

**The potential of eliminating the grain sink for enhancing  
biofuel traits in sweet sorghum hybrids**

by

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B.S., Sabha University, 1994  
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AN ABSTRACT OF A DISSERTATION

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Department of Agronomy  
College of Agriculture

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## Abstract

Sweet Sorghum [*Sorghum bicolor* (L.) Moench] is a type of cultivated sorghum grown primarily for its sugar-rich stalks. Because of its high fermentable sugar content, the crop is widely recognized as an alternative feedstock source for bio-fuel production. The extent to which stalk sugar accumulation occurs may be determined by several factors including the sink size. Grain is the most important sink in sorghum and other grain crops. Three experiments were conducted in this study to determine the extent to which the grain sink can reduce sugar accumulation in the stalks, to test and validate a genetic system that allows development of sterile sweet sorghum hybrids, and to assess the potential of sugar-rich hybrids to overcome stalk rot diseases.

The first experiment, based on 22 sweet sorghum genotypes, was undertaken to study the effect of eliminating the grain sink (removing the head prior to anthesis) on stalk juice yield, sugar accumulation, and biomass. The data showed that the grain sink had a significant effect on all traits measured. Elimination of the grain sink significantly increased °Brix % (17.8%), dry biomass (27.8%), juice yield (23.9%), and total sugar yield (43.5%).

The second experiment was aimed at validating the role of A3 genetic male sterility system for producing sterile sweet sorghum hybrids. Ten sweet sorghum pollinator lines of variable sugar content were selected among the entries included in the previous experiment. The lines were crossed to four A1 and A3 cytoplasmic male sterile (CMS) lines using a Design II mating scheme. The A3 females did not have effective restorers so that the hybrids were expected to be sterile. The parental lines and corresponding hybrids were evaluated for biomass production, °Brix, juice and sugar yield using a randomized complete block design. All A3 hybrids were sterile and did not produce seed when heads were covered prior to pollination. The effect of grain sink represented by the A1 vs. A3 CMS were highly significant for Brix%, biomass, juice, and sugar yield.

Comparison of parents vs. crosses component was highly significant, indicating marked heterosis effect for the traits. Both general (GCA) and specific (SCA) combining ability effects were also significant for all traits, indicating the role of both additive and dominance genetic effects in the inheritance of the characters.

Earlier studies have shown positive relationships between stalk sugar concentration and stalk rot disease resistance in sorghum. Thus, the objective of the third experiment was to study the effects of the CMS mediated differential accumulation of stalk sugar on severity of charcoal rot disease caused by *Macrophomina phaseolina*. The experiment provided an opportunity to test the effect of variable stalk sugar in the same genetic backgrounds. The data indicated that hybrids produced from A3 cytoplasm were more resistant to charcoal rot (7.1cm lesion length) compared to those produced from the A1 hybrids (9.5 cm lesion length). The enhanced resistance of hybrids with higher sugar yield could have significant agronomic advantage in sugar based bio-fuel feedstock production.

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## List of Abbreviations

<b>Abbreviation</b>	<b>Explanation</b>
ANOVA	Analysis of variance
RCBD	Randomized Complete Block Design
HPLC	High-performance liquid chromatography
<i>bmr</i>	Brown midrib
Brix	Total Dissolved Solids in Juice
CMS	Cytoplasmic male sterility
GCA	General combining ability
SCA	Specific combining ability
HP	High parent value
MP	Mid parent value
HPH	High-parent heterosis
MPH	Mid-parent heterosis

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## **Dedication**

I dedicate this dissertation to my loving parents and my supportive lovely wife. I also dedicate this dissertation to the soul of my aunt Khadeja Jebril.

## General Introduction

Bioethanol is a renewable and sustainable liquid fuel alternative that is expected to have a promising future in tackling today's global energy crisis and the worsening of the environmental quality (Aditiya et al., 2016). It is a biodegradable, non-toxic and renewable resource with a potential to minimize particulate emissions in compression ignition engines (Hernandez and Kafarov, 2009). United States is the world's largest producer of bioethanol (Wang et al., 2012). US bioethanol is primarily produced by fermenting maize grain (RFA, 2016). However, the Renewable Fuel Standard (RFS) requires that 60 billion of the 136 billion liters of renewable bioethanol must be produced from non-grain feedstocks by 2022 (EPA, 2015). Therefore, a transition to dedicated bioethanol feedstocks will be required to meet the cellulosic bioethanol production goals established by the RFS (Rooney et al., 2007).

Currently, energy crops are mainly represented by perennial grasses such as switchgrass (*Panicum virgatum L.*), sugarcane (*Saccharum spp.*), sweet/forage sorghum, and miscanthus (*Miscanthus spp.*). Of these, sweet sorghum has emerged as one of the most promising energy crops (Jessup, 2009). Sweet sorghum is of a particular interest because of the large volume of readily fermentable juice that it can produce. Hunter and Anderson (1997) indicate that sugar produced in sweet sorghum has the potential to yield up to 8,000 L ha<sup>-1</sup> ethanol or about twice the ethanol yield potential of maize grain and 30% greater than the average Brazilian sugarcane productivity of 6,000 L ha<sup>-1</sup>.

Sweet sorghum accumulates high concentration of fermentable sugar in soluble form in the stalks which can be converted directly to ethanol without the additional process required by grains to hydrolyze starch before fermentation (Bryan et al., 1981). Sweet sorghums are typically characterized by low grain yields, but high biomass production. The tall juicy stalks, contain 10-

25% sugars (mainly sucrose, glucose, and fructose) near the time of grain maturity (Smith and Frederiksen, 2000). The extractable juice component of sweet sorghum provides a non-food source of readily fermentable sugar comparable to the carbohydrates of maize grain and juice of sugarcane, which are food resources currently used for ethanol production. High biomass production of sweet sorghum also provides a cellulosic source of ethanol in sufficient quantity to further enhance economic opportunities for this crop. Along with the recent worldwide interest in sweet sorghum, widespread agronomic evaluations across the United States have demonstrated that it has wide adaptability and efficient production potential (Smith et al., 1987; Smith & Buxton, 1993).

Sweet sorghum could fit well in areas where sugarcane is currently produced because sugarcane is harvested only during a rather short period primarily between November and February. Harvest of sweet sorghum should be possible from as early as late July for early cultivars until frost hits which is typically after the start of sugarcane harvest (Bradford, 2008). Sweet sorghum can extend the season of sugar mill operation each year to enhance economic viability of this established industry, while contributing to development of the new biofuels industry. Thus, sweet sorghum has been identified as a particularly promising complementary crop for diversification of sugarcane croplands.

Integrating two complimentary bioenergy crops such as sweet sorghum and sugarcane can significantly reduce the cost of producing ethanol (Nguyen and Prince, 1996). The cane milling, and ethanol distillation facilities are a large portion of the cost to produce ethanol from sugarcane or sweet sorghum. Staggering the planting dates of sweet sorghum crops to be harvested before and after the sugarcane crop in the same region will extend the length of time an ethanol plant operates each year and reduces cost per unit of production.

The economic value of sweet sorghum is in the stem and not in the grain as in the grain sorghum. Hence, if photosynthates used in grain formation and development could be diverted into the stems, stem yield and juice quality may be improved. Sweet sorghum stores starch as the principle nonstructural carbohydrate in the grain, but primarily stores sucrose in the stems (Miller and Creelman, 1980). It is speculated that the smaller grain yield in sweet sorghum may be due to competition between elongating stems and pre-anthesis head development (Willey and Basiime, 1973). The present investigation was primarily aimed at determining the impact of manipulating the sink size on enhancing sugar content in alloplasmic isonuclear sweet sorghum hybrids. The specific objectives are:

1. To study the effect of grain sink on stalk juice yield, sugar accumulation of sweet sorghum
2. To determine the potential of A3 cytoplasmic male sterility system to eliminate grain formation in sweet sorghum hybrids and assess its impact on stalk sugar accumulation and agronomic performance.
3. To determine the combining ability for biofuel traits of selected sweet sorghum pollinators lines when tested in the A3 CMS system.

# Chapter 1 - Review of Literature

## Origin and domestication

Sorghum [*Sorghum bicolor* (L.) Moench] has been under cultivation in arid and semi-arid regions of Africa for thousands of years. The north-eastern part of the continent, the Ethio-Sudan region, is widely considered as geographical area of origin (Dhillon et al., 2007; Kimber, 2000; Vavilov, 1951). The wide distribution and high genetic diversity of the crop observed in the continent is an indication not only that Africa is the natural home to sorghum but also its inhabitants have persistent social and economic attachment to this crop. From its home in eastern Africa, sorghum was believed to have spread to other parts of the continent and eventually moved out of Africa to further areas such as India, the Middle East, and China either through trade or human migration (Doggett, 1970; Kimber, 2000).

As the very early domesticated sorghum plants were selected and dispersed, genetic adaptation and intercrossing followed by selection and continued intercrossing in isolated ecosystems gave rise to new and stable sorghum biotypes in almost all places the crop had reached. The cultivation of native cultivars and introduction of cultivars evolved in other locations offered additional opportunity for intercrossing and selection of new, more adapted cultivars. All these domestication events, cultivar evolution, selection, and adaptation were associated with human migrations both on land and water throughout Africa, and later to the Middle East and gradually further to India some 3,000 years ago (Kimber, 2000). This movement and evolution of new cultivars under different environments gave rise to the development of five domesticated sorghum races classified into: bicolor, caudatum, guinea, kafir, and durra, based primarily on the morphology of the spikelet, seed, and panicle (Smith and Frederiksen, 2000).

## **Sorghum in North America**

Like elsewhere, the introduction of sorghum to America was linked to human migration and movement, in this case the movement of native Africans to North America via slave ships during the 17th century. After widespread distribution following the initial introduction, production of sorghum slowly dropped off and eventually disappeared (Maunder, 2002). The next introduction of grain sorghum into America occurred through California in 1874. Shortly after its introduction, the crop became widely distributed in the southern Great Plains and other arid regions of the United States perhaps due to its ability to produce more grain than corn under drought conditions (Smith and Frederiksen, 2000).

Like the grain types, sweet sorghums also were introduced to the US from Africa and also from China in the 1850s (Murray et al., 2009). Throughout the late 1800s and early 1900s ‘Chinese Amber’ and other syrup producing sorghums were more important in the US than grain sorghums, producing millions of gallons of syrup annually (Winberry, 1980). In the early decades of the 20th century, many sweet sorghum varieties were developed for syrup production and most of these varieties exist today. They were developed primarily from six African landraces, MN960 (PI 534165), MN1054 (PI 152965), MN1056 (PI 152967), MN1060 (PI 152971), and MN1500 (PI 154844) (Murray et al., 2009).

## **Sorghum a multipurpose crop**

### **Sorghum as food grain**

Due to its adaptability to dry and high temperature conditions, sorghum is widely cultivated in semi-arid regions of the world. In regions where it is produced, it used for making various types of foods such as breads, fermented or unfermented porridges, couscous, and fried products (Leder,

2004). It is considered as a principal source of energy, protein, vitamins and minerals for millions of poor people in Africa and Asia. In the USA, South America and Australia, the grains are predominantly utilized for animal feed and ethanol production, while a small percentage is used in the production of snacks and gluten-free foods (Taleon et al., 2012). The grain composition of sorghum resembles that of other cereals containing 60-75% carbohydrates, 8-13% protein and 4-6% lipids, and the grains also are used in the production of alcoholic and nonalcoholic beverages such as beer and malts (Adegbola et al., 2013). The stalks are used as source of fuel, building material, and fencing structure (Rooney and Waniska, 2000). The stem and foliage are used for green chop, fodder, hay, silage, and pasture (Dahlberg et al., 2011). The digestibility of sorghum proteins is low, and it is further reduced when wet cooked to process the crop as a food product (Axtell et al., 1981; Duodu et al., 2002; Hamaker et al., 1986). The raw digestibility of sorghum starch is also the lowest among cereals, because the starch granules are strongly associated with endosperm proteins (*kafirins*), restricting the accessibility of  $\alpha$ -amylase to starch (Rooney and Pflugfelder, 1986). This is considered as a negative aspect for animal feeding (Serna-Saldivar and Rooney, 1995), but may be beneficial for human nutrition since it helps to lower the caloric intake (Barros et al., 2012).

### **Sorghum as feed grain**

In the developing world, sorghum is grown as major source of food. In the United States and other western countries, sorghum is used primarily as a feed grain for livestock. It has been reported that 51% of the global sorghum grain is used as feed for livestock, while 49% is for human food and other uses (Maunder, 2002). Other reports show 48% of sorghum grain production is fed to livestock, and its feed value is comparable to maize (Carter et al., 1989; Dowling et al., 2002).



Sorghum is also widely grown for forage or silage with the dried leaves and stems making a valuable roughage for cattle and horses. The well matured plant can be used as green fodder or silage. The kernel of sorghum is somewhat similar to maize, though smaller in size. Whole sorghum grains can be given to sheep, pigs and even poultry but are usually ground for cattle (McDonald et al., 1987; Carter et al., 1989)

### **Sweet Sorghum as biofuel feedstock**

The concern about over reliance on non-renewable fossil fuel and the growing environmental pollution associated with greenhouse gas emissions have increased interest to seek and explore renewable clean energy sources. Biofuels are among the top clean-energy alternatives on the table. Because of its potential to produce massive biomass and the sugar-rich, juicy stalks, sorghum is rated as one of the top candidate feedstock sources for biofuel production. All parts of sorghum can be converted to biofuels. At present, sorghum grain is among the major feedstocks used in the biofuel industry. Furthermore, because of the massive biomass it produces, especially from photoperiod sensitive tropical sorghums, and sugar rich juices from certain variants of sorghum that can be directly converted to liquid ethanol, sorghum has even more potential as a biomass and sugar based bioenergy source. The relatively short growth cycle of the crop and ample knowledge of its agronomy may facilitate its production without the need for further study on production practices. The emphasis in this particular study is to explore and test genetic systems that can maximize sugar and biomass production as biofuel feedstock.

The name “sweet sorghum” was perhaps coined to distinguish varieties of sorghum with high concentration of soluble sugars in the stalk sap or juice compared to grain sorghum, which has relatively less sugar and juice in the stalks. Unlike grain sorghum that is produced for its grain,

the economic value of sweet sorghum lies in its sugar-rich stalks that are exploited in various ways. Apart from the difference in the economically important part of the crop, both grain and sweet sorghums belong to the *S. bicolor* species and can routinely intercrossed and produce fertile hybrids just like grain × grain or sweet × sweet type crosses. Sweet sorghum stores starch as the principle nonstructural carbohydrate in grain, but primarily stores sucrose in the stalk (Miller and Creelman, 1980). In addition to producing carbohydrates that can be easily fermented to ethanol, sweet sorghum has high biomass yield, which is composed of as much as 25% to 50% soluble carbohydrates with the majority of these sugars within the stalk consisting of fructose, monosaccharide glucose, and disaccharide sucrose (Whitfield et al., 2012). The proper stage for greatest sugar concentration in sorghum is at physiological maturity. Total sugar and non-reducing sugars in the juice increase from flowering to physiological maturity whereas, reducing sugars decrease during this period (Channappagoudar et al., 2007). Most of the sugar accumulation in the stalk takes place during inflorescence development (McBee and Miller, 1982) and is accelerated after flowering (Prasad et al., 2007; Almodares et al., 2008).

Typical sweet sorghum cultivars can grow to 2.4-3.0 meters (8-10 feet) in height, can produce up to 30 Mg ha<sup>-1</sup> of dry biomass in favorable environments (Rooney et al., 2007), and can accumulate large amounts of juice in the stalk with a high sugar concentration. Sugar yield varies depending on variety, location, and maturity, but can exceed 4 Mg ha<sup>-1</sup> (Morris and McCormick, 1994). Stalks of sweet sorghum cultivars contain approximately equal quantities of soluble (glucose, fructose and sucrose) and insoluble carbohydrates (cellulose and hemicellulose) (Yu et al., 2012). Stalk juices contain 13-20% total fermentable sugars and thus provide a better source of carbohydrates for the production of fuel ethanol (Woods, 2001). At physiological maturity °Brix (soluble content) of juice from sweet sorghum cultivars can range up to 22.85%, depending on

variety, but before anthesis the plant sugar concentration is estimated at 12.5% (Prasad et al., 2007). The types of sugars and their relative proportion within the stalk varies depending on the variety. However, as the plant matures, sucrose content typically increases, replacing glucose and fructose in the stalk (Whitfield et al., 2012).

Sweet sorghum is characterized by high photosynthetic efficiency and is one of the most promising crops for ethanol production. It can be grown in both temperate and tropical zones in both irrigated and non-irrigated environments. Its C<sub>4</sub> photosynthetic pathway enables it to achieve maximum short-term growth rates (Loomis and Williams, 1963). In addition to sugary juices, sweet sorghum also produces enormous biomass, which contributes to the production of both sugar and cellulosic biomass. Sweet sorghum can outperform corn in terms of total biomass production over short periods. It also has rapid growth and can mature in 3 to 5 months. The top high-yielding varieties can produce well over 60 tons per ha (fresh weight of above-ground biomass) in 5 months under good agronomic conditions (Mastrorilli et al., 1999).

Research into sweet sorghum as an ethanol feedstock is relatively novel when compared to that of more traditional feedstocks such as corn, sugar beet, or sugarcane (Zegada-Lizarazu and Monti, 2012). The fact that sugarcane can be grown only in tropical regions and that the harvestable portion of sugar beets is located underground and requires additional energy inputs makes these crops less attractive as bioenergy feedstocks. Sweet sorghum on the other hand, is both water and nutrient efficient and can be produced within a relatively short period of time compared to other bioenergy feedstock sources (Whitfield et al., 2012).

Swanson and Parker (1931) reported that stalk juiciness in sweet sorghum is controlled by a single recessive gene. In agreement with this, Ayyangar et al. (1936) suggested a single dominant gene conferring the non-sweet characteristic. Later studies provided support for the presence of

multiple genes with additive effects (Li et al., 2004). A recent study indicates that both sugar and juice accumulation are the result of additive genes with dominance effects (Godoy and Tesso, 2013). Another study by Ritter et al. (2007) reported that stalk sugar is controlled by recessive genes. It appears that, depending on the background, multiple genes of various modes of action control stalk sugar in sweet sorghum.

Several studies have shown significant differences among genotypes with regards to mean sugar content and associated traits. Mean °Brix percent in sorghum ranges between 6.48 and 20.68 at maturity, and 7.24 to 18.48 at anthesis (Makanda et al., 2009). Juice yield and composition are highly variable depending on genotype. Mohite and Sivaraman (1984) reported a composition of 60% sucrose, 33% glucose and 7% fructose in the sweet sorghum. Prasad et al. (2007) reported that sweet sorghum juice is made up of anywhere between 50% to 60% sucrose, 25% to 35% glucose, and 7% to 20% fructose. Sugar content in the sorghum stalk is a quantitative character determined by polygenes, with the genes controlling lower sugar content appear partially dominant, and low heritability of sugar content (Hongtu and Xide, 1989).

### **The development of hybrid technology in sorghum**

Hybrid sorghum production utilizes a unique genetic system, cytoplasmic male sterility (CMS), that allows commercial production of hybrid seeds. This requires the use of a tri-parent system, the cytoplasmic male sterile seed parent line (A-line), the female parent maintainer line (B-line), and the fertility restorer line (R-line) used as the pollinator parent in hybrid seed production. Sterility is a function of the joint action of sterile cytoplasm and a defective nuclear gene that is incapable of restoring male fertility in sterile cytoplasm. Hence, seed production from such plants is possible only through the use of maintainer lines (B-lines) that have normal cytoplasm but

inactive fertility restorer genes. The functional fertility restoration gene can override the effect of sterile cytoplasm and produce functional pollen.

The CMS first discovered in onion and was exploited for the production of hybrid seeds before the trait was even discovered in other crops (Jones, 1943). Since then, CMS has been used extensively in a number of other crop species including corn, sorghum, pearl millet, sugar beet, sunflower, rice, and carrot. In sorghum, CMS was first discovered in the 1940s. The male sterility in CMS plants is considered to be the result of a mutation in a mitochondrial gene that interacts with the nuclear fertility restorer gene to result in male sterility. In addition to the CMS, sterility is governed by the allelic state of the fertility restorer gene. Genotypes carrying the recessive form of the fertility restorer gene that also carry the CMS mutation in the cytoplasm are incapable of producing fertile pollen. But when such genotypes occur in normal cytoplasm they will produce fertile pollen. Hence, selected lines carrying the recessive form of fertility restorer genes (B-lines) are crossed to CMS lines to produce sterile hybrids. Repeated backcrossing with pollen from the selected B-line will lead to the development of A-line version of the B-line which is used as a seed parent for hybrid production (Hanson and Conde, 1985).

The mechanism leading to male sterility in CMS lines is not very clear. But it has been suggested that normal anther development is prevented through interaction among substances in the anther and organelles within plants with altered cytoplasm. Additional regulatory substances may be involved to determine the extent of sterility and the environmental effect on the expression of sterility (Flavell, 1974). The CMS is associated with the expression of novel, chimeric open reading frames (*orf's*) encoded by mitochondrial genome. The chimeric *orf's* carry recognizable segments of essential mitochondrial gene coding novel proteins that interfere with the expression of normal gene (Schnable and Wise, 1998; Hanson and Bentolila, 2004). The CMS trait is of

special significance in agriculture as it allows large scale production of hybrid seeds. When CMS plants are crossed to normal B-lines, the resulting progeny will invariably be male sterile. Therefore, restoration of male fertility is essential for obtaining fertile F1 hybrids.

### **The three-parent hybrid breeding system in sorghum**

Just as male sterility is crucial for seed production, restoration of fertility is critical for commercial production of grains from hybrid crops. Thus, in order to deploy CMS systems to develop commercial hybrids, it is essential to have an effective restorer line (Acquaah, 2007). Fertility restoration relies on nuclear genes that suppress or compensate for mitochondrial dysfunction and restores fertility in CMS plants known as fertility restorer (*Rf*) genes (Schnable and Wise, 1998). Genotypes carrying the wildtype allele for the fertility restorer gene can restore fertility in CMS genotypes. The classical method to identify restorer sources is to cross the lines to known CMS females and evaluate the hybrids for male fertility. However, there are several sources that are not complete restorers known as partial restorers. Hence, breeders need to exercise judgement to determine if a given pollinator line is an effective restorer. As with CMS, fertility restorer alleles are known for almost all well characterized CMS system, but the mechanism of action has not been determined definitely for any of them (Li et al., 1998) apart from attempts in a few studies. Schertz (1994) reported fertility restoration is under single or two gene controls or could be polygenic based on the type of cytoplasm used. For example, *ogu* CMS of *Raphanus* (Ogura, 1968) and '*pol*' CMS of *B. napus* (Fang and McVetty, 1989) are restored by single nuclear genes. The T-cytoplasm of maize, PET cytoplasm of sunflower, and T-cytoplasm of onion need two unlinked genes for fertility restoration (Dill et al., 1997). Restorer genes are specific to each CMS system and are usually introgressed from the cytoplasm donor species. Hence, restorer genes also are used

to differentiate CMS systems within the same species (Bellaoui et al., 1999). Although different restorer genes can be found for a given CMS system, a single restorer capable of fertility restoration in two systems is very rare (Bhat et al., 2005). In sorghum, fertility restoration depends on nuclear backgrounds of both male and female parents (Schertz et al., 1989). The A1 cytoplasm requires the action of two complementary nuclear male-fertility restorers (*Rf1* and *Rf2*), similar to T-cytoplasmic maize (Klein et al., 2001). Both *Rf1* and *Rf2* alleles exhibit dominant gene action, and a dominant allele at each locus is necessary for fertility restoration (Maunder and Pickett, 1959). Unlike the A1, the A3 CMS has not been commercially utilized for hybrid seed production because of the low frequency of restorer genes among sorghum lines (Worstell et al., 1984; Bosques-Vega et al., 1989). Genetic analyses of fertility restoration in the A3 source of CMS lead to the determination that restoration conferred by the male-fertile source of the cytoplasm, IS1112C, was through a gametophytic mechanism requiring complementary action of two restoring alleles designated *Rf3* and *Rf4* (Tang et al., 1998; Pring et al., 1999).

In sorghum there are several CMS systems identified designated as A1, A2, A3, A4, etc., and they all have their own unique restorers, except A3 that appears to have only few restorers. Commercial exploitation of F1 hybrids in the crop also started after the discovery of the A1 system in mid-1950s (Quinby, 1974). This discovery allowed a cost effective way to produce F1 hybrid seeds. Once large scale hybrid seed production and marketing became possible, then the hybrid technology got accepted rapidly by sorghum producers, and open pollinated cultivars were effectively replaced by hybrids in a period of less than ten years (Maunder, 2002).

The first CMS was discovered, characterized, and described by Stephens and Holland (1954) as an interaction between milo/durra cytoplasm and a kafir nuclear background, and was designated as A1 CMS. Sorghums with A1 cytoplasm have small pointed anthers and normal

meiosis, but microspores remain uninucleate and abort (Singh and Hadley, 1961). Restorers for the A1 CMS are common and are estimated to be in approximately 68% of the sorghum lines from the USDA-Texas Agricultural Experiment Station Sorghum Conversion Program (Torres-Cardona et al., 1990; Bosques-Vega et al., 1989). As a result, most global sorghum hybrid production depends on the A1 system. The A2 cytoplasm was first reported by Schertz (1977). The source of the original A2 cytoplasm is IS1266C, which is from the Caudatum Nigrican group (Guinea race) from Ethiopia. The source of nuclear genes and the maintainer is IS5344C, which is in the Roxburghii group (Guinea Race) from India. Miller (1986) and Miller et al. (1992) released the seed parents with this CMS system A2Tx632, followed soon after by A2Tx636 and A2Tx637, respectively. Miller (1984) released RTx432 for restoration of fertility in A2 CMS in grain sorghum hybrids. Quinby (1980) reported the sterility-inducing cytoplasm from the line IS1112C and designated it as A3 cytoplasm. The A2 CMS system has effective restorers and is the major CMS system used in commercial hybrid seed production in China. The A3 cytoplasm system was introduced with the release of A3Tx398 (Schertz, 1984). Since that time, several groups have released seed parents with A3 CMS (Pedersen and Toy, 1997; Pedersen et al., 1997). Fertility restorers of A3 CMS are much rarer; only three (0.7%) were found (SC426, SC835, and SC273) in sorghum lines screened by Torres - Cardona et al. (1990) and Bosques Vega et al. (1989). As a result, the A3 CMS system is not being used in commercial hybrid seed production. Others such as A4, A5, A6, 9E and KS have been described in sorghum elsewhere (Rao, 1962; Hussaini and Rao, 1964; Webster and Singh, 1964; Ross and Hackerott, 1972; Schertz and Ritchey, 1978; Worstell et al., 1984).

The product of a cross between two unrelated parents (F1 generation) is called a “hybrid”. Three parents are essential for creating a commercial F1 hybrid using the CMS system, the R line



(pollen parent, homozygous for nuclear restorer genes with normal or male fertile cytoplasm), the A-line (female or seed parent, homozygous for nuclear non-restorer gene with male-sterile cytoplasm), and the B-line (maintainer of A-line, homozygous for nuclear non restorer genes with normal cytoplasm) (Acquaah, 2007). All these must be continually available for use in hybrid production. The need for three parents comes from the genetic behavior of sorghum and the need for a commercially viable seed production system. The male sterile (A) lines lack the *Rf* gene in their nucleus which restores fertility to the sterile cytoplasm. Hence the need for a B-line, which is genetically identical to the A-line apart from the fertility, in order to make sure that the A-line is continually available. The A-line is the seed parent that is used in the seed production field, and F1 hybrid seeds are harvested from this A1 plant. Because it is male sterile, any seed produced on the A1 plant must be the result of fertilization by the pollen from the nearby R- plant. The only difference between A and B lines is the male sterility inducing cytoplasm, where A has defective cytoplasm and B has normal cytoplasm, the two lines are called “iso-cytoplasmic” (Rooney and Smith, 2000). The R-line is the male parent in commercial hybrid seed production. It is genetically different from the iso-cytoplasmic A- and B-lines, and it carries the dominant fertility restorer *Rf* gene necessary in the restoration of male fertility in its hybrids with the A-cytoplasm (Acquaah, 2007).

### **Effect of CMS systems on economic sorghum hybrid traits**

The effect of cytoplasmic male sterile systems on the performance of sorghum hybrids has been varied. When comparing hybrids possessing A1, A2, and A3 cytoplasm, Maves and Atkins (1988) found that A2 and A3 CMS hybrids reached 50% flowering later, had reduced number of seeds per panicle, and reduced grain yield compared to A1 CMS hybrids. Secrist and Atkins (1989)

found no significant differences in grain yield between A1 and A2 CMS hybrids, but they reported a 6% reduction in grain yield in A3 CMS hybrids compared to A1 hybrids.

Moran and Rooney (2003) have reported that A1, A2, and A3 cytoplasm had no effect on plant height and had minimal effect on days to flowering. However, grain yield in the A3 cytoplasmic background was significantly reduced compared with A1 and A2 cytoplasm-based hybrids. Karper and Quinby (1963) reported an increase in stem sugar from 15 to 17% when male-sterile plants did not set seed vs. when cross-pollination and seed set was allowed. In forage sorghum, Pedersen and Toy (1997) tested the effect of A1 and A3 cytoplasm in forage hybrids of sorghum × sudangrass, and they found no differences associated to cytoplasm alone for maturity, height, and dry matter yield.

### **Combining ability and test cross performance**

Combining ability of inbred lines is a determinant of the potential usefulness of an inbred line in hybrid combination and the final evaluation of inbred lines can be best determined by hybrid performance. The concept of combining ability was developed by Sprague and Tatum (1942) and two terms to explain this concept were introduced general and specific combining ability (GCA and SCA, respectively). GCA is a measure of additive genetic effects, which are responsive to selection. On the other hand, SCA is a measure of the residual, dominance, epistatic, and interaction effects of genes (Betran et al., 2003; Aguiar et al., 2003). The knowledge of combining ability of the parents and the inheritance of the traits provide useful information to guide selection of parents and designing breeding schemes (Makanda et al., 2010; Amiruzzaman et al., 2011).

Panhwar et al. (2008) defined combining ability as the ability of parents or cultivars to combine amongst each other during the process of hybridization so that favorable genes or

characters are transmitted to their progenies. On the other hand, Singh et al. (2013) defined combining ability as the relative ability of a genotype to transmit its desirable performance to its crosses. The inbred lines that display higher value for the trait of interest when crossed to series of other lines are said to have favorable combining ability (Qu et al., 2012). Beil and Atkins (1967) reported that both general and specific combining ability effects were important in the expression of grain yield in Sorghum. GCA effects were greater than SCA effects for number of panicles per plants, weight of 100 seeds, and the number of seeds per panicle. They concluded that in the selection of lines for use in hybrid combinations, greatest progress could be made by first selecting on the basis of general combining ability of the lines with further selection guided by evaluation for specific effects.

Combining ability for non-grain traits also were explored primarily in forage improvement. GCA for four male sterile sorghum varieties was evaluated by crossing with four sudangrass cultivars, and the result showed that all the F1 hybrids were taller than the parental female genotypes (perhaps three or two dwarf type) with fewer tillers and showed the highest specific combining ability for fresh yield (Tarumato, 1970). Desai et al. (1980) reported a good GCA and SCA for yield and SCA variance was higher than GCA variance for dry matter and tillers per plant, but negative GCA effects were observed for plant height. Makanda et al. (2009) reported significant general combining ability (GCA) effect for stem brix and associated traits implying the importance of additive gene action. Baocheng et al. (1986) reported GCA effects to be more important (10-26 times) than SCA effects. However, these results do not necessarily give general indication of the behavior of these genes at different environments and in different genetic backgrounds.

## The use of the hybrid technology for designing dedicated sorghum based biofuel feedstock

### High biomass feedstock hybrids

Biomass sorghums are managed to maximize lignocellulosic biomass yields (McKendry, 2002). They are tall (3.5–5 m) with dry, pithy stems and produce high dry matter yields. Biomass sorghum genotypes are specifically photoperiod sensitive (PS) because delaying maturity has particular advantages in the production of biomass (McCollum et al., 2005). Tall stature and delayed flowering favor biomass production, but these traits make seed production difficult, especially in long day environments. To produce photoperiod-sensitive seed, seed production can occur in lower latitudes where short day lengths and long growing seasons provide flexibility. Alternatively, they can be made with a seed production system that enables the production of photoperiod sensitive hybrids regardless of day length (Rooney and Aydin, 1999; Mullet et al., 2010). This system manipulates the sorghum maturity loci *Ma1*, *Ma5*, and *Ma6* to use early maturing, photoperiod-insensitive parental lines to produce photoperiod-sensitive hybrids with delayed flowering. With a dominant allele at each of these loci, flowering is delayed until day lengths are  $\leq 12$  h 20 min. By crossing a photoperiod-insensitive parental line that is homozygous recessive at one of these loci with a parent having the opposite allelic configuration (*ma1ma1Ma5Ma5ma6 ma6/Ma1 Ma1ma5ma5Ma6Ma6*), photoperiod-sensitive hybrid progeny (*Ma1ma1Ma5ma5Ma6ma6*) are obtained. Besides facilitating seed production, producing biomass sorghums as hybrids also may permit the exploitation of high-parent heterosis for increasing biomass yields. High-parent heterosis is (HPH) the superior performance of a hybrid compared to its best parent. High-parent heterosis is used for increasing yield and has been exploited in many crops for decades (Duvick, 1999). In sorghum, the existence of HPH for grain yield is well established and extensively used

in commercial production (Quinby, 1963; Duvick, 1999; Axtell et al., 1999). High yields reduce the fixed costs per unit of energy, particularly land and transportation costs (Epplin et al., 2007).

### **High °Brix sweet sorghum hybrids**

Current opportunities to produce ethanol from sweet sorghum are limited by seed stock of acceptable cultivars. Traditional cultivars produce low yields of seed on tall plants that are difficult to harvest mechanically. Utilizing a hybrid production system based on cytoplasmic male sterility, well established in grain sorghum and forage sorghum production, would ease the seed production limitations of the current sweet sorghum cultivars (Pfeiffer et al., 2010). Female seed parents can be selected for greater seed yields, increased sugar concentration in the stalks, and combining ability to develop hybrids that produce large amounts of fermentable sugar. In addition to making seed production more reliable, sorghum hybrids typically express a moderate level of heterosis. However, the expression of heterosis for stem biomass yield may imply an enhancement of total sugar yield (Makanda et al., 2009, Pfeiffer et al., 2010). Because sugar yield is higher in sterile hybrids due to avoidance of sink competition, the preferential use of the A3 cytoplasm in which most male lines do not affect fertility restoration has been suggested (Pfeiffer et al., 2010).

### **Heterosis in Sweet sorghum**

Heterosis has been defined as superiority of the F1 hybrid over both of its parents (Singh, 2003). The term heterosis was first used and defined by Shull (1952) as the superiority of heterozygous genotypes with respect to one or more characters in comparison with the corresponding homozygotes. Khan et al. (2009) defined heterosis as the difference between a hybrid mean and its two parents. Falconer (1981) showed that heterosis is expressed when some level of dominance

is present at a locus affecting the trait. Heterosis can be described as either mid-parent heterosis (MPH) and high-parent heterosis (HPH). MPH is the performance of the offspring compared with the average performance of the parents, and HPH is the performance of the offspring compared with the best parent in the cross (Lamkey and Edwards, 1999). Because the goal is to enhance a given trait, high parent heterosis is of high priority to breeders and producers. Superior performance of hybrids is only meaningful if it has increased value over the better parent (Blum et al., 1977).

Many studies have been carried out to understand the genetic and physiological basis of heterosis. The most evident expression of heterosis in sorghum was increased vegetative growth and higher grain production associated with extreme lateness (Bartel, 1949; Stephens and Quinby, 1952). In sweet sorghum, very low high parent heterosis for maturity and brix, and moderate values for plant height have been observed. Greater levels of heterosis were observed for grain yield, stalk yield, and juice yield, which was highly variable (Meshram et al., 2005). A recent work by Corn (2009) suggests the involvement of several genes affecting biofuel traits in sweet sorghum. In this study with a consisted fixed set of parents, HPH ranged from -24% to 7% for stem brix, and 27% to 43% for stem biomass, indicating that multiple genes are responsible for these traits. The wide range of variability of °Brix, percent sucrose, stalk yield, and biomass yield indicate the great potential for genetic improvement to produce high yielding sweet sorghum hybrids with high sucrose percent (Reddy et al., 2005). The predominant role of non-additive gene action for plant height, stalk diameter, brix, stalk yield, and extractable juice yield indicates the importance of breeding for heterosis for improving these traits (Reddy et al., 2005; Sankarapandian et al., 1994).

## **Standability and stalk rot in biofuel feedstock sorghums**

Standability or lodging resistance is an important consideration in grain sorghum production. It is particularly so in mechanized farming in that lodged plants are not picked up by harvest machines and directly account for grain loss. Several factors undermine standability including the inherent anatomical characteristics of the plant, plant height and the occurrence of stalk rotting pathogens. Stalk rots have been recognized as leading causes of lodging in commercial grain production. Charcoal rot caused by *Macrophomina phaseolina* (Tassi) Goid., the most aggressive type of stalk rot in sorghum, is an economically important disease worldwide (Mughogho and Pande, 1983). It is a disease of great destructive potential, especially when vigorous and maturing crops face terminal drought stress (Edmunds et al., 1964; Odvody and Dunkle, 1979). Charcoal rot has been reported from all the ecologically diverse areas of sorghum culture in the tropics, subtropics, and temperate regions (Tarr, 1962). In the United States, stalk rot is a common problem in the southern states and in the central Great Plains extending from Texas to Kansas (Edmunds et al., 1964; Edmunds and Zummo, 1975). There are also reports of stalk rot incidence as far north as Nebraska (Duncan, 1983).

Stalk rot is systemic in nature, characterized by degradation of pith tissue near the base of the stalk as a result of senescence of the stalk pith cells (Tesso et al., 2012), resulting in reduced transportation of nutrients and water, and breakage of the stalk at the zone of infection causing lodging (Hundekar and Anahosur, 1994). The incidence of stalk rot diseases is generally associated with high temperature, drought stresses, and senescence (Rosenow and Clark, 1995; Tesso et al., 2004) and is more severe when drought and high temperature stress occurs during grain development followed by wet, cool conditions near physiological maturity.

Little research on stalk rot diseases of sweet sorghum has been reported (Dogget, 1988; Zummo, 1986). These diseases pose a serious constraint for yield and quality of sweet sorghum juice and bagasse (Funnell-Harris et al., 2014). In particular, stalk rot diseases can reduce biomass and are associated with lodging, which reduces the harvestable yield (Bean et al., 2013; Funnell-Harris et al., 2014; Tesso et al., 2005).



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## **Chapter 2 - Effect of the grain sink on stalk juice yield, sugar accumulation, and biomass of sweet sorghum**

### **Abstract**

Sweet Sorghum [*Sorghum bicolor* (L.) Moench] is a type of cultivated sorghum grown primarily for its sugar-rich stalks. Because of its high fermentable sugar content, the crop is widely recognized as industrial raw material for syrup and table sugar production and as an alternative feedstock source in biofuel industries. The extent to which stalk sugar accumulation occurs may be determined by several factors including the sink size. Grain is the most important sink in sorghum and other grain crops, however, there is opportunity to eliminate the grain sink to facilitate sugar accumulation in the hybrids. Before engaging in the development of such system, it is important to determine the potential of the grain sink removal on stalk sugar yield. The aim of this study was to determine the effect of eliminating the grain sink on biomass, juice yield, and sugar accumulation in the stalk. The experiment consisted of two factors. The variety factor consisted of 22 genotypes of variable sugar content, and the sink factor consisted of a with and without grain treatment. The experiment was arranged in split plot with a randomized complete block design with variety assigned to the main plot and the sink effect to the subplot units. The study was conducted in four replications and three environments 2013, 2014, and 2015 in Manhattan Kansas. Treatments without grain sink were applied by manually removing the panicles prior to anthesis. At physiological maturity, all plants within a pre-marked one-meter section in each plot were harvested and used to determine °Brix, biomass, juice, and sugar yield. Results showed that the genotype effect was significant ( $P \leq 0.01$ ) for all traits. Elimination of the grain sink prior to



anthesis significantly ( $P \leq 0.05$ ) increased °Brix by 8%, biomass yield by 12%, sugar yield by 17%, and total sugar by more than 20%, but had minimal effect on juice yield.

Key words: *Sorghum bicolor*, fermentable sugar, grain sink, Brix, biomass yield, physiological maturity.

## Introduction

Sorghum is a warm season cereal with outstanding potential for biomass accumulation. Due to this and its relative tolerance to drought, low input requirements and ability to accumulate a high volume of sugary juice (15-23%) (Miller and Creelman, 1980; Woods, 2001; Sarath et al., 2008; and Gnansounou et al., 2005), sorghum is recognized as one of the most promising feedstock sources for biofuel production. Sweet sorghums in particular are of significant interest in that they produce readily fermentable sugar and can be produced more easily than sugarcane. Sweet sorghums are reported to produce 23% more fermentable sugars while requiring 37% less nitrogen fertilizer, and 17% less irrigation water than maize (Hills et al., 1990). Unlike sugarcane, sweet sorghum is easily established using seeds and is ready for harvest in a single season, offering better returns on a unit area basis (Prasad et al., 2007; Grassi, 2001).

Typical sweet sorghum cultivars grow from 2.4 to 3.0 meters (8-10 feet) height and can produce up to 30 Mg ha<sup>-1</sup> of dry biomass per acre in favorable environments (Rooney et al., 2007). They also produce a large volume of sugary juice, which consists of sucrose, glucose, and fructose in relative proportions of 70, 20 and 10%, respectively (Wu et al., 2010). The relative concentration of sugars and juice volume, however, may vary depending on varieties, management conditions, and the grain sink size (Pfeiffer et al., 2010; Erickson et al., 2011; Hills et al., 1990; Grassi, 2001). These differences may be responsible for reported variation in ethanol yields among sweet sorghum feedstocks (Putnam et al., 1991; Pfeiffer et al., 2010).

The two major sink sources in sweet sorghum are the stalk and the panicle (grain). In sweet sorghum, the stalk, which is the main growing center, serves as the major sink. The panicle or the grain sink develops later during reproductive growth and, depending on the inherent genetic potential for grain formation, this sink can be significant enough to affect stalk sugar accumulation. However, the accumulation of dry matter in both sinks take place at the same time with both reaching the maximum at physiological maturity (Djanaguiraman et al., 2005). Sweet sorghum photo-assimilates are first used for plant growth and development during early vegetative stages. Afterwards, when the internodes have elongated, stems transition to sugar storage organs where most of the accumulated carbon is stored as sucrose (Lingle 1999; Almodares et al., 2008; Almodares and Hadi, 2009; Slewinski, 2012). It has been proposed that stored sugars in the stem are used to buffer photoassimilate supply to the grains during plant growth and development (Batta and Singh, 1986; Slewinski, 2012).

Grain yield in sweet sorghum has not been found to be correlated with juice yield, but it has been negatively correlated with sugar concentrations (Murray et al., 2008; Ritter et al., 2008; Shiringani et al., 2010). Sweet sorghums produce relatively low grain sink because of the competing need for photosynthate between stem elongation and head development during pre-anthesis growth phases. After anthesis, much of the photosynthates are directed to filling grain with some of the assimilates accumulated prior to anthesis also being available for filling grain as required (Rajendran et al., 2000). Hence, although sweet sorghums tend to have stronger sink in the stalk compared to grain types, the reproductive sink also can be significant. Therefore, eliminating this sink or reducing its strength should theoretically lead to further accumulation of sugars in the stalk. Routine observations made in sorghum and maize crops support this theory. In Africa, stalks of corn plants with barren ears are routinely selected and harvested for chewing by

children because of its sweet taste from accumulation of sugar that otherwise should have been translocated to the grain. Similarly, cane sorghum with little grain sink are sold at premium price vs. others because of the extra sweetness of the stalk. In sorghum a genetic system exists that allows production of sweet sorghum hybrids where the grain sinks are totally eliminated. This may provide an opportunity to further increase sugar accumulation in sweet sorghum cultivars and increase the value of sweet sorghum as a feedstock source for biofuel production or use by the table sugar industry. However, before embarking on the development and deployment of such system, it is important to experimentally validate the value of eliminating the grain sink to increase sugar accumulation. Therefore, the objective of this study was to determine the impact of eliminating the reproductive sink on total fermentable sugar yield and sugar composition in sweet sorghum.

## **Material and Methods**

### **Genetic materials**

A total of 22 sweet sorghum genotypes were used in this study (Table 2.1). These included fifteen sweet sorghum genotypes of diverse genetic background, some of which are commonly grown for syrup production in the United States or as a forage crop for livestock, while others have been recently identified as having elevated stalk sugar. The remaining seven were the *brown midrib 6* and *brown midrib12* versions of four popular genotypes, namely Kansas collier, Rox orange, Early hegari, and Atlas (only *bmr6*).

## **Experimental design and management**

The experiment was conducted using a Randomized Complete Block Design (RCBD) arranged in split-plots with four replications. The whole plots consisted of genotypes planted in paired rows of five-meter length spaced 0.75 m apart with a 0.6 m alley at the end of each plot. The sub-plot consisted of the grain sink treatment, with and without grain. The experiment was established by directly seeding 3 g treated (Maxim 4FS, Apron XL, Concept III, and colorant) seeds of the genotypes using a cone planter. Fertilizer nitrogen (urea) and phosphorous (di-ammonium phosphate, DAP) were applied at the rates of 90 kg ha<sup>-1</sup> and 40 kg ha<sup>-1</sup>, respectively. Pre-emergence weeds were controlled with 0.55 kg ha<sup>-1</sup> Atrazine TM, 0.76 kg ha<sup>-1</sup> Dual II Mg TM, and, 0.16 kg ha<sup>-1</sup> CallistoTM. Post-emergence weeds were controlled by hand weeding, and this practice was used to keep the field weed-free throughout the seasons. Prior to anthesis, all heads from main plants and tillers in one of the two rows in each plot were eliminated by manually clipping the panicles with garden shears while the other row remained intact. These formed the subplot treatment units of with and without grain sink. The experiment was conducted in three environments during the 2013 and 2014 seasons at the Ashland Bottoms Agronomy Research Farm and in 2015 at the Agronomy North Farm near Manhattan, KS.

## **Data collection and analysis**

The fields were closely supervised throughout the experiment periods, and notes were taken as necessary. Data were collected on a range of characteristics including both agronomic and biofuel traits. Important agronomic parameters collected, including plant height, lodging score, and days to flowering. Plant height was measured as length of the plant from the ground to the top of the panicles. The severity of stalk lodging was visually scored on a scale of 1 to 5 with a score of 1

representing no lodging to 10% lodging, 5 representing 90% lodging to complete lodging, with values in between representing different lodging severities between 10 and 90%. Days to flowering were recorded as the number of days from planting to the day when 50% of the plants in each plot had reached half bloom.

The biofuel traits were determined at physiological maturity. Here all plants within a one-meter long section of the plot were harvested by cutting at the base of the plant, 5 cm from the soil surface. The harvested plants were bundled and immediately taken to the laboratory where they were separated into panicles, stems, and leaves, which were weighed separately. The stems were then crushed in a three-roller sorghum press to extract the juice and the pressed stems were weighed. The volume and weight of extracted juice was immediately recorded and the °Brix was determined using a hand-held refractometer (Atago U.S.A, Inc., Bellevue, WA, USA). A 15 ml juice sample was obtained from each experimental unite and were frozen for HPLC analysis of sugars. Briefly, each trait was estimated as:

- 1- Fresh, dry Biomass, and juice yield were calculated from a sample taken at harvest using the formula:

$$(\text{weight of sample from 1m}) / (\text{sample row area in m}^2) \text{ then calculated as Mg ha}^{-1}.$$

- 2- Juice extractability (%): juice extractability in percent was calculated using the data of total weight of the fresh stalks using the formula:

$$\text{Juice extractability (\%)} = [(\text{Juice weight} / \text{Fresh stalk yield}) * 100]$$

- 3- Sugar yield was estimated using the formula developed by Corn (2009)

$$\text{Sugar yield (Mg ha}^{-1}\text{)} = 0.95 * \text{juice yield (Mg ha}^{-1}\text{)} * 0.97 * 0.873 * (\text{°Brix}/100)$$

This formula approximates sugar values assuming commercial sugar extraction rates whereas;

0.95 extraction efficiency; 0.97 adjusts for using the first expressed juice to represent all juice (for every 100 parts brix in the first roller juice, there are approximately 97 parts in the whole juice of stalk); 0.873 accounts for percent fermentable sugars present in the brix.

Fresh panicles, leaves, and pressed stems were oven dried at 120°C for 10 days to determine dry biomass.

### **Compositional analysis**

Juice samples for compositional analysis were collected from each plot for the first two replications of the 2012 and 2015 environment experiments and immediately placed in refrigerator. The frozen samples were thawed, and 1.5 ml of the juice samples were collected into 2 ml Eppendorf tubes. The samples were then centrifuged at 3000 rpm for 10 min. and were filtered through 0.2 µm hydrophilic PTFE syringe filters (Millipore, Billerica, MA) membrane prior to HPLC analysis. The samples were analyzed by high-performance liquid chromatography HPLC (Agilent, Santa Clare, CA) equipped with an RCM monosaccharide column (300 × 7.8 mm; Phenomenex, Torrance, CA) and a refractive index detector (RID, Santa Clare, CA). The mobile phase was 0.6 mL min<sup>-1</sup> of double-distilled water, the oven temperature was 80°C, and the cell temperature was 40°C. Retention time for sucrose, glucose, and fructose are 9.03, 11.04, and 12.05 min, respectively. The sucrose, glucose, and fructose present in the 10 µL injected sample were calculated using the peak area and equation of the corresponding standard curve ( $Y = aX + b$ ,  $R^2 > 0.99$ ). Sugar content (g) in the original sample was calculated as:  $C = X \times V$  (g); where  $X$  = concentration (g/L) in the 10 µL injected sample and  $V$  = total volume of sweet sorghum juice samples (L).

## **Statistical analysis**

The data were subjected to analysis of variance (ANOVA) performed using a PROC MIXED COVTEST procedure in SAS (version 9.4) as appropriate for the design. The environment and replicates were treated as random effect parameters. The variety, sink size and the interaction between them were treated as fixed effects. Means for significant effects were separated using the LSD method. Pearson correlation coefficients were estimated from combined environment data to determine the degree of association between all traits using PROC CORR procedure in SAS (version 9.4).

**Table 2.1.** Origin and GRIN identification number of parental sweet sorghum lines used for this study.

No.	Genotypes	Identification no.	Place of origin/source
1	Atlas	NSL 3986	Texas
2	Atlas <i>bmr 12</i>	PI 636763	Nebraska
3	Atlas <i>bmr 6</i>	PI639708	Nebraska
4	Dale	PI 651495	Mississippi
5	Early hegari	PI 665029	Texas
6	Early hegari <i>bmr 6</i>	PI639706	Nebraska
7	Kansas collier	PI 586540	Australia
8	Kansas collier <i>bmr 12</i>	PI639705	Nebraska
9	Kansas collier <i>bmr 6</i>	PI639704	Nebraska
10	M81E	PI 653411	Mississippi
11	MN4179	PI302131	Portugal
12	Masuda	PI193073	Japan
13	No.8	PI257602	Ethiopia
14	//	PI 185672	Delhi India
15	Kaoliang	PI195754	China
16	Red Amber	PI17548	Texas/Kansas
17	Rox orange	PI 641836	Georgia
18	Rox orange <i>bmr 12</i>	PI639703	Nebraska
19	Rox orange <i>bmr 6</i>	PI639702	Nebraska
20	Sugar drip	PI146890	Zaire
21	Topper 76-6	PI583832	Georgia
22	Tracy	669651	Mississippi



## Results

### Analysis of variance

The combined analysis of variance for all traits is presented in Table 2.2. As expected, the genotype effect was highly significant ( $P \leq 0.01$ ) for all traits measured. Similarly, the environment and genotype  $\times$  environment interaction effects were significant except for lodging and juice extractability for both environment and genotype  $\times$  environment interaction effects and for sugar yield for the environment effect (Table 2.2). Likewise, the effect of the grain sink was highly significant for all traits except for juice yield and juice extractability. The grain sink  $\times$  genotype and grain sink  $\times$  environment interaction effects were significant for lodging and brix score, while juice extractability, juice yield, and sugar yield were significant for the grain sink  $\times$  genotype interaction effect only. The three-way interaction effect, grain sink  $\times$  genotype  $\times$  environment, was also significant for °Brix, fresh and dry biomass, as well as juice and sugar yield (Table 2.2).

### The effect of genotypes on agronomic and biofuel traits

The combined mean for agronomic and biofuel parameters among genotypes is presented in Table 2.3. As indicated in the ANOVA in Table 2.2, days to flowering, plant height, and lodging score were significantly different among genotypes, and this reflects the inherent differences in the backgrounds of the genotypes. Moreover, there also were marked difference in these traits among entries of similar background. Plant height in wild-type genotypes of Atlas, Kansas collier, Rox orange, and Early Hegari was greater than their *bmr* versions. It is not clear whether this was due to insufficient backcrossing during the creation of the sister lines or if the *bmr* alleles have an effect on internode elongation. Days to flowering also was different between *bmr* lines and their wild type counterparts, but all were within the range of the entries. The result for lodging score,

however, was interesting in that it clearly depicted the impacts of *bmr* mutations on stalk strength. All wild type genotypes were less prone to lodging compared to their respective *bmr* mutants, with *bmr6* seemingly having more impact on standability than *bmr12*.

Mean fresh and dry biomass among genotypes ranged from a low of 24.51 and 8.54 Mg ha<sup>-1</sup> in Early Hegari to 61.75 and 22.11 Mg ha<sup>-1</sup> in Topper 76-6, respectively, with the across entry mean for the two traits being 40.99 and 14.63 Mg ha<sup>-1</sup> in that order. The popular sweet sorghum variety M-81 E had the second highest fresh and dry biomass yield of 60.36 and 21.16Mg ha<sup>-1</sup>. Thus, the highest fresh and dry biomass yields were 50% and 51% greater than the average, and the lowest yields were 40 and 42% less for fresh and dry biomasses, respectively. Likewise, the juice and sugar yields were least, 12.54 and 1.14 Mg ha<sup>-1</sup>, respectively, again in the *bmr6* version of cultivar Early Hegari, but the greatest juice yield of 32.03 Mg ha<sup>-1</sup> was obtained in M81E followed by 29.93 Mg ha<sup>-1</sup> in Topper 76-6. The greatest sugar yield was also obtained in Topper 76-6 followed by M81E and Dale. The mean juice and sugar content across entries was 20.65 and 2.52 Mg ha<sup>-1</sup>, respectively (Table 2.3). Similarly, the highest juice yield was 55% higher and sugar yield 50% higher than the average while the lowest readings were 39% and 55% lower than the average for juice and sugar yield, respectively. The range for brix across genotypes was narrower spanning from a low of 11.15% (30% lower than the average) to a high of 19.2% (21% higher than the average). Unlike the biomass and juice yield where the same group of genotypes consistently performed high, °Brix showed a slightly different pattern. The traditional sweet/forage sorghums like Atlas, Early hegari, Rox orange, and Kansas collier seem to have greater °Brix than the rest of the entries including the popular M 81E. However, because of their relatively lower biomass and juice yield, the total sugar from these genotypes was less.

### **The effect of sink on biofuel traits**

The central aim of this study was to determine the effect of reduced grain sink size on accumulation of stalk sugar and cellulosic biomass as biofuel feedstock. The data from this study is expected to provide practical evidence for justifying efforts to develop sterile sweet sorghum hybrids as dedicated feedstock source for biofuel production. As shown in Table 2.2, sink removal had a significant effect on both sugar and biomass related traits. Although plant height was reduced by an average of 22.34 cm (an average panicle length) in plants where heads were removed (Table 2.4), removal of the heads seem to have a positive impact on biofuel traits (Table 2.4). The results clearly showed that removing the grain sink prior to flowering significantly increased both sugar and biomass yields in sorghum. Both fresh and dry biomass were greater from plants with removed heads, 46 vs. 35.97 Mg ha<sup>-1</sup> for fresh biomass and 16.41 vs. 12.84 Mg ha<sup>-1</sup> for dry biomass. Similarly, grain sink elimination increased juice yield 23.9% (22.84 vs. 18.44 Mg ha<sup>-1</sup>) compared to the score from intact plants. Likewise, sugar yield and °Brix were 43.5% and 17.8% greater in sink removed plots (2.97 vs. 2.07 Mg ha<sup>-1</sup> for sugar yield and 17.4 vs. 14.55% for °Brix). In addition to increasing biomass, sugar yield and °Brix, removing the sink reduced propensity for lodging by 22% (Table 2.4).

### **Correlation between biofuel traits**

Correlation between the measured traits depicted the expected pattern (Table 2.5). Fresh and dry biomass and juice and sugar yield were significantly and positively correlated with plant height and days to flowering. Lodging score was not significantly correlated with any of the traits. Many of the feedstock traits also had significant correlations with each other with fresh biomass having a significant correlation with dry biomass, juice yield and sugar yield. Dry biomass also was

significantly correlated with both juice yield and sugar yield while juice yield and sugar yield correlated at  $r = 0.93$ . °Brix however was significantly correlated only with sugar yield (Table 2.5).

**Table 2.2.** Combined analysis of variance for agronomic traits, biomass yield, sugar and juice quality components of the sweet sorghum genotypes evaluated at Manhattan, KS during 2013, 2014, and 2015 sowing season.

Source of variation	Mean squares									
	df	Day to 50% flowering	lodging	Plant height (cm)	°Brix (%)	Fresh biomass (Mg ha <sup>-1</sup> )	Dry biomass (Mg ha <sup>-1</sup> )	Juice extractability (%)	Juice yield (Mg h <sup>-1</sup> )	Sugar yield (Mg h <sup>-1</sup> )
Environments (E)	2	1541.65**	1.79	28625*	62.12	4440.63*	552.46*	1262.15	1891.81*	21.27
Block/E	9	3.51	0.44	688.58	5.21	153.18*	28.99*	115.84*	29.47	0.70
Genotypes (G)	21	2305.40**	1.56**	67925**	132.23**	2448.61**	293.43**	2622.66**	706.26**	13.50**
G × E	42	62.85**	0.33	2949.69**	27.08**	495.40**	62.13**	333.67**	149.45**	3.19**
Error a	189	4.39	0.16	481.92	2.68	48.13	8.85	52.38	14.64	0.38
Sink size (H)	1	4.68	42.04*	65883*	885.80*	13268*	1689*	158.51	2563.71	103.97*
H × E	2	2.65	2.50*	956.69	28.47*	17.47	4.99	498.60*	158.34*	5.13**
H × G	21	3.33	0.96*	316.09	9.49*	55.89	6.65	35.09	20.20	0.40
H × G × E	42	6.19	0.28	297.53	4.81**	39.94**	6.43*	24.76	15.13**	0.26**
Error b	189	1.11	0.23	319.19	0.79	15.02	2.92	22.90	4.27	0.10

\*, \*\* statistically significant at  $P \leq 0.05$  and  $P \leq 0.01$  probability, respectively.

**Table 2.3.** Combined mean performance of sweet sorghum genotypes for agronomic traits, biomass yield, sugar and juice quality components, evaluated at Manhattan, KS during 2013, 2014, and 2015 sowing season.

Genotypes	Day to flowering	Lodging	Plant height (m)	°Brix (%)	Fresh biomass (Mg ha <sup>-1</sup> )	Dry biomass (Mg ha <sup>-1</sup> )	Juice extractability (%)	Juice yield (Mg ha <sup>-1</sup> )	Sugar yield (Mg ha <sup>-1</sup> )
Atlas	71	1.29	260.88	15.20	47.77	16.61	43.32	22.98	2.64
Atlas <i>bmr 12</i>	63	1.33	223.92	18.68	39.15	14.03	51.72	19.45	2.72
Atlas <i>bmr 6</i>	70	1.54	219.25	15.50	38.87	13.99	35.17	20.60	2.40
Dale	87	1.00	311.33	16.84	52.99	17.60	50.08	28.96	3.65
Early hegari	68	1.13	151.83	12.91	32.16	10.89	34.64	15.89	1.58
Early hegari <i>bmr 6</i>	71	1.17	130.67	11.15	24.51	8.54	33.46	12.54	1.14
Kansas collier	66	1.00	235.46	18.45	38.32	14.37	36.33	20.64	2.86
Kansas collier <i>bmr 12</i>	72	1.54	227.13	17.78	34.74	12.59	31.83	17.56	2.44
Kansas collier <i>bmr 6</i>	71	1.67	218.88	19.20	32.13	12.08	35.44	15.83	2.33
M 81E	93	1.29	336.71	15.35	60.36	21.06	50.33	32.03	3.65
Masuda	63	1.21	251.83	15.28	46.95	15.35	37.33	24.68	2.86
MN 4179	69	1.63	243.79	16.13	33.16	11.15	35.57	17.30	2.16
No.8	89	1.21	263.96	16.52	55.79	19.77	37.75	27.49	3.52
PI185672	87	1.75	301.71	12.51	45.04	18.73	13.33	21.45	2.15
Kaoliang	72	1.21	239.13	11.18	31.21	13.14	13.07	13.49	1.18
Red Amber	78	1.00	136.08	14.88	27.39	10.85	33.08	12.55	1.44
Rox orange	69	1.75	217.79	16.17	36.66	12.50	34.69	18.39	2.25
Rox orange <i>bmr 12</i>	67	1.71	217.42	19.16	40.62	14.21	39.71	20.55	2.96
Rox orange <i>bmr 6</i>	64	1.17	186.63	18.31	36.14	12.17	47.96	18.30	2.52
Sugar drip	67	1.42	218.46	16.03	43.57	14.56	45.15	21.96	2.66
Topper 76-6	94	1.00	285.83	16.64	61.75	22.11	44.39	29.93	3.79
Tracy	68	1.38	288.33	14.85	42.54	15.60	21.63	21.64	2.45
Mean	74	1.34	234.86	15.85	40.99	14.63	36.64	20.65	2.52
LSD	1.81	0.33	16.19	1.44	5.89	2.17	4.69	3.35	0.51

**Table 2.4.** Combined mean of the effect of head removal treatment on agronomic traits, biomass yield, sugar and juice quality components of sweet sorghum genotypes evaluated at Manhattan, KS during 2013, 2014, and 2015 sowing season.

Head treatment	Lodging	Plant height (cm)	°Brix (%)	Fresh Biomass (Mg ha <sup>-1</sup> )	Dry biomass (Mg ha <sup>-1</sup> )	Juice extractability (%)	Juice yield (Mg ha <sup>-1</sup> )	Sugar yield (Mg ha <sup>-1</sup> )
No grain sink	1.05	223.69	17.14	46	16.41	37.18	22.84	2.97
With grain sink	1.61	246.03	14.55	35.97	12.84	36.08	18.44	2.07
Mean	1.34	234.86	15.85	40.99	14.63	36.64	20.65	2.52
LSD	0.08	4.65	0.37	1.84	0.62	ns	ns	0.13
Difference	-22%	-22.34cm	17.8%	27.9%	27.8%	3%	23.9%	43.5%

LSD = Least significant difference; ns= not significant.

**Table 2.5.** Pearson correlation coefficients between agronomic traits, biomass yield, sugar and juice quality components of sweet sorghum genotypes evaluated at Manhattan, KS during 2013, 2014, and 2015 sowing season.

Traits	Correlation ( <i>r</i> )								
	Grain sink	Lodging	Plant height (cm)	°Brix (%)	Fresh Biomass (Mg ha <sup>-1</sup> )	Dry biomass (Mg ha <sup>-1</sup> )	Juice extractability (%)	Juice yield (Mg ha <sup>-1</sup> )	Sugar yield (Mg ha <sup>-1</sup> )
Day to flowering	NC	-0.20 <sup>ns</sup>	0.52*	-0.20 <sup>ns</sup>	0.66**	0.74**	0.03 <sup>ns</sup>	0.63*	0.41*
	C	-0.15 <sup>ns</sup>	0.58*	-0.11 <sup>ns</sup>	0.63*	0.71*	0.05 <sup>ns</sup>	0.57*	0.49*
Lodging	NC	1	0.11 <sup>ns</sup>	0.13 <sup>ns</sup>	-0.12 <sup>ns</sup>	-0.11 <sup>ns</sup>	-0.26 <sup>ns</sup>	-0.11 <sup>ns</sup>	-0.07 <sup>ns</sup>
	C	1	0.00*	0.23 <sup>ns</sup>	-0.26 <sup>ns</sup>	-0.23 <sup>ns</sup>	-0.17 <sup>ns</sup>	-0.27 <sup>ns</sup>	-0.18 <sup>ns</sup>
Plant height (cm)	NC		1	0.14 <sup>ns</sup>	0.81**	0.85**	0.04 <sup>ns</sup>	0.80**	0.66*
	C		1	0.08 <sup>ns</sup>	0.80**	0.83**	0.06 <sup>ns</sup>	0.79**	0.73*
°Brix (%)	NC			1	0.30 <sup>ns</sup>	0.19 <sup>ns</sup>	0.57*	0.34 <sup>ns</sup>	0.69*
	C			1	0.08 <sup>ns</sup>	0.04 <sup>ns</sup>	0.58*	0.12	0.46*
Fresh Biomass (Mg ha <sup>-1</sup> )	NC				1	0.96**	0.43*	0.98**	0.88**
	C				1	0.95**	0.35 <sup>ns</sup>	0.97**	0.90**
Dry biomass (Mg ha <sup>-1</sup> )	NC					1	0.21 <sup>ns</sup>	0.92**	0.79**
	C					1	0.17 <sup>ns</sup>	0.88**	0.80**
Juice extractability (%)	NC						1	0.49*	0.61*
	C						1	0.42*	0.58*
Juice yield (Mg ha <sup>-1</sup> )	NC							1	0.90**
	C							1	0.94**

NC= Grain sink; C= No grain sink.

\*, \*\* statistically significant at  $P \leq 0.05$  and  $P \leq 0.01$ , respectively.



### **The effect of grain sink removal on sugar composition**

The combined analysis of variance for sugar components is presented in Table 2.6. The data showed that both genotype and sink effects were highly significant ( $P \leq 0.01$ ) for component traits except the sink effect for fructose. Genotype  $\times$  environment (G  $\times$  E) effects were not significant for any traits. Genotype  $\times$  sink and sink  $\times$  environment interaction effects were significant only for sucrose and total sugars, but the three-way genotype  $\times$  sink  $\times$  environment interaction effect was significant for glucose and total sugar.

Mean yield for total sugar across treatments was 1.55 Mg ha<sup>-1</sup>, the largest proportion, (63%), of which was attributed to sucrose while (21%) and (16%) contributed by glucose and fructose, respectively (Table 2.7). Total sugar from intact plants was 1.239 Mg ha<sup>-1</sup> compared to 1.858 Mg ha<sup>-1</sup> in treatments where the grain sink was removed. Removal of the grain sink increased total sugar concentration across genotypes by an average of 50%. All the three sugar components also showed similar increases when the grain sink was removed but to a different degree, with the greatest increase of 76% for sucrose with fructose and glucose showing only 15% increase (Table 2.7).

Mean sugar yield of genotypes across the grain sink treatments is presented in Table 2.8. Regardless of the sink effect, genotypes Dale and M81E had the greatest total sugar yield among genotypes followed by Topper 76-6 and Atlas. All genotypes with relatively greater total sugar also had greater sucrose, glucose, and fructose concentration, showing that these traits are interrelated and that improvement for one trait can lead to improvement in the others. Similarly, genotypes with medium or low total sugar also had sugar components in that range.

Analysis of relationships between sugar components showed that many of the traits were interrelated (Table 2.9). °Brix was significantly correlated with sucrose (0.75) but no significant

relationship with glucose or fructose. Sucrose in turn was highly correlated with both glucose and fructose, while the two glucose and fructose showing the highest correlation (0.93) (Table 2.9). None of these traits had a negative relationship with another.

**Table 2.6.** Combined analysis of variance for the effect of genotypes and head removal treatments on sugar components of sweet sorghum genotypes evaluated at Manhattan, KS during 2013 and 2015 sowing season.

Source of variation	Mean squares				
	df	Sucrose (Mg h <sup>-1</sup> )	Glucose (Mg h <sup>-1</sup> )	Fructose (Mg h <sup>-1</sup> )	Total sugars (Mg h <sup>-1</sup> )
Environment (E)	1	3.702	0.199	0.011	6.141
Block/E	2	0.975*	0.0006	0.009	0.762
Genotype (G)	21	1.401**	0.553**	0.363**	5.462**
G × E	21	0.271	0.038	0.016	0.488
Error a	42	0.260	0.030	0.028	0.567
Grain sink (H)	1	12.781*	0.088*	0.053	16.848*
H × E	1	0.436*	0.0001	0.007	0.536*
H × G	21	0.098*	0.014	0.006	0.203*
H × G × E	21	0.075	0.007*	0.004	0.113*
Error b	42	0.041	0.003	0.003	0.047

\*, \*\* statistically significant at  $P \leq 0.05$  and  $P \leq 0.01$ , respectively.

**Table 2.7.** Combined mean of the effect of head removal treatment on sugar components of sweet sorghum genotypes evaluated at Manhattan, KS during 2013 and 2015 sowing season.

Head treatment	Sucrose (Mg h <sup>-1</sup> )	Glucose (Mg h <sup>-1</sup> )	Fructose (Mg h <sup>-1</sup> )	Total sugars (Mg h <sup>-1</sup> )
Grain sink	0.705	0.299	0.234	1.239
No grain sink	1.244	0.344	0.269	1.858
% change	76	15	15	50
Mean	0.975	0.322	0.252	1.549
LSD	0.115	0.042	ns	0.170

LSD = Least significant difference; ns= not significant.

**Table 2.8.** Combined mean performance of the effect of head removal treatment on sugar components of sweet sorghum genotypes evaluated at Manhattan, KS during 2013 and 2015 sowing season.

Genotype	Sucrose (Mg h <sup>-1</sup> )			Glucose (Mg h <sup>-1</sup> )			Fructose (Mg h <sup>-1</sup> )			Total sugars (Mg h <sup>-1</sup> )		
	NC	C	Mean	NC	C	Mean	NC	C	Mean	NC	C	Mean
Atlas	0.81	1.54	1.17	0.48	0.58	0.53	0.35	0.44	0.40	1.64	2.56	2.10
Atlas <i>bmr 12</i>	1.26	1.73	1.50	0.30	0.34	0.32	0.19	0.24	0.22	1.75	2.31	2.03
Atlas <i>bmr 6</i>	0.62	1.20	0.91	0.29	0.26	0.27	0.21	0.21	0.21	1.12	1.67	1.39
Dale	1.34	2.15	1.74	0.96	0.95	0.95	0.87	0.89	0.88	3.17	3.98	3.58
Early hegari	0.22	0.92	0.57	0.19	0.22	0.20	0.19	0.27	0.23	0.60	1.41	1.01
Early hegari <i>bmr 6</i>	0.06	0.41	0.24	0.10	0.09	0.10	0.07	0.06	0.07	0.24	0.56	0.40
Kansas collier	1.14	1.69	1.41	0.12	0.11	0.12	0.11	0.09	0.10	1.37	1.89	1.63
Kansas collier <i>bmr 12</i>	0.80	1.00	0.90	0.09	0.10	0.10	0.07	0.08	0.08	0.95	1.18	1.07
Kansas collier <i>bmr 6</i>	0.97	1.23	1.10	0.15	0.19	0.17	0.10	0.13	0.11	1.22	1.54	1.38
M 81E	1.28	2.12	1.70	0.88	1.17	1.03	0.60	0.78	0.69	2.76	4.07	3.42
Masuda	0.54	1.15	0.85	0.36	0.51	0.43	0.27	0.39	0.33	1.17	2.06	1.61
MN 4179	0.71	1.47	1.09	0.31	0.38	0.34	0.33	0.30	0.32	1.34	2.16	1.75
No.8	0.84	1.52	1.18	0.39	0.46	0.43	0.26	0.33	0.29	1.49	2.31	1.90
PI185672	0.48	0.74	0.61	0.12	0.12	0.12	0.09	0.09	0.09	0.70	0.95	0.82
Kaoliang	0.12	0.38	0.25	0.05	0.08	0.07	0.04	0.06	0.05	0.21	0.52	0.37
Red amber	0.16	0.57	0.37	0.11	0.11	0.11	0.05	0.10	0.07	0.32	0.78	0.55
Rox orange	0.70	1.06	0.88	0.30	0.22	0.26	0.21	0.17	0.19	1.21	1.46	1.34
Rox orange <i>bmr 12</i>	0.90	1.28	1.09	0.22	0.26	0.24	0.17	0.20	0.18	1.29	1.74	1.51
Rox orange <i>bmr 6</i>	0.93	1.34	1.13	0.19	0.20	0.19	0.13	0.13	0.13	1.24	1.67	1.46
Sugar drip	0.41	1.18	0.80	0.34	0.32	0.33	0.30	0.25	0.27	1.05	1.75	1.40
Topper 76	0.78	1.74	1.26	0.52	0.75	0.64	0.46	0.61	0.53	1.76	3.10	2.43
Tracy	0.45	0.95	0.70	0.12	0.16	0.14	0.10	0.12	0.11	0.67	1.22	0.95
Mean	0.71	1.24		0.30	0.34		0.23	0.27		1.24	1.86	
LSD ( <i>P</i> =0.05)	0.2			0.04			NS			1.54		

NC= Grain sink; C= No grain sink.

LSD = Least significant difference; NS= not significant.

**Table 2.9.** Pearson correlation coefficients between sugar and juice quality components of sweet sorghum genotypes evaluated at Manhattan, KS during 2013 and 2015 sowing season.

Trait	Correlation ( <i>r</i> )				
	Grain sink	Sucrose (Mg h <sup>-1</sup> )	Glucose (Mg h <sup>-1</sup> )	Fructose (Mg h <sup>-1</sup> )	Total sugars (Mg h <sup>-1</sup> )
Brix	NC	0.75**	0.13 <sup>ns</sup>	0.13 <sup>ns</sup>	0.47*
	C	0.46*	-0.04 <sup>ns</sup>	-0.03 <sup>ns</sup>	0.21 <sup>ns</sup>
Sucrose (Mg ha <sup>-1</sup> )	NC	1	0.59*	0.55*	0.86**
	C	1	0.78**	0.77**	0.94**
Glucose (Mg ha <sup>-1</sup> )	NC		1	0.97**	0.91**
	C		1	0.97**	0.94**
Fructose (Mg ha <sup>-1</sup> )	NC			1	0.88**
				1	0.93**

NC= Grain sink; C= No grain sink.

\*, \*\* statistically significant at  $P \leq 0.05$  and  $P \leq 0.01$ , respectively.

## Discussion

Breakthroughs in science witnessed during the last century have impacted human life in many ways. Such discoveries involved development of novel techniques and tools for exploiting natural resources to meet human needs. Among these discoveries are the development of techniques and tools for exploiting natural energy sources and make them available for human use. Developments in petroleum engineering has positively affected every sector including mechanized agriculture that is heavily dependent on petroleum products as source of energy. Nevertheless, as a result of numerous competing needs for this resource including transportation fuels and the fact that it is nonrenewable has led to market volatility of petroleum products at different times. At the same time the extensive burning of fossil fuel has threatened the wellbeing of the environment that the need for alternative source of clean and renewable energy has become the ultimate target for powering our planet in the 21<sup>st</sup> century.

Biofuels are considered among the many renewable energy sources receiving global attention. Although plant based fuels have been in use for centuries, the resource has not been exploited at an industrial scale to enter the global power grid. Sorghum is one of the top plant species recognized to serve as feedstock sources for producing biofuels. Grain-based ethanol has already been in use for transportation fuel with nearly 30% of sorghum grain produced in the United States going to ethanol production. Because of its inherent potential to produce large biomass, and its sugar rich stalks and knowledge of its cultivation and management, it is poised to have even bigger impact. Sugars from stalks of sugary genotypes can be easily fermented in to ethanol and opportunities exist to enhance stalk sugar using different breeding techniques.

Sorghum is already recognized for its efficiency for converting CO<sub>2</sub> into sugar, its tolerance to abiotic stresses (drought, water logging, and salinity) and enhanced productivity

(Hallam et al., 2001; Promkhambut et al., 2010). The juice extracted from the fresh stem of sweet sorghum has been shown to contain 15 to 23% soluble fermentable sugar (Rao et al., 2009; Vinutha et al., 2014). This can be increased by reducing the grain sink to further increase sugar accumulation in the stalks and increase cellulosic biomass there by enhance its value as feedstock source for biofuel and table sugar production.

The current results confirmed the presence of genotypic variation for biomass production and sugar accumulation (Table 2.2). Similar results have been reported by several previous investigators (Almodares et al., 1997; Ali et al., 2008; Murray et al., 2008; Wang et al., 2009; Murray et al., 2009). The genotypes included in this study were sweet sorghum types deliberately selected based on existing information. Observing this level of variation for sugar, °Brix and juice yield among a small set of designated sweet sorghum accessions simply tells how robust the sorghum crop is with respect to variability for biofuel traits and the opportunity for enhancing the crop as biofuel feedstock. The early sweet sorghum cultivars such as Kansas collier, Rox orange and Early hegari continue to have greater °Brix compared most of the entries evaluated (Table 2.3). However, they were not necessarily the greatest in terms of total sugar or juice yield, which again shows the opportunity to recombine these traits to further improve the feedstock value of the crop. Cultivars such as Dale and M81E and a new accession from Ethiopia (No.8), though not among the highest in °Brix, ranked near the top in juice and sugar yield. Similar studies in the past have produced a conforming report where genotypes with greater juice yield and lower °Brix were considered better stem sugar yielders than those with lower juice yield and higher °Brix (Makanda et al., 2009). The brown mid rib (*bmr*) versions of the traditional sweet cultivars also were included to determine the potential role of higher lignin on the traits of interest. It appears that lignin has no impact on brix, juice accumulation and extractability, as well as total sugar (Table 2.3), indicating



that, unlike the forage/silage sorghum, energy sorghums do not need to be low lignin types which is a positive element considering high biomass type sweet sorghums because the presence of lignin reduces propensity to lodging.

The key purpose of this study was to determine the potential of deploying sterile sweet sorghum hybrids for improving biofuel traits. A genetic system based on A3 cytoplasmic male sterility (CMS) or a B pollinator on A1 CMS can produce sterile hybrids that can be commercially deployed if removal of the grain sink is proven to contribute to enhancing biofuel traits. Here all of the twenty-two genotypes were grown in double row plots where heads from one of the rows were clipped prior to anthesis to eliminate grain formation. The results showed that removing the grain sink significantly improved dry biomass, °Brix, juice and sugar yield while at the same time reducing lodging (Table 2.4). The increase in °Brix (17%) and sugar yield (43%) in plots without grain sink may be due to much of the photo-assimilates accumulating in the stalk as opposed to the intact rows where they were translocated to fill the grain. The higher biomass (28%) and reduced lodging (-22%) in headless rows could be due to a profuse nodal tiller caused by head removal and the increased stalk girth compared to intact rows. The increase in juice yield (24%), is difficult to explain but may be related to increased stalk thickness that provided more room for juice accumulation. These results are corroborated by reports from previous studies. In an older report by Broadhead (1973), which was later confirmed by other studies (Pfeiffer et al., 2010; and Erickson et al., 2011), removing the heads before grain formation increased °Brix and sugar. Such a change has been attributed to changes in patterns of assimilate partitioning with the stem becoming the predominant alternative sink (Lin and Lin, 1994). The increase in biomass yield in clipped plants found in this study was also consistent with the results obtained by Ferraris (1981).

Similarly, both genotypes and the sink effects were significant for sugar composition (Tables 2.6, 2.7, and 2.8). Differences in sucrose, glucose, and fructose content among the genotypes may be due to differences in the expression of the amylase enzyme, a possible scenario especially given that genotypes with high sucrose had lower glucose, and fructose content. In all cases, sucrose was the most dominant sugar followed by glucose, and this was consistent across genotypes. Moreover, genotypes with greater sucrose content tended to have greater concentration of the other sugars as well, such that selection for total sugar or sucrose only (whichever is easier to measure) can be used to improve other sugar components (Table 2.8). Total sugar is not necessarily related to °Brix, so one has to make proper choice of traits when attempting to improve biofuel traits. Removing the grain sink had a greater effect on sucrose (76% increases) than glucose (15% increases) and the effect on fructose was not significant. Increased sucrose with head removal in this study was similar to other findings where a 13% increase in sugar occurred with head removal in M81E (Erickson et al., 2011) and in similar report by Broadhead (1973) where increases of sucrose content was reported in plants with heads removed at boot stage.

## **Conclusion**

Results of this study confirm the previous findings and expectations that there is significant genotypic effect for accumulation of biomass and biochemical traits (juice and sugar yield) among genotypes. The significant genotype  $\times$  environment interaction observed for many of the traits suggests that optimal environment and management condition be identified for producing sweet sorghum based feedstock sources for biofuel or table sugar production. The most important finding, however, is that removing the grain sink from potentially high sugar and high juice accumulating genotypes can significantly increase juice, biomass and sugar yields, indicating the

potential benefit of deploying non-grain forming sweet sorghum hybrids as feedstock for producing biofuels or table sugars. Future research should explore genetic systems where genetically improved male sterile sweet sorghum hybrids can be produced and marketed to benefit the major players in the industry including growers, seed producers and ethanol/sugar industries.

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## **Chapter 3 - Effect of male sterility inducing cytoplasm on biomass, stalk juice yield and sugar content in sweet sorghum hybrids**

### **Abstract**

Sweet sorghum [*Sorghum bicolor* (L.) Moench] has considerable potential for bioenergy and table sugar production. Previous research and results reported in Chapter 2 of the current study show that reduction of assimilate competition by eliminating the grain sink can increase the quality and quantity of sugar in sweet sorghum. The objective of the current study is to explore the opportunity to deploy the A3 cytoplasmic male sterility (CMS) system to force hybrid sterility and thus increase sugar production. Four seed parent lines in the A1 and A3 CMS systems were intercrossed with 10 diverse sweet/biomass sorghum lines to create 40 hybrids each in A1 and A3 backgrounds. The resulting hybrids and the parents were evaluated in three environments. At the panicle emergence, heads from all A3 hybrid rows were covered prior to anthesis with a pollination bag to exclude pollen from other plants and also to determine the fertility of the hybrids. Data were collected on major agronomic and biofuel traits including brix, juice, biomass, and sugar yields. The biofuel data were collected by manually harvesting all plants from a one-meter section of the plot and separating them into stalk, leaf and panicle components. The stem was pressed to determine the juice yield, and sugar content of the juice was determined. Examination of pollen production and seed set in A3 rows showed that all A3 hybrids failed to set seeds under bag and were sterile. Analysis of the data showed significant differences between the inbred parents and hybrids for both agronomic and biofuel traits. More importantly, there was a significant difference between A1 and A3 hybrids for all of the biofuel traits, with the A3 hybrids invariably having superior values for all traits, indicating potential for deploying the A3 CMS in sweet sorghum



production to enhance sugar yield. Moreover, perhaps due to the stronger stalk achieved due to absence of the grain sink or lower torque imposed from the panicle, the A3 hybrids showed stronger resistance to stalk lodging than the A1 hybrids. The result indicated that the A3 CMS system can be effectively deployed to facilitate production of sterile sweet sorghum hybrids to increase sugar yield from sweet sorghum. The technique can benefit sorghum producers, biofuel industries, and sugar/syrup industries and can contribute to the competitiveness of the sorghum industry.

*Keywords:* *Sorghum bicolor*, cytoplasmic male sterility, grain sink, biomass, juice quality, combining ability

## **Introduction**

Sweet sorghum [*Sorghum bicolor* (L.) Moench] is a type of cultivated sorghum that is recognized as a potential alternative biofuel feedstock source due to its sugar rich, juicy stalks (Wang et al., 2009). Sorghum has many characteristics such as wide adaptability, rapid growth (Reddy et al., 2007), drought tolerance (Tesso et al., 2005), tolerance to waterlogging and salinity (Almodares et al., 2008), and high biomass production (Almodares and Hadi, 2009) that make it attractive both as a food/feed grain as well as for biofuel production.

Global hybrid sorghum production relies on cytoplasmic male sterility system (CMS) to facilitate seed production. Two mechanisms of sterility induction have been identified in CMS systems, primarily based on when the sterility is manifested in relation to meiosis. Sterility induced before meiosis (sporophytic) results in pollen abortion and is restored by the nuclear genotype of the plant. Manifestation of sterility after meiosis (gametophytic) also results in pollen abortion but fertility is restored by the genotype of the individual haploid male gamete. In sorghum, the first

CMS was discovered, characterized, and described by Stephens and Holland (1954). This system, designated as A1 CMS, is based on the interaction of nuclear and cytoplasmic genes. In the A1 system, fertility is restored in a sporophytic manner by one or two dominant fertility restoration alleles (Schertz et al., 1989). Thus, the A1 CMS system allows the production of uniformly male sterile lines that can produce progeny in which fertility is restored through the action of nuclear restorer (*Rf*) genes (Kempken and Pring, 1999). In addition to the original A1 CMS, which is the most commonly used CMS source for sorghum, several other CMS sources e.g. A2, A3, A4, A5, A6, 9E, and KS, have been developed (Quinby, 1981).

The A3 CMS system was introduced with the release of A3Tx398 (Schertz et al., 1984). Since that time, several groups have released seed parents carrying the A3 CMS (Pedersen et al., 1997; Miller et al., 1999). In the A3 CMS, a unique two-gene gametophytic fertility restoration system that requires complementary action of two nuclear restoration genes was identified (Tang et al., 1998). Fertility restoration genes designated as *Rf3* and *Rf4* must be present in an individual male gamete for its viability to be restored. Because the occurrence of such genes in the normal population is rare, the majority of hybrids between A3 females and the commonly existing sorghum lines tend to be sterile because none of the pollen of such hybrids be fertile (Tang et al., 2007; Pring et al., 1999; Tang et al., 1998).

Although this property of A3 CMS makes it difficult to deploy the system in routine grain sorghum hybrid production, the rare fertility restoration tendency of the A3 system will be of significant benefit for developing hybrids in which seed production is not desired. One such specialty crop is sweet sorghum. Sweet sorghums are the type of sorghum that tend to accumulate large quantity of sugar in the stalk, similar to sugarcane, but have a reduced grain sink compared to grain type sorghums. As such, sweet sorghums have been looked at as a transition crop to fill

the feedstock supply gap between sugarcane harvests in the sugar industry. The value of sweet sorghum as a transition or substitute feedstock for sugar production can be increased through enhancing the stalk sugar yield of the crop. One possible avenue would be to reduce or eliminate the grain sink from the sweet sorghum backgrounds such that the assimilate will remain in the stalk to enrich the stalk sugar. In this study we explored the possibility of deploying the A3 CMS system to reduce grain formation and thus increase stalk sugar in sorghum.

Previous studies comparing different CMS systems for agronomic adaptability have shown no marked effect of the system. Lee et al. (1992) compared a specific A1 hybrid with its corresponding A3 hybrid, and detected no significant differences for days to flowering or plant height. Moran and Rooney (2003) evaluated 36 iso-cytoplasmic hybrids that were developed in 3 different cytoplasm (A1, A2, and A3) for agronomic performance, and found that cytoplasm type had no effect on plant height, minimal effect on days to flowering, but a significant reduction in yield was observed in A3 cytoplasm hybrids compared to A1 and A2 hybrids.

Information comparing the agronomic performance and the sugar and juice yield and quality of A1 and A3 sweet sorghum hybrids is limited. The objective of this study was to investigate the effects of A3 cytoplasm on biomass production, juice yield and stalk sugar accumulation and composition and thereby determine its potential for developing sugar based alternative feedstocks for sugar and biofuel production.

## **Material and methods**

### **Genetic materials**

The experimental materials comprised of 4 grain type female parental lines and 10 sweet/high biomass type pollinator lines. The female lines included Tx399, Tx623, Ks57 and N122, each in

two different cytoplasms, A1 and A3. Two of the females, Tx399 and Tx623 were originally developed and released with the A1 CMS system by Miller et al. (1999). Female N122 also were first developed and released with the A1 cytoplasm system by Andrews et al. (1990) and Ks57 released by Pedersen and Toy (1997) also were first developed and released with the A1 cytoplasm system. All of the females were later converted in to the A3 system. The female parents were selected based on their historical significance as commonly used public lines and the availability of A3 cytoplasm versions of the lines. The 10 pollinator lines were selected based on the results of Chapter Two experiment of this thesis and previous study by Godoy and Tesso (2013).

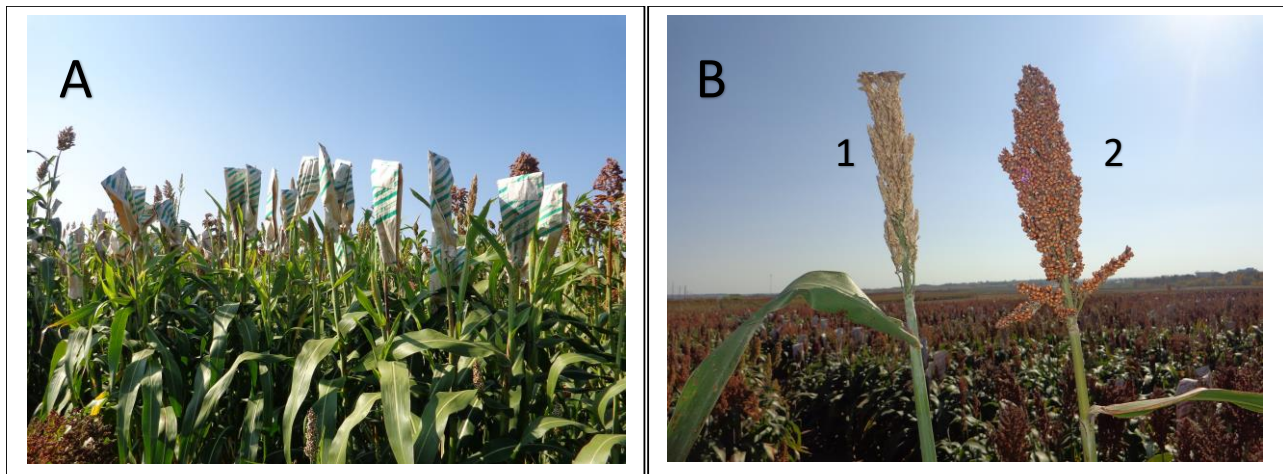
For experimental hybrid synthesis, each of the females were crossed to all of the 10 R-lines (Atlas, Early hegari, Kansas collier, M81E, Masuda, No.8, PI185672, Kaoliang, Rox orange and Sugar drip) in a Design II mating scheme to produce 80 hybrid combinations in A1 and A3 cytoplasm backgrounds. Hybrid seed production was conducted using a partial irrigation facility at KSU Agronomy Research Farm at Ashland Bottoms near Manhattan, KS and at winter nursery (Puerto Rico) during the 2012 and 2013 crop seasons. The hybrids along with the B-versions of the respective female parents and the 10 R-lines, were evaluated at KSU the Research Farm Ashland Bottoms during the 2013, 2014, and 2015 sowing seasons.

### **Experimental design and field procedure**

The experiment was laid in a Randomized Complete Block Design (RCBD) with three replications. The plots consisted of genotypes planted in a single row of five-meter length spaced 0.75 m apart with a 0.6m alley at the end of each plot. The experiment was established by directly seeding 3 g treated (Maxim 4FS, Apron XL, Concept III, and colorant) seeds of the genotypes using a cone planter. Fertilizer nitrogen (urea) and phosphorous (di-ammonium phosphate, DAP)

were applied at the rates of 90 kg ha<sup>-1</sup> and 40 kg ha<sup>-1</sup>, respectively. Pre-emergence weeds were controlled with 0.55 kg ha<sup>-1</sup> Atrazine TM, 0.76 kg ha<sup>-1</sup> Dual II Mg TM, and, 0.16 kg ha<sup>-1</sup> Callisto<sup>TM</sup>. Post-emergence weeds were controlled by hand weeding, and this practice was used to keep the field weed-free throughout the seasons. The experiment was conducted in three environments during the 2013 and 2014 seasons at the Ashland Bottoms Agronomy Research Farm and in 2015 at the Agronomy North Farm near Manhattan, KS.

The plots were routinely supervised throughout the growing period. Prior to flowering panicles from the A3 hybrids were covered with pollination bags to provide isolation from random pollen from nearby plots (Figure 3.1).



**Figure 3.1.** Sweet sorghum hybrids tested at Manhattan Kansas (A) bagged heads of A3 hybrids to maintain isolation (B) A3 hybrids heads (1) bagged heads with no seed set (2) open head outcrossed and set seeds.

### **Data collection**

The fields were closely supervised throughout the experiment periods and notes taken as necessary. Data were collected on a range of characteristics related to both agronomic and biofuel traits. Important agronomic parameters measured included plant height, lodging score, and days to

flowering. Plant height was measured as the distance from the ground to the top of the panicles. The severity of stalk lodging was visually scored on a scale of 1 to 5 with a score of 1 representing no lodging to 10% lodging, 5 representing 90% to complete lodging with values in between representing different lodging severities between 10 and 90%. Days to flowering were recorded as the number of days from planting to the day when 50% of the plants in each plot had reached flowering.

Biofuel traits were determined at physiological maturity. All plants within a one-meter-long section of the plots were harvested at the base 5 cm from the soil surface. The plants were bundled and immediately taken to the laboratory where they were separated into panicles, stems, and leaves, and each component was separately weighed. The stems were then crushed in a three-roller sorghum press to extract the juice, and the pressed stems were weighed. The volume and weight of extracted juice was immediately measured and the °Brix determined using a hand-held refractometer (Atago U.S.A, Inc., Bellevue, WA, USA). Briefly each trait was measured as below:

- 1- Biomass, and juice yield were calculated from a sample taken at harvest using the formula:

(weight of sample from 1m) / (sample row area in m<sup>2</sup>) then calculated as Mg ha<sup>-1</sup>.

- 2- Sugar yield was estimated using the formula developed by Corn (2009)

Sugar yield (Mg ha<sup>-1</sup>) = 0.95 × juice yield (Mg ha<sup>-1</sup>) × 0.97 × 0.873 × (°Brix/100)

This formula approximates sugar values assuming commercial sugar extraction rates whereas; 0.95 extraction efficiency; 0.97 adjusts for using the first expressed juice to represent all juice (for every 100 parts brix in the first roller juice, there are approximately 97 parts in the whole juice of stalk); 0.873 accounts for percent fermentable sugars present in the brix.

Fresh panicles, leaves, and pressed stems were oven dried at 120°C for 10 days to determine biomass yield.

### **Statistical analysis**

The analysis of variance (ANOVA) was performed using a PROC MIXED procedure in SAS (version 9.4) as appropriate for the design. The environment and replicates were treated as random effect parameters. The hybrid effect was further partitioned into male, female, and male × female interaction effects representing general combining ability (GCA) for male and female parents as well as the specific combining ability (SCA) effect. Design II fixed model (Model I) of Hallauer and Miranda (1988) was used to obtain independent estimates of the GCA and SCA. The GCA for each parental line was calculated as the difference between the grand mean of the hybrids and the marginal means for each male and female parent.

Means for significant effects were separated using the LSD method. Pearson correlation coefficients were estimated using data from all combined environments to determine the degree of correlation between all traits using the PROC CORR procedure in SAS (version 9.4).

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 as follow:

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

$SCA_{ij}$  = 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

Mean of the hybrids; and  $GCA_i$  and  $GCA_j$  = General combining ability of parent  $i$  and parent  $j$ .

Significance of the effects was tested using a two-tailed t-test procedure in SAS after rearranging the data set. The result was confirmed by manually computing standard error for GCA for both male and female parents and SCA for the hybrids following the procedure outlined by Cox and Frey (1984).

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. The estimates of heterosis were obtained from average data of three environments using the formula given by Falconer and Mackay (1996):

$$\text{Mid-parent heterosis} = [(F1-MP)/MP] \times 100$$

$$\text{High-parent heterosis} = [(F1 - HP)/HP] \times 100$$

Where,

MP = mid parent value of the particular F1 cross  $[(P1 + P2)/2]$ .

HP = high parent value in the particular F1 cross (P1 or P2).

Test of significance:

The significance of heterosis over mid-parent and high-parent were determined using a t-test (Wynne et al., 1970).

## **Results**

Analysis of the data showed significant differences between hybrids of A1 and A3 CMS systems for all parameters measured. All pollinator lines used in the study failed to restore fertility when crossed with females in A3 CMS system. This was confirmed by failure of the A3 hybrids to produce seeds when heads were covered prior to flowering (Figure 3.1). On the other hand, all the



pollinators restored fertility in the A1 CMS system, confirmed by the fact that all A1 hybrids had normal seed set under pollination bags.

### **Analysis of variance**

Mean squares for the combined analysis of variance across the three environments for biofuel and agronomic traits are presented in Tables 3.1 and 3.3, respectively. Although there were significant environment and entry  $\times$  environment interaction effects for most parameters, the pattern of the effects in an individual environment was generally consistent with the effects in the combined analysis (Appendix 1 and 2).

In the combined analysis, environment, entry, and entry  $\times$  environment interaction effects were highly significant ( $P \leq 0.01$ ) for all biofuel and agronomic traits except the effect for lodging was not significant for any sources of variation except entry (Tables 3.1 and 3.3). Partitioning the entry effect into inbred, hybrid and inbred vs. hybrid components showed that all of them were significant for both agronomic parameters and biofuel traits (Tables 3.1 and 3.3).

Further partitioning of the hybrid effect into male and female components revealed that both male and female parents and male  $\times$  female interaction effects significantly contributed to variability observed among the hybrids (Tables 3.1 and 3.3). The male effect, also referred to as the general combining ability (GCA) for males, was highly significant ( $P \leq 0.01$ ) for all biofuel and agronomic traits. The GCA effect for females also was significant for all traits except for days to flowering. The male  $\times$  female interaction effect, the specific combining ability (SCA), also was significant for all measured traits (Tables 3.1 and 3.3).

Across all environments, the effect of hybrids based on A1 and A3 cytoplasm were significant for all biofuel and agronomic traits except for the effect of A3 hybrid on lodging (Tables

3.2 and 3.4). Cytoplasm effect (A1 vs. A3 hybrids) was not found to be significantly different for any of the agronomic traits except for head weight. Whereas, the effect was significant ( $P \leq 0.05$ ) for all biofuel traits, indicating that low or absence of seed set in A3 cytoplasm hybrids have had a direct effect on stalk sugar accumulation and biomass production (Table 3.2 and 3.4). The cytoplasm  $\times$  environment interaction effect was significant ( $P \leq 0.05$ ) for juice and sugar yield and was highly significant ( $P \leq 0.01$ ) for lodging and head weight (Tables 3.2 and 3.4).

### **The effect of the grain sink on biofuel traits of sweet sorghum hybrids**

The effect of the grain sink is manifested by comparison of the A1 and A3 hybrids because all A3 hybrids failed to produce grain and hence did not have the grain sink. The mean squares for A1 vs. A3 component was highly significant for all the biofuel related traits (Table 3.2). Among hybrids, the across environment °Brix values ranged from 11.2 to 19.8% with mean of 15.6%. When disaggregated by the CMS system, the range of °Brix values in A1 hybrids extends from 11.2 to 17.5% with a mean of 13.8% while the range in A3 hybrids was 14.8 to 19.8% with an average of 17.4% (Table 3.6). The mean °Brix was significantly different between A1 and A3 CMS systems with the A3 hybrids having 26% more °Brix than the A1 hybrids. However, there was no genotype by CMS interaction observed with pollinators M81E, and No.8 consistently produced hybrids with superior °Brix percent in combination with all females of both CMS groups but the percentage was consistently greater in A3 than A1. Mean °Brix percent in the parental lines per se was comparable with that of the A1 hybrids (Tables 3.5 and 3.6) but was markedly less than that of the A3 hybrids (14.7 vs. 17.4). °Brix percent was understandably lower among the seed parents compared to the pollinator parents.

Similar to °Brix, there was wide variation observed for biomass production among the hybrids. The combined across environment biomass yield ranged from 9.19 to 26.5 Mg h<sup>-1</sup> with a mean of 15.4 Mg h<sup>-1</sup>. But it was again different for the different CMS systems with yield among A1 hybrids ranging from 9.2 to 22.8 Mg h<sup>-1</sup> and, among the A3 hybrids from 12.2 to 26.54 Mg h<sup>-1</sup> with mean of 13.4 and 17.3 Mg h<sup>-1</sup> in A1 and A3 hybrids, respectively (Table 3.6). Hybrids of M81E and No.8 that had the greatest °Brix also produced the greatest biomass followed by hybrids of PI185672 in both A1 and A3 backgrounds. Again, the A3 hybrids produced 29.2% more biomass than A1 hybrids. Unlike the °Brix percent where the inbred per se had values comparable to those for A1 hybrids, mean biomass yield was remarkably higher in the hybrids than in the inbred lines. This is a commonly observed effect of heterosis where hybrids tend to accumulate more biomass than inbreds. Nevertheless, lines that had the greatest biomass yield in hybrid combination with the females also had greater relative biomass as inbred per se (Table 3.5 and 3.6).

Among the hybrids, juice yield ranged from 10.31 to 33.27 Mg h<sup>-1</sup> with an average of 19.93 Mg h<sup>-1</sup> (Table 3.6). Hybrids KS57 × No.8, Tx399 × M81E and Tx623 × No.8 in A3 cytoplasmic backgrounds recorded the highest juice yield of 33.3, 31.1, and 30.2 Mg h<sup>-1</sup>, respectively (Table 3.6). Similar to °Brix and biomass, mean juice yield was greater among the hybrids than the inbreds and among the A3 hybrids than A1 hybrids with hybrids No.8 and M81E continue ranking near the top in both A1 and A3 CMS hybrids.

Similar to the juice yield, sugar yield also was higher in the hybrids with an across-environment average of 2.69 Mg h<sup>-1</sup> compared to 1.92 Mg h<sup>-1</sup> in the male and 0.3 Mg h<sup>-1</sup> in the female lines (Tables 3.5 and 3.6). Among the A1 hybrids, sugar yield ranged from 0.9 to 3.4 with an average of 1.9 Mg h<sup>-1</sup> and, the range was 1.6 to 4.8 among A3 hybrids with an average of 2.9

Mg h<sup>-1</sup> (Table 3.6). Hybrids Ks57 × No.8, Tx399 × M81E and Tx623 × No.8 in A3 hybrids recorded the highest sugar yield of 4.8, 4.5 and 4.3 Mg h<sup>-1</sup>, respectively, while the hybrids Tx399 × M81E, Tx623 × No.8 and Ks57 × No.8 recorded highest sugar yield in A1 hybrids with values of 3.4, 3.2 and 3.1 Mg h<sup>-1</sup>, respectively (Table 3.6). Among the parental lines, pollinators No.8 and M81E produced significantly greater sugar yield with a mean of 3.5 and 3.0 Mg h<sup>-1</sup>, respectively (Table 3.5).

### **The effect of A3 system on agronomic traits**

As revealed in the ANOVA, mean agronomic parameters, including days to flowering and plant height, were significantly different among the entries (inbreds and hybrids). However, unlike for other traits, the effect of CMS system was not significant except for panicle weight. Given the variation in plant height and days to flowering among inbreds, variation for those traits among the hybrids was expected. Because differences between A1 and A3 hybrids is largely the result of cytoplasmic mutation and fertility restorer genes in the nucleus, the CMS was not expected to cause difference in the agronomic traits. However, because of poor fertility restoration in A3 cytoplasmic system, seed set was low in A3 hybrids, and thus there was significant effect of CMS on panicle weight of the hybrids (Appendix 3).

**Table 3.1.** Combined analysis of variance for biofuel traits of sweet sorghum genotypes grown at Manhattan, KS during 2013, 2014, and 2015 sowing season.

Source of variation	df	Mean squares			
		°Brix (%)	Biomass Yield (Mg ha <sup>-1</sup> )	Juice yield (Mg ha <sup>-1</sup> )	Sugar yield (Mg ha <sup>-1</sup> )
Environment (E)	2	130.98**	588.22**	7099.91**	100.05**
Replication / E	6	1.51	17.55**	10.47*	0.26*
Entry	97	71.24**	246.01**	403.35**	9.74**
Inbred	17	98.83**	252.19**	498.79**	10.16**
Hybrid	79	49.52**	187.29**	265.46**	7.41**
Male	9	104.65**	1114.93**	1369.83**	31.74**
Female	7	348.96**	434.89**	671.52*	31.08**
Male × Female	63	8.38**	27.27**	62.58**	1.30**
Inbred vs. Hybrid	1	1317.97*	4779.26*	9674.19*	186.87
Entry × E	194	6.65**	16.87**	48.91**	1.05**
Inbred × E	34	6.52*	8.66**	26.72**	0.42**
Hybrid × E	158	5.7**	18.14**	49.32**	1.03**
Male × E	18	12.6**	49.90**	155.34**	3.16**
Female × E	14	4.51*	17.25**	75.75**	1.75**
Male × Female × E	126	4.84**	13.70**	31.24**	0.67**
Inbred vs. Hybrid × E	2	84.38*	56.04	393.46*	13.03**
Error	582	2.25	3.5	2.81	0.1

\*\* , \* statistically significant at 1% and 5% levels of probability.

**Table 3.2.** Combined analysis of variance for biofuel traits of iso-nuclear sweet sorghum hybrids grown at Manhattan, KS during 2013, 2014, and 2015 sowing season.

Source of variation	df	Mean squares			
		°Brix (%)	Biomass Yield (Mg ha <sup>-1</sup> )	Juice yield (Mg ha <sup>-1</sup> )	Sugar yield (Mg ha <sup>-1</sup> )
Hybrid	79	49.52**	187.29**	265.46**	7.41**
A1 hybrids	39	21.49**	132.53**	216.57**	4.13**
A3 hybrids	39	16.82**	175.75**	228.03**	5.62**
A1 vs. A3 hybrids	1	2418.16*	2773.64*	3632.18*	204.62*
Hybrid × E	158	5.7**	18.14**	49.32**	1.03**
A1 hybrid × E	78	6.21**	13.05**	39.79**	0.67**
A3 hybrid × E	78	5.1**	23.28*	55.77**	1.29**
A1 vs. A3 hybrids × E	2	8.85	15.94	169.41*	5.35*
Error	474	2.13	3.86	2.9	0.11

\*\* , \* statistically significant at 1% and 5% levels of probability.

**Table 3.3.** Combined analysis of variance for agronomic traits of sorghum genotypes grown at Manhattan, KS during 2013, 2014, and 2015 sowing season.

Source of variation	df	Mean squares			
		Day to flowering	Lodging	Plant Height (m)	Heads weight (Mg ha <sup>-1</sup> )
Environment (E)	2	4640.98*	4.87	1106819**	205.52**
Replication /E	6	431.18**	2.09**	1472.43**	3.45**
Entry	97	869.62**	1.32**	25760**	78.05**
Inbred	17	772.57**	0.99*	59048**	18.83*
Hybrid	79	877.88**	1.4**	10386**	91.74**
Male	9	7005.41**	5.27**	71287**	18.76*
Female	7	198.86	3.65*	14987*	928.11**
Male x Female	63	77.97**	0.60**	1174.79**	9.23**
Inbred vs. Hybrid	1	1866.58	0.33	674383*	3.15
Entry × E	194	81.42**	0.42**	1927.46**	8.06**
Inbred × E	34	88.71**	0.43*	2560.6**	8.38**
Hybrid × E	158	78.3**	0.42**	1293.76**	8.02**
Male × E	18	130.62**	0.29	2788.06**	7.87**
Female × E	14	141.9**	0.77**	1605.58**	20.33**
Male × Female × E	126	63.75**	0.40**	1045.65**	6.67**
Inbred vs. Hybrid × E	2	204.03	0.47	41226**	5.55
Error	582	20.34	0.19	185.94	0.65

\*\* , \* statistically significant at 1% and 5% levels of probability.

**Table 3.4.** Combined analysis of variance for agronomic traits of iso-nuclear sweet sorghum hybrids grown at Manhattan, KS during 2013, 2014, and 2015 sowing season.

Source of variation	df	Mean squares			
		Day to flowering	Lodging	Plant height (m)	Heads weight (Mg ha <sup>-1</sup> )
Hybrid	79	877.88**	1.4**	10386**	91.74**
A1 hybrids	39	919**	2.14**	9483.67**	18.05
A3 hybrids	39	832**	0.27	11555**	1.53*
A1 vs. A3 hybrids	1	1063.36	17.11	12.27	6483.84*
Hybrid × E	158	78.3**	0.42**	1293.76**	8.02**
A1 hybrid × E	78	60.06**	0.48**	1362.18**	13.38**
A3 hybrid × E	78	96.07**	0.25*	1220.61**	0.57**
A1 vs. A3 hybrids × E	2	96.33	4.52**	1478.51	89.29**
Error	474	22.59	0.19	208.44	0.64

\*\* , \* statistically significant at 1% and 5% levels of probability.



**Table 3.5.** Combined mean performance of sorghum parental lines for biofuel and agronomic traits grown at Manhattan, KS in 2013, 2014, and 2015 sowing season.

Entries	<sup>o</sup> Brix (%)	Biomass yield (Mg ha <sup>-1</sup> )	Juice yield (Mg ha <sup>-1</sup> )	Sugar yield (Mg ha <sup>-1</sup> )	Day to flowering	Lodging	Plant height (m)	Head weight (Mg ha <sup>-1</sup> )
Females								
Tx399	8.72 <sup>b</sup>	5.46	4.66	0.30	67.9 <sup>ab</sup>	1.01	88.2 <sup>b</sup>	4.40
Tx623	9.38 <sup>a</sup>	5.39	4.83	0.32	69.3 <sup>a</sup>	1.00	103.6 <sup>a</sup>	4.35
Ks57	9.00 <sup>ab</sup>	5.21	4.93	0.31	66.0 <sup>b</sup>	1.02	84.2 <sup>b</sup>	5.06
N122	9.57 <sup>a</sup>	4.99	5.06	0.38	70.2 <sup>a</sup>	1.00	78.8 <sup>c</sup>	4.38
<i>Mean</i>	9.17	5.26	4.87	0.33	68.4	1.01	88.7	4.55
<i>LSD (0.05)</i>	1.07	NS	NS	NS	2.6	NS	4.07	NS
Males								
Atlas	15.3 <sup>dc</sup>	14.7 <sup>c</sup>	21.9 <sup>b</sup>	2.6 <sup>c</sup>	81.9 <sup>c</sup>	1.4 <sup>bc</sup>	250.8 <sup>cb</sup>	7.2 <sup>b</sup>
Early hegari	8.4 <sup>f</sup>	6.0 <sup>h</sup>	9.0 <sup>g</sup>	0.6 <sup>g</sup>	68.3 <sup>gf</sup>	1.4 <sup>bc</sup>	149.9 <sup>e</sup>	5.4 <sup>ed</sup>
Kansas collier	17.1 <sup>ba</sup>	9.0 <sup>g</sup>	14.3 <sup>e</sup>	1.8 <sup>ed</sup>	66.7 <sup>g</sup>	1.4 <sup>bc</sup>	217.3 <sup>d</sup>	5.0 <sup>ef</sup>
M81E	17.6 <sup>a</sup>	17.9 <sup>b</sup>	23.5 <sup>b</sup>	3.1 <sup>b</sup>	89.3 <sup>b</sup>	1.2 <sup>c</sup>	311.9 <sup>a</sup>	4.5 <sup>f</sup>
Masuda	15.8 <sup>bc</sup>	11.7 <sup>ed</sup>	16.5 <sup>dc</sup>	1.9 <sup>d</sup>	74.2 <sup>ed</sup>	1.9 <sup>ba</sup>	242.4 <sup>c</sup>	4.6 <sup>f</sup>
No.8	17.5 <sup>a</sup>	23.9 <sup>a</sup>	27.2 <sup>a</sup>	3.6 <sup>a</sup>	96.1 <sup>a</sup>	1.0 <sup>c</sup>	244.4 <sup>cb</sup>	8.5 <sup>a</sup>
PI185672	13.8 <sup>de</sup>	10.0 <sup>egf</sup>	9.3 <sup>g</sup>	1.0 <sup>f</sup>	89.2 <sup>b</sup>	2.1 <sup>a</sup>	245.4 <sup>cb</sup>	5.5 <sup>ed</sup>
Kaoliang	12.6 <sup>e</sup>	12.3 <sup>d</sup>	11.8 <sup>f</sup>	1.1 <sup>f</sup>	82.3 <sup>c</sup>	1.3 <sup>bc</sup>	253.2 <sup>b</sup>	9.0 <sup>a</sup>
Rox orange	13.8 <sup>de</sup>	10.9 <sup>edf</sup>	15.1 <sup>de</sup>	1.5 <sup>e</sup>	77.6 <sup>d</sup>	1.4 <sup>bc</sup>	219.6 <sup>d</sup>	6.0 <sup>cd</sup>
Sugar drip	15.0 <sup>dc</sup>	9.7 <sup>gf</sup>	17.5 <sup>c</sup>	2.0 <sup>d</sup>	71.4 <sup>ef</sup>	1.3 <sup>bc</sup>	223.0 <sup>d</sup>	6.7 <sup>cb</sup>
<i>Mean</i>	14.7	12.62	16.6	1.92	79.71	1.47	235.8	6.22
<i>LSD (0.05)</i>	1.55	1.63	1.84	0.33	3.51	0.59	10.62	0.73

LSD, least significant difference; NS, not significant.

Means within the same column with different letter are significantly different at  $P < 0.05$ .

**Table 3.6.** Combined mean performance of sweet sorghum hybrids for biofuel traits as influenced by male-sterility inducing cytoplasm evaluated at Manhattan, KS in 2013, 2014, and 2015 sowing season.

Hybrids	°Brix (%)			Biomass yield (Mg ha <sup>-1</sup> )			Juice yield (Mg ha <sup>-1</sup> )			Sugar yield (Mg ha <sup>-1</sup> )		
	A1	A3	Diff.	A1	A3	Diff.	A1	A3	Diff.	A1	A3	Diff.
Tx399 × Atlas	13.2 <sup>h-n</sup>	17.9 <sup>d-i</sup>	**	12.2 <sup>hgi</sup>	12.2 <sup>w</sup>	NS	17.8 <sup>j</sup>	20.8 <sup>lmn</sup>	NS	1.8 <sup>lmn</sup>	2.7 <sup>j-n</sup>	*
Tx399 × Early hegari	13.7 <sup>f-k</sup>	17.4 <sup>g-j</sup>	**	12.7 <sup>fg</sup>	16.7 <sup>i-n</sup>	NS	18 <sup>ij</sup>	22.7 <sup>ijk</sup>	NS	2 <sup>lk</sup>	3.1 <sup>g-j</sup>	*
Tx399 × Kansas collier	13.4 <sup>f-n</sup>	16.5 <sup>i-m</sup>	*	10.7 <sup>j-n</sup>	13.1 <sup>r-w</sup>	NS	14.5 <sup>o-s</sup>	18.3 <sup>op</sup>	NS	1.5 <sup>o-r</sup>	2.3 <sup>qpo</sup>	NS
Tx399 × M81E	16 <sup>a-e</sup>	18.9 <sup>a-d</sup>	*	21.3 <sup>b</sup>	21.9 <sup>feg</sup>	NS	28.3 <sup>a</sup>	31.1 <sup>b</sup>	NS	3.4 <sup>a</sup>	4.5 <sup>b</sup>	*
Tx399 × Masuda	12.4 <sup>l-p</sup>	15.8 <sup>n-q</sup>	*	12 <sup>g-j</sup>	16.9 <sup>i-m</sup>	*	14.5 <sup>p-s</sup>	19.8 <sup>omn</sup>	*	1.4 <sup>p-s</sup>	2.5 <sup>l-p</sup>	*
Tx399 × No.8	14.7 <sup>d-h</sup>	19.8 <sup>a</sup>	**	22 <sup>ba</sup>	24.7 <sup>bac</sup>	NS	26.1 <sup>cb</sup>	28.7 <sup>c-f</sup>	NS	2.9 <sup>edc</sup>	4.3 <sup>cbd</sup>	**
Tx399 × PII85672	13.3 <sup>g-n</sup>	15.5 <sup>n-q</sup>	*	18.3 <sup>dc</sup>	22.1 <sup>d-g</sup>	NS	23.5 <sup>ed</sup>	25.8 <sup>gh</sup>	NS	2.3 <sup>ihj</sup>	3 <sup>hji</sup>	NS
Tx399 × Kaoliang	12.8 <sup>k-o</sup>	15.5 <sup>n-q</sup>	*	12.4 <sup>hg</sup>	18.8 <sup>ih</sup>	*	12.5 <sup>tu</sup>	15.1 <sup>sr</sup>	NS	1.2 <sup>str</sup>	1.8 <sup>ts</sup>	NS
Tx399 × Rox orange	13.1 <sup>i-n</sup>	16.4 <sup>k-n</sup>	*	9.4 <sup>mn</sup>	13.1 <sup>r-w</sup>	NS	13.5 <sup>ts</sup>	16.5 <sup>qr</sup>	NS	1.3 <sup>p-s</sup>	2 <sup>qrs</sup>	NS
Tx399 × Sugar drip	14.2 <sup>f-k</sup>	17.7 <sup>e-i</sup>	**	10.2 <sup>l-n</sup>	13.5 <sup>q-w</sup>	NS	16.1 <sup>k-n</sup>	19 <sup>on</sup>	NS	1.7 <sup>l-n</sup>	2.5 <sup>l-o</sup>	NS
Tx623 × Atlas	14.6 <sup>d-i</sup>	17.5 <sup>g-j</sup>	*	11.7 <sup>g-k</sup>	13.4 <sup>q-w</sup>	NS	11.5 <sup>vu</sup>	14.8 <sup>sr</sup>	NS	1.3 <sup>p-s</sup>	1.9 <sup>trs</sup>	NS
Tx623 × Early hegari	12.7 <sup>k-p</sup>	18 <sup>d-i</sup>	**	12.8 <sup>fg</sup>	17.2 <sup>ijk</sup>	*	21.3 <sup>gf</sup>	23.9 <sup>ij</sup>	NS	2.1 <sup>kj</sup>	3.2 <sup>hg</sup>	*
Tx623 × Kansas collier	14.4 <sup>e-j</sup>	17 <sup>i-m</sup>	*	10.8 <sup>i-m</sup>	14.7 <sup>n-t</sup>	NS	15.9 <sup>k-o</sup>	19.8 <sup>omn</sup>	NS	1.7 <sup>l-n</sup>	2.5 <sup>l-p</sup>	NS
Tx623 × M81E	16.5 <sup>bac</sup>	19.2 <sup>bac</sup>	*	19.6 <sup>c</sup>	24.1 <sup>bdc</sup>	*	25.9 <sup>cb</sup>	27.5 <sup>gf</sup>	NS	3.2 <sup>ba</sup>	3.9 <sup>ed</sup>	NS
Tx623 × Masuda	13 <sup>i-n</sup>	17.9 <sup>d-i</sup>	**	11.2 <sup>i-l</sup>	12.6 <sup>vwu</sup>	NS	14.2 <sup>qrs</sup>	21.1 <sup>lmk</sup>	*	1.4 <sup>p-s</sup>	2.8 <sup>j-m</sup>	**
Tx623 × No.8	16 <sup>a-d</sup>	19.2 <sup>bac</sup>	*	17.8 <sup>d</sup>	25 <sup>ba</sup>	**	22 <sup>f</sup>	30.2 <sup>cbd</sup>	**	2.6 <sup>egf</sup>	4.3 <sup>cb</sup>	**
Tx623 × PII85672	12.8 <sup>k-o</sup>	15.1 <sup>qpo</sup>	*	22.9 <sup>a</sup>	23.9 <sup>b-d</sup>	NS	26.9 <sup>b</sup>	28.1 <sup>ef</sup>	NS	2.6 <sup>ghf</sup>	3.2 <sup>hg</sup>	NS
Tx623 × Kaoliang	12.3 <sup>l-p</sup>	15 <sup>qp</sup>	*	13.1 <sup>fg</sup>	14.3 <sup>p-v</sup>	NS	12.6 <sup>tu</sup>	15.3 <sup>sr</sup>	NS	1.1 <sup>st</sup>	1.7 <sup>ts</sup>	NS
Tx623 × Rox orange	13.7 <sup>f-k</sup>	17.4 <sup>g-j</sup>	**	10.9 <sup>i-m</sup>	14.1 <sup>q-w</sup>	NS	15.1 <sup>m-q</sup>	19 <sup>on</sup>	NS	1.5 <sup>m-p</sup>	2.5 <sup>m-p</sup>	*
Tx623 × Sugar drip	13.7 <sup>f-k</sup>	18.3 <sup>b-h</sup>	**	11.9 <sup>g-j</sup>	17.7 <sup>ij</sup>	*	19.3 <sup>ih</sup>	28.4 <sup>efd</sup>	**	2 <sup>lk</sup>	3.9 <sup>ed</sup>	**
Ks57 × Atlas	14.7 <sup>d-h</sup>	17.6 <sup>g-i</sup>	*	14.2 <sup>fe</sup>	14.7 <sup>o-t</sup>	NS	21.6 <sup>gf</sup>	22.2 <sup>ljk</sup>	NS	2.5 <sup>igh</sup>	2.9 <sup>h-k</sup>	*

Ks57 × Early hegari	11.3 <sup>po</sup>	17.1 <sup>i-m</sup>	**	12 <sup>g-j</sup>	15 <sup>m-r</sup>	NS	17.1 <sup>kj</sup>	19.5 <sup>omn</sup>	NS	1.5 <sup>o-r</sup>	2.6 <sup>k-n</sup>	*
Ks57 × Kansas collier	12 <sup>m-p</sup>	18.7 <sup>b-f</sup>	**	10 <sup>mln</sup>	14.8 <sup>m-s</sup>	*	15.3 <sup>m-q</sup>	22.6 <sup>ijk</sup>	*	1.3 <sup>p-s</sup>	3.2 <sup>hgi</sup>	*
Ks57 × M81E	17 <sup>ba</sup>	19.3 <sup>bac</sup>	*	15.6 <sup>e</sup>	20.2 <sup>hg</sup>	*	22 <sup>f</sup>	27.3 <sup>gf</sup>	*	2.8 <sup>edf</sup>	4 <sup>ced</sup>	*
Ks57 × Masuda	12.1 <sup>m-p</sup>	16 <sup>m-p</sup>	**	10.5 <sup>j-n</sup>	14.7 <sup>n-t</sup>	*	16.3 <sup>klm</sup>	21.1 <sup>lmk</sup>	NS	1.5 <sup>n-q</sup>	2.6 <sup>l-o</sup>	*
Ks57 × No.8	15.8 <sup>b-e</sup>	19.4 <sup>ba</sup>	**	17.5 <sup>d</sup>	26.5 <sup>a</sup>	**	25.6 <sup>cb</sup>	33.3 <sup>a</sup>	*	3.1 <sup>bac</sup>	4.8 <sup>a</sup>	*
Ks57 × PI185672	14.4 <sup>e-j</sup>	17.4 <sup>g-j</sup>	*	17.9 <sup>d</sup>	21.6 <sup>fg</sup>	NS	24.9 <sup>cd</sup>	25.9 <sup>g</sup>	NS	2.7 <sup>d-f</sup>	3.4 <sup>fg</sup>	NS
Ks57 × Kaoliang	12.7 <sup>k-p</sup>	15.6 <sup>n-q</sup>	*	10.3 <sup>l-n</sup>	12.4 <sup>vw</sup>	NS	12.4 <sup>tu</sup>	18.9 <sup>op</sup>	*	1.1 <sup>st</sup>	2.2 <sup>qpr</sup>	*
Ks57 × Rox orange	13.6 <sup>f-m</sup>	17.8 <sup>d-i</sup>	**	11.6 <sup>g-k</sup>	12.9 <sup>s-w</sup>	NS	13.6 <sup>trs</sup>	18.9 <sup>op</sup>	*	1.4 <sup>p-s</sup>	2.5 <sup>m-p</sup>	*
Ks57 × Sugar drip	12.2 <sup>l-p</sup>	15.7 <sup>n-q</sup>	*	10.2 <sup>l-n</sup>	16.3 <sup>j-n</sup>	*	16.4 <sup>kl</sup>	24.1 <sup>ih</sup>	*	1.5 <sup>o-r</sup>	2.8 <sup>i-l</sup>	**
N122 × Atlas	15.9 <sup>b-e</sup>	18.4 <sup>b-g</sup>	*	12.3 <sup>hgi</sup>	16.1 <sup>j-p</sup>	NS	12.4 <sup>tu</sup>	18.6 <sup>op</sup>	*	1.5 <sup>n-q</sup>	2.5 <sup>l-o</sup>	*
N122 × Early hegari	12.8 <sup>k-o</sup>	18.3 <sup>b-h</sup>	**	9.7 <sup>mln</sup>	12.3 <sup>vw</sup>	NS	12.7 <sup>tu</sup>	17.2 <sup>qp</sup>	NS	1.2 <sup>str</sup>	2.4 <sup>npo</sup>	*
N122 × Kansas collier	13 <sup>j-n</sup>	18.8 <sup>a-e</sup>	**	9.8 <sup>mln</sup>	14.6 <sup>o-u</sup>	*	13.3 <sup>ts</sup>	21.2 <sup>lmk</sup>	*	1.2 <sup>sqr</sup>	3 <sup>hji</sup>	**
N122 × M81E	17.5 <sup>a</sup>	18.7 <sup>a-f</sup>	NS	17.8 <sup>d</sup>	22.3 <sup>fed</sup>	*	22.5 <sup>ef</sup>	29.3 <sup>b-e</sup>	*	3 <sup>bdc</sup>	4.1 <sup>cd</sup>	*
N122 × Masuda	13.8 <sup>f-k</sup>	18.2 <sup>c-h</sup>	**	10.8 <sup>i-m</sup>	17.1 <sup>i-l</sup>	*	14.9 <sup>n-r</sup>	20 <sup>omn</sup>	*	1.5 <sup>n-q</sup>	2.7 <sup>j-n</sup>	*
N122 × No.8	15 <sup>c-f</sup>	18.3 <sup>b-h</sup>	*	15.1 <sup>e</sup>	22.7 <sup>c-f</sup>	**	15.9 <sup>k-p</sup>	18.6 <sup>op</sup>	NS	1.8 <sup>lm</sup>	2.6 <sup>k-n</sup>	NS
N122 × PI185672	14.8 <sup>d-g</sup>	16.2 <sup>l-n</sup>	NS	17.8 <sup>d</sup>	25.9 <sup>ba</sup>	**	20.2 <sup>gh</sup>	30.3 <sup>cb</sup>	**	2.3 <sup>ij</sup>	3.7 <sup>fe</sup>	**
N122 × Kaoliang	11.9 <sup>pno</sup>	14.8 <sup>q</sup>	*	10.5 <sup>j-n</sup>	15.3 <sup>k-q</sup>	*	10.3 <sup>v</sup>	14.5 <sup>s</sup>	NS	0.9 <sup>t</sup>	1.6 <sup>t</sup>	*
N122 × Rox orange	13.1 <sup>i-n</sup>	17.2 <sup>h-l</sup>	**	9.4 <sup>mn</sup>	15.1 <sup>l-r</sup>	*	15.4 <sup>m-q</sup>	19 <sup>op</sup>	NS	1.5 <sup>n-q</sup>	2.4 <sup>npo</sup>	*
N122 × Sugar drip	11.2 <sup>p</sup>	18.5 <sup>b-g</sup>	**	9.2 <sup>n</sup>	12.7 <sup>t-w</sup>	NS	15 <sup>m-q</sup>	18.8 <sup>op</sup>	NS	1.2 <sup>sqr</sup>	2.6 <sup>l-o</sup>	*
<i>Mean</i>	13.8	17.4		13.4	17.3		17.7	22.2	**	1.9	2.9	**
<sup>a</sup> LSD (0.05)	1.6	1.1		1.5	2.1		1.3	1.8		0.3	0.3	
<sup>b</sup> LSD (0.05)		0.32			0.71			0.88			0.13	

A1, sorghum hybrids in A1 cytoplasm background; A3, sorghum hybrids in A3 cytoplasm background.

<sup>a</sup>LSD least significant difference between hybrids within same CMS system.

<sup>b</sup>LSD least significant difference between A1 and A3 CMS systems.

Means within the same column with different letter are significantly different at  $P \leq 0.05$ .

## **Combining Ability**

The ANOVA tables 3.1 and 3.3 depict both general combining ability (GCA) effects for both male and female parents included in the test and also a specific combining ability (SCA) effect referred to as the male  $\times$  female interaction. Both GCA and SCA were significant ( $P \leq 0.01$ ) for the majority of biofuel and agronomic traits, except female GCA effects for days to flowering. The GCA  $\times$  environment interaction also was significant ( $P \leq 0.01$ ) for these traits except male GCA  $\times$  environment effect for lodging. The SCA  $\times$  environment interaction was significant ( $P \leq 0.01$ ) for all traits (Tables 3.1 and 3.3).

### **Cytoplasmic effects on General Combining Ability (GCA) Effects**

The estimates of cytoplasmic effects on GCA for female and male lines with respect to biofuel and agronomic traits are presented in Tables 3.7 and 3.8.

### **GCA effect for biofuel traits**

Most of the pollinator lines included in the test had significant GCA for one or more of the biofuel traits with few genotypes consistently expressing positive GCA for the trait indicating their value for use directly as a hybrid parent or as a parental source for breeding sugar-based biofuel feedstock cultivars.

For °Brix percentage, none of the female parents had significant GCA in either A1 or A3 CMS systems except Tx399 had significant negative GCA in the A3 CMS system. As shown in their hybrid means, pollinator lines M81E, No.8, and Atlas showed significant and positive GCA for °Brix in both A1 and A3 CMS systems. On the other hand, male lines Masuda and Kaoliang showed significant negative GCA for the trait in both A1 and A3 CMS systems. Few other

pollinators, such as Early hegari, Rox orange and sugar drip exhibited significant negative GCA in A1 and were not significant in A3 CMS system, but GCA for others (PI185672 and Kansas collier) had the opposite result (Table 3.7).

For biomass production, female parents Tx623 followed by Tx399 had significant positive GCA, but the other two had significant but negative GCA for the same trait in A1 CMS system. No female parents were significant in A3 except KS57 that had significant negative GCA (Table 3.7). On the other hand, all pollinator lines had significant GCA for the trait. The three lines, M81E, No.8, and PI185672, that produced high biomass hybrids in both A1 and A3 CMS systems, also had the highest positive and significant GCA for the trait. All other pollinators had significant negative GCA for the trait with lines such as Rox orange, Kansas collier and Sugar drip having the greatest negative GCA (Table 3.7).

The GCA for juice and sugar yield in A1 and A3 CMS systems were generally similar. Female lines Tx623 and Ks57 exhibited highly significant positive GCA for both juice and sugar yield in both A1 and A3 CMS backgrounds. Further, female Tx399 exhibited significant positive GCA in A1 CMS but was negative in A3 (Table 3.7). In contrast, female N122 exhibited highly significant negative GCA in both A1 and A3 CMS background. As noted for other traits, pollinator lines M81E, No.8 and PI185672 were found to have significant positive GCA for both sugar and juice yield in both A1 and A3 CMS backgrounds while all the reimaging pollinator had significant negative GCA for both juice and sugar yield in both CMS systems (Table 3.7).

### **Heterosis for biofuel and agronomic traits**

Heterosis is a genetic mechanism where the performance of an F1 hybrid between a given parental lines is considerably superior from the performance of both of the parents (high parent heterosis)

or average performance of the parents (mid-parent heterosis). In this study both mid-parent and high-parent heterosis were observed for various traits studied. Tables 3.10 and 3.11, and Appendix 5 and 6 show the list of hybrids and the heterosis for various traits in both A1 and A3 backgrounds.

Heterosis was markedly different for the two CMS systems, at least for some of the traits. For °Brix MPH ranged from -12.4 to 30.3% in A1 hybrids with the lowest and highest recorded in N122 × Sugar drip and the highest in Tx399 × Early hegari. Ten A1 hybrids exhibited positive significant MPH for the trait while other were either non-significant or significant but negative. In A3 hybrids, the average MPH for °Brix ranged from 23.4 to 60.7%, much larger than that of A1 hybrids. All hybrids had highly significant positive MPH (Table 3.10). On the other hand, HPH among A1 hybrids ranged from -29 to 26.5% with only 7 of them being positive and significant. The majority of the A3 hybrids exhibited positive and significant HPH except for two entries Tx399 × Kansas collier and Tx623 × Kansas collier, that had negative and significant HPH of -3.2% and -0.7%, respectively (Table 3.11).

For biomass yield, all hybrids outperformed the mean of their parents with MPH among A1 hybrids ranging from 4.5 to 79.2%. Out of 40 hybrids, 16 had significant positive MPH with N122 × No.8 having the smallest and Tx623 × PI185672 having the greatest MPH value. Among the A3 hybrids, all of the hybrids showed significant positive MPH for biomass yield except hybrids Ks57 × Kaoliang, Tx623 × Atlas, and Tx399 × Atlas (Table 3.10). Several hybrids produced relatively less biomass than the high parent with HPH ranging from -36.8 to 35.5%. Only 12 A1 hybrids out of 40 had significant positive HPH while the others were either not significant or negative. Although the majority of the A3 hybrids expressed positive and significant HPH with the maximum HPH of 89.8%, a few had negative HPH of as low as -17.1% (Table 3.11).

Similar to °Brix, both positive and negative MPH were observed for juice yield with values for A1 hybrids ranging from -13.8 to 96.5% (Table 3.10). Hybrid Tx623 × PI185672 exhibited highest significant positive MPH followed by Ks57 × PI185672, Tx399 × PI185672 and Tx623 × Early hegari. On the other hand, hybrids of Atlas and No.8 (Tx623 × Atlas, N122 × Atlas and N122 × No.8) showed negative heterosis. Unlike in the A1 hybrids, MPH in A3 hybrids was all positive and ranged from 10 to 137.1% with hybrids Ks57 × PI185672, Tx399 × PI185672, and Tx623 × Early hegari having the highest and significant positive MPH for the trait, and hybrids N122 × Atlas and N122 × No.8 having the lowest (Table 3.10). HPH for the trait ranged from as low as -47.4 to 36.2% in A1 hybrids and -32.3 to 99.5% in A3 hybrids. Only ten hybrids showed significant positive HPH in A1 cytoplasm while most A3 hybrids had positive and significant HPH for the trait.

MPH for sugar yield was positive in both A1 and A3 hybrids though it was significantly greater in the A3 hybrids. The range was 10.9 to 101% in A1 hybrids and 33.2 to 183.5% in A3 hybrids. Hybrids Tx623 × Early hegari, Tx399 × Early hegari, Ks57 × PI185672, and Tx623 × PI185672 recorded the highest significant positive MPH in both A1 and A3 backgrounds, while N122 × Atlas and N122 × No.8 were the lowest in both CMS systems (Table 3.10). HPH for sugar yield, however, ranged from as low as -49.8 to 27.4% in A1 hybrids and from -26.1 to 111.9% in A3 hybrids. Again, most A3 hybrids exhibited highly significant positive HPH and only three hybrids showed significant negative HPH (Table 3.11).

Heterosis for agronomic traits is widely known for sorghum, and the current study agrees with the general observation for the species. For days to flowering, high parent heterosis tends to be negative since they reach anthesis earlier than the latest parent note necessarily earlier than the average of the parents. In this study MPH ranged from -8.6 to 27.2% and -4.3 to 26.8% in A1 and

A3 hybrids respectively. Hybrids tend to be taller than their inbred parents. Hence MPH for plant height was all positive and ranged from 20.5 to 89.7% and 26.7 to 92.6% among A1 and A3 hybrids, respectively (Appendix 6). Since A3 CMS has very few restorers, most of the A3 hybrids were sterile, and hence there was no significant difference in head weight among A3 hybrids. But among A1 hybrids, MPH for head weight ranged from -7.5 to 98.7% with hybrids N122 x Kansas collier, N122 x Masuda, Tx399 x M81E and Tx399 x Masuda having the highest positive MPH (Appendix 6).

### **Genotypic and phenotypic correlation for biofuel and agronomic traits**

Correlations among some biofuel and agronomic traits are presented in Table 3.12. Highly positive significant correlation was shown between days to flowering and plant height, biomass yield, juice yield and, sugar yield, but it was significantly negative with lodging for both A1 and A3 hybrids. There was also a significant positive correlation between days to flowering with °Brix for both cytoplasms. Further, days to flowering was positively correlated with head weight for A3 hybrids but negatively correlated for A1 hybrids, which indicates that this traits association affected by cytoplasm (Table 3.12).

Lodging score was significantly and negatively correlated with biomass yield but exhibited non-significant correlation with head weight in both A1 and A3 hybrids. Significant negative correlation also was recorded between lodging and plant height, °Brix, juice and sugar yield for A1 hybrids but it showed non-significant correlation with these characters in A3 hybrids. Highly positive significant correlation was shown between plant height and biomass yield, juice and sugar yield in A1 and A3 hybrids. Moreover, plant height recorded significant positive correlation with °Brix at A1 hybrids and non-significant correlation in A3 hybrids. Further, the correlation between



plant height and head weight was significantly positive for A3 hybrids and did not show such correlation in A1 hybrids (Table 3.12).

Highly significant and positive correlation was observed between °Brix, juice, and sugar yield in both A1 and A3 highly positive correlation was shown between °Brix and biomass yield for A1 hybrids, but the correlation in A3 it was not significant. Biomass yield was significantly and positively correlated with juice and sugar yield and juice yield, was significantly and positively correlated with sugar yield in both A1 and A3 hybrids (Table 3.12).

**Table 3.7.** Estimates of general combining ability (GCA) effects of parental lines for biofuel traits evaluated at Manhattan, KS during 2013, 2014, and 2015 sowing season.

Entries	°Brix (%)			Biomass yield (Mg ha <sup>-1</sup> )			Juice yield (Mg ha <sup>-1</sup> )			Sugar yield (Mg ha <sup>-1</sup> )		
	A1	A3	Diff.	A1	A3	Diff.	A1	A3	Diff.	A1	A3	Diff.
Females												
Tx399	-0.11	-0.30*	NS	0.71*	-0.04	*	0.80**	-0.40*	**	0.07*	-0.07*	**
Tx623	0.19	0.01	NS	0.85*	0.36	NS	0.80**	0.64**	NS	0.09*	0.07*	NS
Ks57	-0.2	0.01	NS	-0.41*	-0.41*	NS	0.83**	1.19**	NS	0.08*	0.16**	*
N122	0.11	0.29	NS	-1.14**	0.09	**	-2.42**	-1.43**	**	-0.25**	-0.16**	*
Males												
Atlas	0.82**	0.40*	NS	-0.80**	-3.23**	**	-1.86**	-3.08**	**	-0.10*	-0.40**	**
Early hegari	1.15**	0.25	**	-1.59**	-2.03**	NS	-0.41*	-1.35**	**	-0.18**	-0.10*	NS
Kansas collier	-0.56**	0.29	**	-3.06**	-3.01**	NS	-2.93**	-1.69**	**	-0.43**	-0.20**	**
M81E	2.96**	1.59**	*	5.17**	4.78**	NS	7.00**	6.61**	**	1.23**	1.18**	NS
Masuda	-0.96**	-0.47*	*	-2.29**	-2.00**	NS	-2.71**	-1.67**	NS	-0.43**	-0.28**	*
No.8	1.61**	1.72**	NS	4.70**	7.40**	**	4.71**	5.50**	**	0.76**	1.06**	**
PI185672	0.04	-1.39**	**	5.81**	6.03**	NS	6.18**	5.35**	*	0.61**	0.39**	**
Kaoliang	-1.37**	-2.23**	**	-1.81**	-2.13**	NS	-5.74**	-6.24**	*	-0.77**	-1.11**	**
Rox orange	-0.41*	-0.24	NS	-3.09**	-3.54**	NS	-3.27**	-3.84**	NS	-0.42**	-0.58**	*
Sugar drip	-0.98**	0.07	**	-3.03**	-2.26**	**	-0.98**	0.41*	**	-0.27**	0.03	**

\*\* , \* statistically significant at 1% and 5% levels of probability; NS, non-significant.

A1, sorghum hybrids in A1 cytoplasm background; A3, sorghum hybrids in A3 cytoplasm background.

**Table 3.8.** Estimates of general combining ability (GCA) effects of parental lines for agronomic traits evaluated at Manhattan, KS during 2013, 2014, and 2015 sowing season.

Entries	Day to flowering			Lodging			Plant height (m)			Head weight (Mg ha <sup>-1</sup> )		
	A1	A3	Diff.	A1	A3	Diff.	A1	A3	Diff.	A1	A3	Diff.
Females												
Tx399	0.9	0.18	NS	-0.25**	0.01	**	4.58*	5.51**	**	-0.13	0.1	**
Tx623	0.81	-0.01	NS	0.07	0.02	NS	17.36**	19.86**	NS	0.04	-0.13	**
Ks57	-0.75	0.47	NS	0.05	0.03	NS	-6.86**	-2.64*	**	0.17*	0.19*	NS
N122	-0.96*	-0.65	NS	0.14*	-0.06	**	-15.08**	-11.72**	NS	-0.08	-0.16	NS
Males												
Atlas	-5.60**	-3.00**	**	-0.10*	0.18**	**	-21.64**	-19.82**	NS	0.75**	-0.12	**
Early hegari	3.07*	2.06*	NS	-0.13*	-0.1	NS	-25.73**	-26.32**	NS	0.36**	0.19*	**
Kansas collier	-9.43**	-11.11**	*	0.51**	0.18**	**	-16.28**	-17.99**	NS	0.18	-0.26*	**
M81E	12.10**	11.64**	NS	-0.33**	-0.07	**	54.22**	57.98**	NS	0.46**	0.25*	**
Masuda	-6.57**	-5.86**	NS	0.93**	0.23**	**	-16.45**	-9.88**	NS	0.64**	-0.16	**
No.8	11.87**	10.78**	NS	-0.46**	-0.1	**	28.97**	29.59**	NS	0.34**	0.47**	**
PI185672	16.51**	15.97**	NS	-0.27**	-0.1	*	35.33**	48.98**	NS	1.92**	0.20*	**
Kaoliang	-5.04**	-3.36**	*	-0.21**	-0.13*	NS	7.99**	1.4	*	1.16**	0.08	**
Rox orange	-5.96**	-6.33**	NS	-0.13*	-0.02	NS	-22.92**	-36.93**	**	-1.55**	-0.31**	**
Sugar drip	-10.96**	-10.78**	NS	0.20**	-0.07	**	-23.48**	-27.02**	NS	0.25*	-0.33**	**

\*\* , \* statistically significant at 1% and 5% levels of probability; NS, non-significant.

A1, sorghum hybrids in A1 cytoplasm background; A3, sorghum hybrids in A3 cytoplasm background.

**Table 3.9.** Estimates of specific combining ability (SCA) effects of sweet sorghum hybrids for biofuel traits evaluated at Manhattan, KS during 2013, 2014, and 2015 sowing season

Hybrids	<sup>o</sup> Brix (%)		Biomass yield (Mg ha <sup>-1</sup> )		Juice yield (Mg ha <sup>-1</sup> )		Sugar yield (Mg ha <sup>-1</sup> )	
	A1	A3	A1	A3	A1	A3	A1	A3
Tx399 × Atlas	-1.29*	0.32 <sup>a</sup>	-1.10	-1.87*	1.21*	2.07** <sup>a</sup>	-0.06	0.27* <sup>a</sup>
Tx399 × Early hegari	1.13*	-0.02	0.22	1.49* <sup>a</sup>	-0.09	2.22** <sup>a</sup>	0.19	0.31* <sup>a</sup>
Tx399 × Kansas collier	0.31	-0.89	-0.40	-1.20	-1.01	-1.76*	-0.06	-0.39**
Tx399 × M81E	-0.66	0.19 <sup>a</sup>	1.97*	-0.22	2.80**	2.69**	0.22*	0.41**
Tx399 × Masuda	-0.28	-0.85	0.16	1.60* <sup>a</sup>	-1.26*	-0.28	-0.16	-0.08
Tx399 × No.8	-0.56	0.92 <sup>a</sup>	3.20**	-0.02 <sup>a</sup>	2.91**	1.42*	0.20	0.33* <sup>a</sup>
Tx399 × PI185672	-0.43	-0.28	-1.66*	-1.24	-1.13*	-1.30*	-0.21	-0.27*
Tx399 × Kaoliang	0.49	0.61	0.13	3.63** <sup>a</sup>	-0.27	-0.44	0.02	0.03
Tx399 × Rox orange	-0.20	-0.47	-1.62*	-0.63	-1.73*	-1.46*	-0.19	-0.25*
Tx399 × Sugar drip	1.49*	0.47	-0.91	-1.53*	-1.44*	-3.14**	0.04	-0.36** <sup>a</sup>
Tx623 × Atlas	-0.22	-0.32	-1.79*	-1.08	-5.11**	-4.92**	-0.57**	-0.68**
Tx623 × Early hegari	-0.10	0.33 <sup>a</sup>	0.14	1.51* <sup>a</sup>	3.22**	2.44**	0.34*	0.34*
Tx623 × Kansas collier	1.01*	-0.77	-0.34	0.01	0.36	-1.30*	0.20	-0.29*
Tx623 × M81E	-0.46	0.13 <sup>a</sup>	0.15	1.64* <sup>a</sup>	0.45	-1.97**	0.01	-0.26*
Tx623 × Masuda	0.03	0.88 <sup>a</sup>	-0.80	-3.08**	-1.56*	-0.01	-0.15	0.08
Tx623 × No.8	0.45	0.02	-1.18	-0.11	-1.15*	1.85** <sup>a</sup>	-0.09	0.22
Tx623 × PI185672	-1.23*	-0.95*	2.80**	0.17 <sup>a</sup>	2.20**	-0.07 <sup>a</sup>	0.03	-0.21
Tx623 × Kaoliang	-0.32	-0.23	0.67	-1.29*	-0.15	-1.27*	-0.05	-0.15
Tx623 × Rox orange	0.17	0.16	-0.30	-0.07	-0.10	0.05	0.01	0.06
Tx623 × Sugar drip	0.67	0.75	0.66	2.30** <sup>a</sup>	1.84**	5.20** <sup>a</sup>	0.28*	0.89** <sup>a</sup>
Ks57 × Atlas	0.31	-0.22	2.02*	0.98	4.90**	1.92** <sup>a</sup>	0.63**	0.25*

Hybrids	<sup>o</sup> Brix (%)		Biomass yield (Mg ha <sup>-1</sup> )		Juice yield (Mg ha <sup>-1</sup> )		Sugar yield (Mg ha <sup>-1</sup> )	
	A1	A3	A1	A3	A1	A3	A1	A3
Ks57 × Early hegari	-1.09*	-0.61	0.57	0.12	-0.98	-2.50**	-0.29*	-0.37**
Ks57 × Kansas collier	-0.98*	0.91 <sup>a</sup>	0.09	0.94	-0.32	0.94	-0.19	0.26* <sup>a</sup>
Ks57 × M81E	0.48	0.26	-2.55**	-1.51* <sup>a</sup>	-3.50**	-2.69**	-0.34*	-0.29*
Ks57 × Masuda	-0.57	-1.01*	-0.21	-0.18	0.50	-0.65	-0.01	-0.24*
Ks57 × No.8	0.63	0.20	-0.18	2.22** <sup>a</sup>	2.35**	4.39** <sup>a</sup>	0.44**	0.65** <sup>a</sup>
Ks57 × PI185672	0.80	1.37* <sup>a</sup>	-0.88	-1.37*	0.16	-2.85**	0.14	-0.09
Ks57 × Kaoliang	0.45	0.37	-0.88	-2.37**	-0.37	1.76* <sup>a</sup>	-0.04	0.19
Ks57 × Rox orange	0.39	0.60	1.73*	-0.50	-1.61*	-0.64	-0.14	-0.05
Ks57 × Sugar drip	-0.41	-1.87**	0.28	1.67* <sup>a</sup>	-1.12*	0.33 <sup>a</sup>	-0.20	-0.30*
N122 × Atlas	1.20*	0.23	0.87	1.97* <sup>a</sup>	-1.00	0.93	0.00	0.16 <sup>a</sup>
N122 × Early hegari	0.06	0.30	-0.93	-3.12**	-2.15**	-2.16**	-0.24*	-0.27*
N122 × Kansas collier	-0.34	0.75 <sup>a</sup>	0.65	0.24	0.98	2.12** <sup>a</sup>	0.04	0.43** <sup>a</sup>
N122 × M81E	0.64	-0.59	0.42	0.09	0.26	1.98** <sup>a</sup>	0.11	0.14
N122 × Masuda	0.82	0.97*	0.85	1.66*	2.31**	0.95 <sup>a</sup>	0.33*	0.24*
N122 × No.8	-0.51	-1.14*	-1.84*	-2.10**	-4.11**	-7.66** <sup>a</sup>	-0.55**	-1.20** <sup>a</sup>
N122 × PI185672	0.86	-0.15	-0.27	2.45** <sup>a</sup>	-1.23*	4.22** <sup>a</sup>	0.04	0.56** <sup>a</sup>
N122 × Kaoliang	-0.63	-0.74	0.08	0.04	0.79	-0.04	0.07	-0.06
N122 × Rox orange	-0.35	-0.29	0.19	1.21	3.43**	2.06**	0.32*	0.25*
N122 × Sugar drip	-1.75**	0.65 <sup>a</sup>	-0.03	-2.44** <sup>a</sup>	0.73	-2.39** <sup>a</sup>	-0.12	-0.24*

\*\*, \* statistically significant at 1% and 5% levels of probability; a, Significant cytoplasm differences  
A1, sorghum hybrids in A1 cytoplasm background; A3, sorghum hybrids in A3 cytoplasm background.

**Table 3.10.** Estimates of mid-parent heterosis (MPH) of sweet sorghum hybrids for biofuel traits evaluated at Manhattan, KS during 2013, 2014, and 2015 sowing season.

Hybrids	°Brix (%)		Biomass yield (Mg ha <sup>-1</sup> )		Juice yield (Mg ha <sup>-1</sup> )		Sugar yield (Mg ha <sup>-1</sup> )	
	A1	A3	A1	A3	A1	A3	A1	A3
Tx399 × Atlas	10.04	48.82**	20.95	20.71	34.31	56.4	23.96	90.82*
Tx399 × Early hegari	30.30**	60.72**	71.50**	127.06**	86.98*	231.26**	100.51*	181.01**
Tx399 × Kansas collier	4.04	28.14*	47.02	80.34*	53.58	93.59*	36.3	111.79*
Tx399 × M81E	21.61*	44.10**	69.87**	86.99**	84.06**	120.95**	88.67*	163.59**
Tx399 × Masuda	1.36	28.96*	40.02	97.36**	37.58	87.83*	21.62	125.51*
Tx399 × No.8	12.29	50.93**	49.92**	67.94**	64.10*	80.49**	50.64	120.95**
Tx399 × PI185672	18.01	37.44*	73.69**	184.72**	94.38**	135.35**	95.71*	195.69**
Tx399 × Kaoliang	19.81	45.29**	40.07	111.52**	51.54	83.49	69.76	152.97*
Tx399 × Rox orange	15.86	45.71**	15.1	60.37*	36.21	66.45	45.88	123.65*
Tx399 × Sugar drip	19.57	49.06**	34.11	77.90*	45.15	72.02*	50.66	122.80*
Tx623 × Atlas	13.45	36.45**	15.85	33.02	-13.82	10.86	-10.95	33.25
Tx623 × Early hegari	28.10*	57.50**	71.85**	128.29**	93.64**	133.39**	101.02*	183.52**
Tx623 × Kansas collier	4.85	23.42*	50.33	103.60**	66.49	107.53*	59.55	131.68*
Tx623 × M81E	17.84	37.15**	67.93**	107.01**	83.27*	93.99**	87.79*	130.28**
Tx623 × Masuda	-0.59	36.16**	30.89	48.02*	33.68	98.65*	21.96	148.87*
Tx623 × No.8	14.89	37.50**	21.24	70.53**	37.85	88.67**	35.34	120.66**
Tx623 × PI185672	5.55	24.83*	79.29**	129.51**	96.55**	137.19**	97.63**	197.69**
Tx623 × Kaoliang	6.6	30.02*	48.19*	61.30*	51.55	84.19	59.44	142.92
Tx623 × Rox orange	13.29	43.26**	33.4	72.89*	51.4	90.52*	67.61	169.06*
Tx623 × Sugar drip	7.52	43.71**	57.25*	120.77**	73.40*	129.78**	71.48	193.69**
Ks57 × Atlas	21.23	45.29**	42.40*	47.17*	60.70*	65.57*	71.6	104.50*

Hybrids	°Brix (%)		Biomass yield (Mg ha <sup>-1</sup> )		Juice yield (Mg ha <sup>-1</sup> )		Sugar yield (Mg ha <sup>-1</sup> )	
	A1	A3	A1	A3	A1	A3	A1	A3
Ks57 × Early hegari	30.23*	59.30**	70.70*	123.78**	85.37*	180.88*	92.18	180.29**
Ks57 × Kansas collier	-7.71	43.04**	40.57	108.34**	58.87	125.50**	23.53	192.37**
Ks57 × M81E	29.28*	45.52**	35.02*	74.65**	55.05*	92.15**	66.59*	134.37**
Ks57 × Masuda	-2.91	28.77*	24.4	74.86*	52.6	96.97*	33.77	129.41*
Ks57 × No.8	19.41	46.25**	20.24	82.29**	59.41*	107.35**	62.32*	197.8**
Ks57 × PI185672	26.51*	53.02**	72.98**	124.87**	92.64**	134.92**	99.1**	426.58**
Ks57 × Kaoliang	17.04	44.15**	17.67	41.84	48.35	125.86*	59.55	205.64*
Ks57 × Rox orange	18.83	56.11**	44.5	59.99*	35.92	88.28*	51.22	168.95*
Ks57 × Sugar drip	1.62	30.40*	37.1	118.89**	46.5	115.16**	29.54	147.37*
N122 × Atlas	23.43*	42.38**	24.95	63.75*	-8	37.95	2.32	71.23
N122 × Early hegari	28.21*	58.19**	68.57*	119.19**	81.08	129.61*	190.4	195.16**
N122 × Kansas collier	-5.84	36.02**	40.32	108.75**	37.6	118.96*	10.92	168.65**
N122 × M81E	24.70*	33.57**	55.88*	94.58**	57.86*	105.61**	70.30*	135.64**
N122 × Masuda	4.43	38.51**	30.02	105.24**	38.28	86.13*	30.61	135.94*
N122 × No.8	7.02	30.82**	4.58	57.20**	-1.5	15.39	-7.46	33.57
N122 × PI185672	21.66*	33.27*	75.79**	130.43**	89.57**	183.60**	94.9*	197.9**
N122 × Kaoliang	2.78	27.54*	21.74	77.22**	22.35	71.57	23.28	114.38
N122 × Rox orange	7.9	41.40**	17.81	89.87**	52.72	87.68*	58.8	155.63*
N122 × Sugar drip	-12.49	44.62**	25.06	72.95*	33.18	66.47	4.69	117.31*

\*\* , \* statistically significant at 1% and 5% levels of probability.

A1, sorghum hybrids in A1 cytoplasms background; A3, sorghum hybrids in A3 cytoplasm.

**Table 3.11.** Estimates of high-parent heterosis (HPH) of sweet sorghum hybrids for biofuel traits evaluated at Manhattan, KS during 2013, 2014, and 2015 sowing season.

Hybrids	°Brix (%)		Biomass yield (Mg ha <sup>-1</sup> )		Juice yield (Mg ha <sup>-1</sup> )		Sugar yield (Mg ha <sup>-1</sup> )	
	A1	A3	A1	A3	A1	A3	A1	A3
Tx399 × Atlas	-13.59	16.86**	-17.12	-17.29	-18.57	-5.18	-30.91	6.36*
Tx399 × Early hegari	26.56**	33.24**	39.72**	88.08**	100.42*	93.26**	23.83*	109.09**
Tx399 × Kansas collier	-21.44	-3.25*	17.88	44.60*	1.81	28.34*	-20.95	22.83*
Tx399 × M81E	-8.99*	7.85**	18.64**	21.98**	20.47**	32.38**	9.94*	44.41**
Tx399 × Masuda	-21.40	0.00*	2.80	44.91**	-11.75	20.49*	-29.90	29.99*
Tx399 × No.8	-15.87	13.08**	-7.93**	3.14**	-3.89*	5.71**	-18.43	19.63**
Tx399 × PI185672	-3.70	12.16*	38.88**	84.72**	154.28**	95.06**	21.69*	99.06**
Tx399 × Kaoliang	1.23	22.76**	1.10	52.68**	5.64	27.91	7.40	60.05*
Tx399 × Rox orange	-5.54	18.80**	-13.62	20.35*	-10.96	8.80	-12.95	33.47*
Tx399 × Sugar drip	-5.48	17.84**	4.74	38.93*	-8.11	8.90*	-13.41	28.04*
Tx623 × Atlas	-4.65	14.68**	-20.90	-9.18	-47.42	-32.37	-49.88	-25.00
Tx623 × Early hegari	22.20*	30.21**	33.61**	89.88**	137.23**	94.25**	22.41*	111.90**
Tx623 × Kansas collier	-15.66	-0.71*	19.93	62.44**	11.35	38.80*	-6.26	36.11*
Tx623 × M81E	-6.14	9.24**	9.21**	34.62**	10.46*	16.93**	3.73*	27.19**
Tx623 × Masuda	-17.61	12.84**	-4.30	8.22*	-13.56	28.44*	-28.82	45.25*
Tx623 × No.8	-8.38	9.65**	-25.72	4.47**	-18.83	11.09**	-26.19	20.34**
Tx623 × PI185672	-7.41	9.50*	35.58**	84.73**	190.20**	99.62**	19.14**	100.70**
Tx623 × Kaoliang	-2.81	18.54*	6.53*	15.96*	6.73	29.72	2.87	56.73
Tx623 × Rox orange	-0.72	25.54**	-0.33	29.18*	-0.19	25.60*	1.56	63.02*
Tx623 × Sugar drip	-8.96	21.69**	22.22*	82.48**	10.61*	62.53**	-0.24	98.78**



Hybrids	°Brix (%)		Biomass yield (Mg ha <sup>-1</sup> )		Juice yield (Mg ha <sup>-1</sup> )		Sugar yield (Mg ha <sup>-1</sup> )	
	A1	A3	A1	A3	A1	A3	A1	A3
Ks57 × Atlas	-3.71	15.41**	-3.63*	-0.40*	-1.58*	1.39*	-3.84	14.60*
Ks57 × Early hegari	24.05*	32.00**	26.97*	86.31**	90.75*	92.46*	18.36	107.00**
Ks57 × Kansas collier	-29.56	9.16**	10.78	64.19**	6.82	58.34**	-27.84	70.78**
Ks57 × M81E	-2.97*	10.06**	-12.86*	12.72**	-6.21*	16.23**	-8.32*	28.98**
Ks57 × Masuda	-23.86	0.98*	-9.99	26.52*	-0.87	27.96*	-22.36	33.15*
Ks57 × No.8	-9.59	10.73**	-26.78	11.01**	-5.85*	22.47**	-11.76*	34.95**
Ks57 × PI185672	4.51*	26.41**	25.42**	83.79**	168.59**	96.53**	19.42**	101.87**
Ks57 × Kaoliang	0.18	23.37**	-16.25	0.95	5.11	60.03*	2.04	95.48*
Ks57 × Rox orange	-1.93	28.84**	6.79	18.24*	-9.94	24.74*	-8.99	61.85*
Ks57 × Sugar drip	-18.73	4.29*	5.31	68.15**	-6.13	37.87**	-25.05	43.12*
N122 × Atlas	4.14*	20.13**	-16.37	9.60*	-43.38	-15.10	-41.19	-1.59
N122 × Early hegari	21.78*	33.00**	25.13*	83.76**	41.54	91.98*	15.61	105.69**
N122 × Kansas collier	-23.98	9.81**	8.87	61.97**	-6.84	48.24*	-33.00	62.28**
N122 × M81E	-0.32*	6.77**	-0.35*	24.39**	-4.07*	24.95**	-4.24*	32.49**
N122 × Masuda	-13.12	15.23**	-7.15	46.57**	-9.61	21.68*	-21.71	41.43*
N122 × No.8	-14.35	4.70**	-36.80	-5.00**	-41.58	-31.56	-48.74	-26.01
N122 × PI185672	7.17*	17.39*	23.20**	86.76**	118.49**	99.54**	17.89*	104.07**
N122 × Kaoliang	-5.89	16.78*	-14.44	24.55**	-12.62	22.53	-17.07	44.20
N122 × Rox orange	-5.06	24.42**	-14.12	38.41**	1.86	25.18*	-0.60	60.01*
N122 × Sugar drip	-25.61	22.95**	-5.35	30.89*	-14.15	7.31	-37.48	29.78*

\*\* , \* statistically significant at 1% and 5% levels of probability.

A1, sorghum hybrids in A1cytoplasms background; A3, sorghum hybrids in A3 cytoplasm background.

**Table 3.12.** Pearson correlation coefficients between different biofuel and agronomic traits of sweet sorghum genotypes evaluated at Manhattan, KS in 2013, 2014, and 2015 sowing season.

Traits	Cytoplasm	Correlation ( <i>r</i> )						
		Lodging	Plant height (m)	Head weight (Mg ha <sup>-1</sup> )	°Brix (%)	Biomass yield (Mg ha <sup>-1</sup> )	Juice yield (Mg ha <sup>-1</sup> )	Sugar yield (Mg ha <sup>-1</sup> )
Day to flowering	A1	-0.53*	0.73**	-0.32*	0.54*	0.85**	0.75**	0.76**
	A3	-0.42*	0.78**	0.51*	0.15*	0.81**	0.63**	0.57**
Lodging	A1	1	-0.41*	NS	-0.41*	-0.49*	-0.40*	-0.46*
	A3	1	NS	NS	NS	-0.34*	NS	NS
Plant height	A1		1	NS	0.53*	0.82**	0.70**	0.73**
	A3		1	0.42*	NS	0.77**	0.68**	0.62**
Head weight	A1			1	NS	0.52*	0.48*	0.47*
	A3			1	NS	NS	NS	NS
°Brix	A1				1	0.58**	0.53*	0.74**
	A3				1	NS	0.43*	0.65**
Biomass yield	A1					1	0.87**	0.87**
	A3					1	0.79**	0.75**
Juice yield	A1						1	0.95**
	A3						1	0.96**

\*\* , \* statistically significant at 1% and 5% levels of probability; NS, not significant

## Discussion

The central theme for improvement of sweet sorghum for industrial application (biofuel or table sugar production) is increasing stalk sugar accumulation. Two major avenues may lead to realizing this goal, exploiting high sugar alleles deployed in suitable backgrounds and altering the source-sink dynamics with the aim of reducing the grain sink. The current study is focused on estimating the extent to which stalk sugar accumulation could be improved through reducing the grain sink and evaluating its impact on other agronomic characteristics. Ten sweet sorghum cultivars were intercrossed with four seed parent lines in A1 and A3 backgrounds and the resulting 80 hybrids, 40 in A1 and 40 in A3 CMS systems tested across environments.

The analysis of variance indicated that the entry effect and its components are significant for all of the parameters measured (Table 3.1) and this is expected given the variability in genetic backgrounds of the entries. Like most other economically important traits, biofuel parameters collected in this study appear to be largely controlled by additive genes as shown by higher and more significant male and female GCA effects compared to male  $\times$  female interaction (SCA) effects that represent dominance (Table 3.1). Comparison of the hybrid and inbred components of the entry factor shows much larger mean square values, indicating the traits were markedly different in hybrid vs. inbred backgrounds with hybrids always having the larger value for the traits.

A further breakdown of the hybrid effect in to A1 and A3 CMS systems shows that both A1 and A3 hybrids were significantly different for all traits studied (Table 3.2). Comparison of the A1 and A3 systems on the other hand was even more significant (Table 3.2), indicating that elimination of the grain sink may have positive contribution to increasing biofuel production. The results show similar a trend for agronomic traits (Tables 3.3 and 3.4) except the inbred vs. hybrid

effect was not significant for most agronomic traits (Table 3.3), and the A1 vs. A3 hybrid effect was not significant except for head weight (Table 3.4). These results agree with common observations in that A3 hybrids do not produce grain. A3 hybrids were also less prone to lodging due to a sturdy stalk structure built from excess photo assimilates remaining in the stalk and low or no torque developed from the head due to low or no grain formation.

Nevertheless, due to variable genetic background of the test entries, there is significant variation between the test entries, and a breakdown of these into male and female components show that much of the variability comes from the males (Table 3.5). While entries vary for both agronomic and biofuel traits, the variation between grain forming (A1) and non-grain forming (A3) hybrids for biofuel traits was remarkably significant. The A1 and A3 hybrid pairs in this study are of similar genetic background except the A3 CMS system lacks strong restorer genes and hence most of its hybrids are sterile. The difference between A1 and A3 hybrid pairs are thus simply the effect of the grain sink and are genetically similar otherwise.

Across entries mean °Brix percent was 26% higher in A3 hybrids compared to A1 hybrids with the extent of the difference varying between different A1/A3 pairs. Similarly, biomass accumulation, juice yield, and sugar yield in A3 hybrids were 29%, 25%, and 53% greater than in the A1 hybrids (Table 3.6). The greater biomass of the A3 hybrids may be the result of the visibly thicker stalk girth and the extensive nodal tillers that occurred later in the season. There was no evidence of difference in photosynthetic capacity between A1 and A3 hybrids pairs. The wide difference in biomass yield appears to be the result of the excess photoassimilate that was normally destined for grain filling remaining in the stalk and contributing to stalk sturdiness and stimulating further lateral growth as evidenced from the appearance of nodal tillers in the A3 hybrids (Figure 3.1 A). Although A1 hybrids translocated much of post anthesis photoassimilates to the grain, the

contribution of that to total biomass was not as high as that of A3 hybrids, perhaps because formation of grain biomass (various types of starch, proteins, lipids, etc.) requires more energy than building cellulosic biomass (Fisher and Wilson, 1976; Broadhead and Freeman, 1980; Slewinski, 2012).

In addition to CMS effect, the combining ability of the lines involved in the study were evaluated to determine their potential use as breeding parents for biofuel sorghum improvement. General combining ability (GCA) was not of much interest in the females (Table 3.7 and 3.8). However, the pollinator lines exhibited significant GCA effect in both A1 and A3 backgrounds with the results being consistent across the CMS backgrounds. Accordingly, the sweet sorghum line M81E exhibited the greatest positive GCA for all biofuel traits studied followed by No.8, a durra sorghum from east Africa (Table 3.7). Other lines such as PI85672 did not have favorable GCA for °Brix but was among the superior parents for biomass production, juice yield, and sugar yield, indicating that these traits can be independently manipulated in breeding programs. These lines should be of positive value as breeding parents. The most negative GCA for the traits was recorded in the Chinese cold tolerant source Kaoliang. GCA for agronomic traits were markedly different among the males with Sugar drip having the most negative GCA of -10.78 (early flowering), PI85672 producing the latest hybrid followed by No.8 (Table 3.8). M81E, No.8, and PI85672 had the greatest positive GCA for plant height as well as for biomass.

The specific combining ability (SCA) for the crosses was significant (Tables 3.1 and 3.3). For °Brix percent, the highest SCA was recorded for Ks57 x PI85672 in A3 background followed by N122 x Masuda. The SCA for °Brix tends to be greater in A3 CMS than A1 except for few crosses such as Tx623 x Kansas collier, Tx399 x Sugar drip, and Ks57 x Sugar drip. On the other hand, the greatest SCA for biomass 3.63 was recorded for Tx399 x kaoliang followed by 2.45 for

N122 x P1185672, both in the A3 system. For juice and sugar yield, Tx623 x Sugar drip had the greatest SCA followed by Ks57 x No.8 in the A3 CMS background (Table 3.9). Although SCA is an important consideration for choosing specific hybrid combination, it is not useful for choosing breeding parent as it is contributed by dominance effect, and dominance gene action is not heritable (Kenga et al., 2004; Mutengwa et al., 1999; Sharma, 1994).

As observed on the inbred vs. hybrid component of the entry in Tables 3.1 and 3.2, heterosis had a significant effect on expression of these traits. Table 3.10 shows estimates of mid-parent heterosis for each of the hybrids. For °Brix there was positive heterosis for most hybrid combinations in A1 CMS background except five hybrids where heterosis was negative but not significant. Mid-parent heterosis for °Brix in A3 CMS system was positive and significant in all cross combinations with the largest heterosis values approaching 60% and the least 23%. Heterosis for biomass, juice yield, and sugar yield were positive except for Tx623 x Atlas and N122 x No.8 where both juice yield, and sugar yield and for N122 x Atlas for juice yield in A1 CMS was negative. High parent heterosis may provide better information on the performance of the hybrids. For °Brix, only seven hybrids had significant and positive high parent heterosis in A1 CMS and only two had negative high parent heterosis in A3 CMS. The trend is similar for other traits with several hybrids in A3 CMS having positive high parent heterosis compared to the A1 CMS. The results indicate that eliminating the grain sink using A3 or other sterility system from a potential sweet sorghum crop may significantly increase the value of the feedstock for biofuel or sugar production. Apart from increasing sugar yield and component traits, low or no grain sink contributes to agronomic adaptation with the most significant adaptive trait needed in such crop being resistance to lodging. The sterile sweet sorghum hybrids counter lodging either by increasing

stalk girth and improving mechanical strength of the stalk or through reducing the torque that could be imposed as a result of the grain sink on the head.

## Summary

The analysis of variance revealed that genotypes exhibited highly significant differences among themselves for all the traits studied. Inbreds exhibited significant differences for all the traits studied, indicating greater diversity in the parental lines. Highly significant mean squares due to inbred vs. hybrids showed presence of heterosis for all the traits. When the effects of crosses were partitioned into female, male, and female  $\times$  male interaction effects, the effects of females were found to be significant for all the traits except for days to flowering, whereas the effects of males were significant for all the traits studied. These results indicated diversity among the female and male lines for all the traits. The female  $\times$  male interaction effect was found to be significant for all the traits studied, indicating that A1 and A3 hybrids differed significantly for their SCA effects for most of the traits. Thus, despite similarity of the lines with respect to their GCA effects, significant SCA effects of the hybrids is attributable to non-predictable dominance and dominance-based epistasis in the inheritance of most of the traits in sweet sorghum. These results indicated that, irrespective of cytoplasm, the lines have comparable ability to transmit genes with additive effects to their progenies. Significant influence of cytoplasm on MPH and HPH were detected for all traits except for days to flowering and lodging in most of the A1 and A3 hybrids. High level of heterosis were noticed for biofuel traits ( $^{\circ}$ Brix, biomass yield, juice yield, and sugar yield) followed by head weight. In general, A3 hybrids exhibited greater overall MPH and HPH compared with A1 hybrids in all traits except for head weight.

## Conclusion

The study revealed the presence of significant genetic variability for biomass production, brix, juice yield, and sugar concentration among sweet sorghum germplasm. These resources can be effectively exploited for further enhancing sugar yield and increase feedstock available for table sugar or clean energy production.

The current and previous studies indicate that juice yield, brix, and sugar concentration tend to be inherited from one parent implying that these traits are under the control of a few major genes. These phenomena make the sweet sorghum improvement effort with regard to increased brix, juice, and sugar a relatively easy undertaking, which saves time and money. Moreover, the fact that dominant genes play major roles in affecting these traits allows for only one of the parents to have these traits, which again makes the breeding less complicated. The current result also confirms that the grain sink places significant pressure on stalk performance by competing for assimilates. Although the grain is also of value, it is not a desirable trait in dedicated sugar-based biofuel feedstocks.

The implication of the effect of the grain sink on biofuel traits opens a new research venue for developing non-grain forming hybrids. The A3 CMS system used in the current study effectively demonstrated that a genetic system can be deployed to prevent grain production in sugar based feedstocks and as a result increase all components of sugar. In addition to sugar, elimination of the grain sink promoted lateral tillering and stalk girth thereby significantly contribution to cellulosic biomass, another dedicated feedstock source. The increase in stalk strength in non-grain forming hybrids and the absence of grain torque in the panicle significantly contributed to lodging resistance, an important trait in high biomass production system.



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## **Chapter 4 - Relationship between the grain sink and stalk rot disease response in sweet sorghum [*Sorghum bicolor* (L.) Moench]**

### **Abstract**

Sorghum [*Sorghum bicolor* (L.) Moench] grain yield is severely affected by abiotic and biotic stresses during post-flowering stages. Stalk rot caused by *Macrophomina phaseolina* is one of the economically important biotic stresses in major sorghum-growing areas across the world. The objective of this study was to study the effects of the cytoplasmic male sterility-mediated differential accumulation of stalk sugar on severity of charcoal rot disease caused by *M. phaseolina*. Ten sweet sorghum pollinator lines of variable sugar content were selected from the entries included in an experiment examining sorghum as biofuel feedstock for juice yield, sugar content and lignocellulosic biomass. The lines were crossed to four A1 and A3 cytoplasmic male sterile (CMS) lines using a Design II mating scheme. The A3 females do not have effective restorers so the hybrids are expected to be sterile. Before anthesis, six plants at uniform panicle developmental stage from A1 hybrid plots and nine plants from A3 hybrid plots were tagged using two distinct plastic tapes. Six of the tagged plants in A3 hybrid plots were covered with pollination bags to prevent seed set from random pollen and the remaining three were left uncovered. All of the tagged plants were used for pathogen and control inoculation to measure stalk rot disease development. The results of this study indicated that hybrids produced from A3 cytoplasm were more resistant to charcoal rot (7.1 cm lesion length) compared to those produced from the A1 hybrids (9.5 cm lesion length). Therefore, removing the grain sink, which resulted in higher stalk sugar content, may have delayed senescence, and thus the growth and spread of fungus in the stalk was impeded.

Key words: *Sorghum bicolor*, abiotic, *Macrophomina phaseolina*, grain sink, charcoal rot.

## Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is an important cereal crop worldwide serving as source of food and feed (Liu et al., 2009; Miller et al., 1980; Woods, 2001). In developing countries, the crop has wider application where stalks are used as fodder and as building materials. Sweet sorghum is a special type of sorghum that accumulates large amounts of fermentable sugars (10 – 20%) in its stalks, similar to its close relative, sugarcane (*Saccharum spp.*). The sugary juice of sweet sorghum can be extracted and directly fermented into ethanol. The fact that it is established from seeds, unlike sugarcane, and hence can be produced anywhere grain sorghum is produced (Keeney and DeLuca, 1992; Smith et al., 1987), makes it a versatile feedstock source that can be produced anywhere it is needed. Like other sorghums, sweet sorghums are tolerant to drought, water-logged conditions, and saline/alkali soils (Reddy and Reddy, 2003; Ali et al., 2008). They are cultivated in a wide range of environments in Africa, China, USA, India, Mexico, etc., and are well adapted between 40N and 40S latitudes (Dogget, 1988). The crop can be grown and utilized for food, biofuel, fodder, and fiber (Woods, 2001) and is one of the most efficient dryland crops to convert atmospheric CO<sub>2</sub> into sugar (Schaffert and Gourley, 1982).

Although sorghum has a wide range of adaptability and can be grown in diverse ecologies including areas prone to severe abiotic stresses, high temperature, drought, salinity, and flooding (Ejeta and Knoll, 2007), the crop tends to suffer from some pre- and post-flowering drought (Tuinstra et al., 1997; Kebede et al., 2001; Blum, 2004) because it is almost exclusively grown under these conditions. Post-flowering drought stress is associated with stalk rot diseases, which lead to significant lodging and yield loss (Rosenow and Clark, 1995; Tesso et al., 2004). Stalk rot

diseases are characterized by the degradation of pith tissue at or near the base of the stalk, which weakens stem strength and may result in lodging. Infected plants often have damaged vascular and cortical tissues in both the root and stalks that may reduce nutrient and water absorption and translocation, and perhaps lead to breakage of the stalk at the zone of infection causing lodging (Hundekar and Anahosur, 1994). One of the predominant stalk rot diseases is charcoal rot. Charcoal rot is caused by *Macrophomina phaseolina* (Tassi) Goid. and is a serious problem under high soil temperature and low moisture, particularly during the grain filling stage (Hassan et al., 1996). Charcoal rot pathogens can be found in host tissues at various growth stages, but disease symptoms are visible only after flowering and during the grain filling stage (Reed et al., 1983; Khune et al., 1984). Under severe conditions, the disease may lead to complete stalk collapse and crop lodging (Mughogho and Pande, 1984).

The stalk rot problem seems to have become more serious following the introduction of hybrid system that significantly increased grain yield. Stalk rot pathogens often are considered weak, since they infect and take over hosts that are either in environmental stress such as drought or are undergoing physiological stress such as senescence (Tuinstra et al., 1997). The massive movement of assimilates from the stalk to the grain imposes physiological stress on the stalk and this becomes more severe in high yielding cultivars that translocate a greater proportion of assimilates to fill grain.

With the emergence of sorghum as biofuel feedstock requiring high-biomass cultivars, the problems associated with stalk lodging, whether physiological or stalk rot induced, become a primary concern. Increasing feedstock production requires one to grow taller cultivars that tend to have high propensity for lodging. The main theme of this work was to explore the potential of non-grain forming hybrids for sugar production. The fact that such hybrids possibly may be less prone

to lodging due to reduced weight imposed by the grain or less physiological stress that would otherwise occur due to massive movement of assimilates to the grain is a plus for enhancing standability. Another area of interest was to explore the effect of other factors responsible for lodging. Therefore, the main objective of this study was to investigate the reaction of grain-forming and non-grain-forming hybrids to infection by *M. phaseolina*. This pathogen is the causal agent of the most aggressive type of stalk rot, charcoal rot, which is the major cause of lodging in dryland sorghum production in the United States (Bramel-Cox et al., 1988; Leslie et al., 1990; Marasas et al., 2001).

## **Material and methods**

### **Genetic materials**

The experimental material was comprised of 4 female parental lines, Tx399, Tx623, Ks57, and N122, each in two cytoplasmic male sterility systems (CMS), A1 and A3. Each female was crossed to each of the 10 R-lines (Atlas, Early hegari, Kansas collier, M81E, Masuda, No.8, PI185672, Kaoliang, Rox orange, and Sugar drip) using a Design II mating scheme to produce 40 hybrid combinations each for A1 and A3 CMS systems. All hybrid seed production was conducted at the Kansas State University sorghum breeding nursery near Manhattan Kansas and using a winter nursery in Puerto Rico during 2012 and 2013. All of the female parents were developed as B-line selections and converted to A1 CMS (Andrews et al., 1990; Miller et al., 1999). The lines were later converted to A3 CMS by Pedersen et al. (1997) such that the lines are available in both A1 and A3 CMS systems. The hybrids along with their respective B-lines and the 10 R-lines were evaluated at the Kansas State University research farm near Manhattan, Kansas during the 2013, 2014, and 2015 swing seasons.



## **Experimental design and producers**

The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications. The plots consisted of five-meter long single row spaced 0.75 m apart with 0.6 m alley at the end of each plot. The experiment was established by directly seeding 3 g treated (Maxim 4FS, Apron XL, Concept III, and colorant) seeds of the genotypes using a cone planter. Fertilizer nitrogen (urea) and phosphorous (di-ammonium phosphate, DAP) were applied at the rates of 90 kg ha<sup>-1</sup> and 40 kg ha<sup>-1</sup>, respectively. Pre-emergence weeds were controlled with 0.55 kg ha<sup>-1</sup> Atrazine™, 0.76 kg ha<sup>-1</sup> Dual II Mg™, and, 0.16 kg ha<sup>-1</sup> Callisto™. Post-emergence weeds were controlled by hand weeding, and this practice was used to keep the field weed-free throughout the seasons. The experiment was conducted in three environments during the 2013 and 2014 seasons at the Ashland bottoms Agronomy Research Farm and at the north campus agronomy farm in Manhattan, KS in 2015.

Before anthesis, six plants at uniform panicle development stage from A1 hybrid plots and nine plants from A3 hybrid plots were tagged using two distinct plastic tapes in A1 plots and three in A3 plots. Six of the tagged plants in A3 hybrid plots were covered with a pollination bags to prevent seed set from random pollen while the remaining three left open headed. All of the tagged plants were used for pathogen and control inoculation for measuring stalk rot disease development.

## **Inoculum preparation and inoculation**

Fresh culture of *Macrophomina phaseolina* was initiated in potato dextrose agar (PDA) from pure cultures of the pathogen obtained from the laboratory of Dr. Christopher Little at Kansas State University. The choice of pathogen was based on previous reports that this pathogen is one of the

most virulent organisms causing stalk rot diseases in sorghum (Bramel-Cox et al., 1988; Leslie et al., 1990).

The inoculum was initiated by sub-culturing small section (2-3 mm) of the pathogen mat into several fresh potato dextrose agar 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. At 14 days after flowering, three tagged plants from the bagged A3 plots and three from A1 plots were inoculated with a sterile toothpick as the control, and the remaining tagged plants in both A1 and A3 plots were inoculated with *M. phaseolina* pathogen by inserting the infested toothpick into small holes made on the stalk of the plants using a sterile needle. Inoculations were made on the basal stalk about 10 cm above the soil surface.

### **Data collection**

Twenty-eight days after inoculation, entire stalks of the inoculated plants are harvested and scored for disease severity. The scoring was done by longitudinally splitting the stalks and measuring the length of the visible necrotic lesion and counting the number of nodes contained within the lesioned region.

### **Statistical analysis**

The analysis of variance (ANOVA) was performed using the a PROC MIXED procedure in SAS (version 9.4) as appropriate for the design. The environment and replicates were treated as random effect parameters. The hybrid effect was further partitioned into male, female and male × female interaction effects representing general combining ability (GCA) for male and female parents as well as the specific combining ability (SCA) effect. Design II fixed model (Model I) of Hallauer

and Miranda (1988) was used to obtain independent estimates of the GCA and SCA. The GCA for each parental line was calculated as the difference between the grand mean of the hybrids and the marginal means for each male and female parent.

Mean of significant were separated using the LSD method. Pearson correlation coefficients were estimated from combined environments data to determine the degree of correlation between all traits using PROC CORR procedure in SAS (version 9.4).

## **Results**

### **Analysis of variance**

The combined analysis of variance for A1 and A3 hybrids response to infection by *M. phaseolina* is presented in Table 4.1 and 4.2. The ANOVA revealed that entry effect was highly significant ( $P \leq 0.01$ ) for both lesion length and nodes crossed. Partitioning the entry effect into inbred and hybrids also showed both components to be highly significant for both parameters, indicating the inherent variation for stalk rot disease response among the parental lines (Tables 4.1 and 4.2). Further partitioning of the hybrid effect into male and female components showed that both parents contributed to the disease response variation observed among the hybrids, but the contribution by male parents appear to be greater, indicating more diversity for the trait among the males (Tables 4.1 and 4.2). The male and female effects, also referred to as the GCA for males, and GCA for females was highly significant ( $P \leq 0.01$ ) for male parents and significant ( $P \leq 0.05$ ) for female parents for both traits. The female  $\times$  male interaction effect referred to as specific combining ability (SCA) effect was highly significant for both traits in both A1 and A3 hybrids (Tables 4.1 and 4.2).

The interaction between entry and environment was not significant for either disease parameter in A1 hybrids but was significant ( $P \leq 0.05$ ) for nodes crossed among A3 hybrids. The

inbred by environment interaction was not significant for either trait in both A1 and A3 hybrids (Table 4.1 and 4.2). The male and female  $\times$  environment interaction effects were significant for lesion length in A1 hybrids, and both interactions were significant for both disease parameters in A3 hybrids. Likewise, the male  $\times$  female  $\times$  environment interaction was significant for lesion length in A1 hybrids and significant for both lesion length and nodes crossed in A3 hybrids (Table 4.1 and 4.2).

Comparison of the performance of the inbred parents for disease response showed that Tx623, a tropically adapted seed parent, expressed greater susceptibility to the disease among the female parents (Table 4.3). It had mean lesion length of 7.19 cm, significantly greater than all females parents and 59% greater than the overall mean of 4.58 cm for females. Female N122 had the shortest lesion length. The score for nodes crossed 2.56 nodes per plant for Tx623 was significantly greater than other females but was comparable to Tx399. Other females had close to the mean disease score for both lesion length and nodes crossed. Among the male parents, line M81E had the shortest lesion length, indicating the strongest resistance to charcoal rot, followed by No.8. The longest lesion length among males of 15.68 cm was recorded for Early hegari, which was significantly greater than all entries followed by Masuda, PI185672, and Sugar drip, which had almost similar lesion length scores. Again, the score for nodes crossed in these lines was fairly proportional to lesion length, with Early hegari having a score of 2.00 nodes per plants and the others close to 1 node per plant, with Sugar drip having 1.55 nodes crossed. The reaction of other males was intermediate. Overall, the male lines had greater mean lesion length (8.88 cm) compared to the mean values for the females (Table 4.3). This could be largely the effect of differences in plant height rather than difference in disease response per se.

### **The effect of the grain sink on stalk rot disease response of hybrids**

The analysis of variance comparing the A1 and A3 CMS hybrids revealed that disease parameters were highly affected by the grain sink. Although there is significant difference among A1 hybrids as well as within A3 hybrids for these traits, the greater A1 vs. A3 hybrid effect on lesion length was remarkable (Table 4.4). Nodes crossed for this effect was not significant despite greater mean square value. This was due to the large A1 vs. A3 by environment interaction effect, which was used to test the effect of A1 vs. A3 effect.

Comparison of the mean lesion length for A1 and A3 hybrids explain results from the ANOVA that the across-hybrid mean lesion length in A1 hybrids was 9.55 cm compared to 7.10 cm in A3 hybrids, the difference that was statistically significant. Although not statistically significant, mean nodes crossed was also greater in A1 hybrids compared to A3 (Table 4.5). Similarly mean lesion length for control inoculations was greater in A1 hybrids. Mean lesion length from pathogen-inoculated, unbagged A3 hybrids (9.65), however, was comparable with that of A1 hybrids, showing that the seed sink was the primary factor responsible for differences in stalk rot response between A1 and A3 hybrids (Table 4.5). The strongest resistance reaction was observed in hybrids of No.8 and M81E (Table 4.5). All hybrids from these males exhibited the shortest mean lesion length except in crosses with Tx399 and KS57 in both A1 and A3 CMS. The most susceptible hybrids, however, was that of Masuda and Kansas collier, especially in crosses with Tx399 and N122. Other crosses of these lines had intermediate reaction.

As shown in the ANOVA, the GCA effect for both male and female parents was significant in both A1 and A3 hybrids (Tables 4.1 and 4.2). Estimation of the GCA effect for each of the lines showed Tx623 as having the highest negative GCA among females for resistance to the disease in both A1 and A3 CMS (Table 4.6). Its GCA for lesion length was -0.89 cm and -0.78 cm and nodes

crossed were -0.25 and -0.17 in both A1 and A3 CMS backgrounds, respectively, all of which were statistically significant. This line, however, exhibited the greatest mean lesion length when evaluated as an inbred per se. Ks57 on the other hand, expressed the highest GCA for susceptibility in both traits regardless of the CMS backgrounds (Table 4.6).

Among the males, M81E and No.8 had the greatest, significant GCA for resistance both for lesion length and nodes crossed in both A1 and A3 CMS systems (Table 4.6). Though not significant in A1, PI185672 had significant GCA for resistance in A3 background for lesion length and in both A1 and A3 backgrounds for nodes crossed. The lines, Kansas collier and Sugar drip, had the greatest, and significant GCA for susceptibility in terms of both lesion length and nodes crossed in both A1 and A3 backgrounds. Kaoliang expressed significant GCA for susceptibility for lesion length and Rox orange for nodes crossed in both A1 and A3 CMS systems.

### **Genotypic correlations**

Correlations of disease response with agronomic traits are presented in Table 4.7. Highly negative significant correlations were shown between days to flowering with lesion length and number of nodes crossed at both A1 and A3 hybrids. Lodging score was significantly and positively correlated with number of nodes crossed at both A1 and A3 hybrids, but it was not significantly correlated with lesion length in A3 hybrids. Moreover, there was a significant negative correlation between °Brix with lesion length in both cytoplasms but, it was not significantly correlated with nodes crossed in A3 hybrids. Highly significant and positive correlations were observed between lesion length and number of nodes crossed in both A1 and A3 hybrids (Table 4.7).

**Table 4.1.** Combined analysis of variance of sweet sorghum A1 hybrids for reaction to severity of stalk rot infection caused by *Macrophomina phaseolina* evaluated at Manhattan, KS during 2013, 2014, and 2015 sowing season.

Source of variation	df	Mean squares	
		Lesion length (cm)	Nodes crossed (no. plant <sup>-1</sup> )
Environment (E)	2	0.52	0.23
Replication / E	6	7.39	1.26*
Entry	53	49.65**	3.60**
Inbred	13	104.24**	5.84**
Hybrid	39	24.44**	2.48**
Male	9	53.05**	6.46**
Female	7	57.48*	3.79*
Male x Female	27	11.24**	1.01**
Inbred vs. Hybrid	1	323.22	18.46*
Entry × E	106	3.49	0.32
Inbred × E	26	1.71	0.18
Hybrid × E	78	3.39*	0.36
Male × E	18	4.53*	0.29
Female × E	6	3.27	0.63
Male × Female × E	54	2.87*	0.35
Inbred vs. Hybrid × E	2	3.03	0.32
Error	318	4.33	0.334

\*\*,\* statistically significant at 1% and 5% levels of probability

**Table 4.2.** Combined analysis of variance of sweet sorghum A1 hybrids for reaction to severity of stalk rot infection caused by *Macrophomina phaseolina* evaluated at Manhattan, KS during 2013, 2014, and 2015 sowing season.

Source of variation	df	Mean squares	
		Lesion length (cm)	Nodes crossed (no. plant-1)
Environment (E)	2	1.19	1.80
Replication / E	6	2.12	1.23*
Entry	53	40.69**	3.52**
Inbred	13	105.98**	5.22**
Hybrid	39	19.23**	2.24**
Male	9	44.08*	5.02**
Female	3	48.84*	5.83*
Male × Female	27	7.65**	0.92**
Inbred vs. Hybrid	1	29.19	31.37*
Entry × E	106	4.54	0.43*
Inbred × E	26	1.27	0.20
Hybrid × E	78	4.73**	0.48*
Male × E	18	6.08**	0.56*
Female × E	6	15.23**	0.28
Male × Female × E	54	3.11*	0.47*
Inbred vs. Hybrid × E	2	39.77*	1.95*
Error	318	3.78	0.337

\*\* , \* statistically significant at 1% and 5% levels of probability.



**Table 4.3.** Combined mean performance of sweet sorghum parental lines tested against the infection caused by *Macrophomina phaseolina* evaluated at Manhattan, KS during 2013, 2014, and 2015 sowing season.

Entries	Lesion length (cm)	Nodes crossed (no. plant-1)	Control (cm)
Females			
Tx399	4.22 <sup>b</sup>	2.55 <sup>ab</sup>	1.44 <sup>b</sup>
Tx623	7.19 <sup>a</sup>	2.56 <sup>a</sup>	1.92 <sup>a</sup>
Ks57	4.21 <sup>bc</sup>	2.00 <sup>b</sup>	1.20 <sup>b</sup>
N122	2.71 <sup>c</sup>	2.01 <sup>b</sup>	1.14 <sup>b</sup>
Mean ( <i>females</i> )	4.58	2.29	1.42
Males			
Atlas	7.75 <sup>bc</sup>	1.11 <sup>bc</sup>	2.41 <sup>a</sup>
Early hegari	15.68 <sup>a</sup>	2.00 <sup>a</sup>	2.49 <sup>a</sup>
Kansas collier	8.68 <sup>bc</sup>	1.01 <sup>c</sup>	2.33 <sup>a</sup>
M81E	3.41 <sup>d</sup>	0.33 <sup>d</sup>	1.27 <sup>b</sup>
Masuda	8.31 <sup>bc</sup>	0.88 <sup>c</sup>	2.34 <sup>a</sup>
No.8	6.87 <sup>cd</sup>	0.22 <sup>d</sup>	2.31 <sup>a</sup>
PI185672	10.48 <sup>b</sup>	0.88 <sup>c</sup>	2.14 <sup>a</sup>
Kaoliang	10.20 <sup>bc</sup>	0.82 <sup>c</sup>	2.46 <sup>a</sup>
Rox orange	7.46 <sup>b</sup>	0.86 <sup>c</sup>	1.56 <sup>b</sup>
Sugar drip	10.05 <sup>bc</sup>	1.55 <sup>ab</sup>	2.31 <sup>a</sup>
Mean ( <i>males</i> )	8.88	0.96	2.16

Means within the same column with different letter are significantly different at  $P < 0.05$ .

**Table 4.4.** Combined analysis of variance of iso-nuclear sorghum hybrids tested against infection caused by *Macrophomina phaseolina* evaluated at Manhattan, KS during 2013, 2014, and 2015 sowing season.

Source of variation	df	Mean squares	
		Lesion length (cm)	Nodes crossed (no. plant-1)
Hybrid	79	35.21**	2.39**
A1 hybrids	39	24.44**	2.48**
A3 hybrids	39	19.23**	2.24**
A1 vs. A3 hybrids	1	1078.55*	5.01
Hybrid × E	158	4.01**	0.44*
A1 hybrids × E	78	3.39*	0.36
A3 hybrids × E	78	4.73**	0.48*
A1 vs. A3 hybrids × E	2	0.109	2.12*
Error	474	2.07	0.31

\*\* , \* statistically significant at 1% and 5% levels of probability.

**Table 4.5.** Combined mean performance of sweet sorghum A1 and A3 hybrids tested against infection caused by *Macrophomina phaseolina* evaluated at Manhattan, KS during 2013, 2014, and 2015 sowing season.

Hybrids	Lesion length (cm)					Nodes crossed (no. plant-1)			Control (cm)		
	A1	A3	A3 ‡	°Diff.	°Diff.	A1	A3	Diff.	A1	A3	Diff.
Tx399 × Atlas	9.8 <sup>e-k</sup>	8.3 <sup>c-f</sup>	10.5 <sup>d-f</sup>	NS	NS	0.9 <sup>e-i</sup>	0.8 <sup>e-i</sup>	NS	2.3 <sup>d-k</sup>	2.6 <sup>ba</sup>	NS
Tx399 × Early hegari	7.2 <sup>np0</sup>	6.3 <sup>mnl</sup>	8.1 <sup>k-o</sup>	NS	NS	0.7 <sup>g-j</sup>	0.2 <sup>klj</sup>	NS	2.3 <sup>d-k</sup>	2.3 <sup>a-h</sup>	NS
Tx399 × Kansas collier	12.3 <sup>ba</sup>	8.0 <sup>c-h</sup>	11.1 <sup>bcd</sup>	**	NS	2.1 <sup>a</sup>	1.1 <sup>b-f</sup>	NS	2.9 <sup>b-e</sup>	2.3 <sup>a-g</sup>	NS
Tx399 × M81E	6.5 <sup>qp0</sup>	5.0 <sup>pq0</sup>	8.1 <sup>k-o</sup>	NS	NS	0.1 <sup>kl</sup>	0.0 <sup>l</sup>	NS	2.1 <sup>h-k</sup>	1.9 <sup>d-j</sup>	NS
Tx399 × Masuda	12.7 <sup>a</sup>	10.4 <sup>a</sup>	13.3 <sup>a</sup>	*	NS	1.6 <sup>bc</sup>	2.0 <sup>a</sup>	NS	2.7 <sup>b-h</sup>	2.4 <sup>a-d</sup>	NS
Tx399 × No.8	9.5 <sup>g-l</sup>	6.7 <sup>j-m</sup>	8.2 <sup>j-n</sup>	*	NS	0.4 <sup>i-l</sup>	0.1 <sup>kl</sup>	NS	2.3 <sup>e-k</sup>	1.8 <sup>ihj</sup>	NS
Tx399 × PII85672	7.8 <sup>nmo</sup>	4.9 <sup>pq0</sup>	9.4 <sup>f-k</sup>	*	NS	0.4 <sup>i-l</sup>	0.2 <sup>klj</sup>	NS	2.5 <sup>c-j</sup>	2.4 <sup>a-g</sup>	NS
Tx399 × Kaoliang	10.0 <sup>d-k</sup>	9.1 <sup>bc</sup>	10.0 <sup>d-h</sup>	NS	NS	0.8 <sup>f-i</sup>	0.9 <sup>d-h</sup>	NS	2.3 <sup>d-k</sup>	2.4 <sup>a-d</sup>	NS
Tx399 × Rox orange	10.6 <sup>c-j</sup>	8.5 <sup>ecd</sup>	10.1 <sup>d-h</sup>	NS	NS	1.3 <sup>ecd</sup>	0.6 <sup>g-k</sup>	*	2.8 <sup>b-g</sup>	2.2 <sup>a-i</sup>	NS
Tx399 × Sugar drip	10.6 <sup>c-j</sup>	9.9 <sup>ba</sup>	12.0 <sup>ba</sup>	NS	NS	1.9 <sup>ba</sup>	1.2 <sup>b-e</sup>	NS	3.1 <sup>ba</sup>	2.2 <sup>a-i</sup>	*
Tx623 × Atlas	7.3 <sup>np0</sup>	5.1 <sup>pq0</sup>	7.7 <sup>m-p</sup>	NS	NS	0.9 <sup>e-i</sup>	0.7 <sup>f-j</sup>	NS	2.2 <sup>f-k</sup>	1.8 <sup>ihj</sup>	NS
Tx623 × Early hegari	8.4 <sup>nml</sup>	5.0 <sup>pq0</sup>	8.0 <sup>l-o</sup>	*	NS	0.4 <sup>i-l</sup>	0.4 <sup>h-l</sup>	NS	2.2 <sup>g-k</sup>	1.6 <sup>j</sup>	NS
Tx623 × Kansas collier	9.5 <sup>h-l</sup>	7.4 <sup>e-l</sup>	10.8 <sup>d-f</sup>	NS	NS	0.8 <sup>f-i</sup>	0.7 <sup>f-j</sup>	NS	2.8 <sup>b-f</sup>	1.8 <sup>f-j</sup>	*
Tx623 × M81E	6.3 <sup>qp</sup>	4.1 <sup>q</sup>	6.7 <sup>op</sup>	NS	NS	0.0 <sup>l</sup>	0.0 <sup>l</sup>	NS	1.4 <sup>l</sup>	1.8 <sup>g-j</sup>	NS
Tx623 × Masuda	9.2 <sup>m-l</sup>	6.8 <sup>i-m</sup>	8.8 <sup>g-m</sup>	*	NS	0.7 <sup>g-j</sup>	0.7 <sup>f-j</sup>	NS	2.1 <sup>kji</sup>	1.7 <sup>j</sup>	NS
Tx623 × No.8	5.6 <sup>q</sup>	4.4 <sup>pq</sup>	6.4 <sup>p</sup>	NS	NS	0.0 <sup>l</sup>	0.0 <sup>l</sup>	NS	2.0 <sup>kjl</sup>	1.8 <sup>h-j</sup>	NS
Tx623 × PII85672	10.4 <sup>c-k</sup>	6.9 <sup>h-m</sup>	9.9 <sup>d-h</sup>	*	NS	0.8 <sup>f-i</sup>	0.3 <sup>i-l</sup>	NS	2.5 <sup>b-j</sup>	2.2 <sup>a-i</sup>	NS
Tx623 × Kaoliang	9.7 <sup>e-l</sup>	7.9 <sup>c-h</sup>	10.0 <sup>d-h</sup>	NS	NS	0.9 <sup>e-i</sup>	0.9 <sup>d-h</sup>	NS	2.2 <sup>g-k</sup>	2.3 <sup>a-h</sup>	*
Tx623 × Rox orange	9.9 <sup>d-k</sup>	8.0 <sup>c-h</sup>	9.8 <sup>d-i</sup>	NS	NS	0.9 <sup>e-i</sup>	1.1 <sup>b-f</sup>	NS	2.3 <sup>d-k</sup>	2.2 <sup>a-i</sup>	NS
Tx623 × Sugar drip	10.4 <sup>c-k</sup>	7.7 <sup>d-k</sup>	10.3 <sup>d-g</sup>	*	NS	1.6 <sup>bc</sup>	1.2 <sup>b-e</sup>	NS	2.6 <sup>b-j</sup>	2.3 <sup>a-h</sup>	NS
Ks57 × Atlas	11.1 <sup>b-e</sup>	7.1 <sup>f-l</sup>	9.6 <sup>d-j</sup>	**	NS	0.9 <sup>e-i</sup>	0.6 <sup>g-k</sup>	NS	2.9 <sup>b-e</sup>	2.5 <sup>bac</sup>	NS
Ks57 × Early hegari	10.3 <sup>c-k</sup>	7.6 <sup>d-k</sup>	10.1 <sup>d-h</sup>	*	NS	1.9 <sup>ba</sup>	1.6 <sup>ba</sup>	NS	3.1 <sup>ba</sup>	2.1 <sup>b-j</sup>	*
Ks57 × Kansas collier	11.5 <sup>bac</sup>	8.7 <sup>bcd</sup>	11.0 <sup>b-d</sup>	*	NS	1.6 <sup>bc</sup>	1.3 <sup>bcd</sup>	NS	3.6 <sup>a</sup>	2.6 <sup>ba</sup>	*

Hybrids	Lesion length (cm)					Nodes crossed (no. plant-1)			Control (cm)		
	A1	A3	A3 ‡	<sup>a</sup> Diff.	<sup>e</sup> Diff.	A1	A3	Diff.	A1	A3	Diff.
Ks57 × M81E	10.0 <sup>d-k</sup>	6.9 <sup>g-l</sup>	10.1 <sup>d-h</sup>	*	NS	0.7 <sup>g-j</sup>	0.7 <sup>f-j</sup>	NS	2.7 <sup>b-i</sup>	2.6 <sup>bac</sup>	NS
Ks57 × Masuda	11.0 <sup>b-g</sup>	8.1 <sup>c-g</sup>	10.8 <sup>d-f</sup>	*	NS	1.3 <sup>ecd</sup>	1.4 <sup>bc</sup>	NS	2.9 <sup>bdc</sup>	2.2 <sup>a-i</sup>	NS
Ks57 × No.8	9.3 <sup>i-l</sup>	7.7 <sup>d-k</sup>	10.2 <sup>d-g</sup>	NS	NS	1.0 <sup>d-h</sup>	1.0 <sup>c-g</sup>	NS	2.6 <sup>b-i</sup>	2.7 <sup>ba</sup>	NS
Ks57 × PI185672	11.0 <sup>b-f</sup>	7.1 <sup>f-l</sup>	10.2 <sup>d-h</sup>	**	NS	1.0 <sup>d-h</sup>	1.1 <sup>b-f</sup>	NS	2.5 <sup>b-j</sup>	2.7 <sup>a</sup>	NS
Ks57 × Kaoliang	10.7 <sup>c-i</sup>	8.7 <sup>bcd</sup>	10.7 <sup>d-f</sup>	NS	NS	0.6 <sup>h-k</sup>	0.8 <sup>e-i</sup>	NS	3.0 <sup>bac</sup>	2.6 <sup>ba</sup>	NS
Ks57 × Rox orange	9.9 <sup>d-k</sup>	7.7 <sup>d-k</sup>	10.7 <sup>d-f</sup>	NS	NS	1.3 <sup>ecd</sup>	1.4 <sup>bc</sup>	NS	2.9 <sup>bdc</sup>	2.2 <sup>a-i</sup>	NS
Ks57 × Sugar drip	10.8 <sup>b-h</sup>	8.1 <sup>c-g</sup>	11.2 <sup>bc</sup>	*	NS	1.4 <sup>bcd</sup>	1.6 <sup>ba</sup>	NS	2.5 <sup>b-j</sup>	2.5 <sup>a-d</sup>	NS
N122 × Atlas	9.6 <sup>f-l</sup>	7.1 <sup>f-l</sup>	9.4 <sup>f-k</sup>	*	NS	1.0 <sup>d-h</sup>	0.9 <sup>d-h</sup>	NS	2.3 <sup>d-k</sup>	2.1 <sup>b-j</sup>	NS
N122 × Early hegari	9.1 <sup>kml</sup>	6.6 <sup>mkl</sup>	9.6 <sup>e-k</sup>	*	NS	1.3 <sup>ecd</sup>	0.7 <sup>f-j</sup>	NS	2.5 <sup>c-j</sup>	2.4 <sup>a-f</sup>	NS
N122 × Kansas collier	11.3 <sup>a-d</sup>	7.8 <sup>c-h</sup>	10.7 <sup>d-f</sup>	*	NS	1.3 <sup>ecd</sup>	1.0 <sup>c-g</sup>	NS	2.2 <sup>g-k</sup>	2.2 <sup>a-i</sup>	NS
N122 × M81E	6.5 <sup>qpo</sup>	5.3 <sup>pno</sup>	8.4 <sup>i=n</sup>	NS	NS	0.0 <sup>l</sup>	0.0 <sup>l</sup>	NS	1.8 <sup>kl</sup>	1.6 <sup>j</sup>	NS
N122 × Masuda	9.0 <sup>kml</sup>	7.0 <sup>g-l</sup>	9.7 <sup>d-i</sup>	NS	NS	1.0 <sup>d-h</sup>	1.0 <sup>c-g</sup>	NS	2.0 <sup>kj</sup>	2.1 <sup>b-j</sup>	NS
N122 × No.8	7.6 <sup>npo</sup>	5.7 <sup>mno</sup>	8.3 <sup>j-n</sup>	NS	NS	0.2 <sup>ikl</sup>	0.3 <sup>i-l</sup>	NS	2.2 <sup>h-k</sup>	2.3 <sup>a-h</sup>	NS
N122 × PI185672	9.1 <sup>kml</sup>	4.8 <sup>pqo</sup>	7.2 <sup>nop</sup>	**	NS	0.4 <sup>i-l</sup>	0.0 <sup>l</sup>	NS	2.3 <sup>d-k</sup>	2.1 <sup>c-j</sup>	NS
N122 × Kaoliang	10.2 <sup>c-k</sup>	6.8 <sup>i-m</sup>	8.7 <sup>h-m</sup>	*	NS	1.0 <sup>d-h</sup>	0.4 <sup>h-l</sup>	NS	2.4 <sup>c-k</sup>	2.3 <sup>a-h</sup>	NS
N122 × Rox orange	9.8 <sup>e-k</sup>	7.4 <sup>e-l</sup>	9.5 <sup>f-k</sup>	*	NS	1.2 <sup>c-f</sup>	1.0 <sup>c-g</sup>	NS	2.5 <sup>b-j</sup>	1.9 <sup>e-j</sup>	NS
N122 × Sugar drip	10.7 <sup>c-i</sup>	7.7 <sup>d-k</sup>	10.3 <sup>d-g</sup>	*	NS	1.1 <sup>c-g</sup>	0.9 <sup>d-h</sup>	NS	2.9 <sup>bdc</sup>	2.4 <sup>a-e</sup>	NS
Mean	9.55	7.10	9.65			0.94	0.78		2.49	2.21	
<sup>a</sup> LSD (0.05)	1.44	1.22	1.51			0.53	0.53		0.60	NS	
<sup>b</sup> LSD (0.05)	0.32		-			NS			NS		

Means within the same column with different letter are significantly different at  $P < 0.05$ .

A1, sorghum hybrids in A1 cytoplasm background; A3, sorghum hybrids in A3 cytoplasm background; A3 ‡, A3 sorghum hybrids in A3 cytoplasm background (open heads).

<sup>a</sup>LSD least significant difference between cytoplasm at same levels of A-line.

<sup>b</sup>LSD least significant difference between A1 and A3 cytoplasm background.

<sup>e</sup>Diff., significant differences between A1 and A3 hybrids; <sup>e</sup>Diff., significant differences between A1 and A3 ‡ hybrids.

**Table 4.6.** Estimates of general combining ability (GCA) effects of sweet sorghum parental lines for stalk rot infection caused by *Macrophomina phaseolina* evaluated at Manhattan, KS in 2013, 2014, and 2015 sowing season.

Entries	Lesion length (cm)			Nodes crossed (no. plant-1)		
	A1	A3	Diff.	A1	A3	Diff.
Females						
Tx399	0.16	0.59*	NS	0.09	-0.06	NS
Tx623	-0.89**	-0.78**	NS	-0.25**	-0.17*	NS
Ks57	1.01**	0.66**	NS	0.23**	0.38**	NS
N122	-0.28	-0.48*	NS	-0.07	-0.15*	NS
Males						
Atlas	-0.10	-0.19	NS	-0.02	-0.05	NS
Early hegari	-0.82*	-0.72*	NS	0.15*	-0.05	*
Kansas collier	1.57**	0.89**	*	0.51**	0.26*	*
M81E	-2.24**	-1.77**	*	-0.74**	-0.60**	NS
Masuda	0.92**	0.96**	NS	0.20*	0.51**	**
No.8	-1.56**	-1.01**	*	-0.52**	-0.41**	NS
PI185672	0.05*	-1.19**	**	-0.27**	-0.35**	NS
Kaoliang	0.61*	1.02**	NS	-0.13	-0.02	NS
Rox orange	0.48	0.77*	NS	0.26**	0.26*	NS
Sugar drip	1.09**	1.25**	NS	0.56**	0.45**	NS

\*\* , \* statistically significant at 1% and 5% levels of probability.

A1, sorghum hybrids in A1 cytoplasm background.

A3, sorghum hybrids in A3 cytoplasm background.

**Table 4.7.** Pearson correlation coefficients between agronomic traits of sweet sorghum genotypes and stalk rot infection caused by *Macrophomina phaseolina* evaluated at Manhattan, KS during 2013, 2014, and 2015 sowing season.

Traits	Cytoplasm	Correlation ( <i>r</i> )	
		Lesion length (cm)	Nodes crossed (no. plant-1)
Day to flowering	A1	-0.39**	-0.39**
	A3	-0.41**	-0.32**
Plant height	A1	-0.12*	-0.19**
	A3	-0.16*	-0.26**
Lodging	A1	0.21**	0.16**
	A3	0.06 <sup>ns</sup>	0.14*
°Brix	A1	-0.30**	-0.26**
	A3	-0.11*	0.001 <sup>ns</sup>
Lesion length	A1	1	0.68**
	A3	1	0.59**

\*\* , \* statistically significant at 1% and 5% levels of probability.

## Discussion

Stalk rot is a serious and wide spread disease of sorghum that reduces both yield and quality of the grain and stalk. The disease is caused by several species of pathogen that occur singly or as complexes. Of all, post flowering stalk rot caused by *M. phaseolina* is the most important disease worldwide. A number of physiological and anatomical characteristics of the host plant has been implicated as modulating the response of genotypes to stalk rot infection. The current study investigated the disease response of sweet sorghum hybrids of diverse genetic background variable sink size to infection by stalk rot pathogen *M. phaseolina*.

The mean disease score rated as lesion length was greater in the hybrids than in the parental lines (Table 4.3 and 4.4). This may be due to the fact that hybrids with larger sink size tended to draw more assimilates from the stalk to fill the grain than the inbreds that had relatively smaller sink size. This is in general agreement with the widely reported thought that the severity of stalk rot infection is greater among high-yielding hybrids than in inbred lines (Seetharama et al., 1991). There were also significant differences among parent lines, hybrids, and between A1 and A3 hybrids (Table 4.3 and 4.4). Although all hybrids were generally higher yielders than inbreds, there was significant difference in yield potential of the hybrids. Thus, the amount of physiological stress imposed due to variable assimilate partitioning could be different in high and low-yielding hybrids leading to differences in their response to stalk rot infection. All of the non-grain forming A3 hybrids had lower infection rates than their grain forming A1 counterparts. Nevertheless, due to the inherent genetic difference between the hybrids, response to infection between A1 and A3 hybrids was different, indicating that assimilate partitioning was just one of the many factors contributing to the stalk rot disease.

Because male parents were more divergent with respect to their reaction to stalk rot with mean lesion length ranging from 3.41 cm contained within 0.33 nodes in the resistant line M81E, to 15.68 cm that crossed 2.00 nodes in the susceptible Early hegari, the greatest proportion of the variation among parent lines should have come from the males. Male lines No.8, Rox orange, and Atlas also expressed lower disease scores with necrotic lesion of 6.87, 7.46, and 7.75 cm, respectively (Table 4.3).

The most relevant information in this study is the difference in the reaction of A1 and A3 hybrids to infection by *M. phaseolina*. All of the 40 A1 hybrids had mean lesion length longer than their corresponding A3 hybrids with the overall mean A1 and A3 hybrids being 9.55 and 7.10 cm, respectively. This conforms with many earlier reports that physiological stress resulting from massive movement of assimilates from the stalk to the grain may weaken the host condition and predispose to infection by stalk rot diseases (Odvody and Dunkle, 1979; Dodd, 1980). Because the A3 hybrids had more sugar and juice in the stalk than their corresponding A1 hybrids, those may be among factors responsible for resistance. Disease score from unbagged A3 hybrids was comparable with that of A1 hybrids, confirming that the greater stalk sugar in the A3 hybrids was indeed the factor responsible for improved resistance. This is an important agronomic implication in sweet sorghum production. In addition to increasing the feedstock value through improving sugar and biomass yield, the use of A3 CMS system has added the benefit of great stalk rot resistance. As reported in the previous chapter, A3 hybrids were more tolerant to lodging because of stronger stalk and less torque from the grain. This plus the improved resistance to stalk rot disease addresses concerns with standability, one of the important considerations in high biomass sweet sorghum production.



## **Conclusion**

In addition to increasing sugar yield and biomass, removal of the grain sink, significantly contributed to increased resistance to stalk rot diseases. Such resistance combined with less propensity for lodging in non-grain forming hybrids greatly contributes to standability. The A3 CMS system has great potential for increasing sweet sorghum productivity and increasing the value of the crop as a transition feedstock in sugar industries or as dedicated sugar-based feedstock for biofuel production.

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## Appendix A - Supplemental information for chapter 3

**Table A.1.** Mean squares for the analysis of variance of biofuel traits for individual test environments.

Source of variation	df	Mean squares			
		Brix	Biomass yield	Juice yield	Sugar yield
Environment one (2013)					
Replication	2	2.14	12.73*	27.81**	0.51*
Entry	97	31.92**	124.75**	151.28**	3.35**
Parent	17	45.68**	122.4**	216.6**	4.42**
Hybrid	79	25.21**	102.9**	113.2**	2.77**
Male	9	52.59**	636**	525.1**	11.04**
Female	7	148.89**	176.5**	174.9**	8.95**
Male x Female	63	7.55**	18.55**	47.53**	0.90**
Parent vs. Hybrid	39	328.7**	1890.3**	2045**	31.20**
A1 hybrids	39	10.36**	68.54**	80.99**	1.17**
A3 hybrids	39	14.76**	115.26**	128.3**	2.98**
A1 vs. A3 hybrids	1	1011**	960.8**	782.1**	56.68**
Environment two (2014)					
Replication	2	1.82	21.47*	2.33	0.07
Entry	97	18.86**	64.51**	139.2**	2.8**
Parent	17	19.34**	77.65**	146.28**	2.73**
Hybrid	79	16.68**	50.08**	113.3**	2.38**
Male	9	33.45**	219.3**	612.1**	11.04**
Female	7	106.3**	119.3**	185.8**	7.05**
Male x Female	63	4.32**	18.21**	34**	0.63**
Parent vs. Hybrid	1	182.8**	980.3**	2065**	36.44**
A1 hybrids	39	9.29**	37.45**	109.4**	1.59**
A3 hybrids	39	5.84**	46.47**	101.9**	2.19**
A1 vs. A3 hybrids	1	727.6**	683.8**	708.3**	40.94**
Environment three (2015)					
Replication	2	0.57	18.44*	1.27	0.18
Entry	97	33.76**	90.49**	210.6**	5.69**
Parent	17	46.85**	69.46**	189.2**	3.84**
Hybrid	79	19.03**	70.59**	137.5**	4.32**
Male	9	102.7**	359.3**	462.1**	15.97**
Female	7	43.81**	173.4**	543.2**	18.21**
Male x Female	63	6.19*	17.90**	43.54**	1.12**
Parent vs. Hybrid	1	975.1**	2020**	6350.6**	145.2**
A1 hybrids	39	14.26**	52.63**	105.7**	2.70**
A3 hybrids	39	6.43**	60.59**	109.3**	3.04**
A1 vs. A3 hybrids	1	696.3**	1160**	248**	117**
Error	194				

\*\* , \* - statistically significant at 1 % and 5% levels of probability, respectively.

**Table A.2.** Mean squares for the analysis of variance of agronomic traits for individual test environments.

Source of variation	df	Mean squares			
		Day to flowering	Lodging	Plant Height	Head weight
Environment one (2013)					
Replication	2	148.18**	0.207*	1902**	8.66**
Entry	97	358.37**	0.87**	11896.4**	37.43**
Parent	17	365.3**	0.319**	24388**	12**
Hybrid	79	354.35**	0.98**	5484**	43.37**
Male	9	2795**	2.88**	38013**	14.08**
Female	7	191.7**	2.60**	6488**	395**
Male x Female	63	23.72*	0.53**	725.4**	8.48**
Parent vs. Hybrid	39	557.71*	1.27*	306109*	0.505
A1 hybrids	39	326.5**	1.43**	5256**	17.15**
A3 hybrids	39	369.2**	0.20**	5828**	0.66**
A1 vs. A3 hybrids	1	855.03*	14.01**	928.2	2731**
Environment two (2014)					
Replication	2	646.7**	3.18**	2315**	0.21
Entry	97	390.7**	0.28*	4778**	20.81**
Parent	17	365.3**	0.35	8339**	9.26**
Hybrid	79	381.03**	0.26**	3323**	23.38**
Male	9	2113**	1.13**	18868**	5.71**
Female	7	163.8*	0.304	3087**	186.4**
Male x Female	63	157.7**	0.141	1128**	7.79
Parent vs. Hybrid	1	1587*	0.011	59258**	13.74
A1 hybrids	39	403.9**	0.28*	3795**	14.21**
A3 hybrids	39	365.4**	0.25*	2934**	0.68**
A1 vs. A3 hybrids	1	95**	0.004	63.03	1266**
Environment three (2015)					
Replication	2	498.5**	2.90*	199.5	1.50*
Entry	97	238.3**	1.02**	33.76**	35.92**
Parent	17	219.3**	1.17*	31441**	14.33**
Hybrid	79	299.09**	1.002**	4166**	41.02**
Male	9	2358**	1.84**	19982**	14.70**
Female	7	127.1**	2.29**	8622**	387.2**
Male x Female	63	24.03**	0.73*	1412**	6.31**
Parent vs. Hybrid	1	129.2	0.001	391468**	0.006
A1 hybrids	39	308.6**	1.38**	3156**	13.45**
A3 hybrids	39	289.3**	0.33	5232**	1.33**
A1 vs. A3 hybrids	1	306**	12.15**	1978	2664.2**
Error	194				

\*\* , \* - statistically significant at 1 % and 5% levels of probability, respectively.

**Table A.3.** Combined mean performance of sorghum hybrids as influenced by male-sterility inducing cytoplasm of their A1 and A3-lines for agronomic traits grown in Manhattan KS during 2013, 2014 and 2015 crop seasons.

Hybrids	Day to flowering			Lodging			Plant height			Head weight		
	A1	A3	Diff.	A1	A3	Diff.	A1	A3	Diff.	A1	A3	Diff.
Tx399 x Atlas	69.9	75.2	NS	1.1	1.2	NS	222.3	222.2	NS	10.1	2.3	**
Tx399 x Early Hegari	79	81.8	NS	1.3	1.1	NS	218.4	223.9	NS	7.8	3.1	*
Tx399 x Kansas collier	66.6	67.3	NS	1.4	1.2	NS	234.8	214.9	NS	8.3	1.9	**
Tx399 x M81E	93.7	91.8	NS	1	1	NS	303.9	286.7	NS	8.8	3.4	**
Tx399 x Masuda	71.3	71.4	NS	1.7	1.7	NS	237.8	217.9	NS	8.8	2.4	**
Tx399 x No.8	87.4	91.9	NS	1	1	NS	276.1	287.8	NS	7.4	2.8	*
Tx399 x PI185672	97	99.7	NS	1	1.1	NS	279.1	281.7	NS	6.2	2.2	*
Tx399 x Kaoliang	76	78	NS	1	1	NS	242.8	227.8	NS	10.4	2.3	**
Tx399 x Rox Orange	74	74.6	NS	1.2	1	NS	227.9	195.1	*	6.4	1.9	*
Tx399 x Sugar Drip	66.2	66.6	NS	1.3	1.2	NS	219.7	206.7	NS	7.6	1.9	**
Tx623 x Atlas	73.8	76.9	NS	1.6	1.6	NS	247.1	243.1	NS	10.1	1.8	**
Tx623 x Early Hegari	87.6	85.2	NS	1.3	1	NS	239.8	244.1	NS	8.5	2.1	**
Tx623 x Kansas collier	70.3	67.9	NS	1.9	1.6	NS	242.4	240	NS	7.8	2.3	**
Tx623 x M81E	83	85.6	NS	1.1	1.1	NS	308.6	317.1	NS	10.4	2.4	**
Tx623 x Masuda	69.7	74.6	NS	2.6	1.3	**	234.7	258	NS	8.7	1.9	**
Tx623 x No.8	93.6	86.6	NS	1	1.1	NS	286.3	280.9	NS	7.5	2.6	**
Tx623 x PI185672	95.1	97.7	NS	1.6	1	*	290.9	307.8	NS	5.3	2	*
Tx623 x Kaoliang	72.2	79.7	NS	1.3	1	NS	284.6	283	NS	10.9	2.8	**
Tx623 x Rox Orange	68.2	72.7	NS	1.7	1.1	*	230.1	212.3	NS	5.5	1.8	*
Tx623 x Sugar Drip	66.8	69.7	NS	1.3	1	NS	226.1	231.9	NS	9	2.1	**
Ks57 x Atlas	68.2	75.7	NS	1.1	1.4	NS	201.9	212.8	NS	10.7	2.6	**
Ks57 x Early Hegari	75.8	83.4	NS	1.7	1.1	*	222.1	209	NS	9.5	2.5	**
Ks57 x Kansas collier	64.9	69.8	NS	2.1	1.2	**	216.8	213.7	NS	8.6	2.1	**



Hybrids	Day to flowering			Lodging			Plant height			Head weight		
	A1	A3	Diff.	A1	A3	Diff.	A1	A3	Diff.	A1	A3	Diff.
Ks57 x M81E	89.4	93	NS	1.3	1.2	NS	287.8	312.1	NS	7.5	2.6	**
Ks57 x Masuda	71.1	75.9	NS	2.6	1.2	**	210.9	228.3	NS	9.4	2.3	**
Ks57 x No.8	88.2	90.8	NS	1	1.1	NS	289.1	275	NS	7.8	3	**
Ks57 x PI185672	95.4	94.8	NS	1	1	NS	278.4	289.8	NS	6.9	2.9	*
Ks57 x Kaoliang	70.8	74.4	NS	1.2	1.1	NS	234.2	235	NS	7.4	2.7	*
Ks57 x Rox Orange	74.1	73	NS	1.1	1.3	NS	206.1	209.8	NS	7.9	2.1	**
Ks57 x Sugar Drip	66.6	70.3	NS	2	1.1	**	201.1	207.8	NS	9.1	2.4	**
N122 x Atlas	74.6	78.8	NS	1.7	1.1	*	208.9	210.4	NS	5.4	2.1	*
N122 x Early Hegari	78.8	76.3	NS	1	1	NS	183.6	185.6	NS	9	2.3	**
N122 x Kansas collier	69.3	69.1	NS	2.4	1.3	**	207.7	227.3	NS	9.3	1.9	**
N122 x M81E	91.1	94.8	NS	1.1	1	NS	283.4	283.9	NS	8.4	1.9	**
N122 x Masuda	70.4	73.2	NS	2.8	1.3	**	217.7	224.1	NS	8.9	2.1	**
N122 x No.8	87.1	92.4	NS	1	1	NS	231.1	242.6	NS	9.2	2.7	**
N122 x PI185672	87.3	90.3	NS	1.2	1.1	NS	259.7	284.6	NS	7.2	3	*
N122 x Kaoliang	69.7	73	NS	1.4	1	NS	237.2	227.7	NS	9.3	1.8	**
N122 x Rox Orange	68.7	73	NS	1.3	1.1	NS	211	202.9	NS	7.3	2.2	**
N122 x Sugar Drip	65.4	68.9	NS	2	1	**	226	213.4	NS	8.6	1.6	**
<i>Mean</i>	77.2	79.6		1.5	1.2		241.7	242		8.3	2.3	
<sup>a</sup> LSD (0.05)	4.3	4.6		0.4	0.4		14	12.8		1	0.2	
<sup>b</sup> LSD (0.05)	NS			NS			NS			0.3		

A1, sorghum hybrids in A1 cytoplasm background; A3, sorghum hybrids in A3 cytoplasm background.

<sup>a</sup>LSD, least significant difference between cytoplasm at same levels of A-line.

<sup>b</sup>LSD, least significant difference between A1 and A3 cytoplasm background.

NS, Non-significant.

**Table A.4.** Estimates of specific combining ability (SCA) effects as influenced by male sterility inducing cytoplasm (A1 and A3) for agronomic traits of sorghum hybrids grown in Manhattan KS during 2013, 2014 and 2015 crop seasons.

Hybrids	Day to flowering		Lodging		Plant height		Head weight	
	A1	A3	A1	A3	A1	A3	A1	A3
Tx399 x Atlas	-2.43	-1.60	0.04	-0.11	10.13*	18.08**	1.81**	-0.89** <sup>a</sup>
Tx399 x Early Hegari	-1.99	-0.10	0.29	0.06	10.32*	26.25** <sup>a</sup>	-0.08	-0.43**
Tx399 x Kansas collier	-1.93	-1.38	-0.24	-0.11	17.21**	8.92*	0.64	-1.10** <sup>a</sup>
Tx399 x M81E	3.65*	0.32	0.15	-0.08	15.82**	4.72	0.80*	-0.12 <sup>a</sup>
Tx399 x Masuda	-0.02	-2.52	-0.44*	0.28 <sup>a</sup>	20.38**	3.81 <sup>a</sup>	0.68	-0.79** <sup>a</sup>
Tx399 x No.8	-2.35	1.29	0.29	-0.06	13.29*	34.22** <sup>a</sup>	0.24	-0.98** <sup>a</sup>
Tx399 x PI185672	2.57	3.87*	0.09	0.06	9.93*	8.72	0.58	-1.29** <sup>a</sup>
Tx399 x Kaoliang	3.12*	1.54	0.04	-0.03	0.93	2.42	1.74**	-1.05** <sup>a</sup>
Tx399 x Rox Orange	2.04	1.07	0.18	-0.14	16.96**	8.08	0.41	-1.09** <sup>a</sup>
Tx399 x Sugar Drip	-0.74	-2.49	-0.05	0.14	9.29	9.72*	-0.13	-1.13** <sup>a</sup>
Tx623 x Atlas	1.54	0.26	0.16	0.20	22.13**	13.61*	1.70**	-1.15** <sup>a</sup>
Tx623 x Early Hegari	6.66**	3.53*	-0.04	-0.08	18.88**	21.11**	0.40	-1.14** <sup>a</sup>
Tx623 x Kansas collier	1.93	-0.63	-0.12	0.20	12.10*	8.66	-0.11	-0.52**
Tx623 x M81E	-6.93**	-5.72**	-0.06	0.01	7.71	9.80*	2.29**	-0.93** <sup>a</sup>
Tx623 x Masuda	-1.59	0.78	0.13	-0.08	4.49	18.55** <sup>a</sup>	0.34	-0.98** <sup>a</sup>
Tx623 x No.8	3.85*	-3.86*	-0.04	0.03	10.74*	1.97	0.19	-0.92** <sup>a</sup>
Tx623 x PI185672	0.77	2.06	0.33*	-0.08 <sup>a</sup>	8.93	9.47*	-0.45	-1.25** <sup>a</sup>
Tx623 x Kaoliang	-0.57	3.39*	0.05	-0.05	29.93**	32.27**	2.00**	-0.39** <sup>a</sup>
Tx623 x Rox Orange	-3.65*	-0.63	0.30	-0.05	6.40	-0.06	-0.68	-0.95**
Tx623 x Sugar Drip	-0.09	0.81	-0.37*	-0.11	2.96	9.58*	1.02*	-0.65** <sup>a</sup>

Hybrids	Day to flowering		Lodging		Plant height		Head weight	
	A1	A3	A1	A3	A1	A3	A1	A3
Ks57 x Atlas	-2.44	-1.44	-0.26	0.08	1.12	5.77	2.13**	-0.71** <sup>a</sup>
Ks57 x Early Hegari	-3.56*	1.28 <sup>a</sup>	0.32*	0.02	25.42**	8.49 <sup>a</sup>	1.32**	-1.08** <sup>a</sup>
Ks57 x Kansas collier	-1.94	0.78	0.13	-0.14	10.64*	4.83	0.64	-1.06** <sup>a</sup>
Ks57 x M81E	1.08	1.25	0.18	0.11	11.14*	27.30** <sup>a</sup>	-0.76*	-1.07**
Ks57 x Masuda	1.42	1.64	0.15	-0.20	4.92	11.38*	0.97*	-0.99** <sup>a</sup>
Ks57 x No.8	0.08	-0.11	-0.01	0.02	37.73**	18.58** <sup>a</sup>	0.29	-0.84** <sup>a</sup>
Ks57 x PI185672	2.67	-1.31	-0.21	-0.09	20.70**	13.97*	1.03*	-0.70** <sup>a</sup>
Ks57 x Kaoliang	-0.44	-2.31	-0.04	0.05	3.81	6.77	-1.63**	-0.80** <sup>a</sup>
Ks57 x Rox Orange	3.81*	-0.78 <sup>a</sup>	-0.24	0.16	6.62	19.88** <sup>a</sup>	1.65**	-0.95** <sup>a</sup>
Ks57 x Sugar Drip	1.25	1.00	0.32*	-0.01	2.17	7.97	1.05*	-0.67** <sup>a</sup>
N122 x Atlas	4.10*	2.79	0.20	-0.17	16.34**	12.52*	-2.96**	-0.81** <sup>a</sup>
N122 x Early Hegari	-0.34	-4.71* <sup>a</sup>	-0.44*	0.00	-4.91	-5.87	1.04*	-0.91** <sup>a</sup>
N122 x Kansas collier	2.71	1.23	0.37*	0.06	9.75	27.57** <sup>a</sup>	1.51**	-0.86** <sup>a</sup>
N122 x M81E	2.96*	4.15*	-0.13	-0.03	15.03*	8.16	0.34	-1.43** <sup>a</sup>
N122 x Masuda	0.96	0.09	0.29	0.00	19.92**	16.24**	0.68	-0.79** <sup>a</sup>
N122 x No.8	-0.82	2.68	-0.10	0.01 <sup>a</sup>	-12.05*	-4.79	1.96**	-0.81** <sup>a</sup>
N122 x PI185672	-5.23**	-4.63*	-0.07	0.11	10.14*	17.82**	1.52**	-0.30** <sup>a</sup>
N122 x Kaoliang	-1.34	-2.63	0.09	0.03	15.03*	8.52	0.56	-1.30** <sup>a</sup>
N122 x Rox Orange	-1.43	0.34	-0.10	0.03	19.73**	22.07**	1.29**	-0.56** <sup>a</sup>
N122 x Sugar Drip	0.35	0.68	0.23	-0.03	35.28**	22.71**	0.74*	-1.10** <sup>a</sup>

\*\* , \* statistically significant at 1% and 5% levels of probability; a, Significant cytoplasm differences.

A1, sorghum hybrids in A1 cytoplasm background; A3, sorghum hybrids in A3 cytoplasm background.

**Table A.5.** Estimates of high-parent heterosis of sorghum hybrids as influenced by male-sterility inducing cytoplasm of their A1 and A3-lines for agronomic traits of sorghum hybrids grown in Manhattan KS during 2013, 2014 and 2015 crop seasons.

Hybrids	Day to flowering		Lodging		Plant height		Head weight	
	A1	A3	A1	A3	A1	A3	A1	A3
Tx399 x Atlas	-14.65	-8.14	-23.08	-15.38	-11.34	-11.39	39.46**	-68.10
Tx399 x Early Hegari	5.61*	9.67*	-7.69	-12.08	17.74*	21.37*	44.08*	-43.26
Tx399 x Kansas collier	-1.96	-0.82	0.00	-15.38	8.03*	-1.12	68.63*	-60.64
Tx399 x M81E	4.85*	2.74*	-18.18	-18.18	-2.57*	-8.09*	96.68**	-23.05
Tx399 x Masuda	-3.89	-3.74	-11.76	-11.76	-1.92	-10.13	92.99**	-48.34
Tx399 x No.8	-9.02	-4.39*	0.00	0.00	12.95*	17.73*	-12.56	-67.00
Tx399 x PI185672	8.72*	11.71*	-30.63	-14.37	13.72*	14.76*	13.48	-59.22
Tx399 x Kaoliang	-7.69	-5.26	-25.00	-25.00	-4.12	-10.05	16.41*	-73.92
Tx399 x Rox Orange	-4.58	-3.87	-15.38	-30.77	3.80	-11.13	5.78	-68.28
Tx399 x Sugar Drip	-7.31	-6.84	0.00	-8.33	-1.49	-7.32	14.96	-72.19
Tx623 x Atlas	-9.91	-6.11	7.69	7.69	-1.46	-3.06	40.25**	-74.96
Tx623 x Early Hegari	16.28*	11.62*	-7.69	-30.77	21.97*	23.86*	56.05*	-60.77
Tx623 x Kansas collier	1.44	-2.08	30.77	7.69	11.55*	10.43	56.75*	-53.69
Tx623 x M81E	-7.09	-4.23	-9.09	-9.09	-1.07*	1.67*	97.78**	-46.39
Tx623 x Masuda	-6.14	0.45	17.29*	-29.41	-3.21	6.42*	89.24**	-57.65
Tx623 x No.8	-2.66*	-9.94	0.00	11.11	17.14*	14.91*	-11.09	-69.01
Tx623 x PI185672	6.60*	9.46*	-15.32	-15.63	18.52*	19.40*	-2.36	-62.90
Tx623 x Kaoliang	-12.28	-3.24	0.00	-25.00	12.37*	11.76*	21.27**	-69.19
Tx623 x Rox Orange	-12.03	-6.30	15.38	-23.08	4.81	-3.29	-9.35	-69.75
Tx623 x Sugar Drip	-6.53	-2.49	0.00	-25.00	1.40	3.99	34.76*	-68.58
Ks57 x Atlas	-16.69	-7.60	-13.08	0.00	-19.49	-15.15	48.18**	-64.42
Ks57 x Early Hegari	6.89	8.11*	31.38	-23.08	20.18*	20.44*	75.54**	-53.74
Ks57 x Kansas collier	-2.67	4.67	46.15*	-15.38	-0.26	-1.69	70.90*	-59.13
Ks57 x M81E	0.12*	4.10*	9.09	0.00	-7.73*	0.07*	48.99*	-49.21
Ks57 x Masuda	-4.19	2.25	35.29*	-35.29	-13.02	-5.82	86.47**	-55.48
Ks57 x No.8	-8.21	-5.55*	0.00	11.11	18.27*	12.50*	-8.33	-64.31

Hybrids	Day to flowering		Lodging		Plant height		Head weight	
	A1	A3	A1	A3	A1	A3	A1	A3
Ks57 x PI185672	6.97*	6.23*	-30.63	-11.63	13.44*	18.06*	27.23	-46.93
Ks57 x Kaoliang	-14.04	-9.58	-8.33	-16.67	-7.50	-7.20	-17.78	-70.22
Ks57 x Rox Orange	-4.44	-5.87	-23.08	-7.69	-6.12	-4.45	31.46	-64.57
Ks57 x Sugar Drip	-6.84	-1.56	16.00*	-16.67	-9.82	-6.83	37.28*	-64.07
N122 x Atlas	-8.96	-3.80	15.38	-23.08	-16.70	-16.08	-25.77	-70.56
N122 x Early Hegari	9.18	8.70	-17.77	-30.77	19.46	23.80	65.58**	-57.01
N122 x Kansas collier	-1.27	-1.58	69.23*	-7.69	-4.45	4.60	87.17**	-61.15
N122 x M81E	1.99*	6.09*	-9.09	-18.18	-9.12*	-8.98*	87.55**	-58.19
N122 x Masuda	-5.09	-1.35	47.06*	-29.41	-10.22	-7.56	94.04**	-54.11
N122 x No.8	-9.36	-3.82	0.00	0.00	-5.45	-0.77*	8.38*	-68.09
N122 x PI185672	-2.12	1.25*	-27.11	-13.37	5.79*	15.93*	31.49	-45.86
N122 x Kaoliang	-15.38	-11.34	8.33	-25.00	-6.32	-10.09	3.76*	-79.55
N122 x Rox Orange	-11.46	-5.87	-7.69	-23.08	-3.90	-7.59	21.25	-63.76
N122 x Sugar Drip	-8.40	-3.58	16.00*	-25.00	1.35	-4.29	28.81*	-75.62

\*\* , \* statistically significant at 1% and 5% levels of probability.

A1 = sorghum hybrids in A1cytoplasm background; A3 = sorghum hybrids in A3 cytoplasm background.

**Table A.6.** Estimates of mid-parent heterosis of sorghum hybrids as influenced by male-sterility inducing cytoplasm of their A1 and A3-lines for agronomic traits of sorghum hybrids grown in Manhattan KS during 2013, 2014 and 2015 crop seasons.

Hybrids	Day to flowering		Lodging		Plant height		Head weight	
	A1	A3	A1	A3	A1	A3	A1	A3
Tx399 x Atlas	-6.68	0.45	-9.09	0.01	31.17	31.1	73.35**	-60.34
Tx399 x Early Hegari	15.99*	20.07*	9.09	-9.09	83.48*	88.05*	58.98*	-37.39
Tx399 x Kansas collier	-1.07	0.08	18.18	0	53.67*	40.65	78.54*	-58.33
Tx399 x M81E	19.15*	16.75*	-10	-10	51.90*	43.29*	98.15**	-22.47
Tx399 x Masuda	0.39	0.55	15.38	15.38	43.82	31.79	96.88**	-47.29
Tx399 x No.8	6.64	12.06*	0	0	66.00*	73.01*	15.11	-56.56
Tx399 x PI185672	20.48*	26.87*	-19.71	-28.57	67.30*	68.83*	25.56	-54.88
Tx399 x Kaoliang	1.18	3.85	-14.29	-14.29	42.21	33.42	56.08*	-65.03
Tx399 x Rox Orange	1.76	2.52	0	-18.18	48.09	26.79	22.34	-63.31
Tx399 x Sugar Drip	-4.94	-4.47	14.29	4.76	41.16	32.81	38.37	-66.53
Tx623 x Atlas	-2.42	1.69	27.27	27.27	39.48	37.22	75.11**	-68.73
Tx623 x Early Hegari	21.20*	23.81*	9.09	-18.18	89.22*	92.63*	73.10*	-56.48
Tx623 x Kansas collier	3.43	-0.16	54.55	27.27	51.11*	49.58	66.87*	-50.7
Tx623 x M81E	4.62	7.84	0	0	48.54*	52.66*	95.90**	-45.67
Tx623 x Masuda	-2.94	3.87	76.92*	-7.69	35.65	49.13*	94.17**	-56.55
Tx623 x No.8	13.10*	4.63	0	11.11	64.56*	61.43*	17.52	-59.04
Tx623 x PI185672	19.97*	23.20*	0	-35.71	66.70*	76.38**	8.6	-58.74
Tx623 x Kaoliang	-4.76	5.05	14.29	-14.29	59.51*	58.64*	63.22**	-58.53
Tx623 x Rox Orange	-7.11	-1.06	36.36	-9.09	42.43	31.43	5.36	-64.84
Tx623 x Sugar Drip	-5.13	-1.03	14.29	-14.29	38.48	42.02	62.95*	-62
Ks57 x Atlas	-7.74	2.33	-9.09	18.18	20.53	27.03	74.32**	-58.15
Ks57 x Early Hegari	12.82	24.23*	36.36	-9.09	89.75*	78.55*	81.52**	-52.17
Ks57 x Kansas collier	-2.18	5.19	72.73*	0	43.77	41.71	72.77*	-58.68
Ks57 x M81E	15.16*	19.74*	20	10	45.30*	57.59*	58.26*	-46.05
Ks57 x Masuda	1.43	8.24	76.92*	-15.38	29.12	39.8	95.70**	-53.27
Ks57 x No.8	8.84	11.99*	0	11.11	75.93*	67.34*	14.8	-55.3

Hybrids	Day to flowering		Lodging		Plant height		Head weight	
	A1	A3	A1	A3	A1	A3	A1	A3
Ks57 x PI185672	19.98*	22.12*	-19.71	-35.71	68.92*	75.80*	31.95	-44.97
Ks57 x Kaoliang	-4.57	0.37	4.76	-4.76	38.82	39.28	5.05	-61.95
Ks57 x Rox Orange	3.25	1.7	-9.09	9.09	35.7	38.11	43.01	-61.46
Ks57 x Sugar Drip	-3.15	2.34	71.43*	-4.76	30.92	35.26	55.93*	-59.19
N122 x Atlas	-1.97	3.58	36.36	-9.09	26.77	27.71	-7.52	-63.32
N122 x Early Hegari	13.71	10.18	-18.18	-18.18	60.54	62.29	83.21**	-52.43
N122 x Kansas collier	1.3	0.97	10.0**	9.09	40.26	53.55	98.74**	-58.75
N122 x M81E	14.21*	18.80*	0	-10	45.11*	45.34*	89.53**	-57.75
N122 x Masuda	-2.46	1.38	92.31*	-7.69	35.52	39.54	98.55**	-53.04
N122 x No.8	4.74	11.16	0	0	43	50.09*	42.98*	-57.9
N122 x PI185672	9.55	13.31*	-21.43	-28.57	60.18*	75.53*	45.88	-39.93
N122 x Kaoliang	-8.67	-4.3	23.81	-14.29	42.9	37.15	39.40*	-72.53
N122 x Rox Orange	-7.07	-1.2	9.09	-9.09	41.45	36.01	40.59	-57.97
N122 x Sugar Drip	-7.61	-2.75	71.43*	-14.29	49.78	41.46	55.40*	-70.59

\*\* , \* statistically significant at 1% and 5% levels of probability.

A1 = sorghum hybrids in A1 cytoplasm background; A3 = sorghum hybrids in A3 cytoplasm background.