# SORGHUM IMPROVEMENT AS BIOFUEL FEEDSTOCK: JUICE YIELD, SUGAR CONTENT AND LIGNOCELLULOSIC BIOMASS

by

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## Abstract

Sorghum [*Sorghum bicolor* (L.) Moench] is listed as one of the potential feedstock sources for biofuel production. While sorghum grain can be fermented into ethanol in a similar way as maize, the greatest potential of the crop is based on its massive biomass and sugar rich juices. Thus development of the crop as alternative energy source requires improvement of these traits. The objectives of this study were (1) to determine the mode of inheritance of traits related to ethanol production and identify suitable genetic sources for use in breeding programs, and (2) to evaluate the potential of low lignin mutations for biomass feedstock production and assess biotic stress risks associated with deployment of the mutations. The study consisted of three related experiments: (i) estimating the combining ability of selected sweet and high biomass sorghum genotypes for biofuel traits and resistance to stalk lodging, (ii) determine the impact of brown mid-rib mutations on biofuel production and their reaction to infection by *Macrophomina phaseolina* and *Fusarium thapsinum*, and (iii) assess the reaction of low lignin mutants to green bug feeding. In the first experiment six sorghum genotypes of variable characteristics (PI193073, PI257602, PI185672, PI195754, SC382 and SC373) were crossed to three standard seed parent lines ATx3042, ATx623 and ATx399. The resulting hybrids and the parents were evaluated at four locations, three replications during 2009 and 2010 seasons. Data were collected on phenology, plant height, juice yield, °brix score and biomass production. In the second experiment, two brown mid-rib mutations (*bmr6* and *bmr12*) and their normal versions were studied in four forage sorghum backgrounds (Atlas, Early Hegari, Kansas Collier and Rox Orange). The experiment was planted in four replications and at 14 d after flowering five plants in a plot were artificially infected with *F. thapsinum* and another five with *M. phaseolina*. The plants were harvested and rated for disease severity (lesion length and nodes crossed). Another

five normal plants in each plot were harvested and used to determine biofuel traits (juice yield, °brix score and biomass). In the third experiment, a subset of entries evaluated in experiment II and three tolerant and susceptible checks were tested for greenbug feeding damage. Biotype K greenbug colony was inoculated to each genotype using double sticky foam cages. Feeding damage was assessed as percent chlorophyll loss using SPAD meter. There was significant general combining ability (GCA) effect among the male entries for juice yield, stem °brix and biomass production indicating that these traits are controlled by additive genes. Lines PI257602 and PI185672 in particular, had the highest GCA for all the traits and should serve as excellent breeding materials. There was no significant difference among the *bmr* mutants and between the *bmr* and normal genotypes for both stalk rot and greenbug damage. In conclusion, juice yield, °brix and biomass are largely controlled by additive genes and hence are amenable to genetic manipulation. The *bmr* mutations despite their impact on lignin content do not increase risk of attack by stalk rot pathogens and greenbugs and thus can be deployed for biofuel production without incurring losses to these factors.

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# Introduction

The increasing price of oil and gas due to decreasing supply of fossil fuels has created a worldwide need to identify and develop alternative sources of energy.  Moreover, the heightening of global warming as a consequence of excessive fossil fuel burning increased the importance of new and eco-friendly sources of energy (Rooney et al., 2007).  In the United States, the energy bill enacted in 2005 mandated the increased use of renewable fuels. Encouraged by the new policy, many energy companies took the opportunity to explore and develop novel sources of renewable energy. Biofuels are among the priority energy alternatives being pursued. Starch-based ethanol from maize and sorghum grains is already being used as transportation fuel.  However, the overall contribution of the grain based ethanol to the total energy demand is very low that even if all of the maize and sorghum grains were converted to ethanol, it would still fall far short of the demand at this time. Therefore, other sources including, cellulosic biomass and sugary juices from stalks of crops such as sorghum and sugarcane are of significant importance to increasing bioethanol production.

Sweet and biomass sorghums in the United States are primarily produced for syrup production (Hill et al., 1990) and as forage and silage especially in the dairy industry (Oliver et al., 2005; McCollum et al., 2005). The growing need for renewable energy sources derived both by the declining supply of fossil fuel and the growing environmental concern has elevated the value of these crops and they have become among the major feedstock sources for biofuel production. Both the dry matter produced from high biomass sorghums and the sugary juice extracted from sweet sorghum stalks are among the major raw materials for bio-fuel production. Sorghum possesses unique genetic traits, low lignin mutations that can reduce the cost and at the same time increase the efficiency of converting lignocellulosic biomass to fermentable sugar

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(Saballos et al., 2008).  Also, the sugary juices can be directly fermented to ethanol in much the same way as sugarcane. In fact, sweet sorghum juice contains high percentages of reducing sugars which prevents crystallization and thus increases fermentation efficiency up to 90% (Ratnavathi et al., 2004a). The growing period, water requirement and management costs are four times lower in sweet/biomass sorghum than in sugarcane (Reddy et al., 2007; McCollum et al., 2005).

However, both production of the crop and exploitation of its potential as biofuel source of require development of unique cultivars suited for this use.  Germplasm sources carrying priority biofuel traits needs to be identified and the behavior of these traits in the selected sources carefully understood before they are included in breeding programs. Moreover, introduction of new germplasm sources may affect the pest and disease dynamics. Although previous research on grain sorghum improvement has successfully managed threats posed by major insect pests, diseases and environmental stresses, including downy mildew, greenbugs and drought/stalk rot induced lodging, it is likely that many of the germplasm sources targeted for biofuel sorghum improvement may not possess these traits. Therefore, this work primarily focuses on understanding of the mechanism of inheritance of selected biofuel traits such as stalk juice yield, stem sugar content (°brix), biomass production, and resistance to lodging and identification of potential germplasm sources for use in breeding program. It is also aimed at evaluating the potential risks of stalk rot included lodging and damage by greenbug feeding associated with deployment of low lignin mutations.

# Chapter 1 - Literature Review

## Improving Sweet sorghum for biofuel production

Sweet sorghum also called 'sorgo' in the United States is a special purpose sorghum (*Sorghum bicolor* (L.) Moench) known for its sweet juicy stems (Harlan and deWet, 1972). In the tropical regions, sweet sorghums are grown for fresh chewing of the sucrose rich stalks, and more recently for ethanol production, especially in Brazil and India (House et al., 2000). One of the most important characteristics of sweet sorghum is its adaptability to varying climatic and soil conditions (Ali et al., 2008). Just like grain sorghum, sweet sorghums are also tolerant to marginal conditions including drought, water logging and saline/alkali soils (Reddy, 2003). Naturally adapted to hot and dry conditions, all sorghums generally require less water and thrive well under low input conditions and this makes them the most preferred crop in the semi-arid tropics where the largest acreage of the crop is concentrated (Rooney et al., 2000).

Sweet sorghum is considered better suited for ethanol production compared with sugarcane (Huligol et al., 2004). Firstly, it has higher proportion of reducing sugar which prevents crystallization of sugars and thus results in improved fermentation efficiency that can be as high as 90% is certain cultivars (Ratnavathi et al., 2004a). Furthermore, the bagasse after the extraction of sugary juice has higher biological value than that of sugarcane when fed to animals as it is richer in proteins, micronutrients and minerals (Seetharama et al., 2002). Studies have shown that its relatively short growing period (about 4.5 months), lower water requirements (about 8000 $m^3$ $ha^{-1}$over two crops) (Soltani and Almodares, 1994) and management cost that is four times less than sugarcane (Reddy et al., 2007) makes sweet sorghums a crop of high potential for biofuel production. Because of its high biomass, reasonably good grain yield and of

course the sweet stalks, sorghum is often considered as having greater potential than maize (Hills et al., 1987; Rothman and Calle, 1983).

The growing need to develop alternative energy sources to replace fossil fuels sparked interest in the use of sorghum as one of the major feedstock sources. As a result, research activities to improve sorghum for biofuel production have increased in recent years. Breeding programs are aiming at developing sweet sorghum hybrids with improved juice yield and sugar content among others.  Although, hybrid grain sorghum has been around since the early 1960s, there has not been much effort exerted to develop sweet sorghum hybrids. Most of the sweet sorghum varieties under production at present are pure-line or open pollinated type apart from the sorghum × sudangrass hybrids grown for forage and silage.

Earlier work by Clark (1987) described the heritability of fermentable carbohydrates in sorghum stalks as complex and thus breeding for the trait would require intensive effort. Nonetheless, success in developing sweet sorghum hybrids has already been achieved in India and these hybrids are now being used for ethanol production in that region (Mandke, 2007).  In the United States, sweet sorghum germplasm improvement and hybrid development efforts in a number of public and private programs are beginning to show promising results (Rooney et al., 2007). Ritter et al. (2008) and Guiying et al. (2000) reported that stalk sugar is under the control of recessive genes with additive and dominance effects.  Ayyangar et al. (1936) suggested a single dominant gene conferring the non-sweet character.  Swanson and Parker (1931) reported that stalk juiciness was can be controlled by single recessive gene which seems to agree with this observation. But later studies, provided support for the existence of multiple genes with additive effects (Li et al., 2004).  Continuous variation in the amount of extractable juice is observed in juicy genotypes and inbred progeny of juicy × dry lines, suggesting multiple genes may be

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involved in controlling the trait (Saballos, 2008).  Makanda et al. (2009) reported significant

general combining ability (GCA) effect for stem °brix and associated traits implying the

importance of additive gene action. Similarly, the recent work by Corn (2008) suggests the

involvement of several genes affecting the biofuel traits in sweet sorghum background. In this

study that consisted fixed set of parents, better parent heterosis ranged from -24% to 7% for stem

°brix, and 27% to 43% for stem biomass production indicating that multiple genes are

responsible for these traits. However, these results do not necessarily give general indication of

the behavior of these genes at different environments and in different genetic backgrounds.

The primary step in hybrid production is proper selection of seed and pollinator parents.

Combining ability estimates have been widely used by breeders to predict the suitability of a

given line for use in breeding for a given trait. General combining ability (GCA) is the average

performance of a line estimated on the basis of the performance of its progenies generated from a

cross of that line to several other lines (Falconer, 1989). Specific combining ability (SCA) is the

deviation in performance of a specific hybrid from the sum of the overall mean of entries

involved and the general combining ability of its two parental lines.  GCA of each parent should

be examined when the objective is to develop superior genotypes, while SCA effects provide

information about the performance of hybrids (Cruz and Regazzi, 1994).  The differences in

GCA are mainly due to additive genetic effects and higher order additive interactions, while the

differences in SCA are attributed to the non-additive dominance and other types of epistasis

(Falconer, 1989).  Given this information, the breeder can select desirable parents or determine

the breeding procedure that will efficiently improve the performance of the traits of interest

(Dudley and Moll, 1969).

# Brown Midrib Mutations: their role for forage production and cellulosic biofuel feedstock

Besides the grain, sorghum is also grown as one of the major forage crops in the United States (Oliver et al., 2005). The interest in the growing use of sorghum as forage/silage seems to come from the natural characteristics of the crop. Its high biomass, sweet stalks, faster growth rates and good regrowth are among the merits of sorghum as forage crop. Like most sorghum types, forage sorghums also utilize water more efficiently, yield greater biomass and provide acceptable yields under limited water and nutrient conditions (Sanderson et al., 1992; Pederson et al., 2002). Three types of forage sorghums are grown: sudangrass, sorghum and sorghum × sudangrass hybrids. Sudan grass forage is fine stemmed, leafy and tillers profusely. It produces little seeds and has very quick regrowth. Sorghum (forage type) has sweet juicy stems, higher dry matter yields, large stalks, sparse tillers and limited regrowth capacity. Although they have potential to produce significant grain, there is considerable variation among different genotypes with grain yield ranging from 0 to 40% of the dry matter yield. Sorghum × sudangrass hybrids are intermediate in texture but retain high yield potential of the sorghum parent. Their leaf to stem ratio is lower than 50%. They are utilized for green chop or silage and as coarse hay for overwintering cattle (Pederson et al., 2002). The introduction of low lignin mutants also known as brown midrib (*bmr*) sorghums in to forage sorghum production has remarkably improved the value of forage or silage through improving dry matter digestibility (Akin et al., 1986; Grant et al., 1995; Wedig et al., 1987). Silage from brown midrib sorghum with and without protein supplements was shown to significantly increase milk yield in lactating cows (Frenchik et al., 1976; Keith et al., 1979; Oba and Allen, 1999). However, the effect of *bmr* mutations on forage quality varies depending on background (Cherney et al., 1991). The low lignin content in *bmr*

genotypes are resulted from altered lignin chemical composition (Bucholtz et al., 1980; Cherney et al., 1991; Vogel and Jung, 2001). The *bmr* mutants are characterized by the reddish-brown coloration of the vascular tissue of the leaf blade, leaf sheath and stem (Saballos et al., 2008). The *bmr* phenotype becomes apparent once the plants reach four-leaf stage and begins to fade as the plants approach physiological maturity (Porter et al., 1978).

The first *bmr* lines were developed through artificial mutagenesis (Porter et al., 1975); but several spontaneous mutants have been discovered later. *Bmr6, bmr12,* and *bmr18* happen to occur in backgrounds that are agronomically more acceptable and thus have been widely utilized in forage sorghum breeding program (Fritz et al., 1988). Allelic examination on these mutants revealed that *bmr12* and *bmr18* were allelic (Bittinger et al., 1981) but *bmr6* and *bmr12* occupy independent loci (Gupta, 1995). Several other *bmr* alleles are also allelic to one of these and the *bmr2* allelic group. Recent research has identified two separate enzymes that exhibit reduced activity as the result of the *bmr* mutations. *Bmr12, bmr18* and *bmr26* contain premature stop codons in the lignin biosynthetic enzyme caffeic acid O-methyl-transferase (COMT) (Bout and Vermerris, 2003). The *bmr6* is associated with reduced cinnamyl alcohol dehydrogenase (CAD) activity (Bucholtz et al., 1980; Pillonel et al., 1991). CAD is a member of the alcohol dehydrogenase family of proteins that catalyzes the conversion of the hydroxycinnamoyl aldehydes into alcohols prior to their incorporation into lignin polymers. Reduced CAD activity results in increased digestibility on dry weight basis, altered cell wall architecture, reduced lignin level and the incorporation of phenolic aldehydes into lignin in sorghum and maize (Pillonel et al., 1991; Provan et al., 1997; Halpin et al., 1998; Marita et al., 2003; Shi et al., 2006; Palmer et al., 2008).

The same biological events that led to improved dry matter digestibility in *bmr* sorghums have also been shown to contribute to improved efficiency of converting lignocellulosic biomass in to fermentable sugar (Dien et al., 2006; Chen and Dixon, 2007). Fermentation of ethanol from lignocellulosic sources requires a pretreatment step to remove the enzyme resistant lignin to provide access to the hydrolysable cell wall components (cellulose and hemi-cellulose) (Moiser et al., 2005a; Moiser et al., 2005b; Corredor et al., 2009). The low lignin content of *bmr* genotypes should thus reduce the negative effect of lignin in the conversion of biomass into ethanol and thereby improve ethanol yield or reduce the cost of producing ethanol by avoiding or reducing the need for the pretreatment step (Dien et al., 2009). Previous studies have shown that *bmr* sorghums have improved conversion rate, and yielded higher fermentable sugar than their wild-type versions. Glucose yields from biomass crops with *bmr6* and *bmr12* alleles were reported to be higher by 27% and 23%, respectively, compared to their wild-types. Conversion of cellulose to ethanol of pre-treated sorghum biomass was also improved by 22% and 21% for *bmr6* and *bmr12*, respectively (Dien et al., 2009).

## Association between brown midrib mutation and stalk rot resistance

### *Fusarium stalk rot*

Fusarium stalk rots, caused by multiple *Fusarium spp.*, have been among the major diseases of sorghum in the United States (Edmonds and Zummo, 1975; Duncan, 1983; Leslie et al., 1990; Jardine and Leslie, 1992). The disease is also important in many other places where the crop is cultivated including Africa (Frowd, 1980; Zummo, 1980; Omar et al., 1985), India (Khune et al., 1984) and Australia (Henzell et al., 1984). Isolates of the former *F. moniliforme*

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complex cited as the major causal agent of Fusarium stalk rot has been recently re-established as independent species including *F. verticillioides, F. andiyazi, F. thapsinum, F. brevicatenulum, F. pseudoanthophilum, F. pseudonygamai, F. proliferatum and F. nygamai* (Leslie, 1991; Klittich et al., 1992; Klittich and Leslie, 1992; Leslie, 1992; Leslie, 1995; Marasas et al., 2001). Many of these pathogens do attack sorghum but recent studies confirm *F. thapsinum* as the most aggressive pathogen of sorghum (Tesso et al., 2005; Tesso et al., 2011). In Kansas, average yield reduction due to Fusarium stalk rot is estimated to be 4% although losses may reach up to 50% in areas where disease pressure is very high. Disease severity is very significant especially in low temperature areas with wet conditions that follow prolonged dry periods (Dodd, 1980; Hassan et al., 1996).

The most visible symptom of stalk rot is lodging. As the pathogen enters through the roots, they eventually advance to above ground tissues and infect the stalks. Stalks infected with stalk rot show red, pinkish or brownish coloration in the infected tissue (Zummo, 1980; Reed et al., 1983). These infections lead to vascular and cortical tissue damage thereby reducing water and nutrient absorption and translocation (Hundekar and Anahosur, 1994). As the disease progresses, damaged tissues disintegrate and weaken thereby leading to lodging (Zummo, 1984). *Fusarium spp.* also releases mycotoxins and secondary metabolites which affects grains especially maize when stored or ensiled. These fungi cause grain-molding and contamination which could result to lethal diseases especially to animals that fed to these grains.

The effect of *bmr* mutations on resistance or susceptibility to *Fusarium spp.* have not been widely studied. Funnell et al. (2005) evaluated the seeds of six elite sorghum lines with *bmr6* and *bmr12* mutations together with their wild-type parents against *Fusarium* and *Alternaria spp.* They found seeds of *bmr* lines from two genetic backgrounds had fewer colonies

of both pathogens compared to their wild-type versions.  In a related study, Funell-Harris et al.

(2010) found that *F. incarnatum-F.equiseti* species complex (FIESC) commonly isolated from

wild-type and *bmr6* grains were not detected in *bmr12* grains but it is not known whether this has

resulted from difference in genetic backgrounds or the *bmr* mutations themselves.  But few

evidences point out the contribution of precursors involved in lignin biosynthetic pathway to

disease defense (Nicholson et al., 1992).  However, there has been not much focus on the effect

of *bmr* mutations on *Fusarium* induced stalk damage in sorghum.


### *Charcoal rot*


    *Macrophomina phaseolina* (Tassi) Goid is the causal organism of charcoal rot.  It attacks

over 500 plant species in nearly 100 families (Leslie, 2002).  Charcoal rot is prevalent in areas

with persistent, hot and dry climates.  Yield losses of up to 64% have been recorded due *M.*

*phaseolina* infection (Pande et al., 1991).  Although lodging at maturity is a useful diagnostic

characteristic of the disease, other symptoms include decayed roots, plant death, and abnormal

peduncles with poor-quality grain.

    In the absence of host plants, sclerotia of *M. phaseolina* inoculum remain as debris in the

soil but will eventually germinate as soon as they get in contact with root exudates. Their port of

entry to host plants can be provided by lesion nematodes, particularly *Pratylenchus hexincisus*

(Leslie et al., 1996).  At present, the only practical means of controlling the disease are crop

management and the use tolerant germplasm.  Crop management aims at reducing fungal

inoculum through crop rotation and moisture conservation practices have proven effective

(Mughogho et al., 1991; Tuisntra et al., 2002).

There are only a handful of studies that focused on the reaction of brown midrib sorghum to charcoal rots. Though most post flowering drought tolerant hybrids tend to have resistance to *Macrohomina* and the resulting lodging, no concerted efforts have been made to develop stalk rot resistant hybrids or germplasm. This is primarily due to the complexity of the disease and lack of effective inoculation technique that mimics natural infection. However, few studies conducted using the classical toothpick inoculation procedure and a modified liquid inoculation technique have shown extensive variability for resistance to *Macrophomina* and the potential for genetic improvement of the trait (Bramel-Cox et al., 1998; Tesso et al., 2005). But none of these studies addressed the effect of *bmr* mutations on charcoal rot incidence. One of the reasons for reluctance in deployment of *bmr* traits in forage/silage and now in biofuel sorghums is the fear that the weakened stem as a result of low lignin concentration may predispose the plants to attack by stalk rotting organisms, *Macrophomina* and *Fusarium*. The reaction of seven *bmr* mutants (*bmr2*, *bmr6*, *bmr7*, *bmr12*, *bmr18*, *bmr22* and *bmr28*) and their wild-type counterparts to infection by *M. phaseolina* was evaluated recently. Although there was significant difference between the different mutations which is perhaps due to the background effect, the mutations did not affect severity of stalk rot disease (Tesso and Ejeta, 2011). Low lignin mutants of maize (*bm*1 through *bm*4) included in the same study were also not particularly vulnerable to infection by *Macrophomina*.

# Impact of brown mid rib mutation on greenbug feeding

Greenbug, *Schizaphis graminum* (Rondani), has been a major pest of sorghum frequently causing sever crop damage and economic losses until resistance genes were deployed in the 1980s (Harvey and Hackerott, 1969; Wilde and Tuinstra, 2000). Although there were reports of greenbug infestation is Kansas as early as 1916 (Hays, 1922), it was not until 1968 that it becomes a serious pest in sorghum. Several greenbug biotypes have evolved over time including biotype C, E, I, and K. They all are known to cause injury to sorghum at all stages of growth (Porter et al., 1982; Harvey et al., 1991, 1997). Although biotype E was the most abundant in Kansas for which effective resistance trait was deployed, biotype K was reported to have significantly damaged biotype E-resistant sorghums since 1990 (Harvey et al., 1991). This biotype poses serious threat to the overall sorghum acreage in the Great Plains where most of the sorghum is only resistant to biotype E (Bowling et al., 1994). But the pest has not occurred to an epidemic proportion in the past and hence not much emphasis has been paid to develop germplasms resistant to this particular biotype. But with the use of sorghum as biofuel feedstock increasingly becoming important, the need to look at reaction of these potential sources to various pests including greenbug becomes necessary. Since most biofuel sorghums are of different germplasm pool from the grain sorghum, many of the useful traits imbedded in the grain type sorghums including pest and disease resistance and tolerance to abiotic stresses may not be as widely available in biofuel sorghums.

Greenbugs feed by removing phloem sap and at the same time inject substances that induce chlorosis (Girma et al., 1998). Greenbug damage is characterized by a dark red spot at the feeding site, surrounded by an area of pale and yellow discoloration (Reese and Schmidt, 1986). Damaged leaves begin to die, turning yellow and then brown from the outer edges. Yield

reductions due to infestation at booting, flowering, and kernel development stages could be high

depending on greenbug abundance, length of time greenbugs have infested the plants, and plant

health.  Many greenbugs occurring on booting and older plants reduce yield by reducing seed

number and size (Teetes et al., 2000).  The damage in sorghum tissue, especially in chlorophyll

can be quantified by destructive method or by using the SPAD chlorophyll meter (Girma et al.,

1998).

Resistance to greenbug includes tolerance, non-preference and antibiosis and that the

three may occur in the same or different genotype (Schuster and Starks, 1972).  Greenbug

damage may also vary depending on temperature and the nutritional state of the infected plants

(Schweissing and Wilde, 1979).  Bowling et al. (1996) indicated that antibiosis and antixenosis

as the major mechanisms of resistance to greenbug biotype I.  Puterka et al. (1995) studied the

genetics of greenbug virulence using biotypes C, E and F and using three greenbug resistant

sorghum cultivars acquired from different sources.  They found that both dominant and recessive

genes were responsible for resistance to the different biotypes.  Campbell et al. (1982) reported

that greenbug resistance in sorghum is associated with the phloem. Greenbugs probing on

resistant lines showed a significantly reduced imbibition of phloem sap compared to those which

fed on susceptible lines indicating that certain chemicals in the phloem or phloem sap may be

associated with resistance to the pest.  No information is available on the reaction of *bmr* mutants

to greenbug feeding. Since the insects feed by sucking the phloem sap, the role of lignin to

minimize the attack may not so high.  Nevertheless, it may still play some role in restricting the

piercing and access to the sap. Regardless, there is significant concern among growers that

reduced mechanical strength as a result of low lignin content may predispose *bmr* mutants to

increased damage by insect pests.

# Chapter 2 - GENETIC ANALYSIS OF BIOFUEL TRAITS IN SWEET SORGHUM

## INTRODUCTION

The increasing global demand for renewable alternative fuel sparked interest in the use of plant species for biofuel production and many agricultural crops have been identified as potential feedstock sources.  Although sugar from sugarcane in Brazil and starch from maize in the United States have been the major feedstocks for bioethanol production, these raw material bases which also have to be used for feed, food and other needs, will not be sufficient to meet the increasing demand for fuel ethanol (Hahn-Hagerdal et al., 2006). This necessitates the exploitation of other sugar and lignocellulosic feedstock sources.

Sorghum is listed as one of potential crops for use as dedicated feedstock source for biofuel production (Rooney et al., 2007). The extended knowledge of the agronomy and genetic structure of the crop, its annual nature and tolerance to major biotic and a-biotic stresses makes it best suited among several candidate feedstock sources. All parts of sorghum, the grain, the sugary juice from the stem and the biomass can be converted to ethanol.

Sweet sorghum is particularly important for biofuel production because conversion of the juice to ethanol is less complicated (Almodares et al., 2009). The juice can be easily extracted and directly fermented to ethanol. Although sweet sorghums are widely grown in Africa and also used for syrup production in parts of United States, not much effort was made to improve sugar yields of the crop. Sugar content in common sweet sorghum cultivars may range from 14.32-22.85% (Almodares and Sepahi, 1996) of which 43.6-58.2% is soluble sucrose, glucose and

fructose (Billa et al., 1997).   There exists significant diversity in traits important to biofuel

production among sweet sorghum lines; hence, the opportunity for improvement is tremendous

(Rooney et al., 2007). Sweet sorghum germplasm also has wide genetic variability for

morphological characteristics such as plant height, stalk girth and maturity that may have direct

relationship with sugar content (Reddy et al., 2005).

Compared to grain sorghum, sweet sorghums feature more rapid growth, higher biomass

production, wider adaptation, and have greater potential for ethanol production (Reddy et al.,

2007).  Like other sorghums, sweet sorghums are tolerant to drought, water-logging conditions

and saline/alkali soils (Reddy and Reddy 2003; Ali et al., 2008).  They require less water and

nutrient and hence are widely cultivated in the semi-arid tropics in Sub-Saharan Africa and India

(Rooney et al., 2000). Many of the sweet types are cultivated as multipurpose crop because they

can be grown simultaneously for production of grain from its head, forage from its green foliage

and sugar from its sweet juice. They are often tall and accumulate high biomass, thus the baggase

after juice and sugar extraction can also be converted to ethanol in much same way as

lignocellulosic feedstocks.  These typical characteristics make sweet sorghum the most versatile

feedstock sources for biofuel production. However, exploitation of this potential requires

improvement of these component characteristics. Previous studies have shown significant

genetic variability for major biofuel characteristics (Almadores et al., 1994a; Almadores and

Sepahi, 1996; Mikanda et al., 2009). Successful improvement of the traits require understanding

of the underlying genetic basis for this variation. Therefore, this study was initiated to address

the following objectives: 1) estimate the combining ability of selected sweet sorghum lines for

juice yield, stem °brix percent, and biomass production and thereby determine the genetic

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mechanisms controlling the traits; and (2) to identify promising lines that can be utilized in the breeding programs to improve these characteristics.

## MATERIALS AND METHODS

### *Genetic materials*

Six sorghum genotypes were crossed to three standard seed parents in a Design II mating scheme to produce 18 $F_1$ hybrids. The hybrids along with inbred parents were evaluated at Ashland bottoms, Kansas State University research farm near Manhattan, KS during the 2009 and 2010 main seasons. The six pollinator lines (PI193073, PI257602, PI182672, PI195754, SC382 and SC373) were selected based on the results of a preliminary screening experiment conducted in 2008. PI193073 has high juice yield but low brix while PI257602 has both high juice and high $^o$brix. PI195754 is juicy but has low $^o$brix and PI185672 has medium juice and high $^o$brix. All four are tall and produce high biomass. PI656095 and PI534088 have intermediate juice but low $^o$brix score. All the three females are standard B-lines from the US public breeding programs and all have intermediate to low juice and low $^o$brix. Description of major characteristics of the parental lines is presented in Table 1.

Table 2.1. Origin and pedigree of parental sorghum lines used for this study.

| PI No. | Common name | Origin | Pedigree | stalk sweetness/juiciness |
|---|---|---|---|---|
| PI656095 | SC373 | | Conversion | Medium, watery |
| PI534088 | SC382 | Nigeria | Conversion | Medium, watery |
| PI193073 | Masuda | Japan | Landrace | Juicy, watery |
| PI257602 | No. 8 | Ethiopia | Landrace | Juicy, sweet |
| PI185672 | - | India | Landrace | Medium, sweet |
| PI195754 | - | India | Landrace | Low, watery |
| PI655975 | BTx399 | USA | Improved line | Low, watery |
| - | BTx623 | USA | Improved line | Low, watery |
| PI655989 | BTx3042 | USA | Improved line | Low, watery |

## *Experimental Design and Management*

A randomized complete block design (RCBD) with three replications was used. Plots were 5 m long single rows spaced 0.75 m apart. At planting, approximately 3g were directly seeded into the rows. Twenty days after emergence the plants were manually thinned to 20 cm spacing between plants. The experimental plots were supplied with ammonium polyphosphate (APP) and urea ammonium nitrate (UAN) applied at the rate of 45.5 kg N ha$^{-1}$ and 13.6 kg P$_2$O$_5$ ha$^{-1}$, respectively. For weed control, the plots were sprayed with Bicep Lite II Magnum (a.i. 0.82 kg atrazine ha$^{-1}$ and 1.03 kg S -metolachlor ha$^{-1}$) and Calisto (a.i. 0.22 kg mesotrione ha$^{-1}$) prior to planting. Post emergence weeds were controlled by hand weeding and this practice was used to keep weeds off the field throughout the seasons.

## Data collection and analysis

Data for plant height, days to flowering and maturity were collected on plot basis. Plant height was measured as the length of the plant from the base to the tip of the panicle. Days to flowering was recorded as the number of days from planting to when half of the plants in a plot reached half bloom stage; while days to maturity as the number of days between planting to black layer formation in the lower $1/3^{rd}$ section of a panicle. Lodging was scored using a 1 to 5 scale with a score of '1' means no lodging and '5' means $> 75\%$ plants in a plot has lodged. Juice yield, °brix score and biomass measurements were conducted on individual plant basis. At about 15 d after flowering, ten plants in each plot were randomly tagged using tagging tapes. At physiological maturity the tagged plants were carefully harvested and used for measuring biomass, juice yield and °brix score. For juice extraction, leaves and heads were removed from each plant and the stems were loaded into a Sukra sugarcane crusher (Figure 2.1A). Juice yield was obtained by measuring the total juice extracted from all ten plants. Stem sugar concentration (°brix score) was determined using an Atago hand-held digital refractometer PAL-1 (Atago USA, Inc., Bellevue, WA, USA) (Figure 2.1B). To avoid carryover effects, both the crusher and Atago refractometer were rinsed with distilled water and dried with tissue paper after each sample. After juice extraction, the entire sample was collected and oven dried at $120^{o}C$ for 10 days to determine sample dry weight. Biomass was thus measured as the dry weight of all ten plants (stalk and head) harvested from each plot.

Figure 2.1. Sukra sugarcane crusher (A) and Atago digital handheld refractometer (B)

used for juice extraction and determining sugar content.

## Statistical Analysis

Data were analyzed using statistical analysis systems version 9.1.3 (SAS 2003). Entry, male, female and male $\times$ female interaction effects were determined in all analyses. In the combined data, replication, environment and interactions with environment were treated as random effects while other factors were treated as fixed effects. Treatment effects were partitioned in to inbred and hybrid and their effects and that of inbred vs. hybrid were determined for all parameters. Hybrid effect was further partitioned in to male, female and male $\times$ female interaction effects representing general combining ability (GCA) for male, GCA for female and specific combining ability (SCA) effects. The effects for the different sources of variation were tested using appropriate error terms as shown in the expected mean square (Table 1.2). General combining ability for each line was computed as the difference between the mean performance of the progeny of a given line and the overall mean of the hybrids. Significance of GCA for each line was tested using a two tailed test in SAS and was confirmed using the procedure outlined by Cox and Frey, 1984; Kearsey and Pooni, 1996). Specific combining ability was computed as the deviation of the value of a given cross from the sum of the grand mean and GCA of the lines involved in that cross, i.e.

$$SCA_{ij} = X_{ij} - (\mu + GCA_i + GCA_j)$$

where:

SCA$_{ij}$ = specific combining ability of a cross between parent $i$ and parent $j$;

X$_{ij}$ = the observed value of the cross between parents $i$ and $j$; $\mu$ = the overall

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mean of the hybrids; and $GCA_i$ and $GCA_j$ = General combining ability of

parents $i$ and $j$, respectively.

Mid parent and high parent heterosis were computed to estimate the performance of the

hybrids in relation to the mean and the best parents for each trait. These were computed using the

following formula:

Mid-parent heterosis $= \dfrac{F1 - Mid\ P}{Mid\ P}\ x\ 100$

High parent heterosis $= \dfrac{F1 - High\ P}{High\ P}\ x\ 100$

where:

Mid P or mid-parent $=$ is the mean performance of the parents of a particular

hybrid for specific trait;

High P or high parent $=$ is the mean performance of the best parent of a particular

hybrid for a specific trait;

$F_1$ or hybrid $=$ is the mean performance of a hybrid for a specific trait.

Table 2.2. Expected mean square table for over location combined analysis.

| Source of Variation | Df | Expected Mean Squares |
|---|---|---|
| Location (L) | 2 | |
| Location (Rep) | 6 | |
| Entry (E) | 26 | $\sigma^2_e + 3\sigma_{EL} + 9\sigma^2_E$ |
| Inbred (I) | 8 | $\sigma^2_e + 3\sigma_{IL} + 9\sigma^2_I$ |
| Hybrid (H) | 17 | $\sigma^2_e + 3\sigma_{HL} + 9\sigma^2_H$ |
| Female (F) | 2 | $\sigma^2_e + 3\sigma_{FML} + 18\sigma^2_{FL} + 54\sigma^2_F$ |
| Male (M) | 5 | $\sigma^2_e + 3\sigma_{FML} + 9\sigma^2_{ML} + 27\sigma^2_M$ |
| F x M | 10 | $\sigma^2_e + 3\sigma_{FML} + 9\sigma^2_{FM}$ |
| H vs. I (T) | 1 | $\sigma^2_e + 36\sigma_{TL} + 108\sigma^2_T$ |
| Entry x L | 52 | $\sigma^2_e + 3\sigma_{EL}$ |
| I x L | 16 | $\sigma^2_e + 3\sigma_{IL}$ |
| H x L | 34 | $\sigma^2_e + 3\sigma_{HL}$ |
| F x L | 4 | $\sigma^2_e + 3\sigma_{FML} + 18\sigma^2_{FL}$ |
| M x L | 10 | $\sigma^2_e + 3\sigma_{FML} + 9\sigma^2_{ML}$ |
| F x M x L | 20 | $\sigma^2_e + 3\sigma_{FML}$ |
| I vs. H x L | 2 | $\sigma^2_e + 36\sigma_{TL}$ |
| error[a] | 136 | $\sigma^2_e$ |
| Error | 208 | $\sigma^2_e$ |

# RESULTS

The combined analysis of variance for all the traits and all possible sources of variation is presented in Table 2.3. The entry effect was highly significant for all biofuel traits (juice yield, °brix, sugar yield and biomass), phenology (days to flowering, maturity) and plant height. Partitioning the entry effect into inbred and hybrid components also revealed that both components were significant for all traits with hybrid effect being much higher than the inbred except for plant height and °brix score. The inbred vs. hybrid component shows that the difference in relative contribution of the inbred and hybrids to the total variation among the entries was significant with hybrids accounting for much of the variation except for days to flowering and lodging score where the effects of both hybrids and inbred were comparable. But further partitioning of the hybrid effect in to female, male and female × male interaction shows slightly different results. The female effect also referred to as the GCA for females was significant $(P \leq 0.05)$ only for juice yield, °brix score, days to flowering and plant height. Whereas the effect of GCA due to males unlike that of females was highly significant for all the traits measured. Also male parent lines contributed the most to the variation among the hybrids as shown in their larger mean squares compared to the female parents. The female × male interaction effect also referred to as specific combining ability effect was significant only for juice and sugar yield with the contribution of SCA effect to the overall variation among the hybrids being markedly low. The interaction between environment and entry, hybrids and their components were significant for almost all traits except plant height. The effects for lodging score were significant only for entry × location and inbred × location effects and F × M × L interaction effect was not significant for all traits.

Days to flowering and maturity among the entries ranged from about 60 d in ATx3042 × SC382 to 91 in PI257602 and from 105 d in BTx3042 to 146d in ATx3042 × PI257602, respectively (Table 2.4). On average, the male parents took longer time to reach half bloom compared with the females that were bred to fit to specific growing window. Similar to the grain sorghum, most of the male parents took shorter time to reach flowering stage when grown in hybrid combination than as inbred per se except PI185672. Likewise the difference in plant height among the entries was very wide. The female parents were two or three-dwarf lines and hence are short. While the two converted males (SC373 and SC382) are also semi dwarf types, the other four males are original landraces not manipulated for height and are more than 2.5 m tall. Because of the hybrid vigor and most importantly the reconfiguration of the height genes in hybrid combinations, most of the hybrids are taller than both inbred parents. The dwarf converted males also produced hybrids that are taller than either of the parents despite that both parents are two or three-dwarfs. Variation for lodging score appears to correlate with height. All the dwarf seed parents had mean lodging score of less than 1.15 indicating that they are more resistant than the tall males with a mean lodging score of 1.89. But despite the fact that they are taller in height, the hybrids had lower lodging score than the male parents. Two male parents in particular (PI185672 and PI195754) that expressed the tallest height in hybrid combinations were also the most tolerant to lodging. This is because these hybrids tend to have larger basal stalk girth and stiff stalks and it appears that hybrids that combine these traits tend to be more tolerant to lodging despite their tall stature.

Performance of the entries with respect to biofuel traits is presented in Table 2.5. Juice yield among the entries ranged from as low as 400 ml in SC382 to a high of about 4000 ml in ATx3042 × PI1257602. The result for °brix percent is similar that the values ranged from about

8% in the females and converted males to about 17% in PI257602 and PI185672 and their

hybrids. Similar to the juice and °brix percent, total sugar was lowest among the females and

converted males with a mean of about 40 ml to a high of about 500 ml in PI257602 and its

hybrids. Given their short height, all of the females and converted males had the least biomass of

about 0.6kg as compared to over 2.8kg recorded in the hybrids of PI185672 and PI257602.

     Although the scores were generally low, BTx623 among the females appeared to have

more juice, higher °brix percent and total sugar than the other females. While the juice yield may

be explained by its relative tallness, the °brix percent certainly reflect the inherent difference

from the other females.  But since all of them are grain type sorghums none of these readings are

close to generating interest in the use of these materials for biofuel crop improvement. The

difference among the males for all the three traits, however, was remarkable.  While the

converted males were as low as the seed parents with respect to these traits, genotypes PI193073

and PI257602 for juice yield, brix percent and sugar yield, and PI257602 and PI185672 for

biomass had the highest readings.  Almost all of the positive traits in the male parents were

translated in to the hybrids that many of the lines that had among the highest reading for juice,

°brix, total sugar and biomass as inbred *per se* had even higher scores for these traits when tested

in hybrid combinations. The mean performance of the hybrids for juice, total sugar and biomass

was 42, 32, and 38% higher than that of the males and 192, 525 and 214% higher than the

females. In addition, the hybrids also had 82% higher °brix than the females.  Some of the male

lines combined more than one desired traits both as inbred and in hybrid combinations.

PI193073 and PI257602 seemed to have combined superior alleles for juice yield and percent

°brix, and PI257602 and PI185672 combined excellent lodging tolerance and high biomass

production while PI257602 combined all of the measured traits including juice yield, $^{o}$brix,

biomass and tolerance to lodging (Table 2.5).

Average number of days to flowering and maturity among females was shortest for

crosses of ATx3042 compared with the other females (Table 2.6). Thus it has a negative and

significant GCA of -1.9 and -1.1 for flowering and maturity, respectively. Crosses of ATx399

took longer time, 73 d and 124 d, to reach flowering and maturity, respectively, and hence had

positive and significant GCA of 1.34 and 1.44 for days to flowering and maturity, respectively.

The performance of the third female, Tx623 was comparable to the overall mean and hence its

GCA for both traits was not significant. Days to flowering and maturity among male parents

range from 50-63 d in the crosses of SC382 to 83-88 d in that of PI185672. Mean days to

flowering and maturity among the crosses of both male and female parents are the same but the

range is much wider among the males. This was reflected in the ANOVA where the relative

contribution of the male and females parents to variations among the hybrids for these traits was

remarkably higher for the males. Lines SC382 and SC373 had the highest negative GCA for both

traits. Two of the non-converted males PI193073 and PI195754 also had negative and significant

GCA for both traits while PI257602 and P185672 had the highest and significant GCA. With

respect to plant height, all non-converted males that perhaps carry wild type alleles for many of

the height genes were significantly taller than the others. As a result all of them had positive

GCA for plant height with PI257602 and PI185672 being significant. Though their GCA was

negative and significant, crosses of the converted males were remarkably taller (> 2.5m)

compared to their value as inbred (about 1.5m) despite the fact that the females parents were also

dwarf (< 1.5m) (Tables 2.4 and 2.6).  But results for lodging score among do not agree. Hybrids

of the tall sweet males, despite their high biomass and height, had better standability than that of

the converted males. The performance of PI185672 was striking that all of its hybrids had the lowest lodging score resulting in significant GCA for tolerance to lodging. This line also had the most significant GCA for height indicating tall and high biomass hybrids can be deployed without incurring losses to lodging provided that suitable backgrounds are identified.

The performance of the hybrids with respect to biofuel traits was mainly influenced by the male parents though the females also had significant effect on juice yield and °brix percent. Accordingly, mean juice yield and °brix score among the males ranged from 350 ml to 2690 ml and from 7.03 to 17.28%, respectively (Table 2.5). The variation for the traits among female parents was much narrower. Hybrids of PI257602 followed by PI193073 gave the highest juice yield while the converted lines SC382 and SC373 had the lowest. There was similar trend for °brix percent except the switch of ranks among the top males. PI257602 had the highest significant GCA for both juice yield and °brix percent followed by PI193073 for juice yield and PI18562 for °brix percent. Again PI257602 produced the highest total sugar and the lowest was recorded among converted lines followed by PI195754. With regard to biomass, all the tall parents gave above average mean biomass yield except PI193073. PI185672 produced the highest biomass followed by PI257602. Specific combining ability effect was significant only for juice yield and total sugar and these were very small compared to the male GCA effects.

Positive and significant mid-parent and high parent heterosis were observed in most of the hybrid combinations both for stalk juice and biomass yield with average high parent heterosis for the two traits being 41 and 52%, respectively. The average mid parent heterosis for °brix score was 25% but was close to zero for high parent heterosis. However, certain high biomass hybrid combinations had positive and significant high parent heterosis but crosses of the highest °brix parents did not (Table 2.8).

Table 2.3. Combined analysis of variance for biofuel traits of the genotypes grown at Ashland Bottoms, Manhattan, KS in 2009 and 2010.

| Source of Variation | Df | Juice | °Brix | Sugar Yield | Biomass | Days to Flowering | Days to Maturity | Plant Height | Lodging |
|---|---|---|---|---|---|---|---|---|---|
| Location (L) | 2 | 7.22** | 216.19** | 0.10** | 6.09** | 4136.76** | 3548.25** | 0.15 | 4.68** |
| Location (Rep) | 6 | 0.13 | 5.97* | 0.01* | 0.08 | 9.07** | 26.04 | 0.40 | 1.27** |
| Entry | 26 | 9.54** | 119.87** | 0.30** | 5.45** | 761.41** | 2251.88** | 5.52** | 1.93** |
| Inbred (I) | 8 | 5.24** | 155.37** | 0.20** | 3.20** | 776.96** | 1969.80** | 6.79** | 1.88 |
| Hybrid (H) | 17 | 10.29** | 102.15** | 0.33** | 4.49** | 798.83** | 2481.88** | 3.01** | 1.94** |
| Female (F) | 2 | 0.84* | 32.08* | 0.01 | 0.07 | 177.47* | 100.85 | 1.12* | 0.30 |
| Male (M) | 5 | 32.96** | 320.91** | 1.06** | 14.94** | 2625.19** | 8239.03** | 8.94** | 5.53* |
| F x M | 10 | 0.56* | 6.69 | 0.02* | 0.14 | 10.82 | 63.20 | 0.39 | 0.47 |
| H vs. I | 1 | 30.87** | 137.31* | 0.64** | 39.41* | 0.13 | 907.11* | 40.14** | 2.24 |
| Entry x L | 52 | 0.34** | 6.42** | 0.09** | 0.37** | 42.78** | 58.72** | 0.29 | 0.82* |
| I x L | 16 | 0.24** | 5.07** | 0.01** | 0.17* | 36.42** | 93.43* | 0.09 | 1.18* |
| H x L | 34 | 0.39** | 7.33** | 0.01** | 0.45** | 48.27** | 43.77** | 0.40 | 0.65 |
| F x L | 4 | 0.16 | 2.87 | 0.01 | 0.28 | 12.20 | 36.66 | 0.13 | 0.40 |
| M x L | 10 | 0.86** | 14.61* | 0.02* | 1.01** | 147.43** | 60.71 | 0.60 | 1.01 |
| M x F x L | 20 | 0.19 | 4.76** | 0.01* | 0.21* | 5.63 | 34.72** | 0.39 | 0.53 |
| I vs. H x L | 2 | 0.15 | 1.82 | 0.05 | 0.51 | 1.30 | 25.98 | 0.15 | 0.67 |
| Error | 145 | 0.11 | 2.49 | 0.01 | 0.11 | 71.32 | 26.30 | 0.27 | 0.55 |
| CV | | 20.60 | 12.29 | 25.94 | 18.20 | 2.40 | 4.23 | 19.96 | 41.86 |

*, ** - significant and   highly significant at $P \leq 0.05$ and $P \leq 0.01$ levels of probability, respectively.

Table 2.4 Across location mean performance of genotypes for major morphological traits.

| Name | Days to Flowering | Days to Maturity | Plant Height (m) | Lodging |
|---|---|---|---|---|
| *Females* | | | | |
| BTx3042 | 61.44 | 105.33 | 105 | 1.33 |
| BTx399 | 67.78 | 108.00 | 112 | 1.00 |
| BTx623 | 69.33 | 110.78 | 128 | 1.11 |
| Mean | 66.19 | 108.04 | 115 | 1.15 |
| LSD$_{(0.05)}$ | 0.99 | 0.67 | 40 | 0.43 |
| *Males* | | | | |
| PI193073 | 69.50 | 115.25 | 268 | 2.22 |
| PI257602 | 90.44 | 141.56 | 286 | 1.89 |
| PI195754 | 69.22 | 124.00 | 289 | 1.56 |
| PI185672 | 81.89 | 142.67 | 325 | 1.67 |
| SC382 | 62.88 | 106.63 | 144 | 1.67 |
| SC373 | 69.57 | 107.71 | 155 | 2.33 |
| Mean | 73.92 | 122.97 | 250 | 1.89 |
| LSD$_{(0.05)}$ | 1.14 | 8.18 | 25 | 0.86 |
| *Hybrids* | | | | |
| ATx3042 x PI193073 | 66.67 | 113.56 | 349 | 2.11 |
| ATx3042 x PI257602 | 78.22 | 146.22 | 330 | 1.67 |
| ATx3042 x PI195754 | 66.33 | 110.33 | 330 | 1.56 |
| ATx3042 x PI185672 | 83.00 | 145.11 | 353 | 1.56 |
| ATx3042 x SC382 | 60.25 | 108.38 | 195 | 2.33 |
| ATx3042 x SC373 | 60.67 | 105.33 | 219 | 2.33 |
| ATx399 x PI193073 | 68.44 | 113.13 | 287 | 1.33 |
| ATx399 x PI257602 | 81.89 | 142.13 | 330 | 1.89 |
| ATx399 x PI195754 | 68.11 | 113.56 | 280 | 1.56 |
| ATx399 x PI185672 | 88.00 | 145.22 | 340 | 1.22 |
| ATx399 x SC382 | 63.33 | 115.00 | 193 | 2.22 |
| ATx399 x SC373 | 65.67 | 113.11 | 207 | 2.44 |
| ATx623 x PI193073 | 68.00 | 112.89 | 295 | 2.00 |
| ATx623 x PI257602 | 82.89 | 145.22 | 334 | 1.67 |
| ATx 623 x PI195754 | 68.33 | 112.56 | 304 | 1.44 |
| ATx623 x PI185672 | 85.44 | 145.56 | 372 | 1.00 |
| ATx623 x SC382 | 59.78 | 105.33 | 232 | 2.33 |
| ATx623 x SC373 | 65.13 | 108.38 | 264 | 2.56 |
| Mean | 71.12 | 122.28 | 290 | 1.85 |
| LSD$_{(0.05)}$ | 1.82 | 3.55 | 57 | 0.65 |

Table 2.5. Across location mean performance of genotypes for biofuel traits.

| Name | Juice (ml)[‡] | Brix (%) | Sugar Yield (ml) | Biomass (kg)[‡] |
|---|---|---|---|---|
| *Females* | | | | |
| BTx3042 | 550 | 6.40 | 30 | 0.58 |
| BTx399 | 620 | 6.23 | 40 | 0.71 |
| BTx623 | 740 | 7.69 | 50 | 0.69 |
| Mean | 634 | 6.77 | 40 | 0.66 |
| LSD$_{(0.05)}$ | 110 | 1.50 | 0.01 | 0.22 |
| *Males* | | | | |
| PI193073 | 2280 | 15.56 | 360 | 1.38 |
| PI257602 | 2690 | 17.28 | 470 | 2.06 |
| PI195754 | 850 | 14.52 | 120 | 1.43 |
| PI185672 | 1120 | 13.86 | 160 | 2.38 |
| SC382 | 350 | 7.03 | 20 | 0.75 |
| SC373 | 500 | 6.48 | 30 | 1.00 |
| Mean | 1324 | 12.46 | 198 | 1.50 |
| LSD$_{(0.05)}$ | 360 | 1.72 | 60 | 0.33 |
| *Hybrids* | | | | |
| ATx3042 x PI193073 | 2780 | 11.46 | 310 | 1.82 |
| ATx3042 x PI257602 | 4020 | 16.14 | 650 | 2.94 |
| ATx3042 x PI195754 | 1300 | 11.18 | 140 | 2.09 |
| ATx3042 x PI185672 | 2460 | 15.00 | 370 | 3.07 |
| ATx3042 x SC382 | 620 | 7.61 | 40 | 1.08 |
| ATx3042 x SC373 | 750 | 6.68 | 50 | 1.31 |
| ATx399 x PI193073 | 2790 | 14.40 | 390 | 2.04 |
| ATx399 x PI257602 | 2840 | 16.71 | 480 | 2.84 |
| ATx399 x PI195754 | 1430 | 10.69 | 150 | 2.09 |
| ATx399 x PI185672 | 1930 | 15.66 | 300 | 2.92 |
| ATx399 x SC382 | 660 | 9.62 | 60 | 1.16 |
| ATx399 x SC373 | 640 | 9.16 | 60 | 1.24 |
| ATx623 x PI193073 | 2720 | 12.64 | 340 | 1.95 |
| ATx623 x PI257602 | 3330 | 16.96 | 570 | 2.63 |
| ATx 623 x PI195754 | 1400 | 13.03 | 170 | 2.23 |
| ATx623 x PI185672 | 2110 | 17.19 | 360 | 3.14 |
| ATx623 x SC382 | 700 | 8.72 | 50 | 1.30 |
| ATx623 x SC373 | 710 | 8.19 | 50 | 1.49 |
| Mean | 1843 | 12.28 | 252 | 2.07 |
| LSD$_{(0.05)}$ | 338 | 1.41 | 59 | 0.33 |

[‡] - measured from 10 random plants.

Table 2.6. Mean and general combining ability (GCA) of parent lines for morphological traits.

| Parent lines | Days to Flowering | | Days to Maturity | | Plant Height | | Lodging score | |
|---|---|---|---|---|---|---|---|---|
| | Mean | GCA | Mean | GCA | Mean | GCA | Mean | GCA |
| **Female parents** | | | | | | | | |
| ATx3042 | 69.36 | -1.87** | 121.74 | -1.10* | 300 | 10.00 | 1.93 | 0.08 |
| ATx399 | 72.57 | 1.34** | 123.54 | 1.44** | 273 | -17.00** | 1.78 | -0.07 |
| ATx623 | 71.72 | 0.49 | 121.91 | -0.86 | 300 | 10.00 | 1.84 | -0.02 |
| Mean | 71.23 | | 122.38 | | 290 | | 1.85 | |
| LSD$_{(0.05)}$ | 0.74 | | 1.45 | | 23 | | 0.27 | |
| **Male parents** | | | | | | | | |
| PI193073 | 67.70 | -3.53** | 113.20 | -9.19** | 310 | 20.00 | 1.82 | -0.03 |
| PI257602 | 81.00 | 9.77** | 144.62 | 22.24** | 331 | 41.00** | 1.74 | -0.11 |
| PI195754 | 67.60 | -3.64** | 112.15 | -10.23** | 305 | 15.00 | 1.52 | -0.33* |
| PI185672 | 85.48 | 14.25** | 145.30 | 22.92** | 355 | 64.00** | 1.26 | -0.59** |
| SC382 | 61.15 | -10.08** | 109.62 | -12.77** | 208 | -82.00** | 2.30 | 0.45** |
| SC373 | 63.77 | -7.46** | 108.96 | -13.42** | 226 | -62.00** | 2.45 | 0.60** |
| *Mean* | 71.23 | | 122.20 | | 290 | | 1.85 | |
| LSD$_{(0.05)}$ | 1.05 | | 2.05 | | 32 | | 0.37 | |

*, ** - significant and   highly significant at $P \leq 0.05$ and  $P \leq 0.01$ levels of probability, respectively.

Table 2.7. General combining ability of parent lines for biofuel traits as evaluated at Manhattan Kansas during 2009 and 2010 seasons.

| Parent lines | Juice | | Stem Sugar | | Sugar Yield | | Biomass | |
|---|---|---|---|---|---|---|---|---|
| | Mean | GCA | Mean | GCA | Mean | GCA | Mean | GCA |
| *Female parents* | | | | | | | | |
| ATx3042 | 2014 | 171.00** | 11.42 | -0.88** | 264 | 12.00 | 2.07 | 0.00 |
| ATx399 | 1673 | -170.00** | 12.60 | 0.31 | 232 | -20.00 | 2.04 | -0.03 |
| ATx623 | 1849 | 6.00 | 12.88 | 0.59** | 263 | 11.00 | 2.14 | 0.07 |
| Mean | 1843 | | 12.29 | | 252 | | 2.07 | |
| LSD$_{(0.05)}$ | 140 | | 0.58 | | 24 | | 0.13 | |
| *Male parents* | | | | | | | | |
| PI193073 | 2764 | 921.00** | 12.77 | 0.48 | 345 | 93.00** | 1.93 | -0.14 |
| PI257602 | 3419 | 1576.00** | 16.60 | 4.31** | 569 | 317.00** | 2.80 | 0.73 |
| PI195754 | 1376 | -467.00** | 11.63 | -0.66* | 152 | -100.00** | 2.14 | 0.07 |
| PI185672 | 2166 | 323.00** | 15.95 | 3.66** | 345 | 93.00** | 3.04 | 0.97** |
| SC382 | 661 | -1182.00** | 8.69 | -3.60** | 54 | -198.00** | 1.18 | -0.89** |
| SC373 | 698 | -1145.00** | 8.00 | -4.29** | 52 | -200.00** | 1.34 | -0.73** |
| Mean | 1843 | | 12.28 | | 252 | | 2.07 | |
| LSD$_{(0.05)}$ | 195 | | 0.82 | | 34 | | 0.19 | |

*, ** - significant and   highly significant at $P \leq 0.05$ and  $P \leq 0.01$ levels of probability, respectively.

Table 2.8. Mid- and high parent heterosis (%) in hybrid entries evaluated for biofuel associated traits.

| Hybrid | Juice | | Stem Sugar | | Sugar Yield | | Biomass | |
|---|---|---|---|---|---|---|---|---|
| | Mid P (%) | High P (%) | Mid P (%) | High P (%) | Mid P (%) | High P (%) | Mid P (%) | High P (%) |
| ATx3042 × PI193073 | 109.6** | 32.6* | 6.9 | -25.8** | 57.3 | -1.5 | 111.3** | 51.4* |
| ATx3042 × PI257602 | 147.5** | 49.5** | 38.1** | -4.95 | 149.8** | 41.7** | 121.4** | 44.8 |
| ATx3042 × PI195754 | 81.6** | 46.4* | 7.1 | -22.9* | 60.9* | 20.91 | 102.7** | 46.1 |
| ATx3042 × PI185672 | 211.8** | 137.6** | 48.4** | 8.4 | 277.1* | 169.5* | 108.4** | 30.0* |
| ATx3042 × SC382 | 34.1 | 9.2 | 14.6 | 7.6 | 38.8 | 34.8 | 70.2** | 48.5* |
| ATx3042 × SC373 | 67.2** | 45.6* | -3.85 | -14.5** | 64.9** | 30.3* | 86.6** | 59.6* |
| ATx399 × PI193073 | 122.9** | 44.0** | 30.6* | -9.4 | 101.9 | 31.7 | 119.6** | 71.8** |
| ATx399 × PI257602 | 75.2** | 6.6 | 36.2** | -3.3 | 69.5* | 3.4 | 110.1** | 40.7** |
| ATx399 × PI195754 | 105.4** | 68.7** | -3.4 | -26.3** | 75.2* | 36.3 | 97.4** | 49.6** |
| ATx399 × PI185672 | 144.5** | 94.9* | 51.8** | 13.8 | 179.8** | 137.9* | 96.5** | 25.0* |
| ATx399 × SC382 | 40.5* | 8.8 | 49.7* | 39.6* | 89.7 | 81.3** | 65.2** | 46.4* |
| ATx399 × SC373 | 26.3 | 5.0 | 54.1 | 38.8 | 122.0 | 83.6 | 63.1 | 49.3 |
| ATx623 × PI193073 | 98.8* | 34.3 | 7.5 | -20.0** | 65.3 | 9.8 | 112.0** | 70.6* |
| ATx623 × PI257602 | 98.6** | 26.3* | 37.4** | 0.5 | 106.2* | 28.2 | 95.4** | 29.4* |
| ATx623 × PI195754 | 78.7** | 50.0** | 12.2* | -12.3 | 76.8** | 49.9** | 116.6** | 60.4** |
| ATx623 × PI185672 | 150.4** | 97.2** | 56.4** | 20.7* | 238.8** | 167.8** | 113.0** | 35.7** |
| ATx623 × SC382 | 30.4* | -3.5 | 23.5 | 12.3 | 29.9 | 23.8 | 91.0** | 71.8** |
| ATx623 × SC373 | 14.2 | -8.8 | 25.5 | 9.6 | 42.8 | 9.8 | 88.3* | 69.9 |

*, ** - significant and  highly significant at $P \leq 0.05$ and $P \leq 0.01$ levels of probability, respectively.

# DISCUSSION

Sorghum is one of the potential plant species targeted as feedstock source for biofuel production.  As C-4 species, sorghum can efficiently utilize sunlight and atmospheric carbon dioxide for accumulating dry matter and thus produce high grain yield. It is in much better position for use as biofuel crop than any other plant species. It is unique among other feedstock sources in that it can be grown as grain, sugar and biomass crop simultaneously all of which are important feedstock sources. In addition, unlike perennial sources, sorghum based feedstock can be produced without disrupting the existing production system. Apart from its tolerance to drought and marginal soil conditions, sorghum is easily established by seed and its annual nature makes it fit to various rotation systems with other food/feed crops.

The present study focused on characterization of selected sorghum genotypes for use in improvement of the crop for sugar and biomass based feedstock production.  The entries made up of 18 $F_1$ hybrids and their nine parental sources were compared primarily for juice yield, °brix score and biomass production.  The significant variation observed among the entries for these traits is a reflection of the wide genetic variability among sorghum germplasm and indicates the potential for improvement of the traits.  Some of the parental sources and their hybrids had as high °brix score as 17% close to the average score of 15-25% often recoded in sugarcane (Tee et al., 1997). Most importantly, all of the high °brix genotypes had significant and positive GCA for the trait suggesting that it is amenable to genetic manipulation. It is evident from the data that in this specific set of genotypes, the trait is primarily controlled by additive genes though limited interaction effects appear to have played some role as revealed by significant SCA effect.  While the near complete dominant inheritance in some the best sources may eliminate the need to have

the high ºbrix trait in both parents, the quantitative nature of the trait implies that hybrids with superior ºbrix than either of the parents can be developed. The positive high parent heterosis reported in specific genotypes (PI257602) indicated that favorable intra-alleleic interactions can be exploited to enhance the ºbrix score and thus total sugar yield.

The result for juice yield and biomass shows that the traits are controlled by similar genetic mechanisms as °brix. The general combining ability effect of the male parents for both traits is even more significant than for °brix implying that selection for the trait can produce satisfactory results provided that suitable parental sources are used for developing populations. Moreover the average trait values among male and female parental sources is lower than that of the hybrids for both juice and biomass yield showing that there is positive high parent heterosis for the traits.

Although it is naturally self-pollinated, heterosis for both grain yield and biomass production is well recognized in sorghum. The discovery of cytoplasmic male sterility in the 1950 opened avenue to exploit this and starting from the early 1960s commercial production of grain sorghum was fully replaced by hybrids (Stephens and Holland, 1954). Significant proportion of forage production in the United States at present is also based on hybrids. The same genetic mechanism can be exploited to improve juice yield and °brix score in sorghum to enhance the value of the crop for ethanol production. Although the average heterosis for °brix is not as high as that of juice and biomass yield, specific high °brix genotypes tend to express positive heterosis when combined with low °brix parents such as in the crosses of PI257602. This presents opportunity for improving °brix score and increases the value of sorghum as feedstock source. We believe that the positive interaction between PI257602 and the low °brix females can be reproduced among parents of high °brix pedigree leading to an even higher °brix

score in the hybrids provided that compatible parents are selected. Stalk juice and biomass yields where an average heterosis of 46 and 85%, respectively, were obtained in the current study can be exploited more easily. Improvement in juice yield alone without the °brix score can lead to increased total sugar yield per unit area provided that °brix is not negatively affected.

However, the benefits of heavy plant stature (high biomass/juiciness) should be carefully weighed against its negative effect (lodging) when considering feedstock production. Lodging continues to present formidable challenge to biomass sorghum production. In the current study, most of the test entries were at least partially lodged except few semi-dwarf entries. While plant height seems to be the major contributor to both lodging and biomass production, other plant characteristics such as rind thickness and weight and the overall structural integrity of the plant has been reported to play an important role in affecting standability (Thompson, 1963; Esechie et al., 1977). In the present study many of the tall entries had higher lodging score compared to the semi dwarf seed parents. But at least one of the converted semi-dwarf male SC373 had higher lodging score both as inbred as well as in hybrid combinations (Table 2.4). On the other hand, few other tall entries such as PI185672 and PI195754 have much higher standability and were intact at the time of scoring. Moreover, hybrids of these entries especially that of PI185672 ranked first for both biomass production and juice yield and second for °brix score. This shows that, though the understanding of genetic mechanisms and configuration and introgression of the right traits in to suitable backgrounds requires tremendous efforts, hybrids that combine high biomass, juice and high °brix and also improved standability can be developed and deployed for biofuel production.

# CONCLUSION

Our results indicate that improving sorghum for specific biofuel traits such as juice yield, brix score and biomass can be achieved with reasonable effort. All of these traits had significant general combining ability effect indicating that well planned selection schemes can result in significant progress. The fact that the genes governing these traits appear to have dominant mode of inheritance may facilitate the breeding process in that the need to have the high trait alleles in both parents may not be necessary in breeding feedstock hybrids. Nevertheless, combining the different traits in to one background and also enhancing standability for optimal production may be a challenge. But our results show that, though it requires concerted effort, hybrids that combine improved biofuel traits and increased standability can be developed and deployed.

# Chapter 3 - EVALUATION OF LOW LIGNIN SORGHUM MUTANTS FOR BIOFUEL TRAITS AND RESISTANCE TO STALK ROTS AND GREENBUG FEEDING

# INTRODUCTION

Prior to the current interest in its use as biofuel feedstock, biomass sorghums have been under production primarily as livestock feed. Like the grain sorghum, biomass sorghums can be grown on marginal lands that are otherwise unsuitable for other major crops and produce reasonably high dry biomass. The fact that they fit in the traditional cropping systems and their excellent adaptation to drought and low nutrient input make them attractive that forage sorghums are widely produced throughout the country. In 2007, forage sorghum was produced on approximately 6 million acres in the USA with total biomass production of 58 million tons (Dien et al., 2009).

The discovery of the *bmr* mutations further enhanced the value of sorghum as forage/silage crop in that it improved dry matter digestibility (Akin et al., 1986) and thus milk yield in lactating cows (Frenchik et al., 1976). The *bmr* mutants have lower lignin in their stalks, leaves and leaf sheaths and are more digestible than normal sorghums (Oliver et al., 2005; Oliver et al., 2005). As a result *bmr* sorghums became the major components of forage sorghum production for much of the last two decades (Li et al., 2008).

Following their initial discovery among artificially induced mutant populations (Porter et al., 1978), a number of spontaneous *bmr* mutants were identified with a total of about 30 mutations now reported. Among these *bmr6*, *bmr12* and *bmr18* are very well characterized and also incorporated into a number of commercial forage sorghum varieties (Sarath et al., 2008). The *bmr6* family is reported to have been caused due to the reduced activity of cinnamylalcohol dehydrogenase (CAD) which is directly involved in stem lignification (Sattler et al., 2009). Whereas the *bmr12* family has been shown to have resulted from reduced activity of another lignin biosynthetic enzyme caffeic acid *O*-methyltransferase (COMT) which resulted in

reduction of syringal residues and cell-wall bound *p*-coumaric acid (Bout et al., 2003). The

mechanism of reduced lignin in the third *bmr* family (*bmr2*) is not known.

The interest in the use of biomass sorghum as potential lignocellulosic feedstock for

biofuel production seems to have further increased the importance *bmr* mutations as they

facilitate conversion of biomass into fermentable sugar. Production of ethanol from

lignocellulosic sources involves three major steps; pretreatment of biomass, enzymatic

hydrolysis and fermentation (Corredor et al., 2009). These processes are greatly influenced by

physical structure of the biomass. The pretreatment step is needed to remove lignin, an

important component of plant call wall alluded to be a major obstacle to saccharification by

physically shielding the cellulose from enzymatic action (Moiser et al., 2005a; Moiser et al.,

2005b). The deployment of *bmr* mutations in biomass based feedstock production is expected to

serve the same purpose it does to improve dry matter digestibility in forage sorghums. The low

lignin content of *bmr* genotypes should reduce the negative effect of lignin in the conversion of

biomass into ethanol and there by improve ethanol yield or reduce the cost of producing ethanol

by avoiding or reducing the need for the pretreatment step (Dien et al., 2009). Previous studies

have shown that *bmr* sorghums have improved conversion rate, and yielded higher fermentable

sugar than their wild-type versions. Glucose yields from biomass crops with *bmr6* and *bmr12*

alleles were reported to be higher by 27% and 23%, respectively, compared to their wild-types.

Conversion of cellulose to ethanol of pre-treated sorghum biomass was also improved by 22%

and 21% for *bmr6* and *bmr12*, respectively (Dien et al., 2009).

Despite their apparent role in improving dry matter digestibility and conversion of

cellulose to ethanol, the *bmr* mutations have not been widely deployed because of the fear that

the reduced lignin content may predispose the crops to attack by stalk rot pathogens and increase incidence of lodging.

The objectives of this study, therefore, were (1) to evaluate a set of *bmr* sorghums and their wild type versions for resistance to infection by stalk rot pathogens and greenbug feeding; and (2) to assess the impact of genetic backgrounds and their interaction with *bmr* mutations to affect biofuel traits.


## MATERIALS AND METHODS


Two independent experiments were carried out in the field and greenhouse to address different but related topics. The field experiment focused on evaluation of the relative sensitivity of low lignin sorghum mutants to attack by stalk rotting organisms and their contribution to biofuel production. The greenhouse experiment was aimed at evaluating the response of normal and low lignin materials to greenbug feeding.


### *Genetic Materials*


For the field experiments, four forage sorghum germplasm lines (Atlas, Kansas Collier, Rox Orange and Early Hegari) and their *bmr6* and *bmr12* versions were used (Table 2.1).  The brown midrib lines were jointly developed by USDA-ARS and the Agricultural Research Division, Institute of Agriculture and Natural Resources, University of Nebraska and released in January 2005.  The N121 (*bmr6*) and F220 (*bmr12*) were used as *bmr* gene sources and were crossed to the forage sorghum lines. The progenies were repeatedly backcrossed to the recurrent parents Atlas, Kansas Collier, Rox Orange and Early Hegari-Sart and the *bmr6* and *bmr12*

versions of these lines were developed. The genotypes were evaluated for biofuel associated traits and reaction to *Fusarium thapsinum and Macrophomina phaseolina*. This study was conducted at the Kansas State University research farm, Ashland Bottom, near Manhattan, KS and KSU-Harvey County Experiment Field at Hesston during the 2009 main season. The study was repeated during the 2010 main season at Ashland Bottom, and at the KSU-East Central Experiment Field at Ottawa, KS.

Table 3.1. The list and characteristics of the test materials.

| PI Number | Background | Midrib phenotype |
| --- | --- | --- |
| | Rox Orange | wild type |
| PI639702 | Rox Orange | *bmr6* |
| PI639703 | Rox Orange | *bmr12* |
| | Kansas Collier | wild type |
| PI639704 | Kansas Collier | *bmr6* |
| PI639705 | Kansas Collier | *bmr12* |
| NSL 4009 | Early Hegari-Sart | wild type |
| PI639706 | Early Hegari-Sart | *bmr6* |
| NSL 3986 | Atlas | wild type |
| PI639708 | Atlas | *bmr6* |
| PI636763 | Atlas | *bmr12* |

## *Experimental Design and Management*

The experiment was conducted in randomized complete block design with four replications. Plot sizes were 5m long single rows spaced 0.75 m apart. Approximately 3g seeds for all genotypes were directly seeded into the rows. At twenty days after emergence, the seedlings were manually thinned to 20 cm distance between plants. Recommended rates of chemical fertilizer, ammonium polyphosphate (APP) and urea ammonium nitrate (UAN) at the rate of 45.5 kg N ha$^{-1}$ and 13.6 kg P$_2$O$_5$ ha$^{-1}$, respectively, were applied. For weed control, the plots were sprayed with Bicep Lite II Magnum (a.i. 0.82 kg atrazine ha$^{-1}$ and 1.03 kg S - metolachlor ha$^{-1}$) and Calisto (a.i. 0.22 kg mesotrione ha$^{-1}$) prior to planting. Post emergence weeds were controlled by hand weeding and this practice was used to keep weeds off the field throughout the seasons.

## *Inoculum preparation and inoculation*

Fresh cultures of *Fusarium thapsium*, and *Macrophomina phaseolina* were initiated in potato dextrose agar (PDA) from pure cultures of the respective pathogens. *Fusarium thapsium* was provided by Dr. Cris Little, Department of Plant Pathology at Kansas State University. *M. phaseolina* strain used in this study was originally collected by Dr. Gary Odvody, Texas A&M University and maintained on PDA at Kansas State University.

Small section of fresh *F. thapsinum* culture was used to initiate a suspension culture using Potato dextrose broth (PDB). The pathogen was incubated at room temperature on a rotary shaker (60 rpm) until the culture was substantially grown to change the color of the media to white yellowish. Conidia were separated from the mycelial mass by straining the suspension

through four layers of cheese cloth. Conidial concentrations were determined by counting the

number of spores under the microscope using a hemocytometer.  Concentration of the conidia

was adjusted to 5 x $10^4$ conidia ml$^{-1}$ using 10mM (pH 7.2) phosphate buffered saline (PBS)

solution. The suspension was kept on ice until inoculation. Detailed procedure for liquid

inoculum preparation is described by Tesso et al. (2004).

For *M. phaseolina,* inoculum was initiated by sub-culturing small sections (2-3mm) of

the fungal mat in to several fresh PDA plates. Then sterile toothpicks were placed on the plate

and incubated at 30°C until the media and the toothpicks were covered with the growing
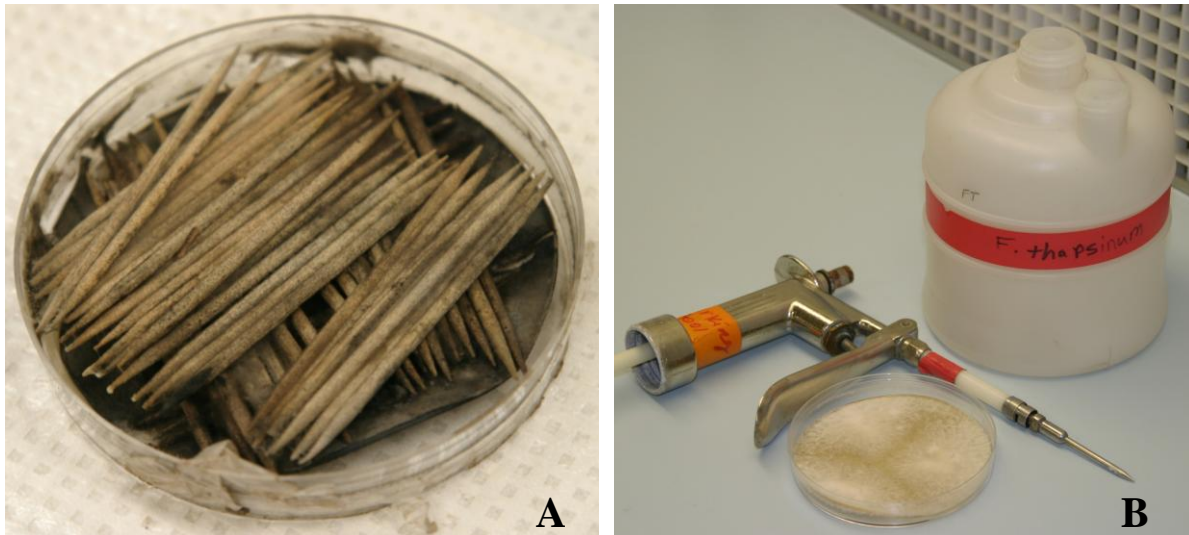
sclerotia (Figure 3.1).



Figure 3.1. Sterile toothpicks covered with *Macrophomina phaseolina* (A) and Idico filler-

plug gun with the stainless steel needle (B).

At flowering, a total of fifteen random plants in each plot were tagged using three distinctly colored plastic ribbons (red, blue and yellow), five plants for each color. At 14 d after flowering the tagged plants were inoculated with the inoculums from each of the pathogen group. Five plants marked with red ribbon were inoculated with liquid inoculum of *F. thapsinum*. A modified syringe, Idico filler-plug gun (Forestry Suppliers, Inc., Jackson, MS) equipped with a stainless needle similar to that described by Toman and White (1993) (Figure 3.1B) was used to inject 1ml inoculum suspension in to the pith of the plants.  The next five plants tagged with blue ribbon were inoculated with *M. phaseolina*. In this case a toothpick inoculation method was used. Infected toothpicks (Figure 3.1A) were inserted into small holes made on the stalk of the plant using a sterile needle. Inoculation with both pathogens was made approximately 10 cm above the ground.

### *Characterization of genotypes for biofuel traits*

The remaining five plants marked with yellow ribbon were carefully harvested at physiological maturity and used for measuring biomass, juice yield and °brix score. Stalk juice was extracted using a Sukra sugarcane crusher. The leaves and heads from the sample plants were removed and the stems loaded into the machine. The juice was collected and the volume determined.  Stem sugar concentration (°brix score) was determined using an Atago hand-held digital refractometer PAL-1 (Atago USA, Inc., Bellevue, WA, USA). Both the crusher and the refractometer were rinsed with distilled water and dried with tissue paper after each sample. After juice extraction, bagasse were collected and dried at $120^{\circ}$C for 10 days and then weighed to

get the dry biomass. Dry biomass was measured as the dry weight of all five plants (stalk and head) harvested from each plot.

### *Greenbug tolerance study*

Due to limited availability of greenbug populations, only nine genotypes including a subset of entries used in the stalk rot resistance study and control genotypes were used. These were Atlas, Rox Orange and their *bmr6* and *bmr12* versions, Redlan (susceptible check), and PI266965 and KS97 (resistant checks). The experiment was conducted in the greenhouse using a randomized complete block design and was repeated three times. Twenty-seven 5L poly-tainer pots were filled with a Metro-Mix 360 growing medium (Sun Gro, Bellevue, WA). Three to five seeds were sown into each pot, and soon after emergence, the seedlings were thinned to one plant per pot. The pots were watered regularly. The plants were fertilized with Miracle Gro every other until inoculation.

At about 40 d after emergence, the seedlings were inoculated with biotype K green bug colony obtained from Dr. John Reese's Lab in the Department of Entomology, Kansas State University. The youngest fully expanded uniformly green leaf was selected for inoculation in each plot. A double-stick, foam leaf cage (Converters, Inc., Huntington Valley, PA) (2x2 cm with a 1.6 cm-diameter hole) was placed on the middle portion of the selected leaves. Biotype K greenbug colony of mixed growth stage reared on susceptible host DK27 and BTx623 were collected into a small plastic cup. About 50 greenbugs, just enough to cover the caged area while feeding, were placed inside each cage (Figure 3.2A). The cage was then covered with an organdy cloth (2x2 cm) to prevent the insects from escaping (Figure 3.2B).

Figure 3.2. Double sticky foam cage used to administer greenbug inoculums (A) to specific

leaf section and an organdy cloth (B) used to contain the bugs within the caged area.

## Data Collection

For the stalk rot resistance study, the infected plants were harvested 35 d after inoculation and rated for disease severity. The plants were split longitudinally and scoring of the disease for both pathogen groups was made by measuring the length of the visible necrotic lesion and counting the number of nodes contained within the lesion.

For the green house experiment, greenbug damage scoring was done 5 d after inoculation. The cages were entirely removed from the leaves and the extent of feeding damage was estimated by measuring the chlorophyll content using SPAD-502 chlorophyll meter (Minolta Camera Co., Ltd., Japan). The readings were taken from the damaged tissue inside the cage and the healthy leaf tissue at both sides of the caged area. Five readings were taken at each site for each leaf and their averages were recorded. Percent chlorophyll loss in the damaged tissue was determined by comparing the reading with the readings from the nearby normal leaf tissue and this was used to estimate the relative tolerance of genotypes. SPAD chlorophyll-loss index was determined by subtracting the SPAD measurement from the greenbug-treated area from that of the control and dividing by the control (Deol et al., 1998).

## Statistical Analysis

Data were analyzed using statistical analysis systems version 9.1.3 (SAS, 2003). Across environment combined analysis was performed considering environments, replications and interactions as random effects and genotypes as fixed effects. Entries were further partitioned by backgrounds and mutations. Analysis of variance was computed using the General Linear Model (GLM) procedure in SAS.

# RESULTS

## *Genotypic response to infection by stalk rot pathogens*

The combined analysis of variance for genotypic response to infection by *F. thapsinum* and *M. phaseolina* is presented in Table 3.2.  Similar information for days to flowering, plant height and biofuel traits are shown in Table 3.3. The data revealed that entry effect for infection by *F. thapsinum* was not significant for lesion length but significant for nodes crossed. But the effect for both lesion length and nodes crossed under *M. phaseolina* was significant (Table 3.2). Partitioning of the entry effect in to mutant and normal backgrounds also showed similar results. There was no significant difference among the mutants with respect to lesion length for both pathogen species but the effect for nodes crossed was significant in both.  Similarly the effect of the normal genotpyes was significant for nodes crossed for *Fusarium* and for both lesion length and number of nodes crossed for *Macrophomina*. On the other hand, the mutant vs. normal effect for both lesion length and nodes crossed for both pathogens was not significant.  Entry $\times$ location effect for both pathogens except lesion length for *Fusarium* and the inteactions of location with components of entry, except nodes crossed for mutant $\times$ location interaction for *Fusarium* and normal $\times$ location interaction for *Macrophomina* were also significant.

The effect of entry and its components were also significant for days to flowering and plant height except the effect of the normal backgrounds for days to flowering was not significant. There was also significant difference between the mutant and normal backgrounds with respect to plant height but not for days to flowering. The effect of location and its interaction with entry and components of entry were significant for both days to flowering and plant height except mutant vs. normal $\times$ location component for plant height (Table 3.3). The

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effect of the entry and its mutant and normal components were significant for all biofuel traits except juice yield and biomass among the mutants. But there was no significant difference between the mutants and the normal genotypes for these traits except biomass. Moreover, the effect of location and its interaction with entry and its normal and mutant components was significant for all biofuel traits except entry × location effect for biomass, mutant × location for °brix and biomass, and normal × location effect for biomass.

Mean lesion length for infection by *F. thapsinum* ranged from about 8 cm in Atlas to 16cm in Kansas Collier with an overall mean of 12.6 cm. This result was consistent across locations. The score for *Macrophomina* also ranged from about 8 cm in Atlas to 18 cm in Early Hegari. The highest and the lowest scores were observed in wild type genotypes for both pathogen groups though the difference for *Fusarium* was not significant at all locations. Unlike for *Fusarium*, genotypic effect at all locations was significant for *Macrophomina*. There was marked effect of location on development of charcoal rot that the Ottawa location sustained the highest infection of 18 cm as compared to about 11 and 13 cm in Hesston and Manhattan. Unlike the other locations, Ottawa soils are sandy in nature and hence drain fast. This along with the high temperature in July may have depleted soil moisture creating ideal condition for charcoal rot infection to take effect. The score for nodes crossed for both pathogen groups and locations was similar to that of lesion length except the effects were significant at all locations for both pathogens (Table 3.5).

Comparison of the mutants and their wild type versions showed that mean lesion length due to both pathogens were not significant between the mutations and also between the mutations and the wild types (Table 3.6). Although *bmr12* appeared to have slightly longer lesion both for *Fusarium* and *Macrophomina*, it was not significantly different from the wild

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type and from *bmr6*. The difference with respect to nodes crossed was significant and was higher in the wild types for both pathogens. On the other hand, the difference with respect to both lesion length and nodes crossed between genetic backgrounds was significant for both pathogens except lesion length for *Fusarium* (Table 3.6). Accordingly, lesion length for *Macrophomina* was highest in Early Hegari and lowest in Atlas. Atlas also had the lowest lesion length for *Fusarium*. The highest and the lowest number of nodes crossed for both pathogens were obtained in Early Hegari and Rox Orange, respectively, for both pathogen species.

### *Phenology, plant height and biofuel traits*

Days to flowering and plant height were significantly different among the entries (Table 3.7). Atlas *bmr12* took the longest time (71 d) to reach half bloom stage while Rox Orange *bmr6* was the earliest to bloom taking only 62 d. For plant height, Kansas Collier was the tallest entry with mean height of 246cm while Kansas Collier *bmr6* being the shortest (151cm). The mutants and their wild type versions were supposed to be of the same height since they are expected to be near-isogenic lines but the mutations and their wild types in all backgrounds markedly differ for both plant height and days to maturity. The difference among the entries for all biofuel traits was significant. The highest juice yield of 750 ml was recorded in Kansas Collier and the lowest of 240ml in Atlas. Whereas, the highest ºbrix score was noted in Rox Orange *bmr6* and the lowest in Early Hegari *bmr6*. The total sugar yield follows the same trend with juice yield and ºbrix score. The highest and the lowest biomass were recorded in Early Hegari and Kansas Collier *bmr12*, respectively. Despite its role in reducing lignin content the *bmr* mutations don't seem to have any effect on all of the biofuel traits. There was no significant difference between the

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*bmr* mutations and between the *bmr* mutations and the wild types for juice yield, °brix score or sugar yield (Table 3.8). Thus the highest juice yields and °brix scores were obtained in Kansas Collier and Rox Orange. Early Hegari was the lowest for °brix score but the highest for biomass yield.

## *Tolerance to greenbug feeding*

Leaf chlorophyll loss rating induced by greenbug feeding was remarkably different among the entries (Table 3.9). The resistant check KS97 had the lowest injury score of 11% followed by the 24% in the other resistant check PI266965. Whereas the susceptible check BTx399 had the highest injury rating of 46%. The other entries, Rox Orange, Atlas and their *bmr6* and *bmr12* versions had intermediate scores but not significantly different. However, it appears that the wild type entries have slightly lower damage rating of about 32% compared with 34% in *bmr6* and 40% in *bmr12* of both backgrounds.

Table 3.2. Combined analysis of variance for reaction to severity of stalk rot infection caused by *Fusarium thapsinum* and *Macrophomina phaseolina* across four locations in Kansas in 2009 and 2010.

| Source of Variation | df | F. thapsinum | | M. phaseolina | |
|---|---|---|---|---|---|
| | | Lesion Length (cm) | Nodes crossed (no. plant $^{-1}$) | Lesion Length (cm) | Nodes crossed (no. plant $^{-1}$) |
| Location (L) | 3 | 175.20 | 1.64* | 401.94 | 7.24** |
| Loc (Rep) | 12 | 47.94** | 0.26 | 45.85* | 0.43 |
| Entry (E) | 10 | 84.58 | 1.96* | 167.36* | 4.12** |
| mutant | 6 | 63.82 | 1.38** | 90.82 | 4.08** |
| Normal (N) | 3 | 150.10 | 3.23** | 345.86** | 4.68* |
| mutant vs N | 1 | 1.58 | 1.19 | 15.82 | 4.20 |
| Entry x L | 30 | 45.46 | 0.29** | 75.93** | 0.78** |
| mutant x L | 18 | 36.35* | 0.16 | 77.83** | 1.15** |
| Normal (N) x L | 9 | 68.53** | 0.51** | 66.61* | 1.23 |
| mutant bmr vs N x L | 3 | 39.82 | 0.64 | 87.57 | 3.61** |
| Error | 95 | 18.99 | 0.15 | 21.14 | 0.35 |

*, ** - Significant at $P \leq 0.05$ and $P \leq 0.01$ levels of probability, respectively.

Table 3.3. Across location combined analysis of variance for biofuel associated traits.

| Source of Variation | df | Days to Flowering | Plant height | Juice | Brix | Sugar Yield | Biomass |
|---|---|---|---|---|---|---|---|
| Location (L) | 2 | 374.98** | 0.40* | 0.65* | 144.75** | 0.03** | 0.18** |
| Loc (Rep) | 8 | 5.27** | 0.03 | 0.01 | 4.45* | 0.0003 | 0.02 |
| Entry (E) | 10 | 97.63* | 2.40** | 0.21** | 38.32** | 0.009** | 0.03** |
| mutant | 6 | 118.37* | 1.93** | 0.07 | 41.52* | 0.004* | 0.007 |
| Normal (N) | 3 | 61.83 | 3.89** | 0.56* | 44.20* | 0.02* | 0.04* |
| mutant vs. N | 1 | 84.16 | 0.72* | 0.005 | 0.07 | 0.0003 | 0.09* |
| Entry x L | 20 | 50.04* | 0.07** | 0.06** | 11.29** | 0.002** | 0.01 |
| mutant x L | 12 | 32.27** | 0.06** | 0.04** | 13.34 | 0.001** | 0.02 |
| Normal x L | 6 | 45.81* | 0.10** | 0.11** | 7.75* | 0.003* | 0.003 |
| mutant vs. N x L | 2 | 374.98** | 0.03 | 0.01 | 9.69 | 0.0004 | 0.003 |
| Error | 70 | 1.35 | 0.02 | 0.01 | 1.75 | 0.00004 | 0.008 |

*, ** - Significant at $P \leq 0.05$ and $P \leq 0.01$ levels of probability, respectively.

Table 3.4. Mean lesion length (cm) among genotypes tested for resistance to *Fusarium thapsinum* and *Macrophomina phaseolina* tested at three locations in Kansas.

| Entry | *F. thapsinum* | | | | *M. phaseolina* | | | |
|---|---|---|---|---|---|---|---|---|
| | Manhattan[‡] | Hesston | Ottawa | Mean | Manhattan[‡] | Hesston | Ottawa | Mean |
| Atlas | 8.12 | 5.73 | 12.79 | 8.68 | 6.75 | 5.37 | 12.44 | 7.82 |
| Atlas *bmr12* | 10.50 | 14.65 | 13.60 | 12.16 | 17.06 | 13.00 | 12.22 | 14.96 |
| Atlas *bmr6* | 11.25 | 10.92 | 13.39 | 11.70 | 11.26 | 10.97 | 11.49 | 11.26 |
| Early Hegari | 18.49 | 4.78 | 14.82 | 14.77 | 16.46 | 6.94 | 31.80 | 18.65 |
| Early Hegari *bmr6* | 13.17 | 7.49 | 8.32 | 10.69 | 18.48 | 8.00 | 16.44 | 15.28 |
| Kansas Collier | 17.55 | 12.26 | 17.57 | 16.14 | 14.73 | 14.43 | 22.92 | 16.83 |
| Kansas Collier *bmr12* | 11.82 | 17.87 | 13.07 | 13.36 | 12.17 | 14.04 | 15.10 | 13.20 |
| Kansas Collier *bmr6* | 11.15 | 5.97 | 9.05 | 9.55 | 8.75 | 6.05 | 19.85 | 11.73 |
| Rox Orange | 11.77 | 12.15 | 12.45 | 12.01 | 10.50 | 11.94 | 11.29 | 11.04 |
| Rox Orange *bmr12* | 17.33 | 13.59 | 12.50 | 15.37 | 13.51 | 14.50 | 28.79 | 16.83 |
| Rox Orange *bmr6* | 15.50 | 12.76 | 15.46 | 14.80 | 16.80 | 14.86 | 16.24 | 16.17 |
| Mean | 13.33 | 10.74 | 13.09 | 12.69 | 13.30 | 11.01 | 18.07 | 13.91 |
| LSD$_{(0.05)}$ | ns | ns | ns | ns | 5.09 | 11.31 | 6.89 | 3.32 |

[‡] – averaged over 2 years.

Table 3.5. Mean nodes crossed among genotypes tested for resistance to *Fusarium thapsinum and Macrophomina phaseolina* testes across three locations in Kansas.

| Entry | F. thapsinum | | | | M. phaseolina | | | |
|---|---|---|---|---|---|---|---|---|
| | Manhattan[‡] | Hesston | Ottawa | Mean | Manhattan[‡] | Hesston | Ottawa | Mean |
| Atlas | 1.51 | 0.39 | 1.35 | 1.17 | 1.51 | 0.44 | 1.35 | 1.18 |
| Atlas *bmr12* | 0.24 | 0.20 | 0.25 | 0.23 | 0.18 | 0.00 | 0.90 | 0.33 |
| Atlas *bmr6* | 0.20 | 0.20 | 0.00 | 0.15 | 0.21 | 0.13 | 0.25 | 0.21 |
| Early Hegari | 1.25 | 0.27 | 0.50 | 0.88 | 1.15 | 0.00 | 3.00 | 1.41 |
| Early Hegari *bmr6* | 0.99 | 0.80 | 0.67 | 0.87 | 1.65 | 0.50 | 2.77 | 1.57 |
| Kansas Collier | 0.50 | 0.00 | 0.25 | 0.30 | 0.31 | 0.05 | 1.70 | 0.61 |
| Kansas Collier *bmr12* | 0.18 | 0.13 | 0.18 | 0.17 | 0.10 | 0.00 | 0.50 | 0.16 |
| Kansas Collier *bmr6* | 1.03 | 0.33 | 0.83 | 0.84 | 0.79 | 0.20 | 1.13 | 0.75 |
| Rox Orange | 0.11 | 0.00 | 0.00 | 0.06 | 0.13 | 0.05 | 0.10 | 0.10 |
| Rox Orange *bmr12* | 0.38 | 0.20 | 0.57 | 0.37 | 0.24 | 0.16 | 0.90 | 0.35 |
| Rox Orange *bmr6* | 0.21 | 0.20 | 0.18 | 0.20 | 0.27 | 0.21 | 0.58 | 0.33 |
| Mean | 0.60 | 0.25 | 0.38 | 0.47 | 0.60 | 0.17 | 1.20 | 0.64 |
| LSD$_{(0.05)}$ | 0.44 | 0.32 | 1.16 | 0.29 | 0.58 | 0.45 | 1.28 | 0.43 |

[‡] – averaged over 2 years.

Table 3.6. Effect of mutations and genetic background on lesion length and number of nodes crossed caused by infection with *Fusarium thapsinum* and *Macrophomina phaseolina*.

| Entry | *F. thapsinum* | | *M. phaseolina.* | |
|---|---|---|---|---|
| | Lesion Length (cm) | Nodes crossed (no. plant $^{-1}$) | Lesion Length (cm) | Nodes crossed (no. plant $^{-1}$) |
| **Mutations** | | | | |
| Bmr12 | 13.63 | 0.26 | 15.10 | 0.29 |
| Bmr6 | 11.74 | 0.50 | 13.52 | 0.71 |
| Wild type | 12.80 | 0.59 | 13.50 | 0.82 |
| Mean | 12.63 | 0.46 | 13.91 | 0.64 |
| LSD$_{(0.05)}$ | ns | 0.21 | ns | 0.31 |
| **Backgrounds** | | | | |
| Atlas | 8.69 | 1.17 | 7.83 | 1.18 |
| Early Hegari | 14.77 | 0.88 | 18.65 | 1.41 |
| Kansas Collier | 16.14 | 0.30 | 16.83 | 0.61 |
| Rox Orange | 12.01 | 0.06 | 11.04 | 0.10 |
| Mean | 12.80 | 0.59 | 13.50 | 0.82 |
| LSD$_{(0.05)}$ | ns | 0.27 | 3.80 | 0.41 |

Table 3.7. Mean performance of genotypes for biofuel traits across locations.

| Entry | Days to Flowering | Plant Height[‡] (m) | Juice (ml) [‡] | Brix (%) | Sugar Yield (ml) | Biomass (kg) [‡] |
|---|---|---|---|---|---|---|
| Atlas | 64.67 | 122 | 240 | 14.03 | 30 | 0.41 |
| Atlas *bmr12* | 71.08 | 224 | 530 | 15.89 | 90 | 0.43 |
| Atlas *bmr6* | 68.17 | 235 | 650 | 15.77 | 100 | 0.48 |
| Early Hegari | 66.92 | 157 | 560 | 13.95 | 80 | 0.54 |
| Early Hegari *bmr6* | 66.92 | 140 | 410 | 12.41 | 50 | 0.42 |
| Kansas Collier | 63.17 | 246 | 750 | 18.03 | 140 | 0.52 |
| Kansas Collier *bmr12* | 68.83 | 231 | 490 | 15.38 | 80 | 0.37 |
| Kansas Collier *bmr6* | 69.91 | 151 | 480 | 14.43 | 70 | 0.47 |
| Rox Orange | 68.25 | 227 | 610 | 16.11 | 100 | 0.45 |
| Rox Orange *bmr12* | 65.50 | 234 | 560 | 17.20 | 100 | 0.39 |
| Rox Orange *bmr6* | 61.58 | 209 | 530 | 18.17 | 100 | 0.42 |
| Overall Mean | 66.80 | 197.80 | 528.20 | 15.60 | 85.50 | 0.45 |
| LSD$_{(0.05)}$ | 0.95 | 10 | 100 | 1.09 | 20 | 0.08 |

[‡] - measured from 5 random plants.

Table 3.8. Effect of mutations and genetic backgrounds on days to flowering, plant height and biofuel traits.

| Entry | Days to Flowering | Plant Height (cm) | Juice yield (ml) | Brix (%) | Sugar Yield (L) | Biomass (kg) |
|---|---|---|---|---|---|---|
| **Mutations** | | | | | | |
| Bmr12 | 68 | 230 | 530 | 16.18 | 90 | 0.40 |
| Bmr6 | 67 | 184 | 520 | 15.21 | 80 | 0.45 |
| Wild type | 66 | 188 | 540 | 15.55 | 90 | 0.48 |
| Mean | 67 | 198 | 530 | 15.60 | 86.70 | 0.45 |
| LSD$_{(0.05)}$ | ns | 19 | ns | ns | ns | 0.04 |
| **Backgrounds** | | | | | | |
| Atlas | 65 | 122 | 240 | 14.03 | 30 | 0.41 |
| Early Hegari | 67 | 157 | 550 | 13.96 | 80 | 0.54 |
| Kansas Collier | 63 | 246 | 750 | 18.03 | 140 | 0.52 |
| Rox Orange | 68 | 227 | 610 | 16.11 | 100 | 0.45 |
| Mean | 66 | 188 | 537.50 | 15.55 | 87.5 | 0.48 |
| LSD$_{(0.05)}$ | ns | 13 | 110 | 1.29 | 0.02 | 0.09 |

Table 3.9. Response of sorghum genotypes of varying brown midrib mutation alleles to greenbug feeding.

| Background | Leaf chlorophyll-loss (%) | | |
| --- | --- | --- | --- |
| | wild type | *bmr6* | *bmr12* |
| Rox Orange | 33.79 | 34.66 | 40.00 |
| Atlas | 30.36 | 33.50 | 39.40 |
| PI266965 | 24.17 | - | - |
| KS97 | 10.80 | - | - |
| BTx399 | 45.92 | - | - |
| $LSD_{(0.05)} = 26.54$ | | | |

# DISCUSSION

Damage by stalk rot diseases and insect feeding are among the important constraints to grain sorghum production worldwide. Apart from reducing yield and quality of grains as a result of impeded photosynthesis, stalk rots can lead to collapse of stalk tissues and thus lodging. Grains on lodged heads are often lost since they are not picked during harvesting and hence percentage yield loss can exceed lodging percent. Since the disease is aggravated by post flowering environmental stress, particularly drought, the obvious successes in the development of post flowering drought tolerant hybrids over the last fifteen years has significantly reduced stalk rot problem. Similarly, a single major gene discovered in the US sorghum working population successfully ended damage by greenbugs (*Schizaphis graminum*) that devastated sorghum production in the 1980s. However, with the apparent need for deployment of *bmr* genes in biomass sorghum improvement, the concern over stalk rotting/standability and insect attack is re-emerging. Because of its impact on lignin concentration, there is well founded concern that deployment of *bmr* genes may expose the materials to attack by these and other biotic agents.

The present study was focused on investigating these concerns. We evaluated the response of two *bmr* mutations and their normal versions in four different genetic backgrounds to infection by common stalk rot pathogens, *F. thapsinum* and *M. phaseolina*. A subset of these genotypes along with tolerant and susceptible checks was also evaluated for reaction to greenbug feeding. Our results showed no evidence of the *bmr* mutations posing any risk of predisposing plants to damage by stalk rot diseases and greenbug feeding. Disease severity among *bmr* mutants was not different from the normal genotypes and was even lower in some of the backgrounds indicating that lignin content has no or little role in affecting plant response to these

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pathogens. The assumption that reduced lignin may aggravate stalk rot diseases has also been challenged by previous investigators. Despite their effect on stalk strength, seven *bmr* sorghum and four *bm* maize mutants were reported to have less stalk invasion by *M. phaseolina* compared to their wild type versions (Tesso and Ejeta, 2011). Similar study on *bmr* and normal grain sorghum lines showed the wild type genotypes as more susceptible to peduncle inoculation by *Fusarium* species than either of *bmr6* or *bmr12* mutants (Funnell et al., 2006). This result also agrees with earlier findings in sorghum where the presumed relationship between lignin deficiency and stalk-rot-induced lodging was refuted (Esechie et al., 1977). Moreover, a study conducted by our group on diverse set of grain sorghum accessions showed no observable difference in stalk rot resistance between stiff (perhaps highly lignified) and weak-stalked genotypes (Tesso et al., 2005).

Our preliminary result on greenbug feeding also shows similar result. In contrast to the presumed assumption, the low lignin mutations did not increase susceptibility to greenbugs. This result may be different for other insect species or different biotypes of this same species. Greenbug feeding involves enzymatic degradation of plant cellwall and sucking of hydrolyzed plant saps (Al-Mousawi et al., 1983) that mechanical resistance offered by lignin is of no significant importance. The fact that the low lignin genotypes sustained lower feeding damage compared with the normal susceptible genotypes BTx623 corroborates this assumption. But this may become an important factor for other insect pests of different feeding habit, especially the chewing insects such as fall armyworm where the feeding mechanism involves mechanical degradation of lignin and plant cell wall component.

The absence of significant difference in disease severity and feeding injury between the normal and low lignin mutants suggests that *bmr* genotypes can be effectively deployed for

production of forage and biofuel feedstocks without incurring losses to stalk rots and greenbug feeding. However, the circumstances under natural conditions may be different. In contrast to artificial inoculation where the inoculums are directly delivered to the target plant part, natural infection involves longer path to initiating infection including passing mechanical defense by lignin.

On the other hand, despite its reported role in facilitating conversion of biomass into fermentable sugar (Dien et al., 2009), the current study shows that lignin has no significant effect on juice yield and °brix score in sweet sorghum germplasm. But since °brix score is not synonymous with sugar content, the *bmr* mutations may have impact on other components of fermentable sugar that are not detected in °brix measurement. But this needs further investigation to determine. However, even if the total sugar yield is not affected by the mutation, the fact that there is no negative impact of *bmr* on either juice yield or °brix, and the fact that it is not associated with aggravated incidence of pests and diseases, maintains the positive role of the *bmr* mutation for biofuel production. The greatest concern in the deployment of the *bmr* genes for biomass production, however, is the marked effect of the mutation on stalk strength. *Bmr* forages have always been noted to be more susceptible to lodging than normal forage crops. Stalk strength measured as rind penetration resistance among a range of *bmr* and normal genotypes also showed the low lignin mutants to be inferior to their normal counterparts (Tesso and Ejeta 2011). The variation in stalk strength in sorghum germplasm indicates the potential to identify suitable background in which the impact of *bmr* on stalk strength can be minimized. In forage breeding leafiness and stalk diameter are among the major considerations since they are related to dry matter intake and digestibility. As a result most forage cultivars tend to have thinner stems and more leafy which upon grain filling increases torque on the slender stems. Superimposing

*bmr* alleles on this may lead to lodging under moderate windy conditions. These traits are of no priority in biomass feedstock production such that selection for thicker rinds and stiff stalks may partly overcome the negative effect of *bmr* genes.

# CONCLUSIONS

Our result confirms that *bmr* alleles, despite their effect on lignin content, do not present any risk of aggravated attack by stalk rot pathogens and greenbug feeding. Thus *bmr* genotypes can be effectively deployed for production of lignocellulosic feedstocks without incurring losses to these agents. However, the result needs to be confirmed under natural conditions. Although, low lignin concentration tends to increase the proportion of the digestible cell wall components, the mutation has no impact on juice yield and °brix percent of genotypes.

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