in sorghum. Crop Science 53, 1056-1065.

Busk, P.K., and Moller, B.L. (2002). Dhurrin synthesis in sorghum is regulated at the transcriptional level and induced by nitrogen fertilization in older plants. Plant Physiology 129, 1222-1231.

Conn, E.E. (1991). The metabolism of a natural product - lessons learned from cyanogenic glycosides. Planta Medica 57, S1-S9.

Crasta, O.R., Xu, W.W., Rosenow, D.T., Mullet, J., and Nguyen, H.T. (1999). Mapping of post-flowering drought resistance traits in grain sorghum: association between QTLs influencing premature senescence and maturity. Molecular and General Genetics 262, 579-588.

Dodman, R.L., Brengman, R.L., and Obst, N.R. (1992). The detection of resistance to stalk rot in sorghum. Proceedings of the second Australian sorghum conference, Gatton, 4-6 February 1992. Volume 2., 260-267.

Duncan, R.R. (1984). The association of plant senescence with root and stalk disease in sorghum. In: L.K. Mughogho (Ed.) Sorghum Root and Stalk Diseases: A Critical Review. Proceedings of the consultative discussion of Research Needs and Strategies for Control of Sorghum Root and Stalk Diseases (Bellagio, Italy, 27 November–2 December 1983). International Crops Research Institute for the Semi-Arid Tropics, Patancheru, India, pp.99–100.

Dykes, L., Rooney, L.W., Waniska, R.D., and Rooney, W.L. (2005). Phenolic compounds and antioxidant activity of sorghum grains of varying genotypes. Journal of Agricultural and Food Chemistry 53, 6813-6818.

Galyuon, I.K.A., Hash, C.T., Bidinger, F.R., Mahalakshmi, V., Folkertsma, R.T., and Howarth, C.J. (2005). Functioning under drought stress of stay-green sorghum developed by marker-assisted selection. Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology 141, S314-S314.

Ganjewala, D., Kumar, S., Devi, A.S., and Ambika, K. (2010). Advances in cyanogenic glycosides biosynthesis and analyses in plants: A review. Acta Biologica Szegediensis 54, 1-14.

Gleadow, R. M., Moldrup, M. E., O'Donnell, N. H., and Stuart, P. N. (2012). Drying and processing protocols affect the quantification of cyanogenic glucosides in forage sorghum. Journal of the Science of Food and Agriculture 92, 2234-2238.

Harris, K., Subudhi, P.K., Borrell, A., Jordan, D., Rosenow, D., Nguyen, H., Klein, P., Klein, R., and Mullet, J. (2007). Sorghum stay-green QTL individually reduce post-flowering drought-induced leaf senescence. Journal of Experimental Botany 58, 327-338.

Haussmann, B.I.G., Mahalakshmi, V., Reddy, B.V.S., Seetharama, N., Hash, C.T., and Geiger, H.H. (2003). QTL mapping of stay-green in two sorghum recombinant inbred populations. Theoretical and Applied Genetics 106, 133-142.

Helsel, D.B., and Frey, K.J. (1978). Grain yield variations in oats associated with differences in leaf area duration among oat lines. Crop Science 18, 765-769.

Henzell, R.G., Dodman, R.L., Done, A.A., Brengman, R.L. and Mayers, P.E. (1984). Lodging, stalk rot, and root rot in sorghum in Australia. In: L.K. Mughogho (Ed.) Sorghum Root and Stalk Diseases: A Critical Review. Proceedings of the consultative discussion of Research Needs and Strategies for Control of Sorghum Root and Stalk Diseases (Bellagio, Italy, 27 November–2 December 1983). International Crops Research Institute for the Semi-Arid Tropics, Patancheru, India, pp. 225–235.

Jenae, L.S., and Mitchell R.T. (2013). Relationship between dhurrin and stay-green in *sorghum bicolor* (L.) Moench. Poster session presented at the annual meeting of the ASA, CSSA, and SSSA, Nov. 3-6, 2013, in Tampa, FL.

Kassahun, B., Bidinger, F.R., Hash, C.T., and Kuruvinashetti, M.S. (2010). Stay-green expression in early generation sorghum *Sorghum bicolor* (L.) Moench QTL introgression lines. Euphytica 172, 351-362.

Kebede, H., Subudhi, P.K., Rosenow, D.T., and Nguyen, H.T. (2001). Quantitative trait loci influencing drought tolerance in grain sorghum (*Sorghum bicolor* L. Moench). Theoretical and Applied Genetics 103, 266-276.

Kumari, M., Pudake, R.N., Singh, V. P., and Joshi, A.K. (2013). Association of stay-green trait with canopy temperature depression and yield traits under terminal heat stress in wheat (*Triticum aestivum* L.). Euphytica 190, 87-97.

Léder, I. (2009). Sorghum and millets. Cultivated plants, primarily as food sources. In: G. Fuleky. (ed.), Encyclopedia of Life Support Systems (EOLSS). UNESCO.

Lieberei, R., Biehl, B., Giesemann, A., and Junqueira, N.T.V. (1989). Cyanogenesis Inhibits Active Defense Reactions In Plants. *Plant Physiology* 90, 33-36.

Liu, Y., Dun, B., Zhao, X., Yue, M., Lu, M., Li, G., Liu, Y., Dun, B. Q., Zhao, X.N., Yue, M. Q., Lu, M., and Li, G. Y. (2013). Correlation analysis between the key enzymes activities and sugar content in sweet sorghum (*Sorghum bicolor* L. Moench) stems at physiological maturity stage. Australian Journal of Crop Science 7, 84-92.

McBee, G.G. (1984). Relation of senescence, nonsenescence, kernel maturity to carbohydrate metabolism in sorghum. In: L.K. Mughogho (Ed.) Sorghum Root and Stalk Diseases: A Critical Review. Proceedings of the consultative discussion of Research Needs and Strategies for Control of Sorghum Root and Stalk Diseases (Bellagio, Italy, 27 November–2 December 1983). International Crops Research Institute for the Semi-Arid Tropics, Patancheru, India, pp. 119–129.

McBee, G.G., Waskom, R.M., Miller, F.R., and Creelman, R.A. (1983). Effect of senescence and nonsenescence on carbohydrates in sorghum during late kernel maturity states. Crop Science 23, 372-376.

Møller, B.L. and Seigler, D.S. (1999). Biosynthesis of cyanogenic glycosides, cyanolipids, and related compounds. In: Singh BK, editor. Plant Amino Acids. Biochemistry and Biotechnology. New York: Marcel Dekker. pp. 563–609.

Nahrstedt A. (1996). Relationships between the defense systems of plants and insects: the cyanogenic system of the moth Zygaena trifolii. In: Romeo JT, edited. Recent Advances in Phytochemistry. Vol. 33. New York: Plenum Press; pp. 217–230.

O'Donnell, N.H., Moller, B.L., Neale, A.D., Hamill, J.D., Blomstedt, C.K., and Gleadow, R.M. (2013). Effects of PEG-induced osmotic stress on growth and dhurrin levels of forage sorghum. Plant physiology and biochemistry 73, 83-92.

Rosenow, D.T. (1984). Breeding for resistance to root and stalk rot in Texas. In: L.K. Mughogho (Ed.) Sorghum Root and Stalk Diseases: A Critical Review. Proceedings of the consultative discussion of Research Needs and Strategies for Control of Sorghum Root and Stalk Diseases (Bellagio, Italy, 27 November–2 December 1983). International Crops Research Institute for the Semi-Arid Tropics, Patancheru, India, pp. 209–217.

Rosenow, D.T., and Clark, L.E. (1981). Drought tolerance in sorghum. In: H.D. Loden and D. Wilkinson (Eds.) Proceedings of the 36th Annual Corn and Sorghum Research Conference (Chicago, IL, 9–11 December 1981), pp. 18–31.

Sanchez, A.C., Subudhi, P.K., Rosenow, D.T., and Nguyen, H.T. (2002). Mapping QTLs associated with drought resistance in sorghum (*Sorghum bicolor* L. Moench). Plant Molecular Biology 48, 713-726.

Selmar, D., Lieberei, R., and Biehl, B. (1988). Mobilization and Utilization of Cyanogenic Glycosides - The Linustatin Pathway. Plant Physiology 86, 711-716.

Subudhi, P.K., Rosenow, D.T., and Nguyen, H.T. (2000). Quantitative trait loci for the stay-green trait in sorghum (*Sorghum bicolor* L. Moench): consistency across genetic backgrounds and environments. Theoretical and Applied Genetics 101, 733-741.

Tao, Y.Z., Henzell, R.G., Jordan, D.R., Butler, D.G., Kelly, A.M., and McIntyre, C.L. (2000). Identification of genomic regions associated with stay-green in sorghum by testing RILs in multiple environments. Theoretical and Applied Genetics 100, 1225-1232.

Tenkouano, A., Miller, F.R., Frederiksen, R.A., and Rosenow, D.T. (1993). Genetics of nonsenescence and charcoal rot resistance in sorghum. Theoretical and Applied Genetics 85, 644-648.

Tesso, T.T., Claflin, L.E., and Tuinstra, M.R. (2005). Analysis of stalk rot resistance and genetic diversity among drought tolerant sorghum genotypes. Crop Science 45, 645-652.

Tesso, T.T., Ochanda, N., Little, C.R., Claflin, L., and Tuinstra, M.R. (2010). Analysis of host plant resistance to multiple Fusarium species associated with stalk rot disease in sorghum *Sorghum bicolor* (L.) Moench. Field Crops Research 118, 177-182.

Thomas, H., and Smart, C.M. (1993). Crops that Stay-green. Annals of Applied Biology 123, 193-219.

Toman, J., and White, D.G. (1993). Inheritance of resistance to anthracnose stalk rot of corn. Phytopathology 83, 981-986.

Tuinstra, M.R., Grote, E.M., Goldsbrough, P.B., and Ejeta, G. (1997). Genetic analysis of post-flowering drought tolerance and components of grain development in *Sorghum bicolor* (L.) Moench. Molecular Breeding 3, 439-448.

VanOosterom, E.J., Jayachandran, R., and Bidinger, F.R. (1996). Diallel analysis of the stay-green trait and its components in sorghum. Crop Science 36, 549-555.

Vetter, J. (2000). Plant cyanogenic glycosides. Toxicon 38, 11-36.

Vijayalakshmi, K., Fritz, A.K., Paulsen, G.M., Bai, G., Pandravada, S., and Gill, B.S. (2010). Modeling and mapping QTL for senescence-related traits in winter wheat under high temperature. Molecular Breeding 26, 163-175.

Walulu, R.S., Rosenow, D.T., Wester, D.B., and Nguyen, H.T. (1994). Inheritance of the Stay-green Trait in Sorghum. Crop Science 34, 970-972.

Wang, A.Y., Li, Y., and Zhang, C.Q. (2012). QTL mapping for stay-green in maize (*Zea mays*). Canadian Journal of Plant Science 92, 249-256.

Xu, W.W., Subudhi, P.K., Crasta, O.R., Rosenow, D.T., Mullet, J.E., and Nguyen, H.T. (2000). Molecular mapping of QTLs conferring stay-green in grain sorghum (*Sorghum bicolor* L. Moench). *Genome* 43, 461-469.

You, S.C., Cho, S.H., Zhang, H., Paik, H.C., Lee, C.H., Li, J., Yoo, J.H., Lee, B.W., Koh, H.J., Seo, H. S., and Paek, N.C. (2007). Quantitative trait loci associated with functional stay-green SNU-SG1 in rice. Molecules and Cells 24, 83-94.

Zagrobelny, M., Bak, S., Rasmussen, A.V., Jorgensen, B., Naumann, C.M., and Moller, B.L. (2004). Cyanogenic glucosides and plant-insect interactions. Phytochemistry 65, 293-306.

Zheng, H.J., Wu, A. Z., Zheng, C.C., Wang, Y.F., Cai, R., Shen, X.F., Xu, R.R., Liu, P., Kong, L.J., and Dong, S.T. (2009). QTL mapping of maize (*Zea mays*) stay-green traits and their relationship to yield. Plant Breeding 128, 54-62.

Tables and Figures

Table 4.1. Sub-sample of Tx642 × Tx7000 RILs with the number and type of *stg* QTL blocks included in the study.

Genotypes	QTL number	Frequency
*Stg*1	1	4
*Stg*2	1	4
*Stg*3	1	4
*Stg*4	1	3
*Stg*1, 2	2	4
*Stg*2, 3	2	2
*Stg*2, 4	2	2
*Stg*3,4	2	4
*Stg*1,3	2	1
*Stg*1,4	2	3
*Stg*1,3, 4	3	4
*Stg*1,2,4	3	4
*Stg*1,2 ,3	3	2
*Stg*1,2,3, 4	4	1
Tx642	all	
Tx7000	0	
Tx430	check	

Table 4.2. Combined analysis of variance for stalk rot parameters, dhurrin and leaf sugars as tested under three environments.

Sources of variation	df	Mean squares					
		M. phaseolina	*F. thapsinum*	Dhurrin	Fructose	Glucose	Sucrose
Block	3	8.5	6.0	6412.4*	15534**	15792**	2207.4**
Env (E)	2	168.5**	29.7**	288.1	255.9	298.7	36.95
Genotype (G)	15	31.6**	27.5**	4249.5**	930.2	859	550.7*
QTL (Q)	5	47.4**	49.9**	8546.2**	495.7	650.2	671.9*
G x E	30	6.0	7.3*	2766.2**	1343.6	1279.4	515.5*
Q x E	10	5.8	6.6	2954.9*	932.7	1006.7	196.6
Error	421	5.4	4.5	1268.1	1021.7	1141.9	268.39

* Significance at $P < 0.05$, **Significance at $P < 0.001$

Table 4.3. Across environment mean disease rating (lesion length) following inoculation with *M. phaseolina* and *F. thapsinum* and leaf sugar content as affected by stg QTL blocks.

	F. thapsinum		*M. phaseolina*			Leaf sugars		
Genotypes	lesion length (cm)	Control (cm)	lesion length (cm)	Control (cm)	Dhurrin	Fructose $\mu g/cm^2$	Glucose $\mu g/cm^2$	Sucrose $\mu g/cm^2$
stg 1	6.0	2.3	4.6	1.7	75.5	106.2	111.0	25.5
stg 1,2	6.3	2.9	5.7	2.3	53.3	108.7	112.8	13.7
stg 1,2,3	6.5	3.0	6.0	2.3	90.6	111.1	120.7	19.2
stg 1,2,4	6.0	2.9	5.6	3.0	80.2	111.2	118.8	18.0
stg 1,3	5.0	2.4	4.2	2.1	39.4	122.6	124.2	11.5
stg 1,3,4	5.4	2.5	4.4	2.0	63.4	100.7	104.6	11.9
stg 1,4	5.5	2.7	5.2	1.7	71.5	101.9	106.3	13.1
stg 1,2,3,4	5.9	3.4	4.4	2.2	47.2	107.4	110.8	17.8
stg 2	7.1	2.9	5.6	2.3	69.1	116.7	118.7	14.3
stg 2,3	7.5	2.8	5.0	2.4	69.3	108.5	113.9	11.1
stg 2,4	6.7	2.8	6.9	2.7	47.9	107.8	114.7	11.6
stg 3	5.6	2.9	4.2	2.3	70.5	94.2	100.9	30.8
stg 3,4	5.9	2.9	4.5	2.2	62.7	101.6	106.8	19.2
stg 4	7.8	3.0	6.2	3.1	76.1	102.5	108.4	17.1
BTx642	3.3	1.6	3.2	1.6	63.9	119.7	124.4	14.2
Tx7000	7.8	3.2	7.8	2.4	40.3	107.7	110.6	11.5
MEAN	6.2	2.8	5.2	2.3	64.6	108.2	113.2	16.6
LSD	1.1	0.6	1.9	0.5	22.9	ns	ns	10.5

[a]LSD- least significant difference; ns –not significant

Table 4.4. Mean lesion length of recombinant inbred genotype groups varying for stay-green QTL as tested against infection by Fusarium thapsinum and Macrophomina phaseolina at Manhattan KS during 2012 crop season.

	F. thapsinum		*M. phaseolina*	
Genotypes	lesion length (cm)	Control (cm)	lesion length (cm)	Control (cm)
stg 1	6.0	2.3	4.2	1.4
stg 1,2	6.2	3.1	4.7	2.8
stg 1,2,3	5.0	2.4	5.7	2.9
stg 1,2,4	5.5	2.6	5.7	2.6
stg 1,3	3.4	2.7	2.6	2.0
stg 1,3,4	4.8	1.3	3.0	2.5
stg 1,4	3.9	2.1	3.2	1.8
stg 1,2,3,4	6.1	2.0	4.3	2.3
stg 2	6.5	3.6	4.9	2.3
stg 2,3	6.7	2.5	3.0	2.5
stg 2,4	6.9	2.6	4.6	2.6
stg 3	4.8	2.7	3.0	2.9
stg 3,4	5.5	1.4	4.1	2.4
stg 4	8.3	2.7	6.6	3.2
BTx642	3.9	1.5	3.4	2.0
Tx7000	7.7	2.8	6.9	2.0
MEAN	5.7	2.4	4.3	2.5
LSD	1.3	0.4	1.3	0.6

[a]LSD- least significant difference; ns –not significant

Table 4.5. Mean lesion length of recombinant inbred genotype groups varying for stay-green QTL as tested against infection by Fusarium thapsinum and Macrophomina phaseolina at Ottawa, KS during 2013 crop season.

	F. thapsinum		*M. phaseolina*			Leaf sugars		
Genotypes	Lesion length (cm)	Control	Lesion length (cm)	Control	Dhurrin µg/cm^2	Fructose µg/cm^2	Glucose µg/cm^2	Sucrose µg/cm^2
stg 1	5.0	2.0	5.6	1.9	73.6	116.0	120.6	19.8
stg 1,2	5.6	2.6	6.4	2.3	42.6	113.8	118.0	11.2
stg 1,2,3	6.6	2.5	5.5	1.7	106.2	113.6	123.7	21.2
stg 1,2,4	6.2	2.7	6.2	2.6	95.4	111.0	119.7	17.3
stg 1,3	4.0	2.4	5.2	2.3	27.3	125.5	123.6	9.8
stg 1,3,4	6.9	2.9	6.4	2.5	56.8	104.4	109.2	13.0
stg 1,4	6.9	2.6	5.6	1.6	74.0	91.0	94.9	9.9
stg 1,2,3,4	5.3	2.6	5.0	2.1	43.1	106.0	109.5	18.1
stg 2	6.8	2.5	6.5	2.3	71.7	125.4	125.2	12.9
stg 2,3	8.6	2.3	6.7	2.3	70.6	116.8	123.2	11.7
stg 2,4	5.8	2.6	8.1	2.3	30.9	106.8	110.3	8.9
stg 3	5.8	2.5	4.4	1.9	63.4	76.0	82.3	49.2
stg 3,4	6.0	2.8	5.1	2.3	76.6	91.5	99.9	20.4
stg 4	7.3	2.9	6.6	2.6	97.2	101.5	108.3	24.3
BTx642	3.7	1.4	3.4	1.4	73.1	108.3	113.9	12.2
Tx430	2.3	1.3	2.5	1.0	52.1	165.9	181.6	6.7
Tx7000	8.1	3.3	8.7	2.9	22.0	96.0	97.4	10.5
MEAN	6.5	2.5	6.3	2.1	65.3	108.8	113.9	16.7
LSD	2.3	0.9	2.5	0.8	30.3	29.2	31.0	15.1

[a] LSD- least significant difference; ns –not significant

Table 4.6. Mean lesion length of recombinant inbred genotype groups varying for stay-green QTL as tested against infection by Fusarium thapsinum and Macrophomina phaseolina at Manhattan, KS during 2013 crop season.

	F. thapsinum		*M. phaseolina*			Leaf sugars		
Genotypes	lesion length (cm)	Control	lesion length (cm)	Control	Dhurrin µg/cm^2	Fructose µg/cm^2	Glucose µg/cm^2	Sucrose µg/cm^2
stg 1	6.8	2.2	3.4	1.8	77.5	96.4	101.5	31.2
stg 1,2	6.7	2.5	5.7	2.0	63.9	103.6	107.6	16.1
stg 1,2,3	6.8	2.8	5.7	2.3	75.0	108.5	117.6	17.2
stg 1,2,4	6.0	2.8	4.7	2.5	65.1	111.4	117.9	18.7
stg 1,3	4.4	2.4	4.3	1.6	51.6	119.7	124.7	13.2
stg 1,3,4	4.1	2.3	3.4	1.6	70.0	96.9	99.9	10.8
stg 1,4	4.5	2.6	5.5	1.6	69.0	112.8	117.7	16.4
stg 1,2,3,4	5.5	2.8	3.6	1.8	51.3	108.7	112.1	17.5
stg 2	7.5	2.7	5.0	2.2	66.5	108.0	112.2	15.7
stg 2,3	6.3	2.8	4.6	2.4	67.9	100.2	104.5	10.4
stg 2,4	7.1	3.0	7.8	3.1	64.8	108.9	119.1	14.2
stg 3	6.4	2.9	4.8	1.9	77.7	112.4	119.5	12.5
stg 3,4	5.8	2.5	4.0	1.9	48.9	111.7	113.6	18.1
stg 4	7.1	2.8	4.9	2.0	55.0	103.5	108.5	9.8
BTx642	2.6	1.6	2.7	1.5	61.7	131.2	135.0	16.2
Tx430	2.5	1.3	2.3	1.0	70.7	95.2	105.3	33.5
Tx7000	7.3	2.6	6.8	2.0	58.5	119.3	123.7	12.6
Mean	6.1	2.5	4.7	2.0	65.3	108.8	113.9	16.7
LSD	1.9	0.7	2.3	0.8	37.7	31.9	33.8	16.6

[a]LSD- least significant difference; ns –not significant

Table 4.7. Pearson correlation coefficients between lesion length, dhurrin and leaf sugars across genotypes with varying stay-green QTLs evaluated against infection by *Fusarium thapsinum* and *Macrophomina phaseolina*.

	Correlation (*r*)					
Traits	MLM[a]	MLF[b]	Dhurrin	Fructose	Glucose	Sucrose
MLM	-					
MLF	0.49***	-				
Dhurrin	-0.05	0.03	-			
Fructose	-0.06	0.00	-0.09	-		
Glucose	-0.05	0.01	-0.03	0.99***	-	
Sucrose	-0.11*	-0.04	0.41***	-0.29***	-0.25***	-

* Significantly different from zero ($P \leq 0.05$);
*** significantly different from zero ($p < 0.001$)
[a] Lesion length for *F. thapsinum*
[b] Lesion length for *M. phaseolina*

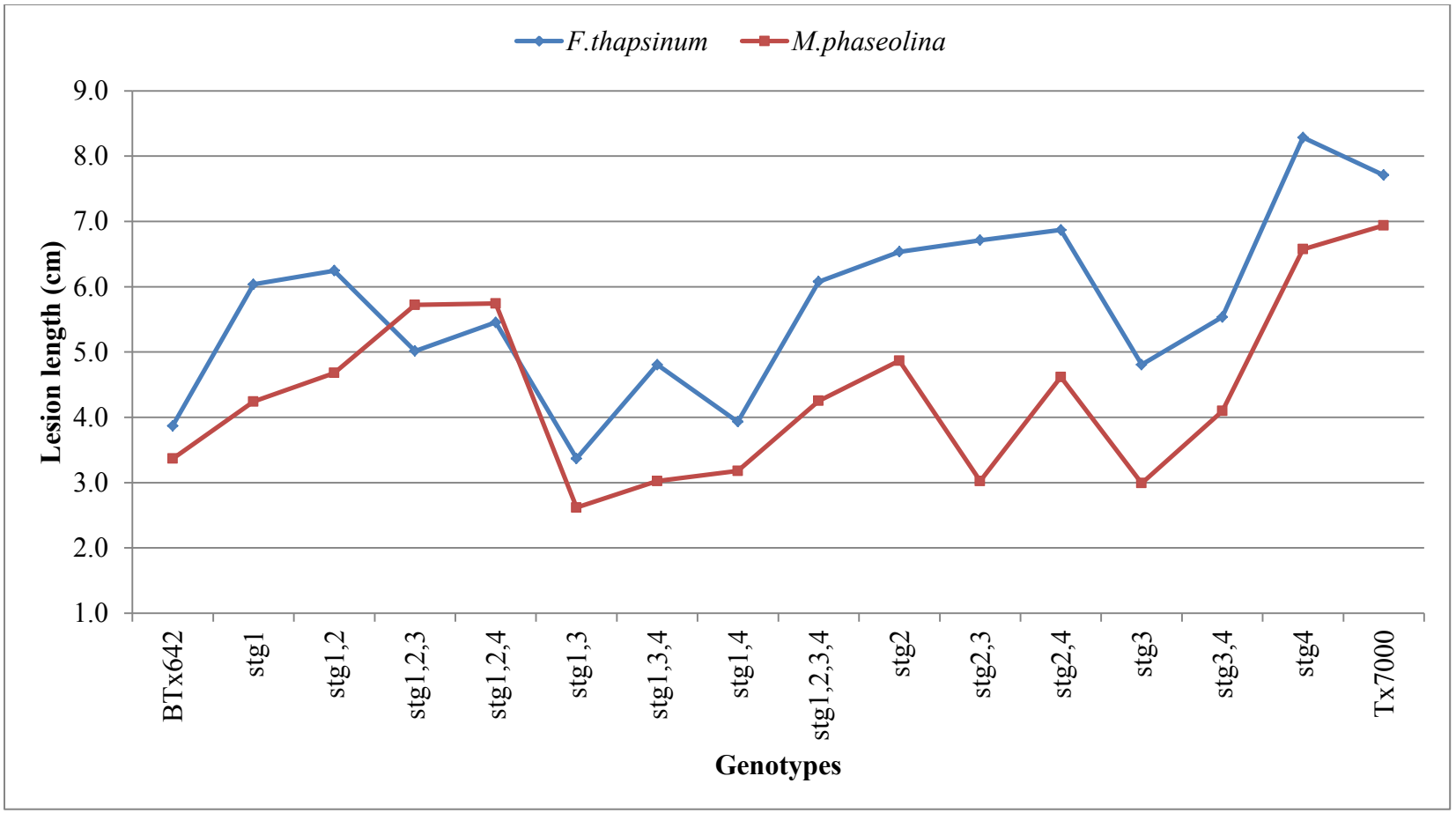

Figure 4.1. Mean lesion length of recombinant inbred lines varying at stay-green QTL loci as compared to the parental checks at Manhattan 2012

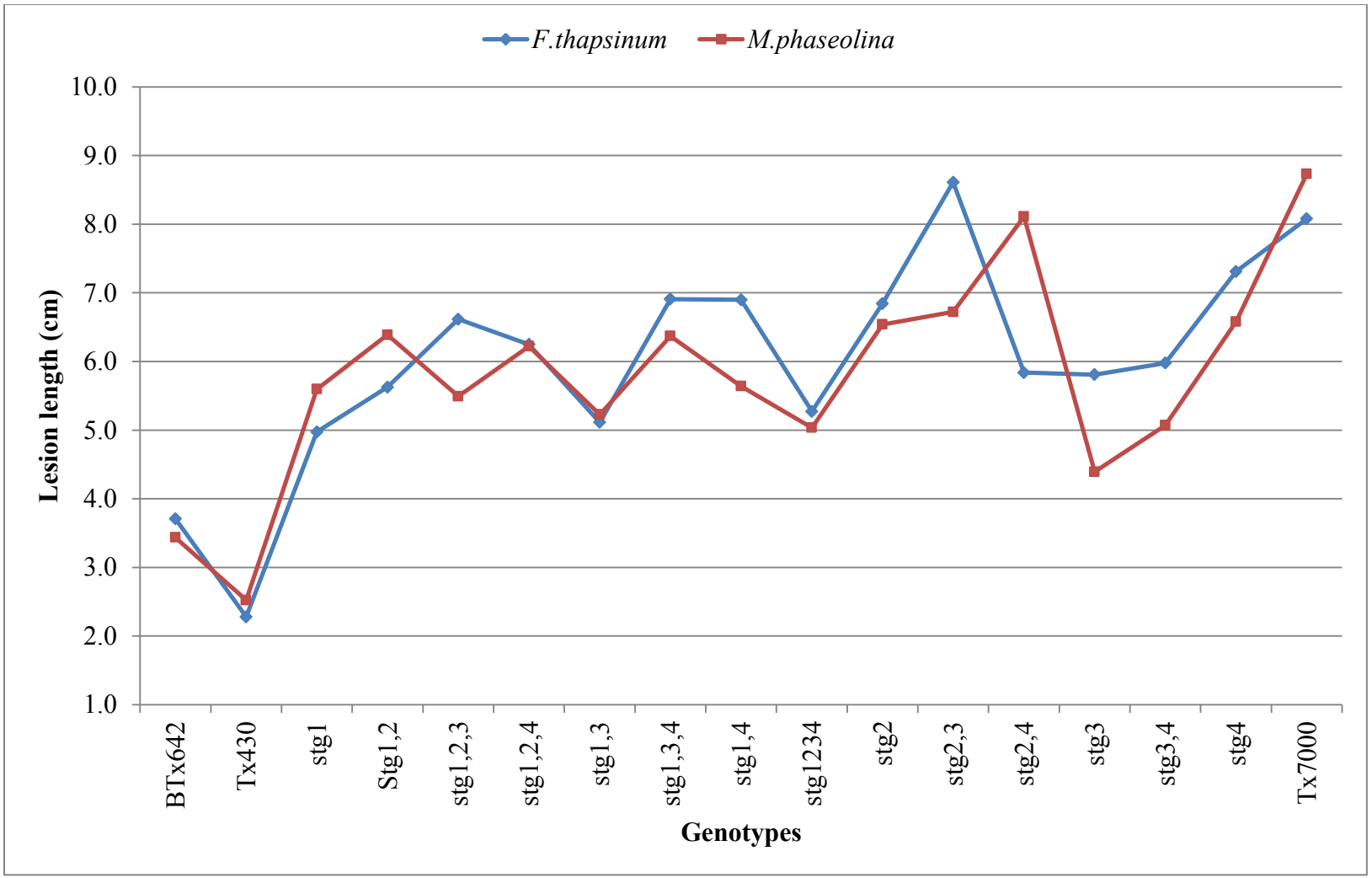

Figure 4.2. Mean lesion length of recombinant inbred lines varying at stay-green QTL loci as compared to the parental checks at Ottawa 2013

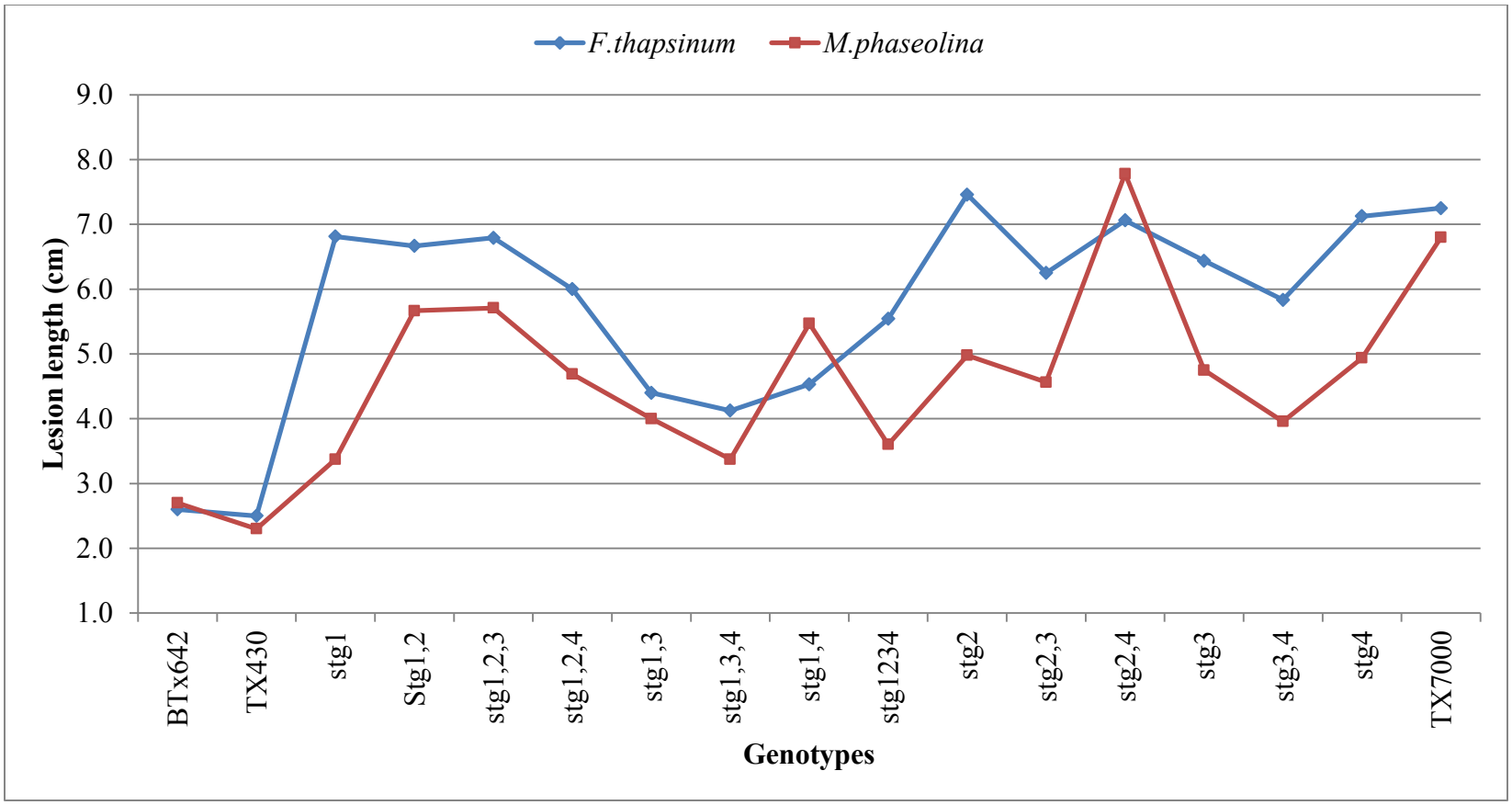

Figure 4.3. Mean lesion length of recombinant inbred lines varying at stay-green QTL loci as compared to the parental checks at Manhattan 2013.

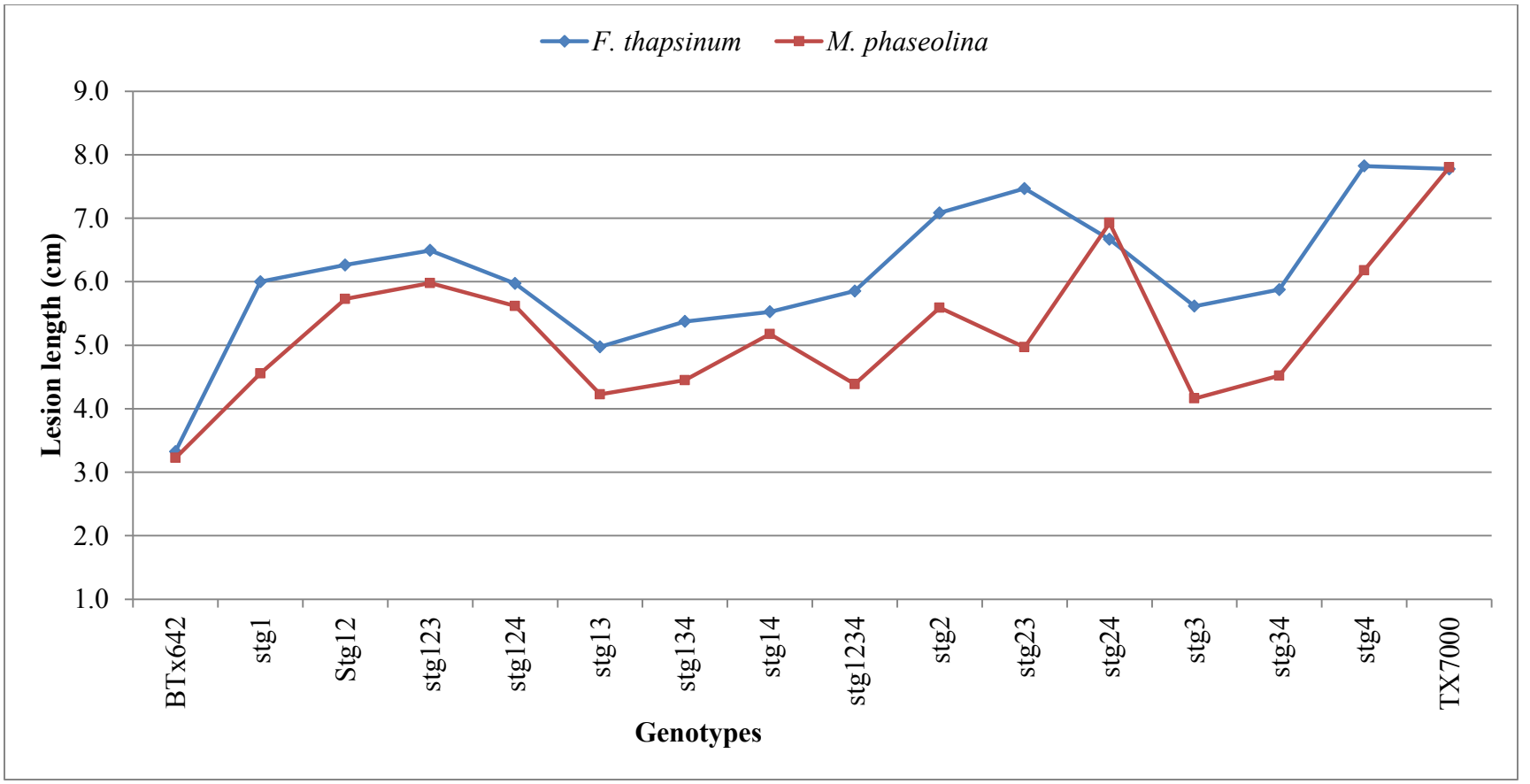

Figure 4.4. Across environment combined mean lesion length of recombinant inbred lines varying at stay-green QTL loci as compared to the parental checks.

Appendix A - Number of nodes crossed and Number of Stg QTL Blocks

Table A.1. Number of nodes crossed following inoculation with *M. phaseolina* and *F. thapsinum* as affected by stg QTL blocks.

	Manhattan 2012		Manhattan 2013		Ottawa 2013		Combined environment	
Genotypes	NNCF[a]	NNCM[b]	NNCF	NNCM	NNCF	NNCM	NNCF	NNCM
stg 1	1.5	0.3	1.4	0.2	0.9	0.9	1.1	0.5
stg 1,2	1.2	0.7	1.5	0.9	1.2	1.3	1.3	1.1
stg 1,2,3	1	0.7	1.4	0.9	1.3	0.8	1.3	0.9
stg 1,2,4	1.3	0.7	1	0.3	1	0.8	1	0.6
stg 1,3	0.1	0	1.2	0.6	1	1	1.1	0.8
stg 1,3,4	1.4	0.2	1	0.6	0.6	1	0.8	0.8
stg 1,4	0.2	0	0.8	0.1	1.8	1.2	1.3	0.7
stg 1,2,3,4	1.4	0.4	0.5	0.9	0.6	0.3	0.6	0.6
stg 2	1.5	0.4	1.7	0.5	1.2	0.9	1.4	0.7
stg 2,3	1.1	0	1.4	0.5	1.8	1.4	1.6	0.9
stg 2,4	1.7	0.6	1.2	1.3	0.6	1	0.9	1.1
stg 3	1.5	0.1	1.4	0.6	1.2	0.3	1.3	0.5
stg 3,4	1.2	0.5	1.2	0.4	1.2	1	1.2	0.7
stg 4	1.9	0.9	1.4	0.5	1.6	1	1.5	0.8
BTx642	0.6	0	0.4	0.2	0.1	0.3	0.2	0.3
Tx7000	0.3	1.3	1	0.3	1.5	2	1.3	1.1
Mean	1.1	0.4	1.1	0.5	1	0.9	1.1	0.7
[c]LSD	ns	ns	ns	ns	ns	ns	ns	ns

[a]Number of nodes crossed by *Fusarium thapsinum*

[b]Number of nodes crossed by *Macrophomina phaseolina*

[c]LSD- least significant difference; ns –not significant

Table A.2. Mean disease rating (lesion length) following inoculation with *M. phaseolina* and *F. thapsinum* and leaf sugar content as affected by the number of stg QTL blocks

	F. thapsinum		*M. phaseolina*			Leaf sugars		
Genotypes	lesion length (cm)	Control	lesion length (cm)	Control	Dhurrin µg/cm^2	Fructose µg/cm^2	Glucose µg/cm^2	Sucrose µg/cm^2
Combined Analysis								
1	5.1	2.3	6.6	2.7	72.7	106.2	110.8	21.5
2	5.3	2.2	6.2	2.8	57.4	108	112.6	13.8
3	5.5	2.5	6	2.8	79.9	108.5	115.9	16.9
4	4.4	2.2	5.9	3.4	47.2	107.4	110.8	17.8
BTx642	3.2	1.6	3.3	1.6	63.9	119.7	124.4	14.2
Tx7000	7.8	2.4	7.8	3.2	40.3	107.7	110.6	11.5
Mean	5.2	2.3	6.2	2.8	64.6	108.2	113.2	16.6
[a]LSD	1	0.4	0.9	0.5	18.7	ns	ns	8.7
Manhattan 2012								
1	6.4	2.8	4.7	2.4				
2	5.8	2.3	4	2.5				
3	5.1	2.5	5.1	2.8				
4	6.1	2.6	4.3	2.6				
Mean	5.8	2.4	4.7	2.4				
LSD	ns	ns	ns	ns				
Ottawa 2013								
1	6.5	2.4	6.3	2.2	75.7	107.9	111.9	24.5
2	6.6	2.6	6.5	2.2	54.5	106.9	111.3	12.5
3	7.1	2.7	6.6	2.2	89.8	110.3	118.6	17.7
4	5.7	2.6	5.4	2.1	43.1	106	109.5	18.1
Mean	6.6	2.6	6.4	2.2	67.7	107.9	113	17.6
LSD	ns	ns	ns	ns	20.4	ns	ns	9.9
Manhattan 2013								
1	7	2.6	4.4	2	69.7	104.5	109.7	18.5
2	6	2.6	5.5	2.1	60.4	109.2	114	15.1
3	5.8	2.6	4.7	2.2	70	106.7	113.3	16.1
4	5.5	2.8	3.6	1.8	51.3	108.7	112.1	17.5
Mean	6.2	2.6	4.9	2.1	64.5	107.3	112.4	16.5
LSD	ns	ns	ns	ns	ns	ns	ns	ns

[a]LSD- least significant difference; ns –not significant