AGRONOMICAL, PHYSIOLOGICAL AND BIOCHEMICAL APPROACHES TO CHARACTERIZE SWEET SORGHUM GENOTYPES FOR BIOFUEL PRODUCTION

by

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M.S., Tamil Nadu Agricultural University, India, 2003

AN ABSTRACT OF A DISSERTATION

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DOCTOR OF PHILOSOPHY

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Abstract

Sweet sorghum (*Sorghum bicolor* L. Moench) is an important bioenergy crop. There is a wide array of genetic diversity in sweet sorghum germplasm collections. However, information on traits associated with sugar yield, optimum harvesting time for maximum sugar yield, effects of abiotic stresses on sugar yield is scarce. The objectives of the present study were: to identify traits that are associated with sugar yield, to determine the optimum harvesting time for maximum sugar yield and to understand the physiological responses of different sweet sorghum genotypes to drought and high temperature. In order to meet these objectives, five independent field and greenhouse studies were conducted. Field experiments were conducted using 280 sweet sorghum germplasm and were evaluated for 2 years. From this study, 30 genotypes representing high and low sugar yielders were selected for the subsequent experiment. We observed a significant variation in physiological, morphological and sugar yield traits associated with biofuel production. In the selection experiment, investigations on the morphological, physiological attributes helped to identify those characters which influence or limit sugar yield in the sweet sorghum. Another field study was conducted to optimize the harvesting time for obtaining highest sugar and juice yields in sweet sorghum. Sweet sorghum variety M81E was harvested at ten growth stages. Our results suggest that the optimum time for harvesting of sweet sorghum cultivar M81E is between milk and hard dough stages when highest sugar yield was observed. Studies on different levels of water stress were studied under greenhouse conditions. Four sweet sorghum genotypes (Awanlek, Smith, Tracy and Wray) were subjected to three water stress treatments (100% pot capacity (PC); 70% PC and 30% PC) for 20 days at early seed filling (Milk) stage. The results showed that genotypes differed significantly for all growth and yield,

biochemical and physiological traits. Severe water stress significantly decreased juice and sugar yields by decreasing net photosynthetic rate, transpiration rate, stomatal conductance and sucrose content in the stem juice. Genotypes Tracy and Wray produced significantly highest brix, stem fresh weight, juice and sugar yield under both irrigated and water stress conditions. In another greenhouse study, we quantified the effects of drought, high temperature, and their combinations on growth, physiology and yield of sweet sorghum genotypes. The same four genotypes above were subjected to four treatments, T_1 - control, T_2 - drought stress, T_3 - high temperature stress and T_4 - combination of drought and high temperature for 16 days after anthesis. The result showed that significant difference was observed for growth and yield traits, physiological traits and non-reducing and total sugar content in juice for genotypes and treatments. Among the genotypes Tracy recorded higher juice and sugar yield. Among the various treatments, combination of drought and high temperature was found to be more deleterious in reducing most of the biofuel traits followed by drought and high temperature stress. The above studies gave significant findings with regards to the identification of superior sweet sorghum germplasm, their tolerance capacity to different abiotic stresses, which allows better selection for the use of bioenergy production.

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Chapter 1 - Review of literature

General Introduction

The production of biofuels (largely ethanol) in the world grew by 13.8% in 2010, and accounted for 0.5% of global primary energy consumption. Today, biofuels represent 3% of the global road transport fuel supply and are expected to account for as much as 9% by 2050 (Calvino and Messing, 2011). Currently, Brazil and the United States are the world leaders in ethanol production (Mussatto et al., 2010). In Brazil, ethanol is fermented from sucrose that accumulates in the stems of sugarcane (*Saccharum officinarum* L.), whereas in the US it is produced from Maize (*Zea mays* L.), which accumulates about 85% starch in its seeds. Although the price of oil could play a significant role in influencing the expansion of biofuels, their production costs will also depend on input costs. Thus, reductions in costs are closely tied to the prices of feedstock commodities. Indeed, for conventional biofuels today (first-generation biofuels), feedstock account for 45–70% of total production costs (Calvino and Messing, 2011). This is especially important for sugarcane-based and corn-based ethanol, where both crops are cultivated under high input conditions requiring significant amounts of water and fertilizers.

Sweet Sorghum (*Sorghum bicolor* (L.) Moench) is considered as an important energy crop for the production of bioethanol due to high biomass, drought tolerance, relatively low input requirements and ability to grow under a wide range of environmental conditions (Steduto et al., 1997; Mastrorilli et al., 1999). Furthermore, in contrast to maize, sweet sorghum accumulate large amount of fermentable sugars in stems (Gnansounou et al., 2005; Vries et al., 2010) which can be easily fermented for ethanol production (House et al., 2000). Despite all the agronomic advantages of sweet sorghum as a bioenergy crop, little scientific effort has been directed in the past toward the elucidation of sweet sorghum traits relevant to biofuel production.

There are many cultivars of sweet sorghum distributed throughout the world, providing a diverse genetic base from which to develop regionally specific, highly productive cultivars (Audilakshmi et al., 2010). Traits like plant height, stem diameter, green biomass, stem sugar content, and stem juice extractability are the major contributors for sweet sorghum's economic importance (Almodares et al., 2006; Almodares et al., 2008). However, these traits are quantitative and polygenic inheritance in nature and are complex to be manipulated directly in breeding procedure (Zou et al., 2011). Therefore, to successfully improve these complex traits, they need to be dissected into smaller morphological, physiological and yield components, which could be easily analyzed and evaluated. Previous studies have suggested that much variability exists in juice quality, sugar content, and juice yield among the U.S sweet sorghum collections (Ali et al., 2008; Murray et al., 2008a; Murray et al., 2009; Makanda et al., 2009). However, information on the extent of variation in growth (plant height and stem diameter), physiology (chlorophyll content and Fv/Fm) and components of stem sugar (°brix, juice yield and stem fresh weight) among sweet sorghum germplasm are limited. Furthermore, correlations between the traits are of great importance in selection process for successful breeding programs. There are no studies that showed direct correlation between physiological traits and sugar yield.

Also, the relationships between morpho-physiological and stem sugar yield traits are not clearly understood. Sugar yield is a quantitative trait, which is the resultant of various traits contributing together during the crop growth, which are interdependent in their development. The inter-relationship between traits can be studied by principal component analysis (PCA), a powerful statistical tool by which the complex traits can be analyzed.

Sweet sorghum harvest plays a significant role in determining juice and sugar yield. The juice sugar yield depends on the plant age of development (Sipos et al., 2009). Sucrose starts to

accumulate in the stem during inflorescence and at later stages competition occurs for carbohydrates between stem and developing grain. At maturity the carbohydrates mobilized from stem and leaves to grains. Sucrose content and grain yield are indicators of how assimilates are partitioned between two sinks (grain and stem). Hence, it is important to determine and optimize the stage of development that provides maximum yield potential for juice and sugar production.

Sorghum is mostly grown under rainfed conditions. Although sorghum is relatively tolerant to individual effects of drought and high temperature, the stress response depends upon the intensity, rate and duration of exposure and the crop growth stages. The physiology, growth, and development of sweet sorghum are different from grain sorghum due to greater need for carbohydrate accumulation in the stem versus the grain sorghum with seeds. The potentiality of sweet sorghum to produce juice for sugar has been exploited to little extent during sensitive stages of crop. Since sweet sorghum exhibits drought resistant C_4 metabolism, emerging studies reveal inability of the crop to tolerate the dual effect of drought and increased temperatures. The impact of water stress (drought), high temperature, and its combination during reproductive stages of crop development are not clearly understood and needs investigation.

The objectives of the present study were to (1) quantify genetic variability for plant height, stem fresh weight, °brix, juice yield and sugar yield in sweet sorghum germplasm; and to identify potential drought tolerant sweet sorghum genotypes, (2) determine the optimum harvest time for obtaining maximum juice yield, (3) obtain information on the various growth and physiological traits influencing sugar yield, (4) quantify effects of water stress on brix, juice and sugar yield, and (5) quantify effects of drought, high temperature and its combination on juice and sugar yield characteristics.

Why sweet sorghum?

The use of sweet sorghum to provide liquid fuels for the transport sector is not a new concept (Rothman et al., 1983). However, the economics of liquid biofuel production are still hotly debated (Bauen, 2000), despite 25 years of large-scale experience in Brazil and USA. According to Sen (1989), *Saccharomyces cerevisia* will convert 1.00 g glucose into 0.51 g ethanol and 0.49 g CO_2 following about a dozen enzymatic steps of the Embden-Meyerhof-Parnas pathway. However, under commercial conditions, the 'loss' of carbon to biomass production is estimated at 5% of the sugar mass and a further 7.5% is estimated to be lost as a result of the production of other chemicals (fuel oils, glycerine, acetic acid, esters, *etc*.). In addition, 1.5% is lost during distillation (Energy Authority of NSW, 1986), and a further 3% is lost during the juice extraction process, either in the bagasse or in the filter mud. Finally 48.9% is lost as CO_2. Therefore, the total amount of sugar that ends up as ethanol on a mass basis is [100-(48.9+3+1.5+7.5+5%)] = 34.1%. The specific gravity of ethanol is 0.789; therefore, 1 g of sugar in sweet sorghum stems will produce 0.432 cc ethanol (0.341/0.789), if used directly for ethanol production. Given that sweet sorghum may be expected to produce 12% sugars (stem fresh weight basis) and a yield of 60 t stems ha^{-1}, an ethanol yield of (60*0.12*0.341 = 2.46 t EtOH) = 3100 litres (819 gallons) ha^{-1} will be produced. Sweet sorghum derived ethanol would be competitive and cheaper than the imported cost of gasoline and the ethanol has a greater value on the world markets. It was expected that the market for biologically derived ethanol to expand from today's level of production as discussed below. In 1998, a total of 8.6 billion gallons of ethanol were produced globally, of which 60% (5 billion gallons) was derived from sugar crops *i.e.* sugarcane and sugar beet. A further 33% (2.8 billion gallons) was produced from grain crops, and the remaining 7% (0.6 billion gallons) from synthetic sources, primarily natural gas. In the U.S., corn is suggested

as a prime candidate for biofuel production, but more ethanol can be produced from an acre of sweet sorghum than an acre of corn. The total ethanol production in 2006 was estimated at about 5.4 billion gallons which is equivalent to 3% of the total U.S. gasoline consumption; to raise ethanol use to 10% in gasoline nationwide would require almost the nation's entire corn crop. Hence there is a great scope for biofuel industry to produce ethanol in near future; at that time, technology for maximizing sweet sorghum stem growth would be of immense use.

Sweet Sorghum is a valuable source of biofuel

The greatest features to use of sorghum for fuel is the presence of two different traits: sweet sorghum is an attractive bioenergy crop that would allow significant increases in the sugar accumulation in the stems and brown midrib (*bmr*) which results in reduced lignin levels for biofuel production.

Sweet sorghum is one of the many types of cultivated sorghum and is highly adaptable cereal crop, which when coupled with its large genetic variability, contributes to its ability to rapidly provide efficient biofuel production from grains, sugar-based and biomass feedstock. The sweet sorghum stalk contains approximately equal quantities of soluble (glucose, fructose and sucrose) and insoluble carbohydrates (cellulose and hemicellulose) (Jasberg et al., 1983). Their juice contains a great quantity of 13-20 per cent total fermentable sugars that can be easily fermented and thus provides a better source of carbohydrates for the production of fuel ethanol (Woods, 2001). Sweet sorghum provides high biomass yields, which is essential for good economic and energy returns. However, unless key biomass "quality" thresholds are attained, sweet sorghum may be too difficult to process without major modifications. These key "quality" parameters are polarity, brix, sucrose purity and preparation index. Under good conditions, sweet

sorghum can outperform corn in terms of total biomass production over short periods. Sweet sorghum's rapid growth and ability to reach maturity in 3 to 5 months, with photo-insensitive character are favorable for its production. High-yielding varieties have now been developed that are capable of producing well over 60 tonnes per ha (fresh weight of above-ground biomass) in 5 months under good agronomic conditions. It is among the most efficient crops requiring less fertilizer, among the crops most tolerant of drought (Mastrorilli et al., 1999), and can be successfully grown in semi-arid regions, making it an efficient user of water (310 kg of water/kg of dry matter) under those circumstances. It has a strong root system and the epidermis of the root is covered by a layer of heavy disilicic till the root grows to ripening and thus makes it still has an enough mechanical intensity during the drought period to prevent the root system to collapse (Li Dajue, 1997). It was observed that although photosynthesis was slightly affected by drought, sweet sorghum juice quality was not affected (Massaci et al., 1996).

Sweet sorghum is a kind of crop with two sinks. Stalk and panicle grew together from the stem elongation in sweet sorghum. Stalk was the main centre of growth but panicle was the secondary one from the elongation to the heading. The panicle became the main centre of growth but stalk was the secondary one after heading. The two kinds of growth matched side by side from the elongation to the maturity at which time the amount of the accumulation of dry matter in stalk and panicle rose to the maximum value simultaneously. Therefore, sweet sorghum was in the state of simultaneous growth of the vegetative and reproductive organs, which lasted above 80 days that accounted for 60% of whole duration of development. Because of the longer time of the growth of stalk, the higher rate of distribution of dry matter to stalk, the final ratio of dry matter among the different organs was stalk> panicle > leaf> sheath (Djanaguiraman, et al., 2005).

Source leaves are the primary site of photoassimilate production, but the plant faces a dilemma with respect to allocation choices for photoassimilate. Many tightly regulated metabolic steps control the accessibility of photosynthetically fixed carbon to the phloem transport system. Control at the source end is governed largely by rates of photosynthetic incorporation of CO_2. A typical higher plant has a myriad of sink tissues that depend on the source leaves for photoassimilates. Recent studies indicated that reproductive sinks represent only a small proportion of potential sinks on a plant (Ma Hongtu and Hua Xiuying, 1986). Hence, there is a scope to improve the vegetative sink (stalk in sweet sorghum). The vegetative sinks, have the unique property of being able to act both as sinks for assimilates and as sources of assimilates for phloem transport, depending on the carbon needs of the plant at a particular growth phase, unlike the terminal sinks (seed) act as sinks only for assimilates. Carbon partitioned to terminal sinks is unavailable for remobilization out of those sinks. Studies indicate that assimilates in the stems start accumulating during their development of the inflorescence. During this period there is no competition between grain development and sugar accumulation. Before anthesis, stem becomes the preferential sink, accumulates more sugar at the expense of growth of apical internodes (Djanaguiraman et al., 2005) and foliar spray of nutrients and PGRs during peak vegetative stage and after anthesis enhanced the source activity enabling the elongation of stem and sugar yield.

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 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 preanthesis head development (Willey and

Basiime, 1973). In sweet sorghum, the sugar mainly sucrose, is accumulated in large amounts in the stem during the development of the inflorescence, when the panicle has formed and is emerging from the boot. During this period there is no competition between grain development and sugar accumulation (Lingle, 1987). Sucrose in the stem may increase or remain constant between the soft dough and the ripe stage of the grain, depending on variety. However, distribution of sugars, starch and acid is not uniform throughout the sweet sorghum stalks (Broadhead, 1972). The four top internodes representing about 18% of the stalk weight are higher in starch, titrable acidity and sucrose than the remainder of the stalk. Internodes near the ground level are higher in invertable sugars (Coleman and Stokes, 1964).

Broadhead (1973) and Ferraris (1981) observed that deheading sweet sorghum increased brix, sucrose and starch, but stems contained less juice than normal plants. In addition, Ferraris (1981) also observed that leaves of deheaded plants remained green for longer and the stems were less prone to lodging. Tillering in sweet sorghum could be profitable if all the tillers produced by crown buds developed to maturity. This would mean an increase in the number of stems and prolonged harvesting period, since, the main shoot matures earlier than tillers. Tillering is also useful in that the roots that develop from the basal nodes lend physical support to the plant and reduce root lodging. However, not all the tillers develop up to marketable size. At high population densities some tillers grow tall and thin and others die due to competition, constituting a loss in economic yield (Ferraris, 1988).

Genetic diversity in sweet sorghum

Sorghum (*Sorghum bicolor* (L.) Moench) is one of the crop species that can survive the harsh climatic conditions of the arid environments (Ritter et al., 2007). *Sorghum bicolor* contains both

cultivated and wild races and possess a significant amount of genetic diversity for traits of agronomic importance (Hart et al., 2001). It is used as a source of grain food, syrup fuel, and feed for livestock. Sweet sorghum, a type of sorghum accumulate high levels of sucrose in the parenchyma of their juicy stems (Murray et al., 2009; Vietor and Miller, 1990), and has recently received attention as a source of biofuel (Rooney et al., 2007). In the US, sweet sorghum has been researched for biofuel for more than 30 years (Lipinsky, 1977), with primary research, development and breeding starting in the late 1970s (Murray et al., 2009) because of the high oil costs and need for alternative energy sources. Under favorable conditions, sweet sorghum is capable of producing up to 13.2 metric tons per hectare of total sugars, which is equivalent to 7682 liters of ethanol per hectare (Murray et al., 2009). Sweet sorghum gene pool creation had not received much attention mainly because it was not considered to be among important crops in the US, and the pedigree information is scarce and incomplete. Most sweet sorghums released in the US were developed by public breeding programs in the 1900s and are mainly open pollinated cultivars (Swanson and Laude, 1934). The improvement was done mainly on syrup, sugar concentration and biomass, with lines primarily selected for improved disease resistance (Murray et al., 2009). Sweet sorghums were introduced to the US as landraces from Africa and China in the 1850s (Murray et al., 2009), and other cultivars were developed later, some with unknown origin. Genetic diversity or knowledge on patterns of diversity of genetic resources, is of great importance (Warburton et al., 2008), and is a key component in crop improvement and plant breeding. The Meridian, Mississippi Station tried curating what may be the world sweet sorghum collection, and when it closed, materials were transferred to the USDA sorghum collection in Griffin, GA (Freeman, 1979). Thus many diversity studies have concentrated on cultivars/lines that are common and known, leaving the vast majority of the collection (genetic

sources) unexploited. In this study we tried to incorporate both the commonly used lines together with rarely used lines, and accessions from other sorghum collections.

Evaluation and characterization of germplasm are the pre-requisite for the utilization of the available diversity in the crop improvement programme. In sweet sorghum, the most useful traits for selection are plant height, stem diameter, brix and juice yield (Ali et al., 2008; Murray et al., 2009). However, these traits are quantitative and polygenic inheritance in nature and are complex to be manipulated directly in breeding procedure (Zou et al., 2011). Therefore, to successfully improve these complex traits, they need to be dissected into smaller morphological, physiological and yield components. It was previously reported that significant difference in brix was observed in the U.S sweet sorghum germplasm (Ali et al., 2008). Genotypic differences for brix and plant height have also been reported in a panel of 125 sweet sorghum collections (Murray et al., 2009). Studies also show much variability exists in sugar content and juice yield among the U.S sweet sorghum collections (Murray et al., 2008a; Makanda et al., 2009). However variation in growth (plant height and stem diameter), physiology (chlorophyll content and Fv/Fm) and components of stem sugar (°brix, juice yield and stem fresh weight) among sweet sorghum germplasm is less investigated than other aspects. Furthermore, correlations between the traits are of great importance in selection process for successful breeding programs.

In sweet sorghum, stalk yield has significant positive correlations with plant height, stem diameter and juice yield (Audilakshmi et al., 2010) and also a strong association of sugar yield with brix was noticed (Pfeiffer et al., 2010). Therefore, selection for stalk yield should be focused on plant height, stem diameter, brix and juice yield. Studies have shown that there was significant negative correlation between grain yields and stem biomass which might eventually lead to reduced sugar yield (Makanda et al., 2009). However, there are no studies that showed

direct correlation between physiological traits and sugar yield. The relationships between morpho-physiological and stem sugar yield traits are important. Sugar yield is a quantitative trait, which is the resultant of various traits contributing together during the crop growth, which are interdependent in their development. It is, desirable to study the association between yield and yield attributing traits since this would facilitate effective selection for simultaneous improvement of one or more yield influencing components.

Timing of harvest in sweet sorghum

The time of harvesting and determination of maturity of sweet sorghum are very crucial in obtaining sweet sorghum with high sugar content and juice yield. Since ethanol can be obtained from juice sugar content, therefore identifying the best stage of harvesting and determining maturity could be beneficial in obtaining high ethanol yield.

The maturity of sweet sorghum can be classified as early-flowering, flowering, late-flowering, early-milk, late-milk, soft-dough, hard-dough, and ripe (Bitzer and Fox, 2000). Sucrose is accumulated in large amounts in the stem during inflorescence development (McBee and Miller, 1982). Hence, there may be a competition for carbohydrates between stem and developing seed. At maturity the sugars (reducing and non-reducing sugars) were mobilized from stem and leaves to grains. The amount of assimilates allocated for sucrose biosynthesis in the stem and grain depends on partitioning. Much of the work regarding carbohydrate production from early stages of growth to maturity has been reviewed by many researchers (Mcbee and Miller, 1982; Ghatode et al., 1991; Hoffman-Thoma et al., 1996). The results showed that the total sugar content varied as the crop approaches maturity. The different stages of maturity also affect the sugar content of sweet sorghum's stem juice. The juice sugar content depended on the plant stage of development, because at the early development stage, fructose is more abundant, whereas

sucrose is dominant after heading (Sipos et al., 2009). At maturity, the sweet sorghum juice sugar content ranged from 10 to 25° Brix (Reddy et al., 2007a). Many studies have already reported that sugar accumulation in the sweet sorghum stalk juice starts from booting stage. Webster et al. (1948) showed a minimal change in total sugar content up to heading, while Hermann (1942) reported that the sugar content increased until head formation. McBee and Miller (1982) found that sucrose increased from the preboot to anthesis. Lingle (1987) reported that sucrose concentration in the stalks increased 7 folds between boot and mid-grain filling stages. Ghatode et al. (1991) noticed that the brix, reducing sugar and non-reducing sugar in the juice decreased when plants were harvested at maturity or ripe stage.

Hills (1990) suggested that the sugar content in the sweet sorghum stalk increases between the milk stages and dough stages for most cultivars. It then starts to decline towards the physiological maturity. Sugar in sweet sorghum begins to accumulate during the early stage of plant development. At the beginning of the harvest, the sugar concentration in sweet sorghum's stem juice is approximately 12.5° Brix and as sweet sorghum reaches maturity the sugar concentration increases up to 17° Brix (Prasad et al., 2007).

Almodares et al. (2007) has reported that during flowering, the sugar content is lowest. This is mainly because of the presence of high acid invertase enzyme during the flowering stage. Hills (1990) reported that for most cultivars, sugar concentration in sweet sorghum's stalk juice starts to increase during the milk stage to the soft dough stage of the seed and then decreases as the seeds become more mature. Hunter and Anderson (1997) cited that sugar content of sweet sorghum's stalk juice is almost double between the dough stage and physiological maturity compared to the sugar content between the milk and dough stages. There was no investigation on

ideal harvesting time of sweet sorghum var M81E for higher sugar and juice yield. Hence, it is important to determine the optimum harvesting time of the sweet sorghum stalk.

Effect of water stress (Drought) in sweet sorghum

Drought stress, one of the multiple environmental stresses, affecting crop productivity and accounting for more than a 50% reduction in yields worldwide (Boyer, 1982). The Great Plains within the United States, including Kansas are suitable for the cultivation of sweet sorghum, but the weather in this region has changed substantially over the last 30 years, which indicates that appropriate agronomic practices need to be identified and recommended for economically productive cultivation of the crop. Although there has been a reduction in rainfall frequency due to uneven precipitation being experienced in the region, the change in weather suggests that extreme conditions like heavy rainfall, heat waves and drought can be expected, particularly in these areas. Water resources are already stressed in the region and the weather projections for the next 7-8 decades indicate that drought will be the major factor affecting most facets of agriculture in these regions.

Generally, sweet sorghum is grown in the semi-arid regions of the world. In these regions, optimum irrigation is vital for maximizing crop yield's because decreasing water supply causes a significant reduction in sorghum biomass and sugar yield (Habyarimana et al., 2004; Vasilakoglou et al., 2010). Ability to produce consistent fermentable sugars under variable environmental factors is necessary to harness sweet sorghum as a potential source of biofuel. The sucrose content in the crop is highly dependent on the environmental conditions, especially during the reproductive, ripening stage as this is the prime factor which determines the actual levels of sugar recovery upon harvest as well as the potential for its exploitation for industrial alcohol production. Also, little is known about the photosynthetic characteristics in sweet

sorghum under drought stress. Mastrorilli et al. (1999) found that temporary soil water stress in sweet sorghum significantly reduced water use efficiency at the early stage, but it had no significant effect on water use efficiency at the late stage. Severe drought stress caused photoinhibition of sweet sorghum and decreased water use efficiency and stem biomass (Tingting et al., 2010).

Drought stress affects various physiological processes such as leaf temperature, leaf chlorophyll, chlorophyll a fluorescence (Fv/Fm), stomatal conductance, transpiration and photosynthesis in various crops (Silva et al., 2007). In studies on sugarcane (*Saccharum officinarum* L.), stem diameter (Da Silva and Da Costa, 2004), and stalk height and cane yield (Inman-Bamber and Smith, 2005) were severely affected by drought conditions. Drought also resulted in morphological changes in sugarcane, which included reduced leaf area, thicker leaves, less responsive stomata and increased ratio of roots to shoots (Hussain et al., 2004). Sugarcane yield decreases by 29.2% and 18.1% respectively in severe and moderate drought stress conditions (Hussain et al. 2004). Drought stress experiments on sugar beet (*Beta vulgaris* L.) have shown adverse effects on both leaf photosynthesis as well as sucrose yields in the mature plants (Monti et al., 2006). They also reported that drought stress in the early growth period was negatively associated with the sucrose content at maturity. Tognetti et al. (2002) observed that optimum irrigation is the key to have higher sugar yields for sugar beet cultivation in semi-arid Mediterranean terrains.

In sweet sorghum, plant height, stem diameter, stem fresh weight, juice yield, brix and stem sugar contents are the most important characteristics for biofuel production (Murray et al., 2008, Pfeiffer et al., 2010). The above established characteristics were obtainable only under optimal irrigation conditions (Vasilakoglou et al., 2010). There are no systematic studies describing

sensitivity of reproductive stage of sweet sorghum to drought stress. Further improvement of drought tolerance in sweet sorghum is still a need for improved biofuel production efficiency. Identification of the most suitable genotypes which are unaffected by drought during the reproductive stages as far as their juice and sugar yields are concerned, as it is this crucial stage for the exploitation of this crop as a potential biofuel source.

Effect of combination of stresses (Drought and High temperature) in sweet sorghum

Drought and high temperature, often occur simultaneously, are important environmental factors restricting plant physiological processes and thereby plant growth (Shah and Paulsen, 2003). Global climate change for instance contains to bring a new reality of environmental effects, presumably increases in global temperature, uneven precipitation, and severe drought in arid and semi-arid areas, on crop productivity (Wigley and Raper, 2001; Chaves et al., 2003). Most studies thus far have focused on crop response to drought and high temperature singly, and few studies have focused on combination of these two stresses. For example, drought and high temperature caused detrimental effects on wheat (*Triticum aestivum* L.), sorghum, barley (*Hordeum vulgare* L.) and various grasses (Savin and Nicolas, 1996; Machado and Paulsen, 2001; Shah and Paulsen, 2003; Xu and Zhou, 2006). However, studies on the effect of these two environmental stresses either singly or in combination is scarce in sweet sorghum.

Drought stress caused significant impact on various sugar yielding crops affecting their yield potentialities. In sugarcane (*Saccharum officinarum* L.), cane yield was decreased by 29.2% and 18.1% respectively in severe and moderate drought stress conditions and led to morphological changes such as reduced leaf area, thicker leaves, less responsive stomata and increased ratio of roots to shoots (Hussain et al., 2004). Drought experiments on sugar beet (*Beta vulgaris* L.) have

shown adverse effects on leaf photosynthetic activities and sucrose yields in mature plants (Monti et al., 2006). Drought stress resulted in reduced root dry weight, leaf water potential and photochemical efficiency in many grass species (Aronson et al., 1987; Carrow, 1996; Perdomo et al., 1996; Huang et al., 1998a).

High temperatures have negative effects on most crops in various ways (Schaffert and Gourley, 1982). Most crops grow well at optimum temperatures which mainly correspond with the optimum photosynthesis levels. High temperatures affect photosynthetic processes (Al-Khatib and Paulsen, 1984) with increased sensitivity of photo-system (PS) II (Xu and Zhou, 2006). High temperature stress causes thylakoid membrane damage and further down regulates PS II photochemistry which led to increased proportion of closed PS II reaction centers (Grove et al., 1986). In addition, leaf chlorophyll degradation is highly correlated with high temperature (Prasad et al., 2009). High temperature stress also causes leaf temperature to rise above air temperature by decreasing transpirational cooling and thus, make the plant more susceptible to photoinhibition (Falk et al., 1996).

Recent studies revealed that plant response to a combination of drought and high temperature is uniquely differently from the effect of individual stress conditions (Rizhsky et al., 2004). While drought remains the single known environmental factor that directly affects plants water status, the severity of drought and high temperature combination is enormously dependant on the prevailing temperatures. Ludlow et al. (1990) reported that combined stresses of drought and high temperature significantly reduced grain yield in sorghum. In addition, combined effects of drought and high temperature strongly affected water relations of both wheat and sorghum (Machado and Paulsen, 2001). As the combined effect of these two stresses are distinct in reality to independent stress effects in other crops, the relationship between drought, high temperature

and their combinations against sugar accumulation in sweet sorghum needs thorough understanding.

In sweet sorghum, the most important traits for biofuel production are plant height, stem diameter, stem fresh weight, juice yield, brix and stem sugar contents (Murray et al., 2008; Pfeiffer et al., 2010) and are determined by the efficient physiological behavior of the plant under different environmental conditions. Previous studies showed that plant height is highly correlated to juice yield and stem fresh weight (Murray et al., 2008). There is also a significant linear correlation between brix and total sugar content of the juice (Audilakshmi et al., 2010). However, optimal growing conditions ensure better plant growth without affecting physiological functions to produce sustainable juice and sugar yield in sweet sorghum (Vasilakoglou et al., 2010).

Sweet sorghum varieties differ in their ability to produce and store sugar in stem (Ali et al., 2008). Mostly, sugar accumulation in stems takes place during inflorescence development (McBee and Miller, 1982) and is accelerated after post anthesis (Prasad et al., 2007; Almodares et al., 2008). Environmental factors such as temperature and water level may greatly determine juice quality and amount. Even though sorghum can withstand moderate high temperatures and drought, occurrence of either drought or high temperature or their combination during early grain filling (milk) stage were not thoroughly studied for their effects on growth, physiology and yield. It is important to understand these effects to predict bioenergy components and selection of genotypes suitable for cultivation under varying stress environments.

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Chapter 2 - Characterization of sweet sorghum (*Sorghum bicolor* L. Moench) germplasm for growth, physiological and bioenergy traits under irrigated and rain-fed conditions

2.0 Abstract

Sweet sorghum (*Sorghum bicolor* L. Moench) is emerging as an important bioenergy crop. The value of the crop as biofuel feedstock is affected by a number of inherent morphological and physiological traits. The objective of this research was to characterize sweet sorghum genotypes and determine genetic variability in traits associated with biofuel production under rain fed and irrigated conditions over two years. Chlorophyll content, photosystem (PS) II photochemical efficiency, (Fv/Fm) at flowering and biomass, juice yield and sugar content at maturity were measured. Rain-fed performance was assessed based on the relative sugar yield reduction in each genotype compared to its performance under irrigated condition. Genotypes showed a large and significant variation for juice, brix and sugar yield and physiological traits (leaf chlorophyll content and Fv/Fm). Mean brix ranged between 6.2 and 20.7%, juice yield between 124.7 and 914.2 (g plant^{-1}); while sugar yield ranged from 17.0 to 118.3 (g plant^{-1}). Compared with irrigated condition, mean values for these traits under rain-fed trials were reduced by 31.7, 34.5 and 62.3%, respectively. The top 27 entries had sugar yield higher than the Mean+2SE. There was positive and significant correlation between sugar yield and plant height, stem diameter, PS II photochemical efficiency (Fv/Fm), juice yield, stem fresh weight and stem dry weight. Overall, genotypic variability was measured in brix and sugar yield can be utilized for the development of sweet sorghum hybrids.

2.1 Introduction

Dwindling fossil fuel reserves and growing demand for energy have resulted in increasing fuel prices, and a search for alternative energy sources such as biofuels. The ideal source for biofuel would be biomass based substances that does not compete with food production. Sweet sorghum (S*orghum bicolor* L. Moench) is a biomass crop grown in the tropical and sub-tropical regions of the world. Sweet sorghum has high sugar content in the parenchyma of stems, which can be fermented for ethanol production (Vietor and Miller, 1990; House et al., 2000). Ethanol can also be produced from dry biomass (cellulose and hemicelluloses) and the grain of sweet sorghum (Habyarimana et al., 2004). Hence, sweet sorghum is becoming a popular energy crop throughout the world (Mastrorilli et al., 1999; Rooney et al., 2007; Vermerris et al., 2007).

Collection and characterization of germplasm is the foremost step in building a gene pool for crop improvement. Development of suitable genotypes is primarily based on the breadth of this pool and the ability to identify sources with the desired traits (Natoli et al., 2002; Ritter et al., 2008; Makanda et al., 2009). In sweet sorghum, the most useful traits for selection are plant height, stem diameter, brix and juice yield (Ali et al., 2008; Murray et al., 2009). It was previously reported that differences in brix were observed in the U.S sweet sorghum germplasm (Ali et al., 2008). Genotypic differences for brix and plant height have also been reported in a panel of 125 sweet sorghum collections (Murray et al., 2009). Earlier studies have indicated that high ethanol production from sweet sorghum could be achieved through increased sugar yield, which has a strong association with stem fresh weight, brix and juice yield (Ravi et al., 1996; Pfeiffer et al., 2010). Recent studies have indicated that sweet sorghum genotypes with taller height and wider stem diameter may have higher sugar yield (Pfeiffer et al., 2010). Despite these and the growing role of sweet sorghum as biofuel feedstock, information on the extent of variation in growth (plant height and stem diameter), physiology (chlorophyll content and

Fv/Fm) and components of stem sugar (brix, juice yield and stem fresh weight) among sweet sorghum germplasm are limited.

Sorghum is a C_4 plant, which has higher water-use efficiency than other grain or sugar crops under both irrigated and drought conditions (Steduto et al., 1997; Gnansounou et al., 2005) and requires relatively low inputs (water and nitrogen) (Mastrorilli et al., 1999). Reports on sugar yielding crops indicated that drought stress decreased stem height, stem diameter, stem biomass and sugar yield in sugarcane (*Saccharum officinarum*) (Da Silva and Da Costa, 2004; Silva et al., 2008) and decreased the leaf and root growth and sugar accumulation in sugar beet (*Beta vulgaris*) (Bloch and Hoffmann, 2005; Hoffmann, 2010). In sorghum drought stress had no influence on total stem sugar accumulation despite decreases in photosynthetic rate (Massacci et al., 1996). However, the impact of drought stress on juice yield is not well documented in sweet sorghums. Thus, identification of suitable genotypes that are capable of accumulating higher juice and sugar yield under drought stress is important task for achieving greater bioethanol production.

We hypothesize that genetic variability exists among sweet sorghum genotypes for brix and juice yield and drought stress decreases both traits. The objectives of this research were (i) to quantify genetic variability for plant height, stem fresh weight, brix, juice yield and sugar yield in sweet sorghum germplasm and (ii) to identify potential drought tolerant sweet sorghum genotypes.

2.2 Materials and Methods

2.2.1 Plant materials

A total of 280 sweet sorghum genotypes were obtained from Plant Genetic Resources Conservation Unit (PGRCU), Griffin, Georgia. These genotypes were originally collected from

diversified origins including Africa, Asia, Australia, Europe, South America and North America and were part of the US historic sweet sorghum collection (Wang et al., 2009). Their identifiers and place of origins are shown in Table 2.1

2.2.2 Experimental site and environmental conditions

All 280 germplasm were evaluated in three environments during 2007 (irrigated) and 2008 (irrigated and rain-fed) at Ashland Bottoms Research Farm near Manhattan, Kansas (Irrigated - 39°08′35.3″N, - 96°37′39.2″W, Altitude: 308 m; Rain-fed - 39°06′54.2″N, - 96°38′10.0″W, Altitude: 323 m). Experiments were conducted on a chase silty clay loam soil (clay 12%, silt 60% and sand 28%; and pH 6.8). The weather parameters during the test seasons are presented in Fig. 2.1.

2.2.3 Crop husbandry

The experimental plots were chisel plowed and planted on 18 May, 2007 and 21 May 2008. In both years plots were fertilized with 90 kg N ha^{-1} as urea. For weed control, the plots were sprayed with Bicep Lite II Magnum (a.i. 0.82 kg atrazine ha^{-1} and 1.03 kg S -metolachlor ha^{-1}) prior to planting. The fields were kept weed free by hand weeding as necessary. In 2007, the experiment was conducted under irrigated condition. Each genotype was evaluated in single row plots of 3 m length with a row spacing of 0.75 m. In 2008, the same 280 sweet sorghum genotypes were planted under two growing conditions (irrigated and rain-fed) with a single row of 3 m length and 0.75 m row spacing. Due to large number of genotypes being evaluated, multiple replications within each environment were not used.

2.2.4 Data collection

Prior to flowering, two random plants for each genotype were tagged using colored tape. All data were collected from these tagged plants. Physiological traits were recorded on attached fully

expanded flag leaves every 15 d beginning at flowering stage. All measurements were taken at midday (between 10:00 and 14:00 h). Leaf chlorophyll content was measured using a self-calibrating chlorophyll meter [Soil Plant Analytical Device (SPAD), Model 502, Spectrum Technologies, Plainfield, IL, USA]. Chlorophyll a fluorescence parameters were measured using pulse-modulated fluorometer (OS5p, Optisciences, Hudson, NH, USA). The photochemical efficiency of photosystem II (PSII) (Fv/Fm) was measured in 30-min dark-adapted leaves (Prasad et al., 2008).

At maturity, growth and yield parameters were measured on tagged plants of each genotype. Plant height was measured as the length of the plant from base of the stem to the tip of the panicle; stem diameter was measured from three regions of the stem bottom (3rd internode), middle (6th internode), and the top (9th internode) using vernier caliper after stripping the leaves and removing leaf sheaths. Data on stem diameter were averaged across regions. The juice from the stalks was extracted and used for recording brix percentage by using digital hand-held refractometer (Digital hand-held pocket refractometer PAL-1, Atago, Bellevue, WA, US). The fresh weight of stems of each sample was recorded and then oven-dried at 60°C for 7 d and dry weight was recorded. The juice yield was obtained by subtracting stem dry weight from stem fresh weight and expressed as g plant^{-1}. The sugar yield was calculated as a product of brix (%) and juice yield.

2.2.5 Data analyses

The experimental design was randomized complete block design. Two plants for each genotype were selected randomly during flowering for recording physiological and yield traits. Growth and physiological traits were recorded in 280 genotypes and means were presented with standard deviation for mean comparison. Among the 280 genotypes, 78 genotypes consistently obtained

juice at harvest in all three environments. Hence, brix and sugar yield were measured and compared in 78 genotypes. The various observations recorded in 78 genotypes from each experiment were analyzed using the Proc GLM procedure of Statistical Analysis Systems, 9.1 (SAS Institute, 2003) using environments and years as replications. To assess the impact of drought stress on various traits including relative sugar yield reduction (RSYR) the mean of irrigated experiment (2008) were used and compared with rain-fed (2008) and expressed as percentage. Genotypes were ranked based on values of RSYR. Pearson's phenotypic correlation coefficients between traits measured were computed using PROC CORR procedure in SAS.

2.3 Results

2.3.1 Genotypic variation

2.3.1.1 Physiological traits

There were differences among genotypes for many of the traits measured (Table 2.2). Leaf chlorophyll content and Fv/Fm ranged from 37 to 63 and 0.413 to 0.810, respectively (Table 2.2). The mean chlorophyll content and Fv/Fm value among the entries were 52 and 0.748, respectively. There was marked variation for both traits that the values ranged from 37 to 63 for leaf chlorophyll content and from 0.413 to 0.810 for Fv/Fm. The highest score for chlorophyll content was recorded in genotype Sugar drip_2 followed by Brawley, Inyagentombi, Mbalwe, Rahmetalla gallabat and IS 12900. While, the lowest chlorophyll content was recorded in MN 2063, followed by MN 1540, Co 1, Opemba nonpha, MN 2109 and MN 2762 (Table 2.3). Thirty two genotypes had mean leaf chlorophyll content values higher than the overall mean plus two standard errors (Mean+2SE). The genotypes, Awanlek, MN 2762, Theis, MN 4564, Saccaline, MN 2363 and Smith had the highest Fv/Fm values (0.79 to 0.78), while genotypes, IS 2131,

Wenabu, Manyoble, Atlas, Masuda Black Seed and No. 8 Gambela had the lowest (0.66 to 0.72) (Table 2.3). Thirty two genotypes had higher than mean+2SE value for Fv/Fm.

2.3.1.2 Growth traits

Significant differences were observed among the genotypes for all the growth traits (Table 2.3). The plant height ranged from 93 to 440 cm and stem diameter ranged from 8 to 27 mm (Table 2.2). The mean plant height and stem diameter was 278 cm and 17 mm, respectively. Plant height, stem diameter, stem fresh weight and stem dry weight differed among the genotypes that consistently produced juice across three environments (Table 2.3). Among the 78 genotypes, the plant height ranged from 153 to 395 cm with a mean of 290. Genotypes, IS 2109, MN 4553, MN 4564, Caxa, Isidomba_2 and Dale_1 were among the tallest. Whereas, Nagad el Mur, HC 41-13, Ames amber, Darso 28, Bargowi and Atlas were some of the shortest plants (Table 2.3). About 34 and 31 sweet sorghum genotypes were found to have a value greater than Mean+2SE and Mean-2SE, respectively.

Stem diameter ranged between 27 and 11 mm with an overall mean of 18 mm (Table 2.3). Genotypes MN 2238, MN 2063, MN 4564, MN 2386, Co 1, MN 4553 and Opemba nonpha had the highest stem diameters. The genotypes HC 41-13, Ames amber, Mbalwe, N 111, Red amber, MN 2894 and Collier had recorded the lowest stem diameter. Twenty nine genotypes had stem diameter greater than Mean+2SE.

Stem fresh weight (g plant^{-1}) varied from 174 to 1190 with a mean of 606 (Table 2.3). The genotype MN 4564 recorded the highest stem fresh weight followed by MN 4566, MN 4553, Wray, Co 1 and MN 2109 (Table 2.3). The lowest stem fresh weight was recorded in Ames amber and followed by Darso 28 and HC 41-13, MN 2894 and Luel. The stem dry weight (g plant^{-1}) ranged from 49 to 334 with a mean of 170 (Table 2.3). Genotypes Caxa, Wray, MN

4564, MN 4553 and MN 4566 recorded the highest stem dry weight and Ames amber, Darso 28, Mbalwe, and Luel recorded the lowest stem dry weight (Table 2.3).

2.3.1.3 Sugar quality and yield traits

Significant differences among genotypes were found for sugar quality and yield traits (Table 2.3). Across 78 genotypes, brix ranged from 6 to 21 with a mean of 13 (Table 2.3). High brix greater than Mean+SE and greater than Mean+2SE was observed in 1 and 45 genotypes, respectively. The genotypes Dura huria, Masuda black seed, Smith, Leoti-Peltier, Tracy_2 and Top 76-6 had high brix (18 to 21) and Sairwa, Iswa, MN 1540, MN 2386, MN 2238 and MN 2363 had low brix (6 to 7) (Table 2.3).

The juice yield (g plant^{-1}) varied from 125 to 914 with a mean of 436 (Table 2.3). Juice yield of greater than Mean+2SE was recorded in thirty genotypes. Genotypes, MN 4566, MN 4564, MN 2109, Co 1, MN 4553, MN 2238, Wray and Sanyagie were among the highest juice yielders. Whereas, Ames amber, HC 4113, MN 2894, Luel, N 111 and Darso 28 were the lowest juice yielders (Table 2.3).

The mean sugar yield (g plant^{-1}) was 56 ranged from 17 to 118 (Table 2.3). Four and 27 genotypes had sugar yields higher than the Mean+SE and Mean+2SE, respectively. Genotype Wray gave the highest sugar yield followed by MN 4564, Caxa, IS 2131, Top 76-6, MN 4553 and Smith (Table 2.3). Whereas, genotype Ames amber had the lowest sugar yield followed by IS 2352, Luel, MN 2894 and HC 41-13 (Table 2.3).

2.3.2 Impact of drought stress

2.3.2.1 Growth, physiology and yield traits

Drought stress appeared to have marked effect on the performance of genotypes as indicated by difference in mean values of various traits measured under rain-fed and irrigated conditions.

Traits such as plant height, stem diameter, chlorophyll content and Fv/Fm were not affected by water supplies as indicated by the minor differences between irrigated and rain-fed conditions (Table 2.4). However, under rain-fed condition brix (37.0%), stem fresh weight (33.7%), stem dry weight (32.9%), juice yield (34.1%) and sugar yield (65.3%) were all reduced compared with irrigated condition (Table 2.4).

2.3.3 Relative sugar yield reduction (RSYR)

The RSYR (%) ranged from 22 to 98 with a mean of 76±16 (Table 2.3). The lowest RSYR was observed in Sanyagie followed by MN 818, Dale_1, Smith, Wray and Caxa (Table 2.3). Genotypes, MN 2386, Ames amber, Luel, MN 2238, Atlas and Iswa had the highest RSYR (>93%) (Table 2.3). There were 23 genotypes that recorded low RSYR (<72%) (Mean-2SE).

2.3.4 Correlation analyses

A positive and significant phenotypic correlation was observed between sugar yield and plant height (Fig. 2.2a), stem diameter (Fig. 2.2b), and PS II photochemical efficiency (Fv/Fm) (Fig. 2.3a). Likewise, various sugar yield traits like juice yield (Fig. 2.3b), stem fresh weight (Fig. 2.3c) and stem dry weight (Fig. 2.3d) were also positively and significantly correlated with sugar yield.

2.4. Discussion

Sweet sorghum genotypes were studied for their sugar yields in irrigated (2007 and 2008) and rain-fed (2008) conditions. There were large variations for plant height, stem diameter, chlorophyll content, Fv/Fm, stem fresh weight, stem dry weight, brix, juice yield and sugar yield among the sweet sorghum genotypes (Table 2.3). Significant genotypic variability for plant height and juice brix among sweet sorghum germplasm was also reported by Almodares et al. (1997), Ali et al. (2008), Murray et al. (2008a), Wang et al. (2009) and Murray et al. (2009).

Plants with greater plant height, stem diameter, Fv/Fm, stem fresh weight and juice yield also produced greater sugar yields (Fig. 2.2 and Fig. 2.3). Similarly, Audilakshmi et al. (2010) reported stem fresh weight to be correlated to plant height, stem diameter and juice yield. Murray et al. (2008) found that plant height was highly correlated with juice yield and stem fresh weight. Pfeiffer et al. (2010) reported that male-sterile sweet sorghum hybrids produced greater stem yield due to taller plants with greater stem diameter. There was significant positive association between stem biomass and plant height and stem diameter of sweet sorghum (Makanda et al., 2009).

Genotypes with higher stem fresh weight produced higher amount of juice that can be immediately fermented to bioethanol. Among the sweet sorghum genotypes studied, Wray had the highest sugar yield which was attributed to increased juice and moderate brix. This genotype also showed minimum reduction in sugar yield when grown under rain-fed condition. This indicates that Wray, besides its desirable biofuel potential, also has greater drought resilience compared with other genotypes. Even though genotype MN 4566 had highest juice yield, its sugar yield was low because of lower brix. Genotypes with moderate brix and high juice yield produced high sugar yields (Table 2.3).

The sugar quality and yield traits in sweet sorghum are the outcome of interaction between genotypes and environmental factors. The rain-fed condition did not have substantial impact on growth (plant height and stem diameter) but physiological traits (chlorophyll content and Fv/Fm) tended to decrease slightly under rain-fed condition (Table 2.4). Vasilakoglou et al. (2011) observed no variation in sugar concentration among six sweet sorghum genotypes under reduced irrigation. Similar situations were also observed by Miller and Ottman (2010). However, the present study found that rain-fed condition decreased brix, stem fresh and dry weights, juice and

sugar yields compared to irrigated condition and this indicated a wide genotype response to drought stress (Table 2.4). Similar results were also observed by Silva et al. (2008) in sugarcane indicating that drought stress had severe impact on plant height, stem diameter, stem weight on the susceptible genotypes than the tolerant genotypes. Reduction in sugar levels (brix) under rain-fed condition might be due to reduced nutrient uptake efficiency at one growth stage or the other.

Correlation analyses indicated that juice yield has greater contribution to higher sugar than the brix suggesting that selection for high sugar yielding genotypes should focus more on juice yield. Given the same brix value, genotypes with greater juice yield produced higher sugar yields (Table 2.3). Makanda et al. (2009) suggested genotypes with higher juice yield and lower brix were considered better stem sugar yielder than those genotypes with lower juice yield and higher brix. The highest performing genotypes also confirmed in the present study that the juice yield is an important trait for selection for higher sugar yield. Murray et al. (2008a,b) reported a significant correlation between brix values and stem juiciness. The juice yield could also be directly related to stem fresh weight. There was a significant and positive correlation between sugar yield and juice yield and stem fresh weight from the present study (Fig. 2.3).

2.5. Conclusions

We found wide genetic variability among 78 sweet sorghum cultivars for plant height, stem diameter, stem fresh weight, brix, juice yield and sugar yield. There were significant, positive correlation between sugar yield and growth (plant height and stem weight), physiological (photochemical efficiency) and bio-energy traits (juice yield). Growth and physiological traits were not affected by the rain-fed condition; however, there were significant reductions in brix, stem fresh weight, juice yield and sugar yield. Among the 78 genotypes, Wray, MN 4564 and

Caxa had higher sugar yield (≥mean+2SE). Genotypes Sanyagie, MN 818 and Dale_1 had lower RSYR indicating their drought tolerant potential with sustainable sugar yield. Sweet sorghum genotype with improved sugar yield can be utilized for genetic improvement.

2.6. Tables and Figures

Figure 2.1 Monthly maximum and minimum mean air temperature and total rainfall during the two years of cropping season (2007 and 2008).

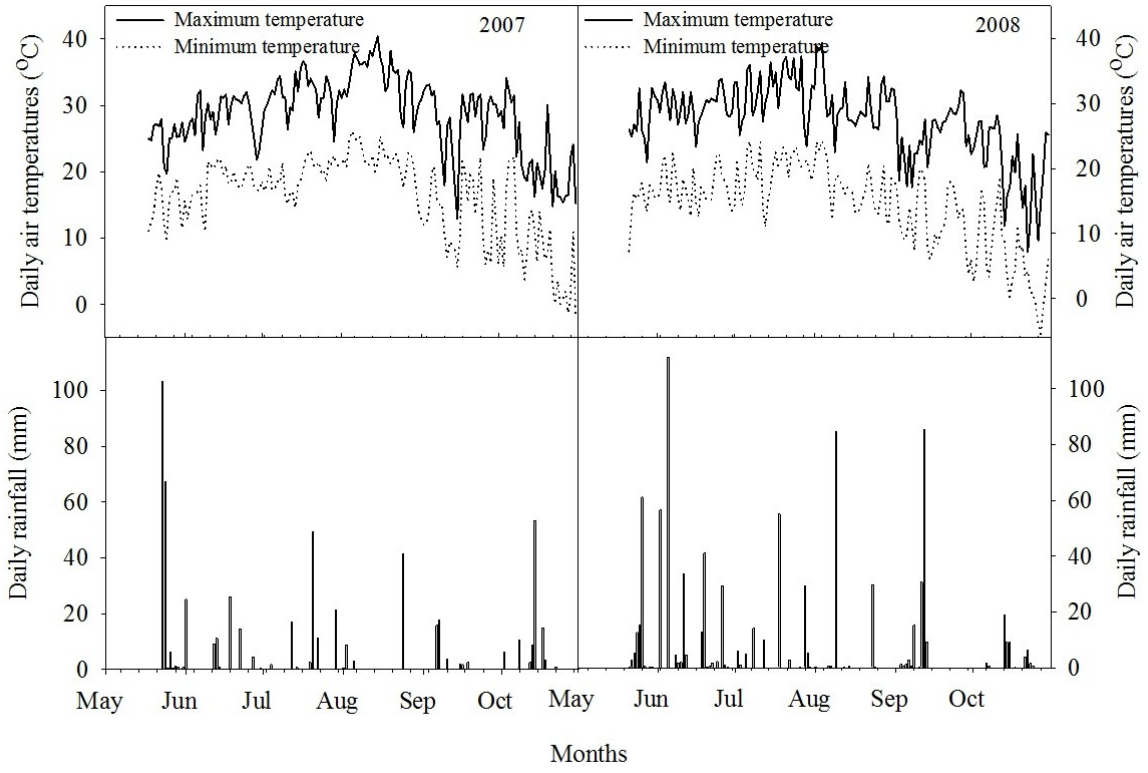

Figure 2.2 Correlation between sugar yield and (a) plant height and (b) stem diameter.

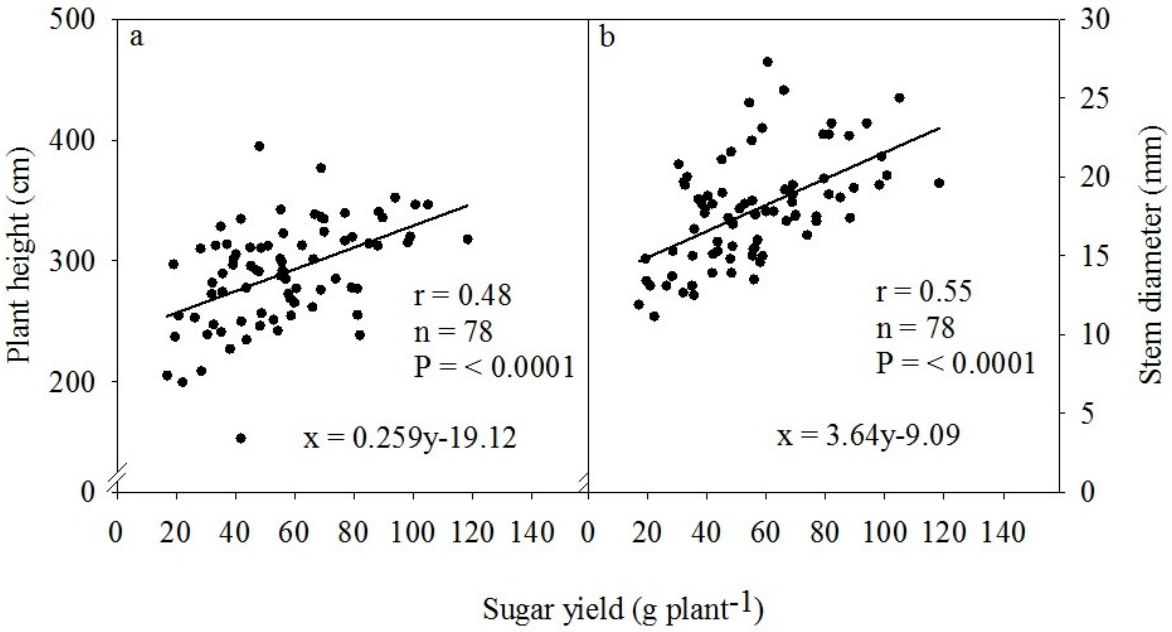

Figure 2.3 Correlation between sugar yield and (a) Photochemical efficiency (Fv/Fm) (b) juice yield (c) stem fresh weight and (d) stem dry weight.

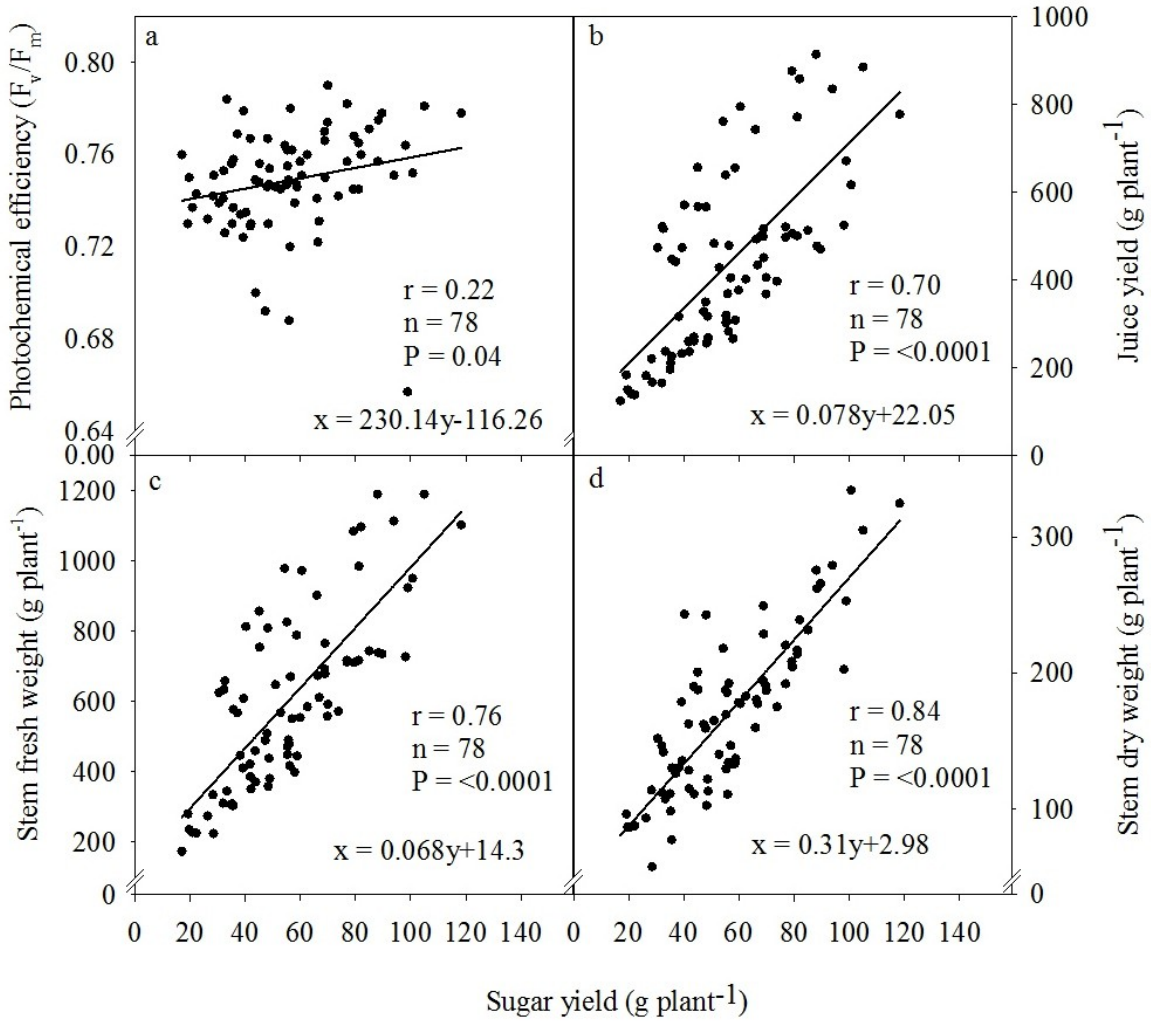

Table 2.1 List of sweet sorghum genotype, designation number and place of origin used in this study.

Genotype	Designation no.	Place of origin
7392	PI173112	Turkey, Artvin
8371	PI173118	Turkey
8493	PI173120	Turkey
Akdari_1	PI182303	Turkey
Akdari_2	PI167352	Turkey
Akdari_3	PI179504	Turkey, Urfa
Akdari_4	PI167047	Turkey
Akdari_5	PI177553	Turkey
Akdari_6	PI170783	Turkey
Aleppo No. 41	PI181899	Syria
Ames Amber	PI641806	*
Andiwo III 57	PI157030	Kenya
Ankolib tequil	PI152596	Sudan
AS-475	PI255348	Sri Lanka
Atlas	PI641807	*
Awanlek	PI152971	Sudan
Ayuak	PI152966	Sudan
B. 35	PI147224	India
Balaka	PI221560	Nigeria
Bangu manguisusu	PI88007	Korea
Bargowi	PI217770	Sudan
Bilichigan	PI152898	India
Brawley	BRAWLEY	*
Bwalimbo	PI155889	Tanzania
Caxa	PI255239	Mexico, Sonora
Chedomba	PI156268	Malawi
Chifungo	PI155924	Zambia
Chikkori	PI152953	Sudan, Kordofan
Chinese amber_1	PI248298	India
Chinese amber_2	PI22913	China
Co. 1	PI266927	India
Co. 4	PI185672	India, Delhi
Collier	PI641862	*
Colman	PI641810	*
Dale_1	Dale	USA
Dale_2	DALE	USA
Darso 28	PI260210	Guadeloupe, Basse-Terre
Della	PI566819	United States, Virginia
DEPAR	PI181077	India
Dhurra No. 7	PI155149	Yemen
Dobbs	PI156463	Tanzania
Dova-benko	PI196600	Taiwan
Dura hegari	PI156884	Zaire

Genotype name	Designation no.	Place of origin
Dura huria	PI156890	Zaire
Duro El jack	PI152923	Sudan, Kordofan
Dwarf ashburn	PI641893	*
Early folger	PI641815	*
Early sumac	PI641817	*
Feterita abdel magid	PI152872	Sudan
Feterita abu derega	PI157804	Sudan
Feterita fayoumi D.S. 10	PI152630	Sudan
Feterita fayoumi D.S. 13	PI152633	Sudan
Feterita fayoumi D.S. 8	PI152629	Sudan
Feterita fulli	PI152650	Sudan
Feterita geshaish	PI152651	Sudan
Feterita gezira	PI152646	Sudan
Feterita la estenzuela	PI201723	Nigeria
Gilgil	PI173808	Turkey
Gishish	PI152671	Sudan
Gonbo	PI155793	Malawi
Grassal	PI154844	Uganda
Gumbilu	PI152998	Eritrea
H.C. 41-13	PI641904	*
Hasesa	PI155543	Zambia
Heger Taie	PI152675	Sudan
Hegiri 1	PI152676	Sudan
Hemaisi red shendi shersher	PI152683	Sudan
Honey No. 6	PI562716	United States
Honey sorghum	PI181080	India
Honey_drip	PI641821	*
Ifube No. 18	PI157033	Kenya
Inyangentombi	PI144134	South Africa, Natal
IS 12807	PI170802	Turkey
IS 12810	PI170805	Turkey
IS 12833	PI175919	Turkey
IS 12849	PI177547	Turkey
IS 12900	PI183086	India
IS 2109	PI193613	Ethiopia
IS 2131	PI196049	Ethiopia
IS 2352	PI218112	Pakistan
IS 2462	PI149830	Somalia
IS 2464	PI149832	Somalia
IS 3986	PI643464	*
IS 81	PI167093	Egypt
Isidomba_1	PI145619	South Africa
Isidomba_2	PI144331	South Africa, Natal

Genotype name	Designation no.	Place of origin
Iswa	PI156423	Tanzania
Italian	PI196597	Taiwan
J56 akouangok	PI154929	Uganda
Jawar_1	PI180004	India
Jawar_2	PI179747	India
Jawar_3	PI173971	India
Jawar_4	PI180005	India
Jerima	PI273465	Nigeria
Jiba	PI145622	South Africa
Juar_1	PI183149	India
Juar_2	PI165532	India
Juar_3	PI180487	India
Juar_4	PI180348	India
Juar_5	PI180489	India
Juar_6	PI180008	India
Juar_7	PI180349	India
Juar_8	PI179749	India
Kabiri	PI154846	Uganda
Kafir pink	PI152692	United States
Kamandri	PI181083	India
Kaoliang	PI195754	China
Karadari	PI174381	Turkey
Keller	KELLER	USA
L28 lawere	PI154943	Uganda
L31 emiroit	PI154944	Uganda
Leoti-peltier	PI642999	*
Longwe	PI155571	Zambia
Luel	PI152714	Sudan
Lwel fadiang	PI152880	Sudan
M_81E	M_81E	USA
Magohe	PI156496	Tanzania
Mahananga	PI152909	Somalia
Maila_1	PI155485	Zambia
Maila_2	PI155556	Zambia
Maila_3	PI156136	Zambia
Malnal	PI152961	Sudan
Malwal aweil	PI152725	Sudan
Malwal tonj	PI152727	Kenya
Manyoble	PI145626	South Africa
MAPIeRA	PI155609	Zambia
MAPIRA	PI155805	Malawi
Masaka	PI155516	Zambia
Masuda black seed	PI193073	Japan
Mbagobago	PI156877	Zaire

Genotype name	Designation no.	Place of origin
Mbalwe	PI155756	Malawi
Merasi	PI152860	Sudan
Merissa (BARI)	PI152733	Sudan
Misali	PI155517	Zambia
MN 12	PI287627	Zimbabwe
MN 1344	PI154787	Uganda
MN 1540	PI155230	Sudan
MN 1615	PI156018	Zaire
MN 1644	PI155885	Tanzania
MN 1921	PI155710	Malawi
MN 1983	PI155767	Malawi
MN 2014	PI156178	Malawi
MN 2030	PI155804	Malawi
MN 2063	PI155899	Malawi
MN 2077	PI155845	Malawi
MN 2089	PI156203	Malawi
MN 2103	PI155902	Malawi
MN 2109	PI156217	Malawi
MN 2161	PI155912	Malawi
MN 2238	PI156352	Zambia
MN 2248	PI156363	Zambia
MN 2277	PI156393	Tanzania
MN 2282	PI156399	Tanzania
MN 2363	PI156487	Tanzania
MN 2386	PI156510	Tanzania
MN 2387	PI156511	Tanzania
MN 2398	PI156525	Tanzania
MN 2418	PI156696	Kenya
MN 2553	PI161586	Liberia
MN 2578	PI162806	Liberia
MN 2635	PI166210	Sierra Leone
MN 2680	PI52606	South Africa, Transvaal
MN 2740	PI92270	China, Beijing
MN 2742	PI177156	Turkey
MN 2751	PI643008	*
MN 2756	PI643013	*
MN 2761	PI643016	*
MN 2762	PI643017	*
MN 2826	PI170787	Turkey
MN 2827	PI170788	Turkey
MN 2838	PI170799	Turkey
MN 2857	PI173121	Turkey
MN 2873	PI176766	Turkey
MN 2894	PI177554	Syria

Genotype name	Designation no.	Place of origin
MN 2939	PI181971	Syria
MN 2972	PI189114	Nigeria
MN 3080	PI196583	Taiwan
MN 3081	PI196584	Taiwan
MN 3089	PI196592	Taiwan
MN 3095	PI196598	Taiwan
MN 3370	PI211633	Afghanistan
MN 4036	PI241566	Papua New Guinea
MN 4052	PI247136	Yugoslavia
MN 4118	PI250232	Pakistan, Punjab
MN 4120	PI250234	Pakistan
MN 4122	PI250521	India, Punjab
MN 4124	PI250582	Egypt
MN 4126	PI250402	Pakistan
MN 4133	PI250897	Iran
MN 4134	PI250898	Iran
MN 4135	PI251672	Yugoslavia
MN 4136	PI253795	Iraq
MN 4137	PI253796	Iraq
MN 4138	PI253986	Syria
MN 4155	PI302120	Belgium
MN 4179	PI302131	Portugal
MN 4243	PI302198	Argentina
MN 4252	PI302122	Portugal
MN 4299	PI302252	China
MN 4330	PI302264	Tanzania
MN 4369	PI302199	Argentina
MN 4553	PI271232	India
MN 4564	PI273953	Ethiopia
MN 4566	PI273955	Ethiopia
MN 4570	PI273959	Ethiopia
MN 4573	PI273963	Ethiopia
MN 4578	PI273969	Ethiopia
MN 48	PI287625	Zimbabwe
MN 600	PI147573	French Guiana
MN 818	PI586443	Hungary
Mokutakususu	PI88000	Korea
Mubeya	PI153871	Kenya
Mubovi	PI155328	Kenya
Muthiikwa	PI155333	Kenya
Muyo	PI155336	Kenya
N100	PI535785	United States, Nebraska
N107	PI535792	United States, Nebraska
N111	PI535796	United States, Nebraska

Genotype name	Designation no.	Place of origin
N98	PI535783	United States, Nebraska
Nagad El mur	PI217691	Sudan
Nagro	PI147026	Egypt
Namuse	PI155760	Malawi
Nefee	PI156252	Malawi
Nerum boer	PI303658	Sudan, Southern
Nkolongo	PI156465	Tanzania
Nkumba	PI154796	Uganda
NO. 5 gambela	PI257599	Ethiopia
NO. 6 gambela	PI257600	Ethiopia
NO. 8 gambela	PI257602	Ethiopia
Ntiboyumba	PI156899	Zaire
Nyagwang No. 56	PI157035	Kenya
Nytwal	PI152751	Sudan
Opemba nonpha	PI156435	Tanzania
P 127 (S.A. 5)	PI154990	Swaziland
Planter	PI641834	*
Popsorghum	PI584989	United States, Texas
Potch 4	PI152755	Sudan
Query 3	PI152764	Sudan
Rahmetalla gallabat	PI152771	Sudan
Ramada	RAMADA	*
Red janpur	PI181074	India
Red losinga	PI641909	Sudan
Red_amber	PI17548	Australia, New South Wales
REX	PI641835	*
Rio_2	PI563295	United States, Maryland
Rutobo	PI156871	Zaire
S. A. 1	PI154987	Swaziland
S. A. 2	PI154988	Swaziland
Saccaline	PI48191	Australia, New South Wales
Sairwa	PI168525	Nigeria
Sanyagie	PI156405	Tanzania
Serere	PI154750	Uganda
Smith	PI511355	United States, Texas
Sonkwe	PI156356	Zambia
Sucre drome	PI197542	Algeria
Sugar drip_2	PI146890	Zaire
Sugar_drip_1	Grif14907	*
Sweet saccaline	PI198885	Australia
Tegevini	PI145632	South Africa
Texas seeded ribbon	PI641848	*
Theis	THEIS	USA

Genotype name	Designation no.	Place of origin
Thok (B)	PI152963	Sudan
Tjolotjo	PI247745	Zaire
Top 76-6	PI583832	United States, Georgia
Tracy	Tracy	USA
Tseta 27/51	PI267476	India
Tugela ferry	PI145633	South Africa
U. g. 6. 7.	PI247744	Zaire
U.T. 23	PI152828	Zaire
V3 nakyeru	PI154962	Uganda
W. 21	PI147200	India
Wad aker red	PI152813	Sudan
Wad fur white	PI152816	Sudan
Waquema	PI155721	Malawi
Waxy club	PI152914	United States
Wenabu	PI154800	Uganda
Wheatland	PI154980	Kenya
Wray	Wray	USA

*unknown.

Table 2.2 Mean and range for different traits of 280 sweet sorghum genotypes averaged over three growing conditions (2007-irrigated; 2008-irrigated and rain-fed).

Traits	Mean	Range	Standard deviation (SD)
Plant height (cm)	278	93-440	64.3
Stem diameter (mm)	17	8-27	3.8
Leaf chlorophyll (SPAD)	52	37-63	6.2
PS II photochemical efficiency (Fv/Fm)	0.748	0.413-0.810	0.03

Table 2.3 Means of growth and bioenergy traits of 78 sweet sorghum genotypes averaged across three growing conditions during 2007 and 2008 at Manhattan, KS.

Genotype	Plant height (cm)	Stem diameter (mm)	Chlorophyll (SPAD)	Fv/Fm	Brix (%)	Juice yield (g plant^{-1})	Sugar yield (g plant^{-1})	Stem fresh weight (g plant^{-1})	Stem dry weight (g plant^{-1})	RSYR† (%)
Ames amber	205.1	11.9	50.6	0.760	12.8	124.7	17.0	173.9	49.1	95
Atlas	234.6	15.3	53.3	0.700	14.5	260.4	43.7	371.5	111.1	93
Awanlek	324.0	17.6	52.1	0.790	15.8	405.3	69.9	592.5	187.1	80
Bargowi	227.1	18.3	51.3	0.734	12.5	316.5	38.2	446.8	130.3	64
Brawley	287.6	15.0	58.7	0.755	17.9	302.4	55.3	472.1	169.6	59
Caxa	346.5	20.1	55.6	0.752	15.5	616.3	100.7	950.8	334.4	51
Co. 1	238.5	23.4	44.7	0.760	9.2	858.2	82.0	1097.4	239.2	58
Collier	328.5	13.1	56.7	0.756	17.5	196.0	35.0	307.3	111.3	74
Colman	310.0	13.7	48.4	0.742	17.4	220.4	28.3	334.6	114.1	73
Dale_1	340.6	17.4	51.0	0.775	18.0	477.0	88.3	739.4	262.3	37
Darso 28	208.8	15.3	57.3	0.751	17.0	166.6	28.5	224.4	57.7	83
Della	299.1	15.4	52.5	0.749	16.6	305.1	55.7	490.8	185.7	82
Dura huria	272.6	14.6	52.8	0.739	20.7	266.0	57.9	398.9	132.9	74
H.C. 41-13	199.5	11.1	55.6	0.743	15.2	137.9	22.2	225.7	87.8	88
Honey No. 6	336.0	19.5	47.7	0.750	14.9	450.8	69.0	679.5	228.7	75
Inyangentombi	284.8	16.0	58.6	0.762	14.1	404.6	57.0	551.3	146.7	61
IS 12810	250.0	15.1	52.4	0.730	16.6	236.3	42.0	351.4	115.1	90
IS 12900	291.3	14.8	57.7	0.746	13.6	349.5	47.9	508.9	159.4	65
IS 2109	394.8	21.6	57.2	0.767	8.0	566.4	48.1	809.2	242.7	82
IS 2131	320.1	21.3	51.5	0.657	12.6	671.1	98.9	924.1	252.9	92
IS 2352	297.1	14.8	52.6	0.730	11.1	183.6	19.2	279.9	96.3	87

Genotype	Plant height (cm)	Stem diameter (mm)	Chlorophyll (SPAD)	Fv/Fm	Brix (%)	Juice yield (g plant^{-1})	Sugar yield (g plant^{-1})	Stem fresh weight (g plant^{-1})	Stem dry weight (g plant^{-1})	RSYR† (%)
Isidomba_2	342.5	18.5	55.8	0.762	16.2	319.6	55.3	449.0	129.4	85
Iswa	239.0	20.8	51.7	0.739	6.2	473.5	30.5	625.4	151.9	93
Jerima	305.6	18.8	52.4	0.735	9.2	570.6	40.2	813.9	243.3	90
Leoti-peltier	246.1	13.9	52.6	0.730	18.4	2557.	48.3	358.4	102.7	64
Luel	237.0	13.4	51.5	0.750	12.4	149.4	19.6	236.2	86.8	93
Mahananga	338.6	17.2	50.5	0.731	14.2	433.9	66.8	611.5	177.6	83
Manyoble	292.0	17.4	56.0	0.692	12.8	327.7	47.2	490.0	162.2	87
Masuda black seed	322.8	15.5	54.9	0.720	18.7	283.2	56.2	417.4	134.1	82
Mbalwe	274.3	12.5	58.1	0.737	15.1	226.2	35.6	303.6	77.43	84
MN 1540	281.8	19.7	44.0	0.753	6.3	521.6	32.2	633.6	112.0	79
MN 1644	301.8	22.3	52.0	0.747	8.1	638.9	55.1	826.3	187.4	79
MN 1921	289.5	16.7	53.8	0.758	7.2	447.0	35.7	577.2	130.2	90
MN 2063	261.6	25.5	37.0	0.741	9.0	742.5	66.0	902.5	159.9	72
MN 2089	312.5	18.0	54.1	0.746	10.9	483.2	51.0	648.4	165.1	64
MN 2109	278.0	22.7	45.3	0.745	8.5	876.1	79.2	1084.6	208.5	71
MN 2161	295.8	19.0	50.2	0.756	7.3	567.1	45.2	754.7	187.6	90
MN 2238	277.0	27.3	53.8	0.751	6.7	795	60.5	972.5	177.5	93
MN 2363	301.3	18.0	50.6	0.779	7.2	473.3	39.4	609.0	135.6	92
MN 2386	242.1	24.7	49.6	0.764	6.6	761.0	54.3	979.1	218.1	98
MN 2756	251.1	18.3	55.1	0.745	12.9	428.3	52.8	568.5	140.2	69
MN 2762	312.6	20.0	45.6	0.784	14.4	237.4	33.3	344.9	107.5	88
MN 2894	254.6	13.1	56.8	0.737	13.9	140.9	20.8	228.0	87.1	91
MN 4135	254.6	15.0	53.5	0.746	17.8	308.1	58.8	445.5	137.3	84
MN 4553	352.3	23.4	51.0	0.751	10.2	834.8	93.9	1114.0	279.1	76
MN 4564	346.6	25.0	49.8	0.781	11.0	885.1	105.0	1190.0	304.9	61
MN 4566	312.6	22.6	51.9	0.757	8.9	914.2	88.0	1189.8	275.6	85

Genotype	Plant height (cm)	Stem diameter (mm)	Chlorophyll (SPAD)	Fv/Fm	Brix (%)	Juice yield (g plant^{-1})	Sugar yield (g plant^{-1})	Stem fresh weight (g plant^{-1})	Stem dry weight (g plant^{-1})	RSYR† (%)
MN 4570	313.6	18.6	52.0	0.769	8.9	441.6	37.1	568.0	126.4	64
MN 600	319.8	19.9	49.2	0.768	15.1	506.4	79.4	711.0	204.6	89
MN 818	316.6	17.5	54.8	0.757	14.6	520.7	76.9	712.9	192.2	28
Mubeya	312.8	17.8	53.2	0.760	15.5	402.0	62.5	585.1	183.1	54
N100	256.5	17.0	50.5	0.754	16.8	267.9	48.8	381.2	113.2	81
N111	272.6	12.6	47.2	0.741	17.7	164.5	32.0	311.3	146.7	91
Nagad El mur	153.0	18.3	50.7	0.729	15.6	258.1	41.8	386.6	128.6	79
Nkolongo	311.0	21.1	51.0	0.748	8.3	656.2	45.0	856.9	200.7	67
NO. 5 gambela	276.1	18.4	56.4	0.770	13.3	498.6	68.8	693.0	194.4	66
NO. 6 gambela	314.3	18.7	47.4	0.771	15.4	512.6	85.0	744.2	231.6	72
NO. 8 gambela	301.3	19.2	55.6	0.722	13.9	493.2	66.3	673.8	180.6	87
Opemba	268.5	23.1	45.0	0.747	8.2	655.5	58.6	788.9	133.5	92
Rahmetalla gallabat	285.1	16.3	57.9	0.742	17.5	397.1	73.8	572.3	175.1	78
Red_amber	253.0	13.1	51.6	0.732	14.8	181.4	26.3	274.8	93.4	77
Rex	296.8	17.7	50.9	0.724	15.6	232.2	39.2	411.1	178.9	89
Rio_1	265.3	17.8	53.5	0.757	15.7	376.5	59.9	554.9	178.4	83
Saccaline	291.0	17.6	52.9	0.780	11.8	478.4	56.4	671.0	192.6	81
Sairwa	247.3	19.5	46.8	0.726	6.2	517.0	32.6	658.8	141.8	80
Sanyagie	255.3	22.7	46.1	0.765	10.6	770.9	81.2	985.1	214.1	22
Smith	335.8	19.3	52.8	0.778	18.7	470.2	89.6	735.9	265.7	39
Sugar drip_2	334.6	13.9	60.0	0.767	15.4	260.1	41.8	422.7	162.6	88
Tegevini	310.5	15.6	49.4	0.747	15.1	316.5	48.6	438.5	122.0	73
Theis	339.5	17.2	52.6	0.782	14.3	497.1	76.9	717.6	220.5	74
Top 76-6	315.1	19.5	51.1	0.764	18.1	524.8	98.1	727.5	202.6	57
Tracy_2	334.8	17.5	57.2	0.774	18.3	367.5	69.8	558.3	190.8	76
Tracy_1	277.7	15.9	53.5	0.749	15.3	269.8	43.6	459.9	190.1	83

Genotype	Plant height (cm)	Stem diameter (mm)	Chlorophyll (SPAD)	Fv/Fm	Brix (%)	Juice yield (g plant^{-1})	Sugar yield (g plant^{-1})	Stem fresh weight (g plant^{-1})	Stem dry weight (g plant^{-1})	RSYR† (%)
Wad fur white	276.8	18.9	54.3	0.745	15.6	500.2	81.1	717.1	216.9	75
Waxy club	241.0	15.0	55.4	0.730	15.7	210.3	35.2	308.9	98.5	85
Wenabu	292.1	13.5	52.2	0.688	14.7	368.7	55.9	479.6	110.8	54
Wray	317.8	19.6	54.0	0.778	15.7	777.0	118.3	1102.7	324.8	40
Mean	289.5	17.9	52.2	0.749	13.4	436.1	56.1	606.2	170.0	75.5
Range	153-395	11-27	37-60	0.66-0.79	6.2-20.7	125-914	17-118	174-1190	49.1-334.4	22-98
SE*	4.80	0.39	0.44	0.002	0.42	23.09	2.57	28.75	6.9	1.8
p value	<0.0001	<0.0001	0.02	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	-
LSD	80.0	3.4	9.2	0.04	5.3	285.1	38.0	346.8	116.7	-

†RSYR, relative sugar yield reduction in rain-fed condition to irrigated condition and not statistically analyzed

*Standard error

Table 2.4 Comparison of irrigated and rain-fed mean values of various traits among 280 sweet sorghum genotypes.

Traits	Irrigated	Rain-fed	% Decrease from irrigated
Plant height (cm)	291.89	278.14	4.7
Stem diameter (mm)	16.82	15.79	6.1
Leaf chlorophyll (SPAD)	52.59	52.24	0.7
PS II Photochemical efficiency (Fv/Fm)	0.754	0.748	0.8
Stem fresh weight (g plant^{-1})	525.36	348.12	33.7
Stem dry weight (g plant^{-1})	164.53	110.40	32.9
Brix (%)	16.06	10.11	37.0
Juice yield (g plant^{-1})	360.84	237.72	34.1
Sugar yield (g plant^{-1})	69.14	24.02	65.3

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Chapter 3 - Effects of harvest time on juice yield of sweet sorghum

3.0 Abstract

Sweet sorghum (*Sorghum bicolor* L. Moench) is an important bioenergy crop, and has the potential to be grown for ethanol production. In sweet sorghum, sugar content changes with development, so it is important to determine and optimize of stage of development that provides maximum sugar yield. The objective of this research was to determine optimum harvest time for obtaining the maximum sugar and juice yield of sweet sorghum. A field experiment was conducted using sweet sorghum variety M81E, and harvested at ten growth stages (flag leaf tip appearance, start of panicle emergence, complete panicle emergence, anthesis, post-anthesis, milk, soft dough, hard dough, physiological maturity and 30 d after physiological maturity). Data on physiological, growth and yield traits were measured at each harvest. The results revealed that the effect of harvest timing was significant for most traits. Sugar yield and juice yield linearly increased from flag leaf appearance to milk stage, thereafter remained constant until hard dough stage. Juice purity, total sugars, and non-reducing sugars were highest at hard dough stage. These results suggest that optimum harvesting time for sweet sorghum is the hard dough stage.

3.1 Introduction

Biofuels, particularly bioethanol can be a potential alternative fuel for consumption. The demand for alternative fuel has gained world-wide attention including the U.S and Brazil which together contribute to 89% of total global ethanol production (Davila-Gomez et al., 2010). Maize (*Zea mays*) and sugarcane (*Saccharum officinarum*) are two major crops for ethanol production (Mussatto et al., 2010). In the U.S., Maize is the major feedstock for grain ethanol. Continuous increase in energy demand necessitates more attention towards alternative feedstock for biofuel production (Solomon et al., 2007). The limitations of cultivating maize for ethanol include increased use of inputs to produce biomass in terms of nitrogen requirements and inefficient conversion of starch to ethanol. In addition use of maize grain for ethanol competes with food and feed demands.

Sweet sorghum (*Sorghum bicolor* L. Moench) is an attractive feedstock for ethanol production (Wu et al., 2010). Sorghum requires less water and nitrogen and better adapted to high temperature and drought stress (Prasad et al., 2007; Almodares and Hadi, 2009). In addition, sweet sorghum has been reported to yield adequate biomass for increased sucrose accumulation in the stem. Ethanol, a high octane fuel, produced by the fermentation of carbohydrates such as glucose and fructose (reducing sugars) and sucrose (non-reducing sugar) in the stem juice (Mastrorilli et al., 1999; Barbanti et al., 2006; Rooney et al., 2007; Teetor et al., 2011). Quality of juice (extractable juice and purity) depends upon many factors such as variety, stage of the crop and other management and environmental factors.

In sweet sorghum, sucrose is accumulated in large amounts in the stem during inflorescence development (McBee and Miller, 1982). Hence, there may be a competition for carbohydrates between stem and developing seed. At maturity the sugars (reducing and non-

reducing sugars) were mobilized from stem and leaves to grain. The amount of assimilates allocated for sucrose biosynthesis in the stem and grain depends on partitioning. Previous studies have reported that sucrose levels start to increase in the stem from preboot to anthesis (McBee and Miller, 1982; Hoffman-Thoma et al., 1996). Ghatode et al. (1991) observed that brix, reducing sugars and non-reducing sugars were found to decrease at maturity. Sucrose content and seed yield are indicators of how assimilates are partitioned between two sinks (grain and stem). Hence, to get maximum stem sucrose content, time of harvest is important.

In sweet sorghum, traits such as plant height, stem diameter and brix can influence juice yield (Ali et al., 2008; Murray et al., 2009). The primary biomass component in sweet sorghum is stem weight, which accounts to 70% of total weight (Dalianis, 1997). Stem weight is correlated with height, diameter, thickness and juiciness (Audilakshmi et al., 2010; Pfeiffer et al., 2010). Murray et al. (2008) found that plant height was highly correlated with juice yield and stem fresh weight. It has also been reported that there is a linear correlation between brix and total sugar content of the juice (Tsuchihashi and Goto, 2004; Audilakshmi et al., 2010; Davila-Gomez et al., 2010).

Harvesting is a critical operation in sweet sorghum. Selection of an optimal harvest date will require a better understanding of the biochemical changes that occur during different stages of plant growth and development. The cultivation of sweet sorghum variety M81E has acquired great commercial importance for ethanol production (Wu et al., 2010) but information on the effect of harvest time from start of flowering to physiological maturity on sugar yield is not known and needs attention. The objective of this research was to determine the optimum harvest time for obtaining maximum juice yield. We hypothesize that sugar yield will increase between milk and dough stages.

3.2 Materials and Methods

3.2.1 Plant material

Sweet sorghum variety M81E used in this study was developed at U.S. Sugar Crops Field Station, Meridian, Mississippi. The variety was selected from the F_2 progeny of the cross 'Brawley' x (Brawley x 'Rio') and released in 1981 and is late maturing (140-150 days).

3.2.2 Experimental site and environmental conditions

The variety M81E was grown under rain-fed condition during 2009 at Ashland Bottoms Research Farm near Manhattan, KS (39°06′54.2″N-96°38′10.0″W, Altitude: 323 m). Soil at the experimental site was a Harney silt loam (clay 16%, silt 64% and sand 20%; and pH 6.3). The daily weather conditions during the 2009 cropping season are presented in Fig. 3.1. The total amount of rainfall received during the cropping season (June-November) was 626 mm. The average maximum day and minimum night time air temperature during crop growing season were 23.7°C and 11.5°C, respectively. The Normal (1971-2000) daily maximum and minimum air temperature for the period is 26.1°C and 11.8°C.

3.2.3 Crop husbandry

The experimental plot was chisel ploughed and the seeds were sown on 18 June in 2009 at 5 cm deep. Plots were fertilized with 90 kg ha^{-1} N as urea. For weed control, the plots were sprayed with Bicep Lite II Magnum (a.i. 0.82 kg atrazine ha^{-1} and 1.03 kg S -metolachlor ha^{-1}) prior to planting. The field was kept weed free by hand weeding as necessary. Plot size was of 9 m x 3 m. Each plot consisted of four rows of 9 m length spaced 0.75 m apart. The harvest treatments comprised of ten growth stages: flag leaf tip appearance (71 d after planting), start of panicle emergence (79 d after planting), complete panicle emergence (85 d after planting), anthesis (92 d after planting), post-anthesis (99 d after planting), milk stage (107 d after planting), soft dough

(117 d after planting), hard dough (129 d after planting), physiological maturity (140 d after planting) and post-physiological maturity (170 d after planting).

3.2.4 Data collection

3.2.4.1 Physiological traits

Physiological traits were recorded on attached fully expanded flag leaves of two different tagged plants per plot before each harvest stage at midday (between 10:00 and 14:00 h). Chlorophyll content was measured using a self-calibrating chlorophyll meter [Soil Plant Analytical Device (SPAD), Model 502, Spectrum Technologies, Plainfield, IL, USA]. Chlorophyll *a* fluorescence parameters were measured using pulse-modulated fluorometer (OS5p, Optisciences, Hudson, NH, USA). Photosystem II (PSII) photochemical efficiency of (Fv/Fm) was measured in 30-min dark-adapted leaves (Prasad et al., 2008). The leaf and stem temperature was measured with a hand-held thermal imager (FLIR ThermaCAM BCAM thermal imaging systems, Janesville, WI, USA). For measurement of stem temperature, three regions of the stem (bottom 3rd internode, middle 6th internode, and the top 9th internode) were used to record temperatures and the average was computed from the values of all three regions.

3.2.4.2 Growth traits

Plant growth traits were recorded from the same two tagged plants, at each time of harvest. Plant height was measured from base of the stem to the tip of the panicle using a measuring tape. Stem diameter was measured from the three regions of the stem (bottom 3rd internode, middle 6th internode, and the top 9th internode) using vernier caliper after stripping the leaves and removal of leaf sheaths. Data on stem diameter was averaged across the stalk. Number of leaves and total number of internodes on the stem were counted. Leaf area was measured with a LI-COR leaf area meter (LI-3100, Li-Cor Biosciences, Lincoln, NE, USA).

3.2.4.3 Yield traits

At each harvest, yield traits were obtained from center 1.5 m of row consisting of 10 plants from each plot, and the stems were stripped. The fresh weight of the panicles, leaves, and stems were recorded. From these data the total fresh biomass comprised of panicles, leaves and stem was calculated. The fresh leaf, panicle and crushed stem were oven-dried at 60°C for 7 d and dry weights were recorded. The total dry biomass was calculated from the oven dried samples. The panicles were threshed by hand to obtain grain yields. Grain harvest index was calculated as the ratio of grain yield to total aboveground dry biomass and expressed as a percentage. Sugar harvest index was calculated as the ratio of sugar yield to total dry biomass and expressed as a percentage.

3.2.4.4 Juice quality and sugar yield

The juice from 10 plants was extracted using a motor operated three roller sugarcane crusher (Sukra sugarcane crusher, Coimbatore, Tamil Nadu, India). After juice extraction, juice volume was used to calculate juice yield. The total soluble brix of extracted juice was determined using a digital hand-held refractometer (Digital hand-held pocket refractometer PAL-1, Atago, Bellevue, WA, USA) and expressed in percentage. The sugar yield was calculated as a product of brix (%) and juice yield. The juice samples were frozen and stored -24°C freezer for further analysis.

Total sugars and reducing sugars were estimated in the extracted stem juice. Total sugars and reducing sugars were estimated using procedures of Robertson et al. (1996) and expressed as a percentage. Non-reducing sugar was obtained from the differences of total and reducing sugars and expressed as a percentage. The starch content in the juice was estimated using rapid starch test (Ronaldson and Schoonees, 2004) and expressed as $\mu g\ g^{-1}$ of brix. Juice pH was determined using pH meter (Digital pH meter DPH-1, Atago, Bellevue, WA, USA). Percentage extractable

juice was calculated from the juice yield of the sample and fresh weight of stem portion of the sample. Juice purity was derived from the ratio of non-reducing sugar to brix and expressed in percentage.

3.2.5 Data analyses

The experiment was conducted using a randomized complete block design with four replications. The data were subjected to the analysis of variance for each trait using the general linear model procedures in statistical analysis software version 9.1 (SAS Institute, 2003). Treatment means were compared using L.S.D at 5% level of probability.

3. 3 Results

3.3.1 Physiological traits

Leaf temperatures ranged from 30.8°C to 23.5°C during different harvest periods. The trend of stem temperature followed similar to that of leaf temperature and ranged from 28.4°C to 20.9°C (Table 3.1). Photochemical efficiency (Fv/Fm) increased from flag leaf stage up to hard dough stage. There was no effect of harvest time on initial fluorescence (F_o). The chlorophyll (SPAD) steadily increased from flag leaf tip appearance until hard dough stage (Table 3.1).

3.3.2 Growth traits

Plant height of this variety was 378 cm with 15 internodes at anthesis (Table 3.2). These values remained similar from anthesis to post-physiological maturity. Total leaf number was 14 and leaf area was 5075 cm^2 at post-anthesis stage. The bottom 3rd internode diameter continuously increased and reached 19.8 mm at milk stage (Table 3.2). The 6th and 9th internode diameter also increases from flag leaf stage and reached maximum at hard dough. Similarly, maximum average stem diameter was observed at hard dough stage (Table 3.2).

3.3.3 Yield traits

Stem fresh weight increased from flag leaf stage until hard dough stage with highest percent increase from flag leaf to boot stage (Table 3.3). The subsequent increase at each successive stage was 9.4% (panicle emergence), 11.5% (anthesis), 6.6% (post-anthesis), 6.1% (milk stage) and 3.3% (soft dough). The highest stem fresh weight was obtained at hard dough stage followed by soft dough stage and these values were the highest compared with those obtained during other harvest timing. Harvesting beyond hard dough stage decreased stem fresh weight at physiological maturity and post- physiological maturity (170 d after planting). Stem dry weight markedly increased from flag leaf stage to physiological maturity and thereafter decreased at post- physiological maturity. The grain dry weight increased from anthesis to physiological maturity with a harvest index of 23.0% (Table 3.3). Sugar harvest index, while it decreased at physiological maturity and post-physiological maturity, was similar at milk stage, soft and hard dough stages, respectively (Table 3.3).

3.3.4 Juice quality and sugar yield

The juice pH declined from flag leaf stage up to hard dough stage and then peaked at physiological maturity (Table 3.4). The starch content in the juice increased from flag leaf stage to soft dough stage (Table 3.4). Thereafter starch content decreased at hard dough and physiological maturity. However, starch increased substantially when plants senesced at post-physiological maturity.

The percent extractable juice was similar from flag leaf stage to physiological maturity (Table 3.4). However, lowest extractable juice was obtained when plants were harvested at post-physiological maturity. The data on juice purity percentage revealed increasing trend from flag leaf tip appearance up to panicle emergence, decreased steadily at milk stage, then increased at

soft dough stage. The juice purity was highest at hard dough stage and it declined thereafter at physiological maturity and post-physiological maturity. Brix had an increasing trend from flag leaf up to soft dough stage, then slightly decreased and remained similar between hard dough and physiological maturity, and then peaked at post-physiological maturity (Table 3.5).

Reducing sugars increased from early flag leaf tip appearance stage until post- anthesis stage and thereafter remained similar until physiological maturity (Table 3.5). Harvesting beyond physiological maturity resulted in lower reducing sugars. Non-reducing sugar steadily increased from flag leaf tip appearance, peaked at hard dough stage and steadily decreased thereafter until post-physiological maturity (Table 3.5). The total sugars continuously increased as plants matured with lowest at flag leaf stage and the highest at hard dough stage. A decline in the percent total sugars was observed at physiological and post- physiological maturity (Table 3.5).

Juice yield was highest when plants were harvested during the hard dough stage (Table 3.5). However, juice yield was not different at soft dough stage and milk stage. The lowest juice yield was obtained at flag leaf stage. Harvests beyond hard dough stage, decreased juice yield at physiological maturity and post-physiological maturity. Sugar yield gradually increased from flag leaf tip appearance stage to milk stage, thereafter it was statistically similar until hard dough. Sugar yield decreased at physiological maturity and post-physiological maturity (Table 3.5).

3.4 Discussion

In sweet sorghum, changes in the composition of juice and stem weight influences juice quantity and quality as the crop matures. Our results revealed that maximum sugar yield was observed during hard dough stage, where stem fresh weight was at a maximum (Table 3.3). There were no changes in juice extraction percentage until physiological maturity in spite of gradual increases in stem fresh weight (Table 3.3 and Table 3.4) through continuous

accumulation of carbohydrate in the stem. The increase in stem fresh weight was due to increase in average stem diameter and plant height (Table 3.2 and 3.3). The decrease in fresh weight at physiological maturity was due to leaf loss and dry down. Pfeiffer et al. (2010) stated that taller sweet sorghum hybrids with greater stem diameter produced greater stalk yields. Stem is a much stronger sink for sugar accumulation and fiber content (mainly cellulose and lignin) (Powell et al., 1991; Zhao et al., 2009). Higher Fv/Fm and chlorophyll SPAD at soft dough and hard dough stages indicated that the plant was also physiologically efficient, leading to greater accumulation of carbohydrates in stems.

The significant increase in the sugar yield between milk and hard dough stage was due to increase in juice yield (Table 3.5). However, at maturity a decrease in sugar yield in the stem was measured with a concomitant increase in grain yield (Table 3.5 and Table 3.3). Rajendran et al. (2000) reported the decrease in stem weight was due to mobilization of carbohydrates from stem to developing grain at maturity in sweet sorghum.

In our study, juice brix decreased at physiological maturity (Table 3.5), which indicates enhanced mobilization of soluble sugars to the developing grains resulted in increased grain yield (Table 3.3). Conversely, Almodares et al. (2007) observed highest brix in 'Rio' genotype at physiological maturity. The decrease in starch content in juice from soft dough stage leads to increase in reducing and non-reducing sugars at hard dough stage. Almodares and Hadi (2009) reported that decrease in starch content in juice along with an increase in reducing and non-reducing sugars was due to structural changes in carbohydrates namely starch to sucrose, an important process in sweet sorghum to obtain maximum ethanol production.

The total sugars in the juice increased from flag leaf to hard dough stage (Table 3.5). Hills (1990) reported that the sugar content in sweet sorghum stems increased between milk and

dough stages for most cultivars. It then begins to decline towards physiological maturity. The reducing sugars in juice decreased from hard dough to post-physiological maturity. Almodares et al. (1994) observed high sucrose and total sugar content and low reducing sugar at dough stage in sweet sorghum cultivars, MN1500, Soave, Wray and Vespa. This was attributed to increased use of reducing sugars (glucose and fructose) for various metabolic functions and higher activity of amylase enzyme. At all the stages of harvest, the percentage of reducing sugars differed significantly. The non-reducing sugar percentage, as sucrose, increased from flag leaf tip appearance to hard dough stage and this could be attributed to decreased activity of the enzyme invertase. The increase in non-reducing sugar at hard dough stage was in accordance with the finding of Lingle (1987) and Almodares et al. (2007) in sweet sorghum. Ferraris and Charles-Edwards (1986) also reported that sucrose and soluble solids concentration and yields in sweet sorghum stems were highest at or near grain maturity.

Our present study revealed that there was a dramatic decrease in sugar yield, brix and juice yield with significant increase in grain yield during harvest at physiological maturity. Harvesting 30 d after physiological maturity decreased sugar yield, juice yield but increased brix (Table 3.5). The increase in brix at post-physiological maturity stage was due to concentration of sugar in the juice due to drying and freezing (cool temperature). The juice purity significantly decreased at physiological and post-physiological maturity stages because of decrease in non-reducing sugar content and increase in brix.

3.5 Conclusions

Harvesting plants at hard dough stage resulted the highest brix, total sugars, reducing sugars and non-reducing sugars. The highest level of sugar and juice in the stem was obtained from plants harvested from milk to hard dough stage. We conclude that the optimum harvest

time for maximum juice and sugar yields for the sweet sorghum variety M81E is between milk and hard dough stages.

3.6 Tables and Figures

Figure 3.1 Daily maximum and minimum mean air temperatures and rainfall during the cropping season (2009) at Manhattan, Kansas.

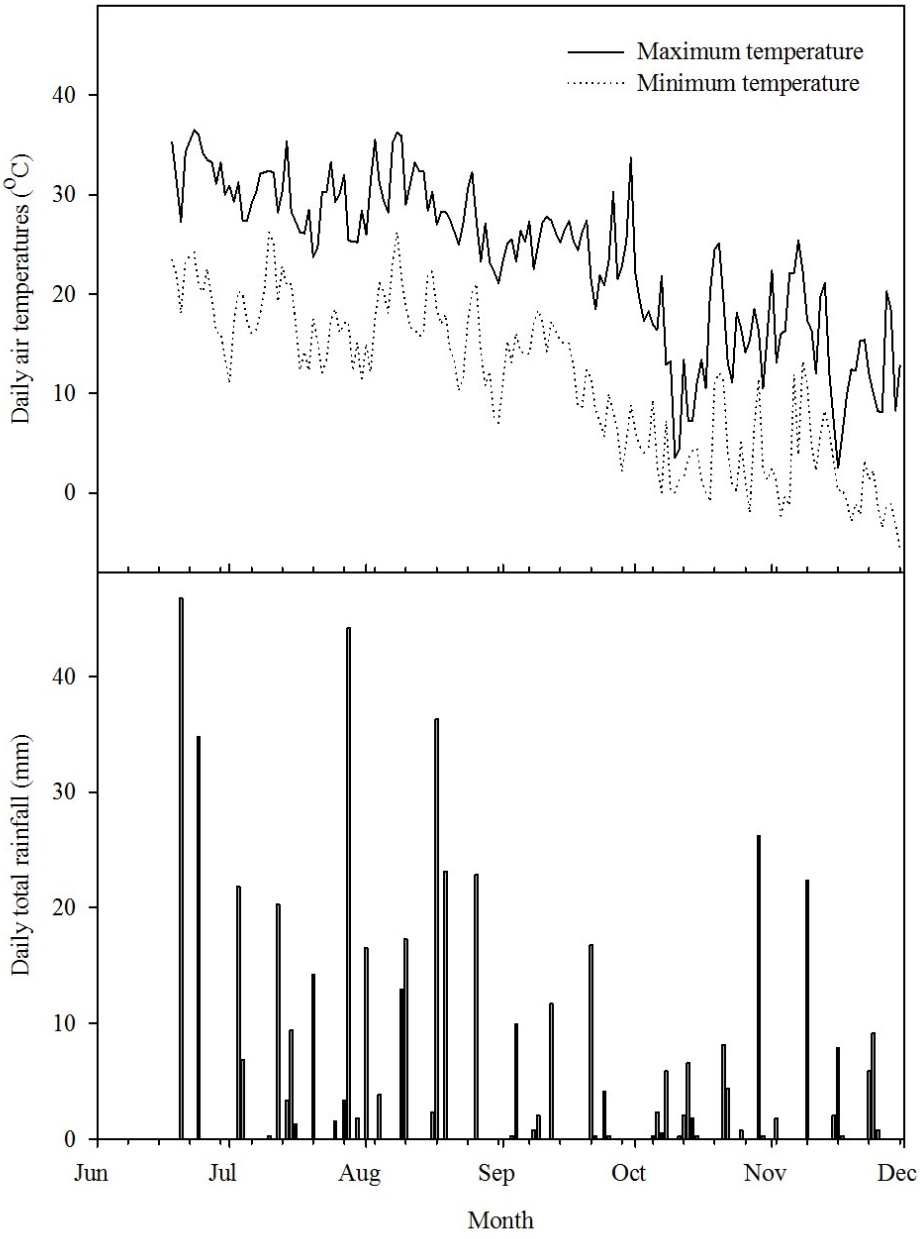

Table 3.1 Effect of different stages of harvesting on physiological traits of sweet sorghum variety M81E grown at Manhattan, Kansas.

Stages of harvesting	Days after planting	Leaf temperature (^{o}C)	Stem temperature (^{o}C)	Fo^{+}	Fm$^{\#}$	Fv/Fm	Chlorophyll (SPAD)
Flag leaf tip appearance	71	30.8^{a}	28.4^{a}	259.0^{a}	961.0^{b}	0.730^{d}	41.8^{e}
Boot (start of panicle emergence)	79	26.9^{b}	26.8^{ab}	247.0^{a}	974.0^{b}	0.746^{c}	43.2^{de}
Complete panicle emergence	85	29.6^{a}	27.8^{ab}	258.5^{a}	1055.5^{b}	0.755^{bc}	45.2^{cd}
Anthesis	92	26.9^{b}	25.4^{bc}	256.5^{a}	1096.0^{b}	0.766^{b}	47.3^{ab}
Post-anthesis	99	25.2^{c}	23.0^{cd}	267.0^{a}	1120.0^{b}	0.762^{b}	47.1^{bc}
Milk	107	24.7^{cd}	23.2^{cd}	252.0^{a}	1089.0^{b}	0.768^{b}	48.5^{ab}
Soft dough	117	23.8^{d}	21.4^{d}	249.5^{a}	1178.5^{ab}	0.788^{a}	49.1^{ab}
Hard dough	129	23.5^{d}	20.9^{d}	293.5^{a}	1405.5^{a}	0.792^{a}	49.3^{a}
Physiological maturity	140	-	-	-	-	-	-
Post-physiological maturity	170	-	-	-	-	-	-
LSD value (P=0.05)		1.3	2.7	NS	NS	0.013	2.0

NS – Non-significant; Means within the same column with different letter are significantly different at P<0.05; - Data not recorded (Leaves were completely dried up at these stages of harvest).

$^{+}$ Fo = initial fluorescence

$^{\#}$ Fm = maximum fluorescence

Table 3.2 Effect of different stages of harvesting on growth traits of sweet sorghum variety M81E grown at Manhattan, Kansas.

Stages of harvesting	Days after planting	Plant height (cm)	No. of internodes plant^{-1}	No. of leaves plant^{-1}	Leaf area (cm^2)	Bottom 3rd internode diameter (mm)	Middle 6th internode diameter (mm)	Top 9th internode diameter (mm)	Average stem diameter (mm)
Flag leaf tip appearance	71	249^{d}	11.1^{b}	13.5^{ab}	4141.6^{b}	16.8^{e}	13.9^{e}	12.2^{d}	14.3^{g}
Boot (start of panicle emergence)	79	314^{c}	13.4^{a}	13.5^{ab}	4368.8^{b}	17.5^{de}	14.7^{de}	12.6^{d}	14.9^{fg}
Complete panicle emergence	85	352^{b}	13.5^{a}	13.8^{a}	4540.7^{ab}	18.1^{cd}	14.9^{d}	12.6^{d}	15.2^{f}
Anthesis	92	378^{a}	14.5^{a}	13.8^{a}	5069.2^{a}	18.4^{cd}	15.3^{cd}	13.4^{c}	15.7^{ef}
Post-anthesis	99	383^{a}	14.5^{a}	13.8^{a}	5074.6^{a}	18.7^{bc}	16.2^{bc}	13.6^{c}	16.1^{de}
Milk	107	383^{a}	14.5^{a}	13.5^{ab}	5036.5^{a}	19.8^{ab}	16.8^{b}	14.0^{bc}	16.8^{cd}
Soft dough	117	383^{a}	14.5^{a}	13.3^{ab}	4017.8^{b}	20.4^{a}	16.9^{b}	14.5^{b}	17.2^{bc}
Hard dough	129	385^{a}	14.5^{a}	12.8^{b}	-	20.5^{a}	17.9^{a}	15.3^{a}	17.9^{ab}
Physiological maturity	140	384^{a}	14.5^{a}	11.3^{c}	-	20.6^{a}	18.4^{a}	15.9^{a}	18.3^{a}
Post-physiological maturity	170	385^{a}	-	-	-	-	-	-	-
LSD value (P=0.05)		11.5	1.3	0.76	609.4	1.13	0.96	0.75	0.80

Means within the same column with different letter are significantly different at P<0.05; - Data not recorded.

Table 3.3 Effect of different stages of harvesting on yield traits of sweet sorghum variety M81E grown at Manhattan, Kansas.

Stages of harvesting	Days after planting	Stem fresh weight (t ha^{-1})	Total fresh biomass (t ha^{-1})	Stem dry weight (t ha^{-1})	Total dry biomass (t ha^{-1})	Grain yield (t ha^{-1})	Harvest index (grain) (%)	Harvest index (sugar) (%)
Flag leaf tip appearance	71	42.7^{h}	52.7^{g}	4.9^{f}	7.2^{f}	-	-	16.9^{d}
Boot (start of panicle emergence)	79	53.2^{g}	64.3^{f}	7.6^{e}	10.5^{e}	-	-	18.6^{cd}
Complete panicle emergence	85	58.2^{f}	70.3^{fe}	8.9^{de}	12.1^{e}	-	-	22.3^{bc}
Anthesis	92	64.9^{de}	79.0^{dc}	10.6^{cd}	16.8^{d}	2.4^{d}	14.3^{f}	22.4^{bc}
Post-anthesis	99	69.2^{dc}	89.6^{ba}	11.8^{bc}	18.7^{cd}	2.8^{d}	14.9^{ef}	23.1^{b}
Milk	107	73.4^{bc}	89.6^{ba}	12.1^{abc}	19.5^{bc}	3.3^{c}	16.7^{de}	29.6^{a}
Soft dough	117	75.8^{ba}	90.9^{ba}	13.1^{ab}	21.3^{ab}	3.9^{b}	18.3^{cd}	28.5^{a}
Hard dough	129	78.8^{a}	95.0^{a}	13.5^{ab}	22.0^{a}	4.2^{b}	19.1^{bc}	27.9^{a}
Physiological maturity	140	67.3^{d}	72.8^{de}	13.6^{a}	21.7^{a}	5.0^{a}	23.0^{a}	22.3^{bc}
Post-physiological maturity	170	62.0^{fe}	68.0^{fe}	11.9^{abc}	18.8^{cd}	3.9^{b}	20.5^{b}	23.4^{b}
LSD value (P=0.05)		4.6	6.2	1.8	2.2	0.45	1.8	4.2

Means within the same column with different letter are significantly different at $P<0.05$; - Data not available (No grain formation at these stages of growth).

Table 3.4 Effect of different stages of harvesting on juice quality traits of sweet sorghum variety M81E grown at Manhattan, Kansas.

Stages of harvesting	Days after planting	Juice pH	Starch ($\mu g\ g^{-1}$)	Extractable juice (%)	Juice purity (%)
Flag leaf tip appearance	71	5.32^{cd}	7.8^{f}	52.2^{a}	34.4^{e}
Boot (start of panicle emergence)	79	5.30^{bcd}	9.0^{f}	51.8^{a}	44.2^{d}
Complete panicle emergence	85	5.37^{b}	10.6^{ef}	54.5^{a}	55.8^{bc}
Anthesis	92	5.32^{bc}	16.51^{de}	50.9^{a}	49.3^{cd}
Post-anthesis	99	5.25^{cde}	21.2^{cd}	52.9^{a}	46.6^{cd}
Milk	107	5.20^{ef}	23.5^{bc}	53.5^{a}	41.9^{de}
Soft dough	117	5.15^{f}	28.4^{b}	52.6^{a}	49.7^{cd}
Hard dough	129	5.22^{def}	20.8^{cd}	53.2^{a}	81.3^{a}
Physiological maturity	140	5.55^{a}	20.6^{cd}	50.5^{a}	64.5^{b}
Post-physiological maturity	170	4.57^{g}	42.7^{a}	41.8^{b}	15.2^{f}
LSD value (P=0.05)		0.08	7.5	5.1	9.3

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

Table 3.5 Effect of different stages of harvesting on brix, reducing sugars, non-reducing sugars, total sugar percentage, juice and sugar yield of sweet sorghum variety M81E grown at Manhattan, Kansas.

Stages of harvesting	Days after planting	Brix (%)	Reducing sugar (%)	Non-reducing sugar (%)	Total sugar (%)	Juice yield (L ha^{-1})	Sugar yield (t ha^{-1})
Flag leaf tip appearance	71	5.4^{h}	2.8^{e}	1.8^{g}	4.7^{f}	22333^{f}	1.2^{f}
Boot (start of panicle emergence)	79	7.0^{g}	5.4^{d}	3.1^{f}	8.5^{e}	27631^{e}	1.9^{e}
Complete panicle emergence	85	8.3^{f}	7.1^{cd}	4.6^{e}	11.7^{d}	31822^{d}	2.6^{d}
Anthesis	92	10.1^{e}	$9.1b^{c}$	4.9^{e}	14.0^{c}	33067^{cd}	3.7^{c}
Post-anthesis	99	11.8^{d}	12.0^{a}	5.4^{de}	17.5^{b}	36667^{bc}	4.3^{b}
Milk	107	14.7^{b}	11.8^{a}	6.1^{d}	18.0^{b}	39244^{ab}	5.8^{a}
Soft dough	117	15.1^{b}	10.4^{ab}	7.5^{c}	17.9^{b}	39933^{ab}	6.0^{a}
Hard dough	129	14.6^{bc}	11.9^{a}	11.8^{a}	23.7^{a}	41867^{a}	6.1^{a}
Physiological maturity	140	14.0^{c}	9.2^{bc}	9.0^{b}	18.3^{b}	34056^{cd}	4.8^{b}
Post-physiological maturity	170	17.0^{a}	2.3^{e}	2.5^{fg}	4.8^{f}	26000^{ef}	4.4^{b}
LSD value (P=0.05)		0.6	2.2	0.99	2.3	3740.2	0.46

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

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Chapter 4 - Morpho-physiological based screening of sweet sorghum genotypes for high sugar yield

4.0 Abstract

Field research was conducted using 30 sweet sorghum genotypes varying in sugar yield, to understand the morpho-physiological basis for sugar yield in sweet sorghum (*Sorghum bicolor* L. Moench) genotypes. The physiological, growth, and yield traits were measured. Correlation studies were undertaken to establish valid relationship between the traits studied and sugar yield. A principal component analysis (PCA) was performed to distinguish genotypes depending on their sugar yield potential as well as to identify traits contributing for high sugar yield. High sugar yielding group produced greater number of green leaves, tall plant stature, and high average stem diameter. Greater number of green leaves coupled with high leaf chlorophyll (SPAD) content resulted in higher quantum yield of photosystem (PS) II (Fv/Fm). As a result of increased photosynthetic performance due to high Fv/Fm, plants produced greater stem fresh weight, which constitutes one of the major determinants of sugar yield in sweet sorghum. High stem weight resulted in greater quantity of extractable juice. The high sugar group accumulated relatively higher total dry biomass than the low sugar group. However, the grain yield was found to be significantly higher in the low sugar yielders in contrast to the high yielders. Further, it was observed that late maturing rather than early maturing genotypes proved to be high sugar yielders. Overall, high sugar yielders had better juice quality in terms of higher brix. The PCA revealed that PC1 and PC2 accounted for 52.38% and 12.25% of the variance, respectively. The component scores indicated genotypes No. 6 gambela and No. 5 gambela were high sugar yielders and genotypes Rahmatella gallabat and Atlas were low yielders. The highest positively

contributing trait for sugar yield was stem fresh weight, whereas grain yield had high negative characteristic.

4.1 Introduction

Sweet sorghum (*Sorghum bicolor* L. Moench.) can be an alternative biofuel feedstock because of its high biomass production and high percentage of easily fermentable sugars mainly sucrose in the juice (Mastrorilli et al., 1999; Barbanti et al., 2006; Rooney et al., 2007). Sweet sorghum is adaptable to a wide range of environmental conditions (Berenguer and Faci, 2001; Almodares and Hadi, 2009), and requires relatively less water and nitrogen fertilizer (Mastrorilli et al., 1999; Barbanti et al., 2006).

Traits like green stalk yield, stalk sugar content, stalk juice extractability and grain yield are the major contributors for sweet sorghum's economic importance (Almodares et al., 2006; Almodares et al., 2008). However, these traits are quantitative and polygenic inheritance in nature and are complex to be manipulated directly in breeding procedure (Zou et al., 2011). Therefore, to successfully improve these complex traits, they need to be dissected into smaller morphological, physiological and genetical components, which could be easily analyzed and evaluated. Previous studies have suggested that much variability exists in juice quality, sugar content, and juice yield among the U.S sweet sorghum collections (Ali et al., 2008; Murray et al., 2008a; Murray et al., 2009; Makanda et al., 2009). The consequences of the phenotypic variation depend largely on the environment. Furthermore, correlations between the traits are of great importance in selection process for successful breeding programs.

Correlation studies are important for both quantitative and qualitative trait improvement programs. In sweet sorghum, stalk yield has significant positive correlations with plant height, stem diameter and juice yield (Audilakshmi et al., 2010) and also a strong association of sugar

yield with brix was noticed (Pfeiffer et al., 2010). Therefore, selection for stalk yield should be focused on plant height, stem diameter, brix and juice yield. Total sugar content could be calculated from the brix because of a significant linear correlation between brix and total sugar content of the juice (Ma et al., 1992). Studies have shown that there was significant negative correlation between grain yield and stem biomass which might eventually lead to reduced sugar yield (Makanda et al., 2009). However, there are no studies that showed direct correlation between physiological traits and sugar yield.

Sugar yield is a quantitative trait, which is the resultant of various traits contributing together during the crop growth, which are interdependent in their development. It is, therefore, desirable to study the association between yield and yield attributing traits since this would facilitate effective selection for simultaneous improvement of one or more yield influencing components. However, due to multicollinearity between yield components, a simple correlation analysis between yield and its components would be misleading. In order to avoid multicollinearity between yield components and reduce the dimension of correlation, a PCA analysis is preferable. PCA is a powerful statistical tool by which relationship between complex traits can be studied. Development of suitable genotypes for sustainable production of sweet sorghum requires large screening of existing genotypes for quality and quantity of juice yield and sugar content. Scientific information on growth and development of sweet sorghum is limited and precludes establishing valid interrelationships for predicting its biomass production and sugar yield. Further, the relationships between morpho-physiological and stem sugar yield traits are not clearly understood.

The present study was conducted to gain an insight into various morphological and physiological traits contribute to sugar yield in sweet sorghum genotypes. Objectives of this

study were to (1) evaluate 30 sweet sorghum genotypes for their sugar yield; and (2) obtain information on the various growth and physiological traits influencing sugar yield. We hypothesize that sweet sorghum genotypes vary for the traits responsible for sugar yield.

4.2 Materials and Methods

4.2.1 Plant materials

A total of 30 genotypes were chosen based on sugar yield data collected from two years of field study using 280 sweet sorghum germplasm collections. Genotypes chosen comprised 15 high sugar yielders and 15 low sugar yielders. Seeds were obtained from the USDA-ARS, PGRCU, Isabela, Puerto Rico. The genotypes and their respective countries of origin selected for the study are presented in Table 4.1.

4.2.2 Experimental site and environmental conditions

All 30 genotypes were evaluated under rain-fed condition during 2009 at Ashland Bottoms Research Farm near Manhattan, Kansas (39°06′54.2″N - 96°38′10.0″W, Altitude: 323 m). Soil at the experimental site was chase silty clay loam (clay 12%, silt 60% and sand 28%; and pH 6.8). The daily weather parameters during the cropping season 2009 are presented in Fig. 4.1. The total amount of rainfall received during the cropping season (May-October) was 588 mm. The average maximum day and night air temperature were 25.4°C and 13.1°C, respectively. The normal (1971-2000) maximum and minimum air temperature for the period is 29.2°C and 14.8°C and normal cumulative rainfall is 497.3 mm.

4.2.3 Crop husbandry

The experimental plot was chisel plowed and planted on 29 May, 2009 at 5 cm deep with 6 m x 2 m plot size. Plots were fertilized with 90 kg N ha^{-1} as urea. For weed control, the plots

were sprayed with Bicep Lite II Magnum (a.i. 0.82 kg atrazine ha^{-1} and 1.03 kg S -metolachlor ha^{-1}) prior to planting. The field was kept weed free by hand weeding as necessary. Each plot consisted of four rows of 6 m length spaced 0.75 m apart.

4.2.4 Data collection

4.2.4.1 Physiological traits

Physiological traits were recorded on attached fully expanded flag leaves of two different tagged plants in each replicate for each genotype from flowering through maturity at 15 d intervals at midday (between 10:00 and 14:00 h). Leaf chlorophyll content was measured using a self-calibrating chlorophyll meter [Soil Plant Analytical Device (SPAD), Model 502, Spectrum Technologies, Plainfield, IL, USA]. The photochemical efficiency of PSII (Fv/Fm) was measured in 30-min dark-adapted leaves (Prasad et al., 2008) using pulse-modulated fluorometer (Pulse-modulated fluorometer OS 30p, OptiScience, Hudson, NH, USA). The leaf temperature was measured with a hand-held thermal imaging camera (FLIR ThermaCAM BCAM, Janesville, WI, USA).

4.2.4.2 Phenology and growth traits

Days to 50% flowering were recorded when 50% plants of each genotype in a plot had flowered. The plant growth traits were recorded from two randomly selected plants per replicate. Plant height was measured from base of the stem to the tip of the panicle using a measuring tape. Stem diameter was measured from the three regions of the stem (bottom 3^{rd} internode, middle 6^{th} internode, and the top 9^{th} internode) using vernier caliper after stripping the leaves and leaf sheaths. Data on stem diameter were averaged across the regions. Total number of green leaves and total internodes on the stem were counted.

4.2.4.3 Juice quality and yield traits

The yield traits were obtained from 5 plants from the interior row was harvested at maturity. Plants were stripped and topped and the fresh weight of the panicles, leaves, and stems were recorded. To extract juice, stems were crushed using a motor operated three roller sugarcane crusher (Sukra sugarcane crusher, Coimbatore, Tamil Nadu, India). After extraction, juice was weighed. The percentage of extractable juice was calculated from the juice volume of the sample and weight of fresh stem and expressed as a percentage. Juice yield was estimated as the volume of juice extracted from the stem. The total soluble solids (Brix) of extracted juice were determined using a brix meter (Digital hand-held pocket refractometer PAL-1, Atago, Bellevue, WA, USA) and expressed in percentage. Juice yield and brix were used to calculate sugar yield. The fresh leaf, panicle and crushed stem were oven-dried at 60°C for 7d and dry weights were recorded. The total dry biomass was calculated from the oven dried samples and expressed in g plant^{-1}. The panicles were threshed to obtain grain yield.

4.2.5 Data analyses

The experimental design was randomized complete block with three replications. The data were subjected to the analysis of variance for each trait using the general linear model of the statistical software by Statistical Analysis Systems (SAS), 9.1 (SAS Institute, 2003). Genotypic means and group means between high and low sugar yielders were compared by least significant differences (LSD) at 5% level of probability. Pearson's phenotypic correlation coefficients between traits measured were computed using PROC CORR procedure in SAS. The PCA was run using the PRINCOMP procedure of the SAS statistical package. The eigenvalue-one criterion was used to retain the principal components that contributed considerable variability. Eigenvectors generated by PCA were used to identify traits that best differentiate genotypes for sugar yield performance.

The first two PCA scores, PC1 and PC2 that accounted for maximum variability of the traits tested, were used to group the genotypes.

4.3 Results

4.3.1 Physiological traits

Significant differences were found for leaf chlorophyll content (SPAD) and PS II photochemical efficiency (Fv/Fm) across the genotypes. The leaf temperature did not differ significantly among genotypes (Table 4.2). The genotype Isidomba had maximum chlorophyll content, while the genotype Colman recorded the lowest (Table 4.3). The high sugar yielding genotype, Tracy, registered higher Fv/Fm ratio while the low sugar yielder, IS 2352 had the minimum ratio (Table 4.2). Groupwise analyses showed marked difference in chlorophyll content and Fv/Fm (Table 4.5). The high sugar yielders as a group contained significantly more chlorophyll than did the low sugar yielding group (Table 4.5). The high sugar yielding group significantly had higher Fv/Fm value as compared to low sugar yielders (Table 4.5).

4.3.2 Phenology and growth traits

There were significant differences among the genotypes for days to 50% flowering, plant height, number of green leaves, number of internodes and average stem diameter (Table 4.3). The high yielding genotypes, Honey No.6, No. 5 Gambela and No. 6 Gambela had the longest duration for 50% flowering and the genotypes, IS 2352 and Ames amber, a low sugar yielders, had shorter period (Table 4.3). The maximum plant height was recorded in a high sugar yielder, Honey No. 6 while the lower sugar yielder Waxy club had the minimum height (Table 4.3). Among the genotypes, the high sugar yielder, MN 4578, recorded maximum green leaf number as against the minimum found in Red amber, a low sugar yielder (Table 4.3). Genotype MN 600, a high sugar yielder had maximum number of internodes at maturity as against the low sugar

yielder, Red amber (Table 4.3). The average stem diameter of individual genotypes showed the high yielder Wray, maintaining a higher value compared to a low sugar yielder, IS 2352 (Table 4.3). Significant differences were also observed between high and low sugar yielding groups (Table 4.5). The data indicated that high sugar yielding group reached longer period to attain 50% flowering whereas the low sugar group had shorter period when 50% of the plants flowered (Table 4.5). Groupwise also, the high sugar yielders significantly differed with low sugar yielders and exhibited higher plant stature as against the low sugar yielders (Table 4.5). The high sugar yielding group had greater green leaf number when compared to the low sugar group (Table 4.5). The high sugar yielders as a group possessed significantly greater number of internodes when compared to low sugar yielders (Table 4.5). The high sugar yielders maintained higher values of average stem diameter compared to low sugar yielders (Table 4.5).

4.3.3 Juice quality and yield traits

Significant genotypic variation was observed for brix, juice yield, sugar yield, extractable juice, grain yield, stem fresh weight and total dry biomass (Table 4.4). Highest brix was recorded in the genotype, Della, a high sugar yielder whereas the lowest percentage of brix was obtained in the genotype, IS 2352, a low sugar group (Table 4.4). The highest juice yield recorded in a high sugar yielder, Honey No. 6 whereas the lowest yield was obtained by the low sugar yielder, MN 2894 (Table 4.4). The high sugar yielders, Wray, Honey No. 6 and Isidomba were found superior as indicated by the mean sugar yield value (Table 4.4). Among low sugar yielders, IS 2352 and Ames amber produced the lowest sugar yields. The highest extractable juice percentage was recorded in a low sugar yielder, IS 2352 while also the low sugar yielder, Rahmatella gallabat recorded the lowest percentage juice extractability (Table 4.4). The genotype belonging to the low sugar group, MN 2894 showed higher grain yield while the high sugar genotype,

Honey No. 6 recorded the lowest (Table 4.4). Genotype Wray, a high yielder, produced highest yield of stem fresh weight (Table 4.4). The two early maturing and low yielders, IS 2352 and Ames amber, produced the lowest stem fresh weights (Table 4.4). Among the genotypes, Awanlek, the high sugar yielder, was found to be highest in respect of total dry biomass (Table 4.4). The two low sugar yielders, Ames amber and Red amber, were recorded lowest total dry biomass.

There also appeared to be marked difference between the two sugar groups for the above traits except extractable juice (Table 4.5). Groupwise mean values indicated significantly higher amount of brix value present in the high sugar group than recorded lowest brix in the low sugar yielders (Table 4.5). Similarly, higher content of juice was obtainable from the high sugar yielders than low sugar yielding genotypes (Table 4.5). The high sugar group registered significantly higher sugar yield than the low sugar group (Table 4.5). The low sugar yielders produced higher grain yield than most of the high sugar yielders (Table 4.5). The high sugar yielding group had significantly higher stem fresh weight as compared to low sugar group (Table 4.5). The high sugar group produced significantly higher dry biomass than the low sugar group as indicated by their mean values (Table 4.5).

4.3.4 Correlation analyses

Correlation studies revealed significant and positive correlation between sugar yield and plant height (Fig. 4.2a), number of green leaves (Fig. 4.2b), and average stem diameter (Fig. 4.2c). Significant positive correlation was observed between sugar yield and days to 50% flowering (Fig. 4.3a), leaf chlorophyll (Fig. 4.3b); Fv/Fm (Fig. 4.3c) and brix (Fig. 4.3d). The yield traits such as stem fresh weight (Fig. 4.4a) and juice yield (Fig. 4.4b) were positively and

significantly correlated with sugar yield while grain yield (Fig. 4.4c) was found negatively associated with sugar yield.

4.3.5 Principal component analyses (PCA)

The results of the PCA of the traits and genotypes are presented in Table 4.6. Considering the Eigenvalue-one criterion, the first three principal components explained 74.08% of the total variation among sweet sorghum genotypes. The first principal component (PC1) had an eigenvalue of 7.334 and explained 52% of the total variation present in the sweet sorghum genotypes. Stem fresh weight, number of green leaves, number of internode, plant height, days to 50% flowering, stem diameter, juice yield, total dry biomass and brix had the highest positive loadings in PC1 while grain yield, extractable juice and leaf temperature had negative loadings (Table 4.6). The second principal component (PC2) had an Eigenvalue of 1.715 and accounted for around 12% of the total variation. The PC2 primarily had a positive loading with extractable juice, leaf temperature, chlorophyll SPAD and Fv/Fm. The third principal component had eigenvalue of 1.322 and contributed 9.44% to the total variation. Component scores for the genotypes evaluated are shown in Table 4.6. Genotypes such as MN 4578, Wray, MN 600, No. 6 gambela, No. 5 gambela and Honey No. 6 had highest positive values for PC1 whereas, genotypes IS 2352, Ames amber, Red amber, Waxy club, Luel and Colman showed negative values for PC1. The group of genotypes with the positive PC2 values were Honey No. 6, IS 2352, Tracy, IS 12900, Isidomba and MN 4135 while genotypes Atlas, MN 2894, MN 4578, Della, Nerum boer and Leolti-peltier had negative PC2 values (Table 4.6). The first two PCs, which contributed to about 65% of the total variance, were graphically presented on a two-dimensional plot (Fig. 4.5). On an average, high sugar yielders were highly correlated to PC1 and were placed on the right of the bi-plot and most of the low sugar yielders correlated to PC2

and were placed on the left of the bi-plot (Fig. 4.5). Fig. 4.6 shows the correlation between the traits and the principal components for the contribution of each trait to the total variance. The first principle component PC1 was significantly and positively associated with most of the traits such as stem fresh weight, number of green leaves and internodes, days to 50% flowering, plant height, average stem diameter, juice yield, total dry biomass, Fv/Fm, brix, and chlorophyll SPAD negatively with grain yield, extractable juice and leaf temperature (Fig. 4.6). PC2 had a significantly and positively correlated to extractable juice, leaf temperature and chlorophyll SPAD and negatively with grain yield (Fig. 4.6).

4.4 Discussion

Recently, breeding for improving biofuel (from juice) and byproducts (from bagasse) is becoming an important breeding objective for sweet sorghum breeders to meet the rapidly increased demand for biofuel production worldwide. It is well known that progress in plant breeding depends on the extent of genotypic variability existed in a population. Significant genotypic variability for plant height, stem diameter, days to anthesis, brix and grain yield among sweet sorghum genotypes was previously reported (Ritter et al., 2008; Ali et al., 2008; Murray et al., 2008a; Wang et al., 2009; Murray et al., 2009; Makanda et al., 2009). In the present study, since the sweet sorghum genotypes was chosen from two diverse sugar yielding groups, most of the measured traits exhibited extensive variability.

The presence of green leaf number contributes assimilates supply to the sink (stem). The green leaf numbers revealed that the genotypes belonging to the high sugar yielding group possessed greater number of leaves. The low sugar yielding genotypes contained fewer number of green leaves which restricted it's assimilate production (Table 4.5). The high sugar yielding genotype, MN 4578, which produced the maximum number of green leaves, also accumulated

the greatest amount of dry matter; while Red amber, a low sugar yielder, produced a low total dry biomass with the least number of leaves (Table 4.3 and Table 4.4). In general plants that accumulated greater biomass had higher sugar yield at maturity. The leaf number and dry matter accumulation in sorghum were positively associated with each other as reported by Berenguer and Faci (2001).

High sugar yielders generally possessed taller stature than the low sugar yielders. Tallness of the plant favored production of greater amount of biomass in the stem portion and this was indicated by the accumulation of greater stem fresh weight in the taller genotypes like MN 4578, MN 600 and No. 6 Gambela (Table 4.3 and Table 4.4). Earlier studies involving sweet sorghum hybrids also brought out a significant role of plant height in contributing to total biomass (Pfeiffer et al., 2010).

The high sugar yielders recorded the highest chlorophyll content and PS II photochemistry (Fv/Fm) whereas low sugar yielders had lowest chlorophyll and Fv/Fm (Table 4.2 and Table 4.5). Evidence is also available to point towards relative chlorophyll content as a rate determining factor in photosynthesis (Anjum et al., 2011). High photosynthetic rate is positively correlated with PS II photochemistry Fv/Fm ratio (Van der Tol et al., 2009). The increase in PS II photochemistry might have increased photosynthetic rate in high sugar yielding group which favored high biomass accumulation and sugar yield. The leaf temperature did not vary between the two sugar yielding groups hence the influence of leaf temperature on sugar yield was minimum (Table 4.5).

The high sugar yielding group produced higher stem fresh weight, juice yield and high brix which were the contributory factors for their high sugar yield (Table 4.4). Conversely, low sugar yielding group had lower stem fresh weight, juice yield and brix resulted in lower sugar

yields. The stem fresh weight could also be directly related to juice yield. Murray et al. (2008 a, b) reported a significant association between brix and juice yield. Since sugar yield is a product of brix and juice yield, these two traits may be considered selection criteria for identifying a sweet sorghum genotype with a potential for high sugar yield.

It is also noticed that high sugar yielders are late maturing enabled the plant to maintain an adequate supply of photoassimilates for a longer duration than in the case of low sugar yielders. It suggests that earliness does not favor higher accumulation of sugar in sweet sorghum (Table 4.3). Stem and grain were all sinks of the photosynthates in terms of the relationship between source and sink in sweet sorghum. After post-flowering, stored photoassimilates from leaf and stem would start to mobilize to the grain development. In sweet sorghum, the maintenance of stem weight depends on how fast this partitioning process occurs in order to favor high sugar accumulation. This is observed from this study that high sugar yielders are known for their low grain yields, partitioning of photoassimilates was less towards grain sink unlike in the case of low sugar yielders where greater proportion of assimilates were diverted for grain as indicated by higher grain harvest index (Table 4.4).

Knowledge of correlation is important to obtain the expected response of other traits when selection is applied to a particular trait of interest in a breeding programme. To determine the degree of association of component traits with sugar yield, the correlation coefficients were estimated considering sugar yield as a dependent trait. Of the various traits, plant height, stem diameter, juice yield and stem fresh weight were positively and highly significantly correlated with sugar yield. Taller plants having more stem biomass and juice could produce more sugar yield. Similar result was reported by Murray et al. (2008a and b). There was a significant

negative correlation between grain yield and sugar yield indicating that panicle acted as a strongest sink for carbohydrates (Fig. 4.4c).

The PCA was performed on the basis of all measured traits (Table 4.6) and genotypes (Fig. 4.5) were subjected to biplot analysis for assessing the relationships among all of component traits. The first two principal components explained about 65% variation among sweet sorghum genotypes for all the traits investigated. The PC1 had higher correlation with stem fresh weight, number of green leaves, internode number, days to 50% flowering, plant height, stem diameter and juice yield (Fig. 4.6). Thus, PC1 can be named as the sugar yield potential and genotypes on this PC1 biplot will be high sugar yielders (Fig. 4.5). PC2 had positive correlation with extractable juice and leaf temperature and negatively associated with grain yield. The PC2 was named as low sugar yield potential which separates low sugar yielders from high sugar yielding genotypes. Thus selection of genotypes that has high PC1 is suitable for high sugar yield characteristics.

4.5 Conclusions

Sugar yield in sweet sorghum is an integration of morpho-physiological traits. High sugar yielders had higher green leaf numbers, tall plant stature, high average stem diameter, higher Fv/Fm and higher stem biomass accumulation, and low grain yield. Whereas, the low sugar yielders had more assimilates in grain. Of the thirty sweet sorghum genotypes investigated in this study, five genotypes, Wray, Honey No. 6, Isidomba, MN 4135 and No. 5 Gambela were identified as the high sugar yielders. In addition, principal component analysis (PCA) made it possible to establish similar groups of genotypes, according to their sugar yielding characteristics, as well as to study relationships among traits associated with sugar yield.

4.6 Tables and Figures

Figure 4.1 Daily maximum and minimum mean air temperature and rainfall during the cropping season (2009).

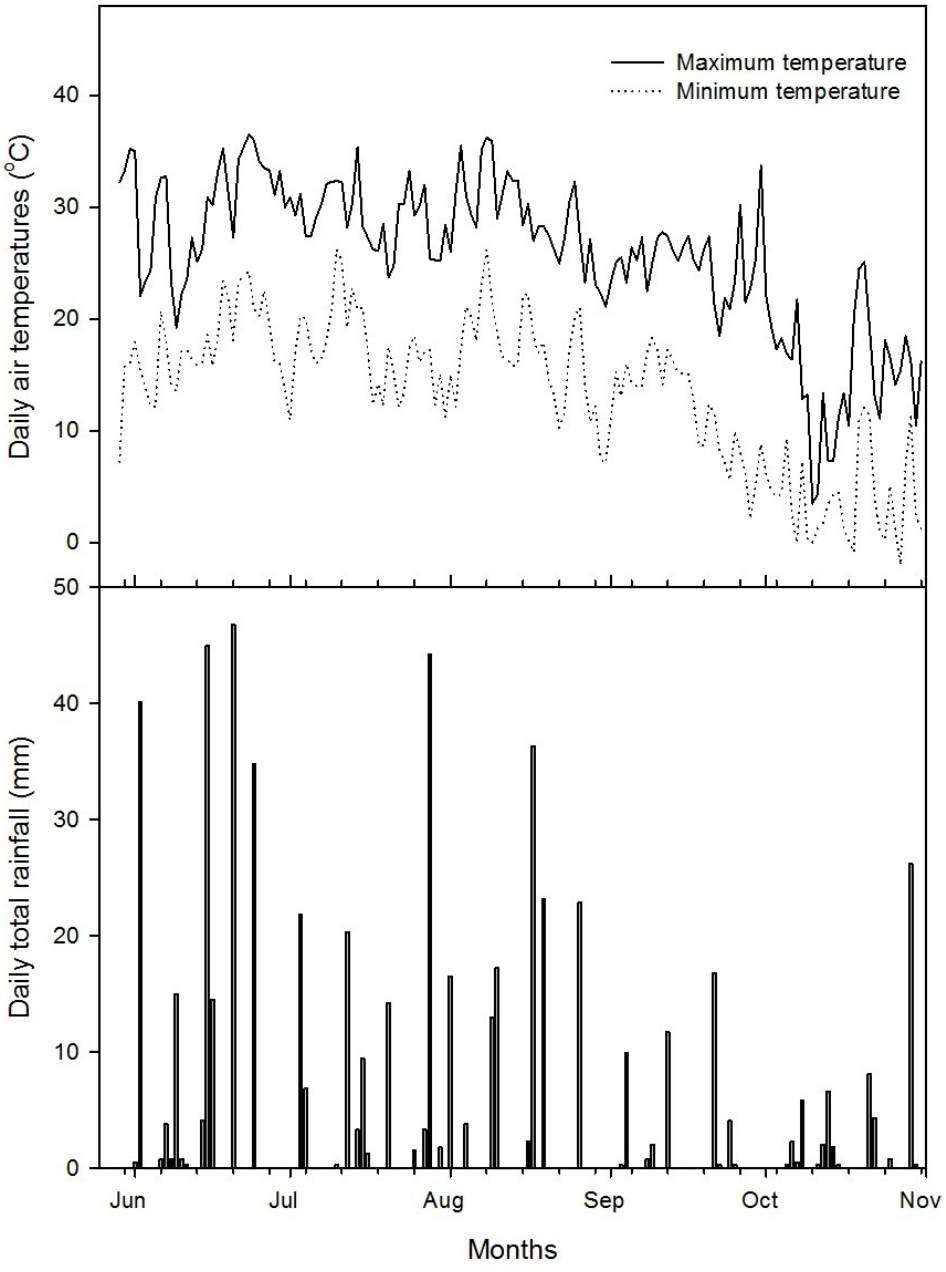

Figure 4.2 Correlation between sugar yield and growth traits. (a) plant height (b) number of leaves and (c) average stem diameter.

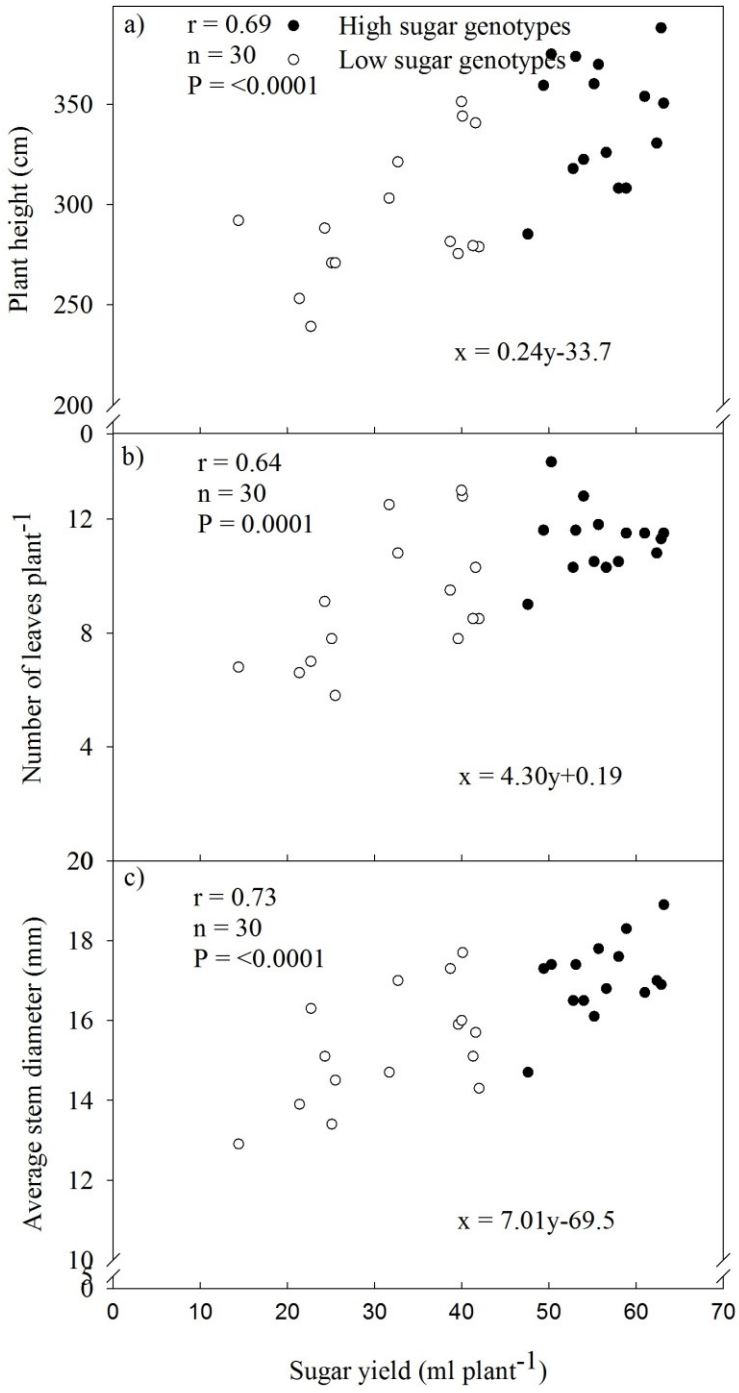

Figure 4.3 Correlation between sugar yield and phenology, physiology and juice quality traits. (a) days to 50% flowering (b) chlorophyll SPAD (c) Fv/Fm and (d) brix.

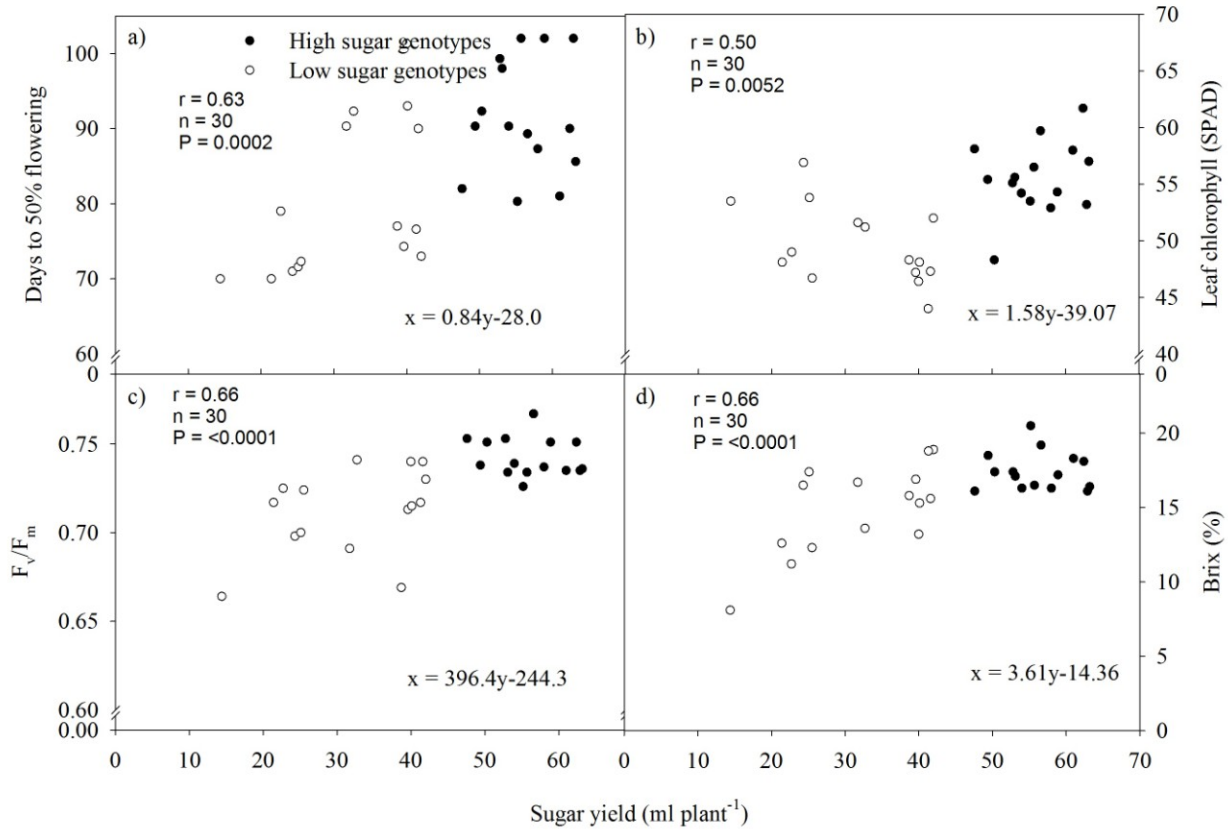

Figure 4.4 Correlation between sugar yield and yield traits. (a) stem fresh weight (b) juice yield and (c) grain yield.

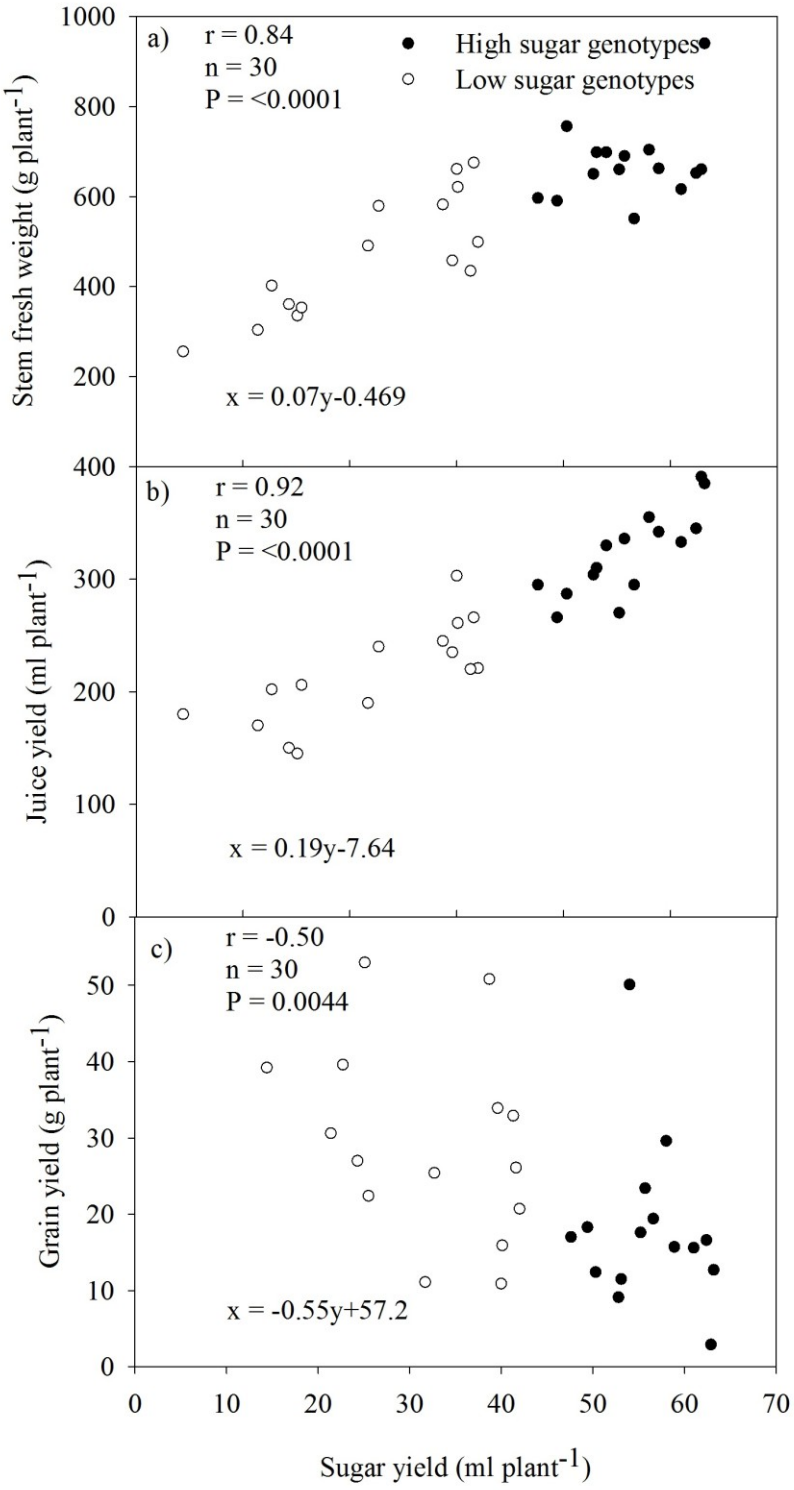

Figure 4.5 Biplot analysis showing 30 sweet sorghum genotypes for principle components (PC1 and PC2).

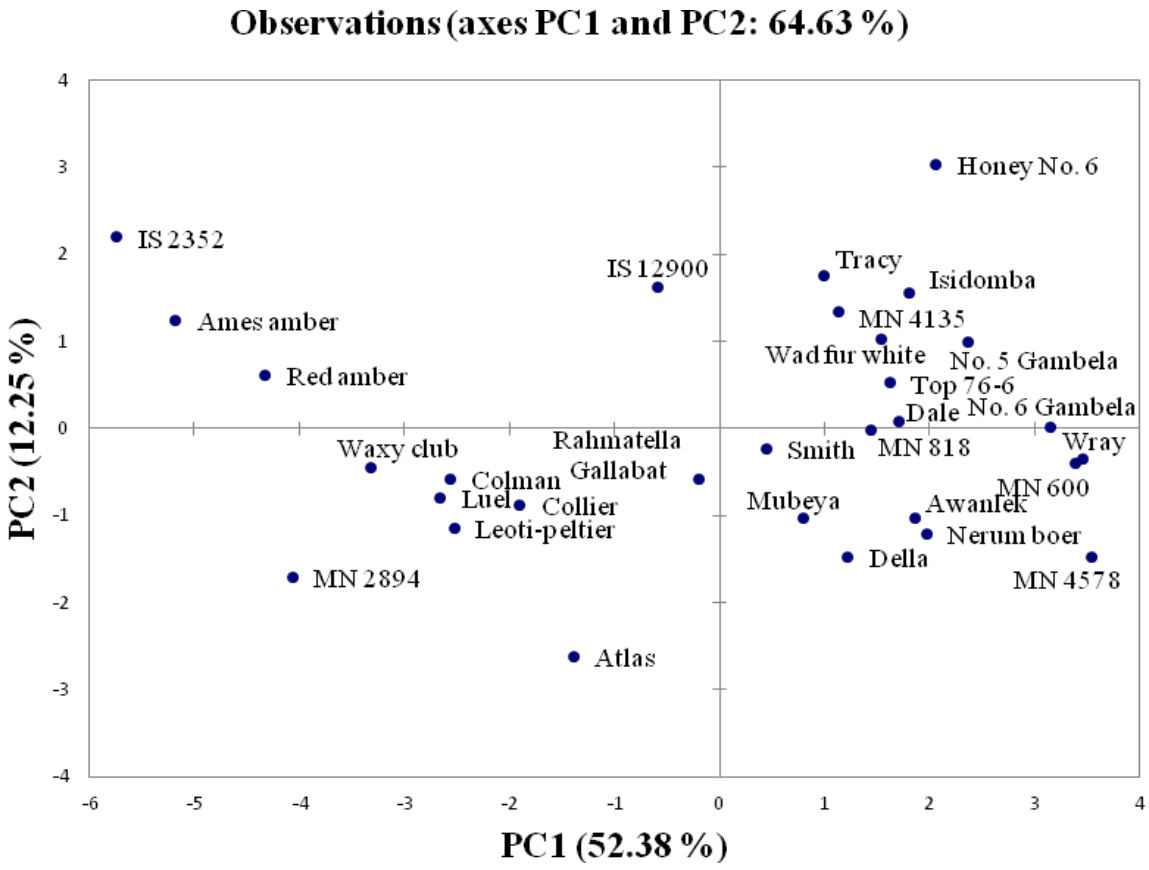

Figure 4.6 Correlations between traits and principal components indicated by thick lines from centre point and showing the direction (angle) and magnitude (length) for maximum chlorophyll (SPAD), PS II photochemical efficiency (Fv/Fm), juice yield (JY), days to 50% flowering (DF), plant height (PH), number of internodes (NI), stem fresh weight (SFW), number of green leaves (NL), average stem diameter (ASG), brix, total dry biomass (TDM), extractable juice (JEX), leaf temperature (LT) and grain yield (GY).

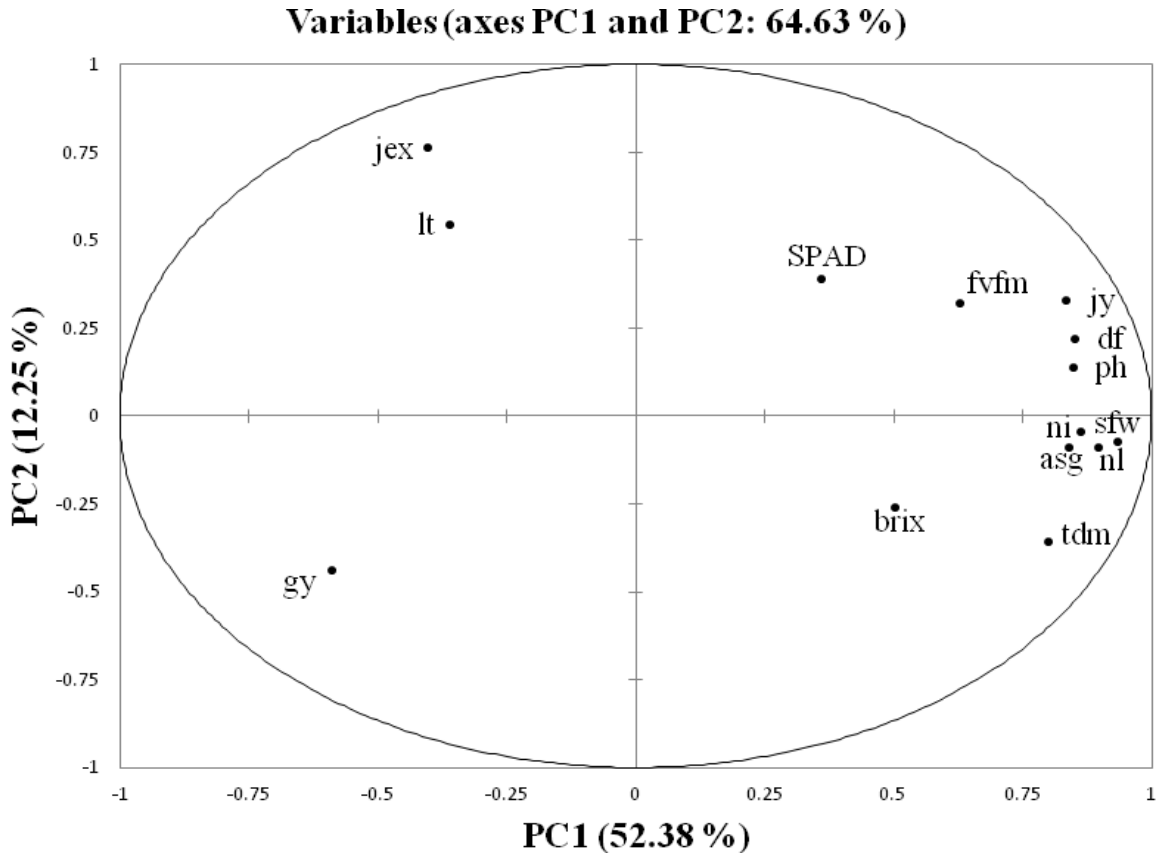

Table 4.1 Sweet sorghum genotypes selected for the study and their respective countries of origin.

High sugar yielders	Origin	Low sugar yielders	Origin
Awanlek	Sudan	Ames amber	*
Dale	USA	Atlas	*
Della	USA	Collier	*
Honey No.6	*	Colman	*
IS 12900	India	IS 2352	Pakistan
Isidomba	South Africa	Leolti-peltier	*
MN 4135	Yugoslavia	Luel	Sudan
MN 4578	Ethiopia	MN 2894	Syria
MN 600	France	Mubeya	Kenya
MN 818	Hungary	Nerumboer	Sudan
No. 5 gambela	Ethiopia	Rahmetalla gallabat	*
No. 6 gambela	Ethiopia	Red amber	Australia
Top 76-6	USA	Smith	USA
Tracy	USA	Wad fur white	Sudan
Wray	USA	Waxy club	USA

*Unknown.

Table 4.2 Genotypic means of various physiological traits of sweet sorghum genotypes grown at Manhattan, Kansas.

Genotypes	Chlorophyll (SPAD)	Leaf temperature (°C)	PS II photochemical efficiency (Fv/Fm)
Ames amber	48.1	29.8	0.717
Atlas	48.3	29.0	0.669
Awanlek	54.2	29.0	0.739
Collier	52.0	28.7	0.730
Colman	44.0	29.5	0.717
Dale	55.4	29.1	0.738
Della	53.5	28.4	0.726
Honey No. 6	53.2	29.4	0.735
IS 12900	58.1	29.6	0.753
IS 2352	53.5	29.7	0.664
Isidomba	61.7	29.1	0.751
Leoti-peltier	47.2	28.5	0.713
Luel	56.9	29.2	0.698
MN 2894	53.8	29.0	0.700
MN 4135	58.0	29.1	0.735
MN 4578	48.3	28.6	0.751
MN 600	55.6	28.5	0.734
MN 818	52.9	29.0	0.737
Mubeya	47.3	29.1	0.740
Nerumboer	48.1	28.8	0.715
No. 5 gambela	54.3	29.2	0.751
No. 6 gambela	56.5	28.7	0.734
Rahmatella gallabat	51.6	29.6	0.691
Red amber	46.7	28.7	0.724
Smith	51.2	29.1	0.741
Top 76-6	55.1	28.7	0.753
Tracy	59.7	29.3	0.767
Wad fur white	46.4	29.6	0.740
Waxy club	49.0	28.9	0.725
Wray	57.0	28.6	0.736
LSD at 0.05 significance level	3.0	NS	0.04

NS – Non-significant.

Table 4.3 Genotypic means of phenology and growth traits of sweet sorghum genotypes grown at Manhattan, Kansas.

Genotypes	Days to 50% flowering	Plant height (cm)	Number of green leaves plant^{-1}	Number of internodes plant^{-1}	Average stem diameter (mm)
Ames amber	70	253.1	6.6	9.5	13.9
Atlas	77	281.5	9.5	12.3	17.3
Awanlek	90	322.5	12.8	14.3	16.5
Collier	73	278.9	8.5	10.3	14.3
Colman	76	279.4	8.5	10.8	15.1
Dale	90	359.4	11.6	14.0	17.3
Della	80	360.2	10.5	12.1	16.1
Honey No. 6	102	388.1	11.3	13.5	16.9
IS 12900	82	285.2	9.0	11.8	14.7
IS 2352	70	292.0	6.8	11.1	12.9
Isidomba	90	330.6	10.8	13.0	17.0
Leoti-peltier	74	275.5	7.8	10.1	15.9
Luel	71	288.2	9.1	11.6	15.1
MN 2894	71	270.9	7.8	10.1	13.4
MN 4135	81	353.9	11.5	13	16.7
MN 4578	92	375.0	14.0	15.0	17.4
MN 600	98	373.8	11.6	16.3	17.4
MN 818	87	308.1	10.5	13.6	17.6
Mubeya	90	340.7	10.3	13.0	15.7
Nerumboer	93	344.1	12.8	15.3	17.7
No. 5 gambela	102	308.1	11.5	13.6	18.3
No. 6 gambela	102	369.9	11.8	14.5	17.8
Rahmatella gallabat	90	303.1	12.5	15.3	14.7
Red amber	72	270.9	5.8	8.6	14.5
Smith	92	321.3	10.8	13.5	17.0
Top 76-6	99	317.9	10.3	12.8	16.5
Tracy	89	325.9	10.3	12.8	16.8
Wad fur white	101	351.3	13.0	14.1	16.0
Waxy club	79	239.1	7.0	10.5	16.3
Wray	85	350.5	11.5	13.8	18.9
LSD at 0.05 significance level	3.9	19.3	1.6	1.4	1.3

Table 4.4 Genotypic means of various juice quality and yield traits of sweet sorghum genotypes grown at Manhattan, Kansas.

Genotypes	Brix (%)	Juice yield (ml plant^{-1})	Sugar yield (ml plant^{-1})	Extractable juice (%)	Grain yield (g plant^{-1})	Stem fresh weight (g plant^{-1})	Total dry biomass (g plant^{-1})	Harvest index
Ames amber	12.6	170	21.4	56	30.6	303.8	132.6	0.23
Atlas	15.8	245	38.7	42	50.8	582.3	224.2	0.23
Awanlek	16.3	330	54.0	47	50.1	698.3	273.7	0.18
Collier	18.9	221	42.0	44	20.7	499.2	170.0	0.12
Colman	18.8	220	41.3	50	32.9	434.8	183.3	0.18
Dale	18.5	266	49.4	45	18.3	590.6	204.8	0.09
Della	20.5	270	55.2	41	17.6	659.9	212.4	0.09
Honey No. 6	16.1	391	62.9	60	2.90	660.5	167.7	0.02
IS 12900	16.1	295	47.6	49	17.0	596.5	190.2	0.09
IS 2352	8.1	180	14.4	70	39.2	255.8	153.9	0.25
Isidomba	18.1	345	62.4	53	16.6	652.3	210.2	0.08
Leoti-peltier	16.9	235	39.6	51	33.9	457.7	164.9	0.21
Luel	16.5	150	24.3	41	27.0	360.9	161.3	0.17
MN 2894	17.4	145	25.1	43	53.0	335.4	165.1	0.33
MN 4135	18.3	333	61.0	54	15.6	616.1	180.9	0.09
MN 4578	17.4	287	50.3	38	12.4	755.9	250.4	0.05
MN 600	17.1	310	53.1	44	11.5	698.3	238.5	0.05
MN 818	16.3	355	58.0	50	29.6	703.8	233.7	0.13
Mubeya	15.6	266	41.6	39	26.1	675.1	228.1	0.11
Nerum boer	15.3	261	40.1	42	15.9	621.0	232.6	0.07
No. 5 Gambela	17.2	342	58.9	51	15.7	662.3	228.6	0.07
No. 6 Gambela	16.5	336	55.7	49	23.4	690.0	257.7	0.09
Rahmatella	16.7	190	31.7	38	11.1	490.9	192.0	0.06
Red amber	12.3	206	25.5	58	22.4	353.0	136.9	0.17
Smith	13.6	240	32.7	42	25.4	579.0	192.9	0.14
Top 76-6	17.4	304	52.8	46	9.1	650.4	194.4	0.05
Tracy	19.2	295	56.6	54	19.4	550.7	188.1	0.11
Wad fur white	13.2	303	40.0	46	10.9	661.2	201.2	0.05
Waxy club	11.2	202	22.7	50	39.6	401.8	171.6	0.23
Wray	16.4	385	63.2	41	12.7	940.0	220.4	0.06
LSD at 0.05 significance level	1.3	39.9	7.0	8.8	6.8	72.3	19.4	0.04

Table 4.5 Group mean values of various traits in high and low sugar yielding sweet sorghum genotypes grown at Manhattan, Kansas.

Traits	High sugar yielders	Low sugar yielders	LSD
Physiology			
Chlorophyll content (SPAD)	55.6[a]	49.6[b]	0.7
Leaf temperature (°C)	28.9[a]	29.1[a]	NS
PS II photochemical efficiency (Fv/Fm)	0.743[a]	0.712[b]	0.01
Phenology and growth			
Days to 50% flowering	91.4[a]	80.1[b]	1.0
Plant height (cm)	341.9[a]	292.7[b]	4.9
Number of green leaves plant^{-1}	11.2[a]	9.1[b]	0.4
Number of internodes plant^{-1}	13.6[a]	11.7[b]	0.3
Average stem diameter (mm)	17.1[a]	15.3[b]	0.3
Juice quality and yield			
Brix (%)	17.4[a]	14.8[b]	0.3
Juice yield (ml plant^{-1})	323.0[a]	215.7[b]	10.3
Sugar yield (ml plant^{-1})	56.1[a]	32.1[b]	1.8
Extractable juice (%)	48.3[a]	47.7[a]	NS
Stem fresh weight (g plant^{-1})	675.1[a]	467.4[b]	18.6
Total dry biomass (g plant^{-1})	216.8[a]	180.7[b]	5.0
Grain yield (g plant^{-1})	18.1[b]	29.3[a]	1.7

NS – Non-significant.

Table 4.6 Eigenvectors, eigenvalue, total variance, cumulative variance, and component scores for 30 sweet sorghum genotypes based on 14 traits.

Traits	Eigenvectors			Genotypes	Component scores		
	PC1	PC2	PC3		PC1	PC2	PC3
Chlorophyll SPAD	0.133	0.297	-0.406	Awanlek	1.866	-1.030	0.724
Leaf temperature	-0.133	0.416	0.402	Dale	1.703	0.084	-0.243
Fv/Fm	0.232	0.244	-0.337	Della	1.213	-1.471	-1.696
Days to 50% flowering	0.314	0.169	0.242	Honey No. 6	2.056	3.031	0.557
Plant height	0.314	0.106	0.091	IS 12900	-0.595	1.620	-0.840
Number of leaves	0.332	-0.068	0.245	Isidomba	1.805	1.555	-1.356
Number of internodes	0.318	-0.034	0.339	MN 4135	1.133	1.341	-1.027
Stem diameter	0.310	-0.068	-0.016	MN 4578	3.547	-1.479	0.577
Brix	0.186	-0.197	-0.522	MN 600	3.379	-0.407	0.111
Juice yield	0.308	0.252	-0.091	MN 818	1.433	-0.015	0.017
Extractable juice	-0.149	0.581	0.005	No. 5 Gambela	2.363	0.983	0.082
Stem fresh weight	0.345	-0.056	-0.035	No. 6 Gambela	3.153	0.016	0.272
Total dry biomass	0.295	-0.273	0.173	Top 76-6	1.621	0.521	-1.035
Grain yield	-0.218	-0.334	0.076	Tracy	0.991	1.751	-1.531
Eigenvalue	7.334	1.715	1.322	Wray	3.460	-0.354	-0.847
Variability (%)	52.38	12.25	9.44	Ames amber	-5.174	1.241	0.495
Cumulative (%)	52.38	64.64	74.08	Atlas	-1.393	-2.620	1.102
				Collier	-1.904	-0.875	-1.953
				Colman	-2.557	-0.580	0.128
				IS 2352	-5.734	2.196	2.116
				Leoti-peltier	-2.524	-1.143	-1.148
				Luel	-2.667	-0.791	-0.564
				MN 2894	-4.064	-1.702	-0.936
				Mubeya	0.789	-1.031	0.823
				Nerum boer	1.965	-1.216	1.588
				Rahmatella Gallabat	-0.201	-0.576	1.893
				Red amber	-4.329	0.613	-0.808
				Smith	0.445	-0.240	0.877
				Wad fur white	1.532	1.018	2.426
				Waxy club	-3.312	-0.444	0.196

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Chapter 5 - Effect of water stress during early grain filling on growth, physiology and yield attributes of sweet sorghum in controlled environment

5.0 Abstract

Sweet sorghum (*Sorghum bicolor* L. Moench) is an important bioenergy crop grown in semi-arid regions of the world. Yield and quality of sweet sorghum are severely affected by occurrences of drought (water stress) during reproductive periods. A greenhouse experiment was conducted to study the impact of water stress on physiology, growth and development of four sweet sorghum genotypes. Genotypes (Awanlek, Smith, Tracy and Wray) were subjected to three water stress treatments (100% pot capacity, fully irrigated; 70% pot capacity, mild stress; and 30% pot capacity, severe stress) for 20 days at the beginning of grain filling (Milk) until hard dough stage. During the stress period, data on physiological traits were recorded at 5 d interval. At grain maturity, growth, yield and bioenergy traits were measured. The results showed that genotypes differed significantly for all physiological, growth and yield, and bioenergy traits. Average stem diameter and grain yield were found non-significant among the genotypes. Treatments showed significant effect on yield, sucrose, and all physiological parameters. The interaction for genotypes and treatments was significant for juice and sugar yields, glucose, fructose, sucrose and all measured physiological traits. Water stressed plants significantly decreased chlorophyll SPAD and Fv/Fm. Severe drought significantly decreased juice and sugar yields by decreasing net photosynthetic rate, transpiration rate and stomatal conductance. Relative to the control plants, sucrose in the stem juice increased significantly under severe stress, whereas the water stress did not affect the levels of glucose and fructose. Genotypes Tracy and Wray produced significantly highest brix, stem fresh weight, juice and sugar yields under

both irrigated and water stress conditions. Severe water stress at milk stage has detrimental effect in reducing most of the bioenergy traits than mild water stress. Among the genotypes, Tracy was found to be relatively more drought tolerant.

5.1 Introduction

Sweet sorghum (*Sorghum bicolor* L. Moench) is an important feedstock for the use of biofuels (from juice) and by-products (from bagasse) (Vermerris et al., 2007; Rooney et al., 2007; Vasilakoglou et al., 2010). Sweet sorghum is characterized by high sugar content primarily sucrose in the plant sap or juice, from which ethanol can be produced (Kamiyama et al., 2009). The sorghum biomass is a rich source of cellulose, hemicelluloses and lignin.

Drought stress is the primary limiting factor of crop productivity, accounting for more than a 50% reduction in yields worldwide (Boyer, 1982). Generally, sweet sorghum is grown in the semi-arid regions of the world. In these regions, optimum irrigation is vital for maximizing crop yield's because decreasing water supply causes a significant reduction in sorghum biomass and sugar yield. Drought stress affects various physiological processes such as leaf temperature, leaf chlorophyll, chlorophyll *a* fluorescence (Fv/Fm), stomatal conductance, transpiration and photosynthesis in various crops (Silva et al., 2007). In the case of sweet sorghum extensive research has been done on agronomic performance for sugar and ethanol yield (Teetor et al., 2011), biomass yield and composition (Zhao et al., 2009), and juice fermentation (Wu et al., 2010), genetic diversity (Ali et al., 2008; Wang et al., 2009), water use efficiency and other photosynthetic characteristics (Cornic and Massacci, 1996; Steduto et al., 1997; Mastrorilli et al., 1999). However, little is known about the physiology, growth and yield of sweet sorghum under drought stress.

In sweet sorghum, sucrose, glucose and fructose contents in stem increase after anthesis and reach a maximum level near post anthesis (Almodares et al., 2008). Hence, environmental condition during reproductive growth stage is an important factor affecting carbohydrate content (Almodares et al., 2007). Drought stress experiments on sugar beet (*Beta vulgaris* L.) have

shown adverse effects on both leaf photosynthesis as well as sucrose yields in the mature plants (Monti et al., 2006). They also reported that drought stress in the early growth period was negatively associated with the sucrose content at maturity. In studies on sugarcane (*Saccharum officinarum* L.), stem diameter (Da Silva and Da Costa, 2004), and stalk height and cane yield (Inman-Bamber and Smith, 2005) were severely affected by drought conditions. Drought also resulted in morphological changes in the sugarcane, which included reduced leaf area, thicker leaves, less responsive stomata and increased ratio of roots to shoots (Hussain et al., 2004). Tognetti et al. (2002) observed that optimum irrigation is the key to have higher sugar yields for sugar beet cultivation in semi-arid Mediterranean terrains.

In sweet sorghum, plant height, stem diameter, stem fresh weight, juice yield, brix and stem sugar contents are the most important characteristics for biofuel production (Murray et al., 2008, Pfeiffer et al., 2010) and are determined by the efficient physiological behavior of the plant under different environmental conditions. Previous studies showed that plant height is highly correlated to juice yield and stem fresh weight (Murray et al., 2008). There is also a significant linear correlation between brix and total sugar content of the juice (Audilakshmi et al., 2010). The above established characteristics were obtainable only under optimal irrigation conditions (Vasilakoglou et al., 2010). There are no systematic studies describing sensitivity of reproductive stage of sweet sorghum to drought stress. Hence, this study aims to achieve a better understanding of the drought stress during early grain filling stage and the effect of changes in the sugar contents and composition. Further improvement of drought tolerance in sweet sorghum is still a need for improved biofuel production efficiency. Identification of the most suitable genotypes which are unaffected by drought during the ripening stages is also crucial for consistent juice and sugar yield. The objectives of this research were to (i) quantify effects of

water stress on brix, juice and sugar yield and (ii) quantify the genotypic difference for brix, juice and sugar yield under various drought stress levels.

5.2 Materials and Methods

5.2.1 Crop husbandry

The experiment was conducted under controlled environment facilities (greenhouse) at the Kansas State University, Department of Agronomy, Manhattan, KS. Four sweet sorghum genotypes namely Awanlek, Smith, Tracy and Wray were grown in 15-L pots filled with soil, sand and vermiculite in the ratio of 2:1:1 by weight. The pots were fully soaked with water and left for 1 d to drain and then five seeds per pot were sown at a 5-cm depth. The soil medium was fertilized with slow-release fertilizer (Osmocote®, Hummert International, Topeka, KS, USA, 14:14:14% N: P: K, respectively) at 5 g per pot before sowing. After emergence (two-leaf stage), a systemic insecticide (Marathon®1% G; Imidacloprid, 1-[(6-chloro-3-pyridinyl) methyl]-N-nitro-2-imidazolidinimine) was applied to each pot at 4 g per pot. Seedlings were thinned to two per pot after 15 d. The plants were grown at a temperature regime of 32/22°C ±3°C day/night, 12 h photoperiod and photosynthetic photon flux density of 800-1400 μmol m^{-2} s^{-1} provided from natural solar radiation and supplemental fluorescent lights. The relative humidity in the greenhouse was set at 80%. Air temperature, relative humidity, and light level were continuously monitored at 20-min intervals throughout the duration of experiment with HOBO data loggers (Onset Computers, Bourne, MA, USA).

5.2.2 Water stress treatments

All plants were grown under fully irrigated conditions (watered daily) from sowing to 10 d after complete anthesis. At the beginning of the milk stage, the pots were subjected to three levels of water stress. These were: 100% pot capacity (fully irrigated; control), 70% pot capacity

(mild water stress) and 30% pot capacity (severe water stress). Water stress treatments were imposed from the beginning of the milk to hard dough stage. The water stress treatments and stage of stress were assigned because sweet sorghum accelerates sugar accumulation in juice during milk stage than at later stages of grain maturity (Almodares et al., 2008c). Initially, pots were weighed and filled with 15 kg of soil. All pots were then completely saturated with water and the excess water was allowed to drain for 1 d. The pot weight was determined after saturation of the soil (pot weight about 17 kg). Then five pots were randomly selected and soils in each pot were completely sun dried and dry weight of the soil was recorded (pot weight about 13 kg each). The difference between the soil weight after drainage and soil weight after complete drying is 100% water holding capacity (water content about 4 kg). From this 100% water holding capacity value 70 and 30% pot capacity were computed. The pot capacity (100, 70 and 30%) was maintained by weighing pots daily and adding the required amount of water during water stress period. The plant weight was deducted from these pots by comparing daily weight of five representative pots without plants maintained separately for the purpose. The duration of water stress was 20 d and then plants were watered daily until final harvest at maturity.

5.2.3 Observations recorded

5.2.3.1 Physiological traits

One uniform plant in each pot was tagged for recording the physiological traits before start of treatments. Measurements were taken on the tagged plants from the flag leaf. After the start of water stress treatments, data on physiological traits (chlorophyll content (SPAD), Spectrum Technologies, Plainfield, IL, USA); chlorophyll a fluorescence (Fv/Fm) (Pulse-modulated fluorometer OS 30p, OptiScience, Hudson, NH, USA); canopy leaf temperature (FLIR ThermaCAM BCAM infrared thermal imaging camera, Janesville, WI, USA) and gas exchange

measurements, (LI-COR 6400 portable photosynthesis system, Lincoln, NE, USA) were measured. Measurements were taken for 20 d on 5 d intervals.

5.2.3.2 Growth traits

At maturity, plant height was measured from base of the stem to the tip of the panicle and was expressed in cm. Stem diameter was measured from the three regions of the stem (bottom 3rd internode, middle 6th internode, and the top 9th internode) using vernier caliper after stripping the leaves and removal of leaf sheaths. Average stem diameter was computed from the mean of the three regions. The number of leaves on the stem was counted. The leaf area was measured by leaf area meter (Model LI-3100, Li-Cor, Inc. Lincoln NE, USA). Stalks were stripped of leaves and topped. The fresh weight of the stem of each sample was recorded. The fresh leaf, panicle and crushed stem were oven-dried at 60°C for 7 d and dry weights were recorded individually. The total dry biomass was calculated from the oven dried samples. The panicles were threshed to obtain grain yield.

5.2.3.3 Bioenergy traits

The stalks were chopped in to small pieces before juice was extracted. The juice from a single plant was extracted by using garlic press. The extracted juice was weighed for calculating juice yield per plant. The brix was recorded on the extracted juice using digital hand-held refractometer (Digital hand-held pocket refractometer PAL-1, Atago, Bellevue, WA, USA). The sugar yield was calculated as the product of brix (%) and juice yield. The juice samples were then kept frozen for carbohydrate (glucose, fructose and sucrose) analysis. Glucose, fructose and sucrose were estimated in the extracted stalk juice using HPLC (Shimadzu Corporation, Japan). The juice samples from each treatment were diluted appropriately and the diluted liquid after centrifugation was filtered through 0.45 micron RC membranes into the HPLC vials and placed

in the autosampler tray (Prominence, SIL-20AC) maintained at 4°C. Sugars were quantified by the binary HPLC system (Shimadzu Corporation, Japan) using the Refractive Index (RI) detector (RID-10A) and Phenomenex RPM monosaccharide column (300 x 7.8 mm, Phenomenex, USA). Deionised water was collected from the Milli Q (Direct Q, Millipore Inc, USA), degassed using ultrasonicator (FS 60, Fisher Scientific) and was used as mobile phase. The column oven (Prominence CTD-20A) was maintained at 80°C, RID at 65°C and the mobile phase was pumped at a flow rate of 0.6 ml/ min through the binary pump (Prominence LC-20AB). Standards of glucose, fructose and sucrose were also run under same conditions. The sugars in the samples were analyzed by comparing area under the peaks of the standards and multiplying with the dilution factor.

5.2.4 Data analyses

The experiment was conducted in a factorial randomized block design (4x3). There were two factors in this experiment. Factor 1 is genotype and has four levels. Factor 2 is water stress with three levels. The experiment consisted of 12 treatments. Each treatment was replicated thrice. Three pots were used for each replication. The data were subjected to the analysis of variance for each trait using the general linear model of the statistical software by statistical analysis software 9.1 (SAS, 2003). Differences among treatment means were compared by least significant differences (LSD) at 5% level of probability.

5.3 Results

5.3.1 Physiological traits

Significant differences were observed among the genotypes and water stress treatments for all physiological traits such as Fv/Fm, chlorophyll SPAD, leaf temperature, photosynthetic rate, stomatal conductance, intercellular CO_2 concentration (Ci) and transpiration rate (Table 5.1). The

interaction between genotype and water stress treatment was significant for most of the traits except intercellular CO_2 concentration (Table 5.1).

The genotype Tracy had maximum chlorophyll SPAD, Fv/Fm, net photosynthetic rate, stomatal conductance and transpiration rate and also showed lower leaf temperature (Table 5.2). Genotypes Wray and Awanlek were intermediate for most of the traits whereas Smith had the lowest values (Table 5.2). Severe water stress significantly decreased chlorophyll SPAD, Fv/Fm, net photosynthetic rate, stomatal conductance, and transpiration rate followed by mild water stress (Table 5.3). In addition, severe water stress significantly increased leaf temperature but the difference was only modest in comparison to mild water stress and also showed higher intercellular CO_2 concentration compared to mild stress and control condition (Table 5.3).

Interaction effects showed that sweet sorghum genotypes experienced a decrease in SPAD, Fv/Fm, net photosynthetic rate, stomatal conductance and transpiration rate under water stress conditions (Fig. 5.1 and Fig. 5.2). Tracy showed highest reduction of SPAD at mild and severe water stress conditions (17.9 and 24.5%, respectively) when compared to its control (Fig. 5.1). Fv/Fm decreased in genotype Smith with a highest reduction of 4.8% under mild stress followed by Wray (3.9%), Tracy (1.8%), and Awanlek (1.3%) (Fig. 5.1). Genotype Tracy experienced highest decrease by 7.0% compared to control under severe water stress.

Net photosynthetic rate was decreased by 23.9% in Tracy and 54.3% in Wray under mild and severe stress respectively (Fig. 5.2). On the other hand, stomatal conductance decreased by 32% in Tracy and 57.8% in Wray under mild and severe stresses respectively (Fig. 5.2). Similarly, mild and severe water stress decreased transpiration rate by 35.1% in Tracy and 57.8% in Wray, respectively (Fig. 5.2).

5.3.2 Growth and yield traits

Genotypes significantly influenced growth and yield traits of sweet sorghum except average stem diameter and grain yield. Water stress caused a significant impact on yield traits but did not affect growth traits. The interaction of genotype and water stress did not significantly influence both growth and yield traits (Table 5.4).

Tracy recorded the maximum plant height, followed by Wray (Table 5.5). Leaf area was maximum in Awanlek and Wray (Table 5.5). Similarly, Awanlek and Wray produced maximum number of leaves plant^{-1} (Table 5.5). Genotype Tracy had maximum stem diameter while minimum was found in Awanlek (Table 5.5). Tracy recorded highest stem fresh weight which was similar to Wray whereas genotype Smith produced lowest stem weight (Table 5.5). Genotype Awanlek recorded significantly highest total dry biomass followed by Tracy (Table 5.5). The grain yield was highest in Tracy and the lowest in Wray (Table 5.5).

Water stress treatments significantly affected stem fresh weight and control had significantly highest stem fresh weight followed by mild stress whereas severe stress significantly decreased stem fresh weight (Table 5.6). Similarly, total dry biomass was highest in control and was followed by mild stress. Severe water stress significantly decreased total dry biomass across the genotypes (Table 5.6). The grain yield was significantly reduced in severe water stress than mild stress and control (Table 5.6).

5.3.3 Bioenergy traits

Significant differences were observed among the genotypes for brix, juice yield, sugar yield, glucose, fructose and sucrose contents. Water stress treatments significantly differed all bioenergy traits except brix, glucose and fructose content. The interactions between genotype and treatment were also significant for all bioenergy traits except brix (Table 5.7).

Among the genotypes, Wray recorded highest brix but statistically similar with Tracy (Table 5.8). The juice yield was significantly highest in Tracy followed by Wray (Table 5.8). Tracy produced highest sugar yield and had higher glucose and fructose contents (Table 5.8). Genotypes Smith and Awanlek recorded lowest sugar yield (Table 5.8). Sucrose content was highest in Wray but was statistically similar with Tracy whereas the lowest sucrose was observed in Smith (Table 5.8).

The fully irrigated plants had the highest juice yield and sugar yield and the lowest was observed under severe stress (Table 5.9). The sucrose content was found to be similar under fully irrigated control and mild stress, however, significantly increased under severe water stress (Table 5.9).

The interaction effects indicated that the juice and sugar yields varied across the genotypes when they were subjected to water stress (Fig. 5.3). Under mild stress, Tracy showed an increase in the juice yield and had the lowest reduction in sugar yield when compared to control. The highest reduction in juice yield and sugar yield was observed in genotypes Smith and Wray, respectively (Fig. 5.3). Under severe water stress, the lowest reduction of juice and sugar yield was observed in Awanlek and Wray, respectively (Fig. 5.3). Whereas, Smith had the highest reduction in juice and sugar yield respectively. Tracy showed 35.2% and 42.9% reduction in juice and sugar yield respectively (Fig. 5.3).

Wray and Smith showed greater reduction in glucose content under mild and severe stress respectively compared to control (Fig. 5.4a) However, genotypes Tracy and Awanlek showed an increase in glucose content by 4.7% and 75.9%, respectively under mild water stress (Fig. 5.4a). Severe water stress caused a decrease in glucose content of Wray and Tracy (Fig. 5.4a).

Similar trend was also observed for fructose content for all the genotypes (Fig. 5.4b). Under mild stress, genotype Wray had the greatest decrease by 53.1%, followed by Smith with 32.4% reduction. Genotypes Tracy and Awanlek again increased by 4.7 and 80.4%, respectively (Fig. 5.4b). Severe water stress showed that Smith had highest reduction at 91.9% in fructose content. Genotypes Wray and Tracy showed 27.6 and 14.1% reductions, respectively (Fig. 5.4b). Under mild water stress, genotypes Smith and Tracy showed reduction in sucrose content as compared to control, whereas Wray and Awanlek had 77.2 and 31.7% increase respectively (Fig. 5.4c). Genotypes Tracy, Awanlek and Wray increased their sucrose content under severe water stress. However, genotype Smith showed reduction in sucrose under severe water stress (Fig. 5.4c).

5.4 Discussion

Water stress in sweet sorghum can cause significant reduction in biomass production including sugar and grain yield. Stage sensitivity studies for understanding the effect of water stress on sweet sorghum revealed that a temporary water stress had severe impact in the water use efficiency at early stage sweet sorghum (Mastrorilli et al., 1999) however, a perennial stress had significant impact at the late stage (Tingting et al., 2010). In this study, water stress at early grain filling (milky) stage in sweet sorghum had significant effect on growth, physiological, biochemical and yield traits. In sweet sorghum, juice yield is a function of both stem juiciness (total stem water content/stem fresh weight) and stem fresh weight. In the present study, Tracy recorded higher juice yield and brix value and was found to have the higher stem fresh weight also. The increased stem fresh weight in Tracy might be due to higher stem tissue water content, plant height and average stem diameter as suggested by Murray et al. (2008). The sugar yield was also significantly higher in the same genotype. Even though, Wray had similar brix as that of Tracy, the sugar yield was low as a result of lower juice yield. Pfeiffer et al. (2010) reported that

greater juice yield and higher sugar content were observed from highest performing sweet sorghum hybrids than pureline varieties. Similar differences in plant height, brix, stem fresh weight, sugar and juice yield traits among the U.S. sweet sorghum collections were reported earlier by Murray et al. (2008; 2009). Grain yield was also increased in genotype Tracy. Simultaneous increases of both sugar and grain yield in Tracy indicated that this genotype was able to recover from water stress during early grain filling stage. The dual-purpose nature of this genotype could be utilized for bioenergy production even under changing environmental conditions. Glucose, fructose and sucrose contents in juice varied significantly among the genotypes. Similar type of genetic difference in sugar accumulation in sweet sorghum lines was reported by Erdei et al. (2009).

Stem fresh weight, total dry weight, brix, juice, sugar and grain yield varied significantly among different water stress levels. The control and mild water stress had similar brix and juice yield, however, sugar yield was increased in control due to increase in stem fresh weight. A similar trend was also observed in grain yield. Under severe water stress, sugar yield and grain yield were drastically decreased when compared to mild stress and control. This was due to decreased photosynthetic rate, stomatal conductance and transpiration rate. water stress affects mainly the photochemical events by affecting photosystem 2 (PS2) both by degradation of D1 and D2 proteins in the PS2 reaction centre leading to lowered electron transport (He et al., 1995). Many experiments revealed that a decrease in stomatal conductance correspond to decrease in photosynthetic rate (Tenhunen et al., 1987; Nilsen and Orcutt, 1996; Chaves and Oliviera, 2004). The stomatal closure and CO_2 deficit in the chloroplasts were also the main causes of decreased photosynthesis under mild and moderate stresses (Flexas and Medrano, 2002).

In this study, the decrease in photosynthetic rate was ascribed to reduction in stomatal conductance, PS II photochemistry (Fv/Fm), chlorophyll content and increase in leaf temperature. In spite of little difference in the leaf temperature of control and water stressed plants, differences in SPAD and Fv/Fm among genotypes were apparent. Water stress significantly decreased Fv/Fm in all four genotypes and may be due to photoinhibition that resulted in separation of light harvesting complex II from the PS II core complex and blockage of the PS II reaction center, which inhibits electron flow from QA to QB (Maxwell and Johnson, 2000). Silva et al. (2007) had also reported reduction in Fv/Fm in sugarcane under water stress conditions. Chlorophyll SPAD also decreased significantly in all the genotypes under different water stress levels. The results are in agreement with Silva et al. (2007), who described a significant decrease of leaf chlorophyll caused by water stress in eight sugarcane genotypes. Decreased or unchanged chlorophyll content during water stress has been reported in shrub species, depending on the duration and severity of water stress (Kpyoarissis et al., 1995). Loss of chlorophyll is also attributed to membrane damage (lower Fv/Fm) (Ristic et al., 2007).

Glucose and fructose levels in juice did not change significantly over the three different water stress levels. However, sucrose content increased under severe stress condition. This may be due to the conversion of reducing sugars into non-reducing sugars resulting in higher content of sucrose. Terzi et al. (2009) reported increased sucrose in the stem juice could be attributed to the plant's growth sustenance during severe water stress periods. A positive association between water stress and sucrose accumulation was also reported in sugarcane genotypes (Inman-Bamber and Smith, 2005). Further, there is also possibility for increased sucrose concentration as a result of decreased water content in the stem as reflected by reduced juice yield.

The interaction effect revealed that Tracy recorded higher juice yield under all water stress levels. This is due to the increased transpiration rate, stomatal conductance and photosynthetic rate associated with this genotype irrespective of the water stress levels (Fig. 5.2). The photosynthetic rate at early grain filling is very critical in maintaining sugar concentration in the stem. While all the genotypes had lower photosynthetic rates, genotype Tracy had maintained a higher photosynthetic rate that resulted in higher sugar yield to meet the sugar demand by the stem at grain filling stage under water stress condition.

The increased sugar yield in the genotype Tracy across the three different stress levels was due to higher juice yield and/or brix value (Fig. 5.3). Increased sugar yield is the outcome of higher stomatal conductance which in turn led to higher photosynthetic rate. The juice brix is also generally an indirect measure of CO_2 assimilation. It is generally regarded that decrease in photosynthesis under water stress conditions could be attributed either to a decrease in stomatal conductance and/or to non-stomatal limitations (Cornic and Massacci, 1996). The relatively higher stomatal conductance of the tolerant genotypes results from mechanisms maintaining a higher leaf water status and hence more open stomata. As a consequence, CO_2 influx towards chloroplast may be longer, thus allowing greater photosynthetic rates under water stress conditions (Kumar, 2005). This was made possible in Tracy with the higher level of stomatal conductance (Fig. 5.2).

Even though glucose and fructose levels were similar in severe water stress between Wray and Tracy, the sucrose content was highest in Wray. This result was in agreement with the finding of Zinselmeier et al. (1995), who showed that water stress decreased activities of both vacuolar and cell wall-bound acid invertases during maize kernel development with parallel reductions in ovary growth and concentration of hexoses.

Genotype Tracy maintained higher value of SPAD and Fv/Fm compared to other genotypes under different water stress levels which was reflected in photosynthetic rate. Similarly, stomatal conductance was highest in Tracy under different water stress levels resulting in higher photosynthetic rate. Moriana et al. (2002) observed a close correlation between stomatal conductance and photosynthetic rate in Olive leaves exposed to water stress. One of the important influences on sugar production is brix, juice yield and leaf net photosynthetic rate. We found that the genotype Tracy had higher brix, juice yield and leaf net photosynthetic rate compared to other genotypes under different water stress levels. As a result, Tracy can be cultivated under water stress environment for higher sugar yield to achieve sustainable bioenergy production.

5.5 Conclusions

Our results showed significant differences among the genotypes for all growth, physiology and bioenergy traits. Overall, across all genotypes severe water stress significantly decreased brix, juice yield, sugar yield, sucrose content, total dry biomass and grain yield. Genotype Tracy produced significantly highest juice and sugar yields under both irrigated and water stress conditions compared to genotypes Wray, Awanlek and Smith. The water stress tolerance of Tracy could be ascertained from the present study, based on significant increase in net photosynthetic rate, stomatal conductance, transpiration rate and intercellular CO_2 concentration. The relatively higher chlorophyll SPAD coupled with smaller decrease in Fv/Fm activity and leaf temperature also supported the tolerant nature of the genotype Tracy. The degree of accumulation of sugars (glucose, fructose and sucrose) varied among genotypes, and genotype Tracy accumulated relatively greater amounts of sugars in the juice than other genotypes.

5.6 Tables and Figures

Figure 5.1 Effect of different water stress levels on (a) chlorophyll content (SPAD) (b) leaf temperature and (c) Fv/Fm of four sweet sorghum genotypes.

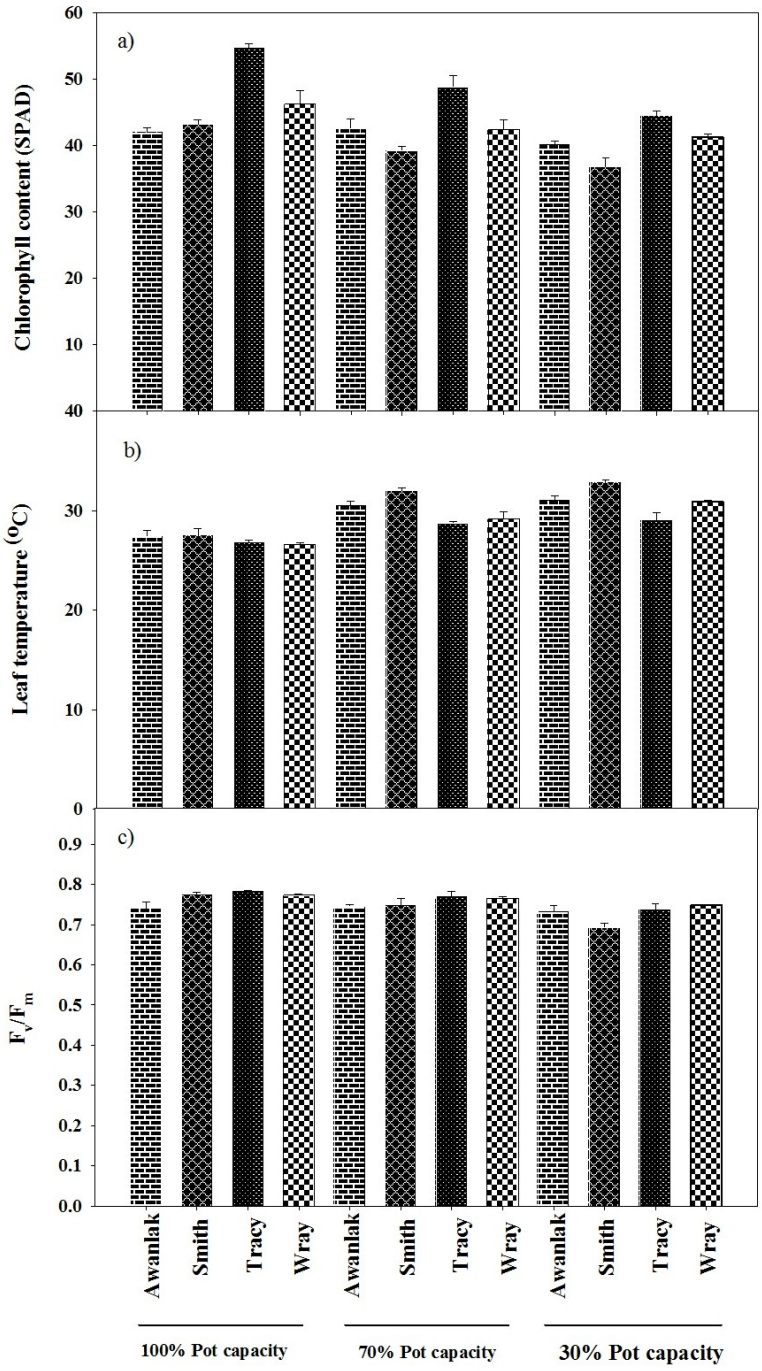

Figure 5.2 Effect of different water stress levels on (a) photosynthetic rate (b) stomatal conductance and (c) transpiration rate of four sweet sorghum genotypes.

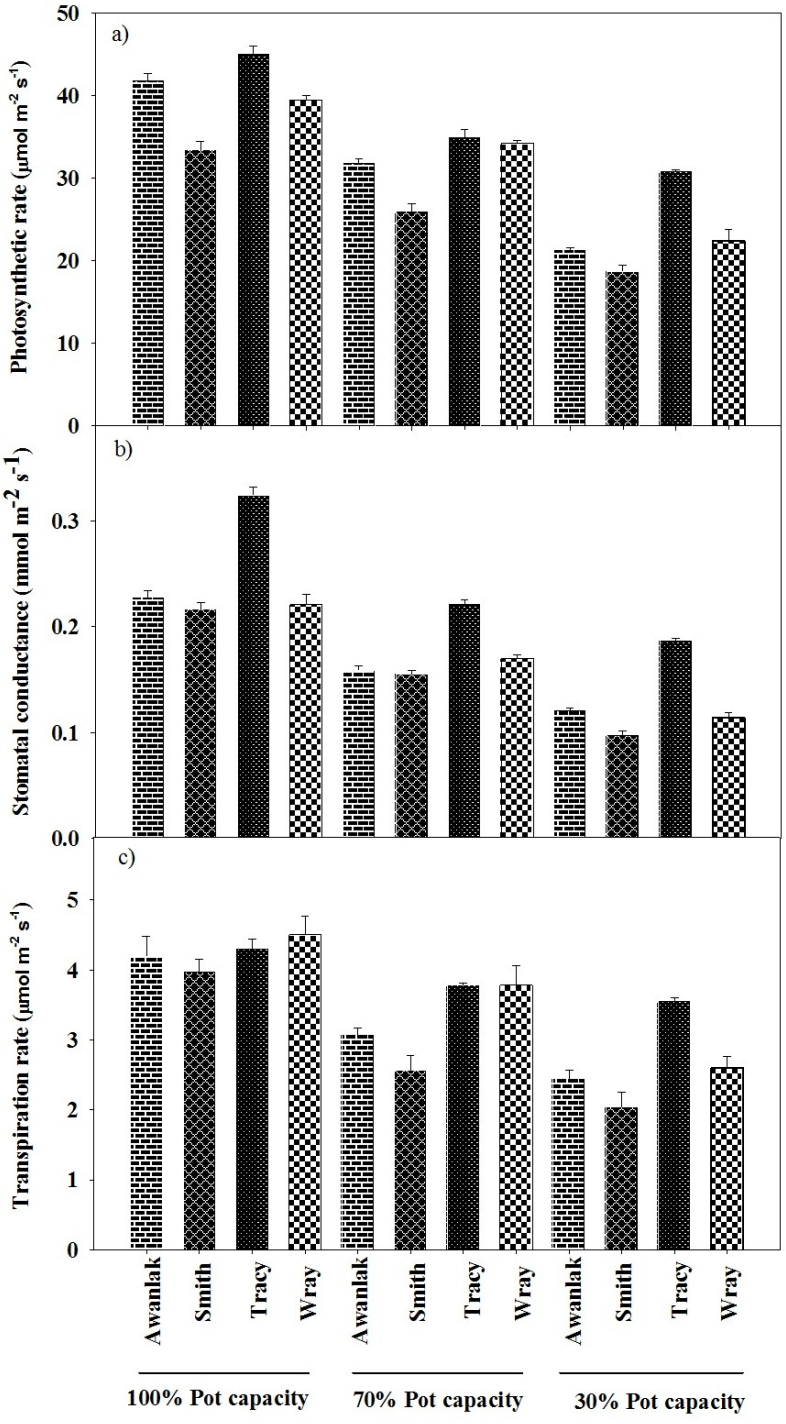

Figure 5.3 Effect of different water stress levels on (a) juice yield and (b) sugar yield of four sweet sorghum genotypes. The vertical bar denotes ±SE of means (n=3).

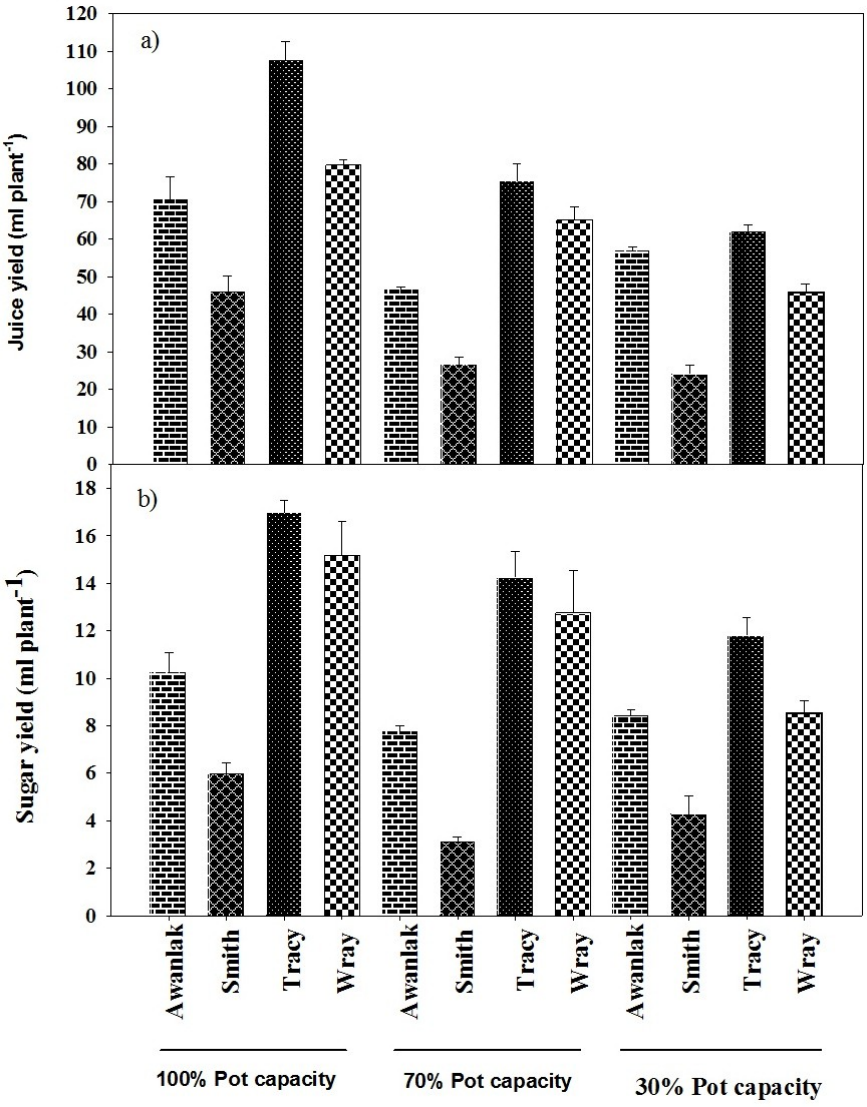

Figure 5.4 Effect of different water stress levels on (a) glucose (b) fructose and (c) sucrose contents in the juice of four sweet sorghum genotypes. The vertical bar denotes ±SE of means (n=3).

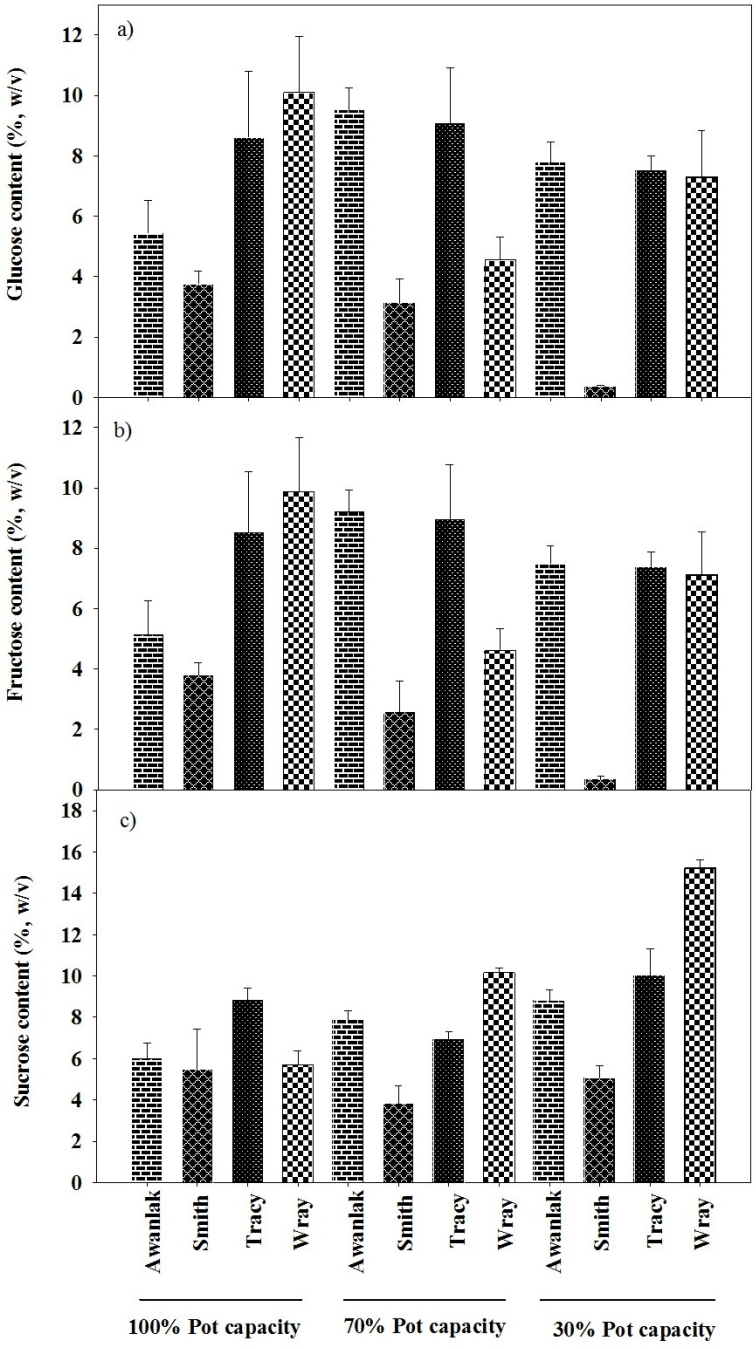

Table 5.1 Significance and P values of the effects of genotypes (G), water stress levels (T) and their interaction (G x T) on physiological traits in different sweet sorghum genotypes.

Physiological traits	Chlorophyll (SPAD)	Leaf temperature (°C)	Fv/Fm	Net photosynthesis (μmol CO_2 m^{-2} s^{-1})	Stomatal conductance (mmol H_2O m^{-2} s^{-1})	Transpiration rate (mmol H_2O m^{-2} s^{-1})
Genotype (G)	<0.0001	<0.0001	<0.01	<0.0001	<0.0001	<0.0001
Treatment (T)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
G*T	<0.05	<0.05	<0.05	<0.01	<0.01	<0.05

Table 5.2 Effect of various sweet sorghum genotypes on physiological traits.

Physiological traits	Genotypes				
	Awanlek	Smith	Tracy	Wray	LSD
Chlorophyll (SPAD)	41.7^{bc}	39.8^{c}	49.4^{a}	43.3^{b}	1.9
Leaf temperature (°C)	29.7^{b}	30.8^{a}	28.2^{c}	28.9^{c}	0.7
Fv/Fm	0.740^{b}	0.739^{b}	0.762^{a}	0.765^{a}	0.01
Net photosynthetic rate (μmol CO_2 m^{-2} s^{-1})	31.7^{b}	26.0^{c}	37.0^{a}	32.0^{b}	1.3
Stomatal conductance (mmol H_2O m^{-2} s^{-1})	0.170^{b}	0.157^{c}	0.245^{a}	0.168^{b}	0.009
Transpiration rate (mmol H_2O m^{-2} s^{-1})	3.2^{b}	2.9^{c}	3.9^{a}	3.6^{a}	0.3

Means within the same row with different letter are significantly different at $P<0.05$. Each data is the average of four independent measurements of each genotype recorded on day 5, 10, 15, and 20.

Table 5.3 Effects of various water stress levels on physiological traits of sweet sorghum genotypes.

Physiological traits	Water stress levels			
	Control (fully irrigated)	70% pot capacity (mild stress)	30% pot capacity (severe stress)	LSD
Chlorophyll (SPAD)	46.6[a]	43.3[b]	40.7[c]	1.7
Leaf temperature (°C)	27.1[c]	30.1[b]	31.0[c]	0.6
Fv/Fm	0.768[a]	0.758[a]	0.729[b]	0.01
Net photosynthetic rate (μmol CO_2 m^{-2} s^{-1})	40.0[a]	31.8[b]	23.3[c]	1.2
Stomatal conductance (mmol H_2O m^{-2} s^{-1})	0.248[a]	0.177[b]	0.130[c]	0.008
Transpiration rate (mmol H_2O m^{-2} s^{-1})	4.25[a]	3.30[b]	2.66[c]	0.27

Means within the same row with different letter are significantly different at $P<0.05$. Each data is the average of four independent measurements of each genotype recorded on day 5, 10, 15, and 20.

Table 5.4 Significance and P values of the effects of genotypes (G), water stress levels (T) and their interaction (G x T) on growth and yield traits in different sweet sorghum genotypes.

Growth and yield traits	Genotype (G)	Treatment (T)	G x T
Plant height (cm plant^{-1})	<0.001	NS	NS
Leaf area (cm^2 plant^{-1})	<0.001	NS	NS
Number of leaves plant^{-1}	<0.001	NS	NS
Average stem diameter (mm plant^{-1})	NS	NS	NS
Stem fresh weight (g plant^{-1})	<0.01	<0.01	NS
Total dry biomass (g plant^{-1})	<0.01	<0.01	NS
Grain yield (g plant^{-1})	NS	<0.01	NS

NS= Non-significant.

Table 5.5 Effect of various sweet sorghum genotypes on growth and yield traits.

Growth and yield traits	Genotypes				
	Awanlek	Smith	Tracy	Wray	LSD
Plant height (cm plant^{-1})	134.4^{c}	131.3^{c}	185.8^{a}	152.1^{b}	11.6
Leaf area (cm^2 plant^{-1})	3526.8^{a}	2261.4^{c}	3020.7^{b}	3316.8^{ba}	428.9
Number of leaves plant^{-1}	12.5^{a}	9.6^{c}	10.6^{b}	11.7^{a}	0.8
Average stem diameter (mm plant^{-1})	8.6^{b}	8.7^{b}	10.8^{a}	9.0^{ab}	2.0
Stem fresh weight (g plant^{-1})	165.0^{b}	116.0^{c}	200.0^{a}	191.0^{a}	15.0
Total dry biomass (g plant^{-1})	392.0^{a}	283.0^{c}	340.0^{b}	297.0^{c}	42.0
Grain yield (g plant^{-1})	29.6^{ba}	27.8^{b}	33.0^{a}	27.5^{b}	3.5

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

Table 5.6 Effects of various water stress levels on growth and yield traits of sweet sorghum genotypes.

Growth and yield traits	Water stress levels			
	Control (fully irrigated)	70% pot capacity (mild stress)	30% pot capacity (severe stress)	LSD
Plant height (cm plant^{-1})	154.7^{a}	152.7^{a}	149.9^{a}	NS
Leaf area (cm^2 plant^{-1})	3076.8^{a}	2814.8^{a}	3055.8^{a}	NS
Number of leaves plant^{-1}	11.5^{a}	11.0^{ba}	10.3^{b}	0.7
Average stem diameter (mm plant^{-1})	9.0^{a}	9.9^{a}	8.8^{a}	NS
Stem fresh weight (g plant^{-1})	212.0^{a}	171.0^{b}	120.0^{c}	15.0
Total dry biomass (g plant^{-1})	412.0^{a}	305.0^{b}	267.0^{c}	36.0
Grain yield (g plant^{-1})	40.0^{a}	28.9^{b}	19.4^{c}	3.0

Means within the same row with different letter are significantly different at $P<0.05$. NS – Non-significant.

Table 5.7 Significance and P values of the effects of genotypes (G), water stress levels (T) and their interaction (G x T) on bioenergy traits in different sweet sorghum genotypes.

Bioenergy traits	Genotype (G)	Treatment (T)	G x T
Brix (%)	<0.01	NS	NS
Juice yield (ml plant^{-1})	<0.0001	<0.0001	<0.01
Sugar yield (ml plant^{-1})	<0.0001	<0.0001	<0.05
Glucose (%, w/v)	<0.001	NS	<0.05
Fructose (%, w/v)	<0.001	NS	<0.05
Sucrose (%, w/v)	<0.001	<0.01	<0.01

NS= Non-significant.

Table 5.8 Effect of various sweet sorghum genotypes on bioenergy traits.

Bioenergy traits	Genotypes				
	Awanlek	Smith	Tracy	Wray	LSD
Brix (%)	15.3[b]	14.0[b]	17.9[a]	19.1[a]	2.25
Juice yield (ml plant^{-1})	58.2[b]	32.3[c]	81.8[a]	63.6[b]	5.51
Sugar yield (ml plant^{-1})	8.83[c]	4.47[d]	14.36[a]	12.16[b]	1.45
Glucose (%, w/v)	7.58[a]	2.42[b]	8.41[a]	7.31[a]	2.0
Fructose (%, w/v)	7.28[a]	2.24[b]	8.29[a]	7.2[a]	2.0
Sucrose (%, w/v)	7.58[b]	4.72[c]	8.62b[a]	9.75[a]	1.59

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

Table 5.9 Effects of various water stress levels on bioenergy traits of sweet sorghum genotypes.

Bioenergy traits	Water stress levels			
	Control (fully irrigated)	70% pot capacity (mild stress)	30% pot capacity (severe stress)	LSD
Brix (%)	15.7[a]	16.8[a]	17.4[a]	1.95
Juice yield (ml plant^{-1})	76.1[a]	60.0[b]	47.3[c]	4.77
Sugar yield (ml plant^{-1})	12.1[a]	10.0[b]	8.3[b]	1.26
Glucose (%, w/v)	6.97[a]	6.57[a]	5.75[a]	NS
Fructose (%, w/v)	6.83[a]	6.34[a]	5.58[a]	NS
Sucrose (%, w/v)	6.51[b]	7.21[b]	10.2[a]	1.3

Means within the same row with different letter are significantly different at $P<0.05$. NS – Non-significant.

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Chapter 6 - Effect of drought, high temperature, and their combination during early grain filling (Milk) stage on growth, physiology and yield of sweet sorghum

6.0 Abstract

Studies on the effect of drought (water stress) and increase in air temperature (high temperature stress) have independently been conducted extensively. At the individual level, these stresses have diverse effects on various crops. Sweet sorghum, an important bioenergy crop, is mostly grown under rainfed conditions. Although sweet sorghum is a relatively more drought and high temperature tolerant compared to other cereals, its physiology, growth and development is not clearly understood by occurrences of drought and high temperature stress independently and combined during early grain filling (milk) stage. This study was conducted to quantify the effects of drought, high temperature (38/28±3°C, day/night)), and their combinations on growth, physiology and yield of sweet sorghum genotypes. Four sweet sorghum genotypes *viz.* Awanlek, Tracy, Wray and Smith were grown in greenhouse with uniform watering at 32/22°C day/night prior to the stress treatment. Thereafter, each genotype was subjected to four treatments (T_1 - control: fully irrigated/optimum temperature; T_2 - drought stress: no irrigation/optimum temperature; T_3 - high temperature stress: fully irrigated/high temperature and T_4 - combination of drought and high temperature, no irrigation/high temperature stress). Treatment was imposed on 10 day after complete anthesis and was continued for 16 days. Physiological traits such as chlorophyll (SPAD), chlorophyll *a* fluorescence (Fv/Fm), leaf temperature and gas exchange measurements, were recorded at 4 d interval. At grain maturity, data on plant height, leaf area, stem diameter, fresh and dry weights of stem, leaves and panicles, and grain were measured. Bioenergy traits include brix, juice and sugar yield were measured. The total and reducing sugars

were also estimated from the extracted juice samples. The results indicated that significant differences were observed for growth and yield traits, physiological traits and non-reducing and total sugar content in juice for genotypes and treatments. The interaction of genotype and treatment showed significance for most of growth and yield traits except for number of leaves and internodes, leaf and panicle fresh weight and stem dry weight. All physiological and bioenergy traits were significantly influenced by genotype and treatment combination. Among the genotypes Tracy recorded significantly higher juice and sugar yield under all stress treatments. The combined drought and high temperature stresses were more deleterious in reducing most of the bioenergy traits than either stress alone. The more reduction in juice and sugar yield caused by combined stresses may be due to non-availability of water and high air temperature which significantly impairs photosynthetic rate and sugar accumulation in the stem.

6.1 Introduction

Sweet sorghum (*sorghum bicolor* L.) has been grown in various parts of the world mostly for use as either grain or forage under varying environmental conditions. Global energy needs have driven sweet sorghum as a popular energy plant throughout the world (Barbanti et al., 2006; Vasilakoglou et al., 2010). Sweet sorghum provides efficient biofuel production from stem juice, grain and whole plant biomass. Sweet sorghum genotypes are superior when compared to other bioenergy crops in terms of higher green biomass, amount of fermentable sugars, fermentation efficiency and percentage of juice brix (Steduto et al., 1997; Rooney et al., 2007). The stem juice is a main source for bioethanol production due to greater quantity of fermentable sugars (Woods, 2001; Akbulut and Ozcan, 2008). Sweet sorghum, a C_4 metabolic plant, is generally grown under rainfed conditions, which are characterized by low water levels and high temperature.

Drought and high temperature, often occur simultaneously, are important environmental factors restricting plant physiological processes and thereby plant growth (Shah and Paulsen, 2003). Global climate change for instance contains to bring a new reality of environmental effects, presumably increases in global temperature, uneven precipitation, and severe drought in arid and semi-arid areas, on crop productivity (Wigley and Raper, 2001; Chaves et al., 2003). Most studies thus far have focused on crop response to drought and high temperature singly, and few studies have focused on combination of these two stresses. For example, drought and high temperature caused detrimental effects on wheat (*Triticum aestivum* L.), sorghum, barley (*Hordeum vulgare* L.) and various grasses (Savin and Nicolas, 1996; Machado and Paulsen, 2001; Shah and Paulsen, 2003; Xu and Zhou, 2006). However, studies on the effect of these two environmental stresses either singly or in combination is scarce in sweet sorghum.

Drought stress caused significant impact on various sugar yielding crops affecting their yield potentialities. In sugarcane (*Saccharum officinarum* L.), cane yield was decreased by 29.2% and 18.1% respectively in severe and moderate drought stress conditions and led to morphological changes such as reduced leaf area, thicker leaves, less responsive stomata and increased ratio of roots to shoots (Hussain et al., 2004). Drought experiments on sugar beet (*Beta vulgaris* L.) have shown adverse effects on leaf photosynthetic activities and sucrose yields in mature plants (Monti et al., 2006). Drought stress resulted in reduced root dry weight, leaf water potential and photochemical efficiency in many grass species (Aronson et al., 1987; Carrow, 1996; Perdomo et al., 1996; Huang et al., 1998a).

High temperatures have negative effects on most crops in various ways (Schaffert and Gourley, 1982). Most crops grow well at optimum temperatures which mainly correspond with the optimum photosynthesis levels. High temperatures affect photosynthetic processes (Al-Khatib and Paulsen, 1984) with increased sensitivity of photo-system (PS) II (Xu and Zhou, 2006). High temperature stress causes thylakoid membrane damage and further down regulates PS II photochemistry which led to increased proportion of closed PS II reaction centers (Grove et al., 1986). In addition, leaf chlorophyll degradation is highly correlated with high temperature (Prasad et al., 2009). High temperature stress also causes leaf temperature to rise above air temperature by decreasing transpirational cooling and thus, make the plant more susceptible to photoinhibition (Falk et al., 1996).

Recent studies revealed that plant response to a combination of drought and high temperature is uniquely differently from the effect of individual stress conditions (Rizhsky et al., 2004). While drought remains the single known environmental factor that directly affects plants water status, the severity of drought and high temperature combination is enormously dependant on the

prevailing temperatures. Ludlow et al. (1990) reported that combined stresses of drought and high temperature significantly reduced grain yield in sorghum. In addition, combined effects of drought and high temperature strongly affected water relations of both wheat and sorghum (Machado and Paulsen, 2001). As the combined effect of these two stresses are distinct in reality to independent stress effects in other crops, the relationship between drought, high temperature and their combinations against sugar accumulation in sweet sorghum needs thorough understanding.

In sweet sorghum, the most important traits for biofuel production are plant height, stem diameter, stem fresh weight, juice yield, brix and stem sugar contents (Murray et al., 2008; Pfeiffer et al., 2010) and are determined by the efficient physiological behavior of the plant under different environmental conditions. Previous studies showed that plant height is highly correlated to juice yield and stem fresh weight (Murray et al., 2008). There is also a significant linear correlation between brix and total sugar content of the juice (Audilakshmi et al., 2010). However, optimal growing conditions ensure better plant growth without affecting physiological functions to produce sustainable juice and sugar yield in sweet sorghum (Vasilakoglou et al., 2010).

Sweet sorghum varieties differ in their ability to produce and store sugar in stem (Ali et al., 2008). Mostly, sugar accumulation in stems takes place during inflorescence development (McBee and Miller, 1982) and is accelerated after post anthesis (Prasad et al., 2007; Almodares et al., 2008c). Environmental factors such as temperature and water level may greatly determine juice quality and amount. Even though sorghum can withstand moderate high temperatures and drought, occurrence of either drought or high temperature or their combination during early grain filling (milk) stage were not thoroughly studied for their effects on growth, physiology and yield.

It is important to understand these effects to predict bioenergy components and selection of genotypes suitable for cultivation under varying stress environments.

The objectives of this research were to (i) quantify effects of treatments on juice and sugar yield characteristics and (ii) quantify genotypic difference for juice and sugar yield characteristics under various stress treatments.

6.2 Materials and Methods

6.2.1 Crop husbandry

This experiment was conducted under greenhouse controlled conditions at the Kansas State University, Department of Agronomy, Manhattan, KS during 2008-2009. Four sweet sorghum genotypes namely Awanlek, Smith, Tracy and Wray were grown in 15-L pots containing Metro-Mix 200 (Hummert International, Topeka, KS, USA) as soil medium. The pots were fully soaked with water and left for 1 d to drain and then five seeds per pot were sown at a 5-cm depth. After emergence (two-leaf stage), a systemic insecticide (Marathon®1% G; Imidacloprid, 1-[(6-chloro-3-pyridinyl) methyl]-N-nitro-2-imidazolidinimine) was applied to each pot at 4 g per pot. Seedlings were thinned to two per pot after 15 d. The soil medium was fertilized with slow-release fertilizer (Osmocote®, Hummert International, Topeka, KS, USA, 14:14:14% N: P: K, respectively) at 5 g per pot before sowing. The plants were grown at a temperature regime of 32/22°C ±3°C day/night, 12 h photoperiod and photosynthetic photon flux density of 800-1400 μmol m^{-2} s^{-1} provided from natural solar radiation and supplemental fluorescent lights. The relative humidity in the greenhouse was set at 80%. Air temperature, relative humidity, and light level were continuously monitored at 20-min intervals throughout the duration of experiment with HOBO data loggers (Onset Computers, Bourne, MA, USA).

6.2.2 Treatment application

All plants were grown under fully irrigated conditions (watered daily) from sowing to 10 d after complete anthesis (milking). Thereafter, each genotype was subjected to four treatments (control: fully irrigated/optimum temperature; drought stress: no irrigation/optimum temperature; high temperature stress: fully irrigated/high temperature 38/28°C and the combination of drought and high temperature, no irrigation/high temperature stress 38/28°C). Plants were randomly given for the stress treatments. The plants were held in a separate greenhouse condition for high temperature stress and combination of drought and high temperature whereas the other two treatments (control and drought stress) were given in another controlled greenhouse conditions. Temperature treatments were maintained at 38/28°C but varied ±3°C during 12-h day and ±2.5°C during 12-h night until completion of stress period. Relative humidity ranged from 40% to 60% under high temperature condition. The drought treatment was imposed by withholding irrigation continuously throughout the stress period. The duration of stress treatments was 16 d and then plants were brought to normal growth conditions. The plants were then rewatered until final harvest at maturity.

6.2.3 Observations recorded

6.2.3.1 Physiological traits

One uniform plant in each pot was tagged for recording physiological traits. Measurements were taken on the tagged plants from the flag leaf. After the start of various stress treatments, data on physiological traits (leaf chlorophyll content (SPAD), Spectrum Technologies, Plainfield, IL, USA); chlorophyll a fluorescence (Fv/Fm, which indicates photochemical efficiency) (Pulse-modulated fluorometer OS 30p, OptiScience, Hudson, NH, USA); canopy leaf temperature (FLIR ThermaCAM BCAM infrared thermal imaging camera, Janesville, WI, USA)

and gas exchange traits, (LI-COR 6400 portable photosynthesis system, Lincoln, NE, USA) were measured. Measurements were taken for 16 d on 4 d intervals.

6.2.3.2 Growth and yield traits

At maturity, plant growth and yield traits were recorded on each plant in each treatment of all replications. Plant height was measured from base of the stem to the tip of the panicle and was expressed in cm. Stem diameter was measured from the three regions of the stem (bottom 3rd internode, middle 6th internode, and the top 9th internode) using vernier caliper after stripping the leaves and removal of leaf sheaths. Average stem diameter was computed from the mean of the three regions. Number of leaves and internodes on the stem were counted. Leaf area was measured by leaf area meter (Model LI-3100, Li-Cor, Inc. Lincoln, NE, USA).

Stalks were stripped of leaves and topped. The fresh weight of the panicle, leaf, and stem of each plant were recorded. From this, total fresh biomass comprised of panicles, leaves and stem was calculated. The fresh leaf, panicle and crushed stem were oven-dried at 60°C for 7 d and dry weights were recorded individually. The total dry biomass was calculated from the oven dried samples. The panicles were threshed to obtain grain yields.

6.2.3.3 Bioenergy traits

The juice from a single plant was extracted by a motor operated three roller sugarcane crusher (Sukra sugarcane crusher, Coimbatore, Tamil Nadu, India). The extracted juice was weighed for calculating juice yield. The brix was recorded on the extracted juice using digital hand-held refractometer (Digital hand-held pocket refractometer PAL-1, Atago, Bellevue, WA, USA). Sugar yield was calculated as the product of brix (%) and juice yield. The juice samples were then kept frozen for carbohydrate (Reducing and Total sugars) analysis. The method described by Balnaco et al. (1995) was employed to estimate reducing and total sugars and it was

expressed as percentage. Non-reducing sugar was obtained from the differences of total and reducing sugars and expressed as percentage.

6.2.4 Data analyses

The experiment was conducted in a factorial completely randomized design (4x4). There were two factors in this experiment. Factor 1 is genotype and has four levels. Factor 2 is stress treatments with four levels. The experiment consisted of 16 treatments. Each treatment was replicated thrice. Three pots were used for each replication. The data were subjected to the analysis of variance for each trait using the general linear model of the statistical software by statistical analysis software 9.1 (SAS, 2003). Differences among treatment means were compared by least significant differences (LSD) at 5% level of probability.

6.3 Results

6.3.1 Physiological traits

There was a significant effect of genotype on all physiological traits leaf chlorophyll content (SPAD), leaf temperature, Fv/Fm, net photosynthesis, stomatal conductance and transpiration rate except intercellular CO_2 concentration (Ci). Treatments showed significant effect for all physiological traits, while the interaction effects revealed significant effects for most of the physiological traits except Ci and transpiration rate (Table 6.1).

Among the genotypes, Tracy recorded significantly highest chlorophyll content and was followed by Wray (Table 6.2). Leaf temperature was significantly higher in Smith and Tracy (Table 6.2). Fv/Fm was significantly higher in Tracy, Wray and Awanlek (Table 6.2). Net photosynthetic rate was significantly highest in Tracy, whereas significantly lowest was recorded in Smith (Table 6.2). Stomatal conductance was similar in Tracy and Wray but was significantly

greater than Awanlek and Smith (Table 6.2). Transpiration rate was significantly higher in Wray and Tracy (Table 6.2).

Among various treatments, drought stress combined with high temperature significantly decreased leaf chlorophyll and Fv/Fm and increased leaf temperature (Table 6.3). Net photosynthetic rate was significantly lower in combination of drought and high temperature, followed by drought stress (Table 6.3). Stomatal conductance was significantly decreased in combination treatment (Table 6.3). Ci was significantly increased under combination of drought and high temperature followed by drought stress (Table 6.3). The transpiration rate was significantly lowest in drought and high temperature combination followed by drought stress (Table 6.3).

The interaction effects revealed genotype Tracy recorded lowest reduction of chlorophyll content under combination of drought and high temperature, drought and high temperature by 10.6, 7.7 and 3.0%, respectively when compared to control (Fig. 6.1). Drought stress increased leaf temperature by 15.9, 14.4, 16.5 and 15.0% in genotypes Awanlek, Smith, Tracy, and Wray respectively. Whereas, high temperature stress slightly decreased leaf temperature of Awanlek, Smith, Tracy and Wray by 10.0, 12.4, 13.4 and 14.1%, respectively. The increase in leaf temperature was highest in Smith in response to combination of drought and high temperature (Fig. 6.2a). Drought stress reduced Fv/Fm in Awanlek, Smith, Tracy and Wray by 8.3, 11.3, 7.7 and 8.3% respectively, whereas, drought combined with high temperature stress drastically reduced Fv/Fm in all genotypes with the lowest reduction in Tracy (Fig. 6.2b). Stomatal conductance was greatly decreased in Awanlek, Smith and Wray, by 44.8, 51.7, and 55.1%, respectively when compared to control, while Tracy (3.4%) showed the lowest reduction (Fig. 6.2c).

High temperature stress did not affect photosynthetic rate of Tracy as compared to control, but decreased in Smith, Awanlek and Wray by 13.7, 9.2 and 4.6% respectively (Fig. 6.2d). Drought stress decreased photosynthetic rate in Smith, Awanlek, Wray, and Tracy genotypes by 52.4, 44.5, 44.5 and 38.7% respectively. Whereas, combination of drought and high temperature greatly decreased photosynthetic rate in Smith, Wray, Awanlek and Tracy by 64.3, 53.7, 46.2, and 43.9% respectively as compared to control (Fig. 6.2d).

6.3.2 Growth and yield traits

Genotypes and treatments significantly influenced all the growth and yield traits, while interaction effects were significant only for plant height, leaf area, average stem diameter, stem fresh weight, total fresh biomass, leaf dry weight, panicle dry weight, total dry biomass and grain yield (Table 6.4).

Among the genotypes, Tracy showed significantly maximum plant height (Table 6.5). Genotype Wray recorded significantly highest leaf area, number of leaves per plant and number of internodes (Table 6.5). Average stem diameter was significantly higher in Wray, followed by Tracy (Table 6.5). Leaf fresh weight was significantly higher in Tracy, followed by Wray (Table 6.5). Among the genotypes, Tracy recorded significantly highest stem fresh weight and total fresh biomass (Table 6.5). Wray was found to record significantly highest panicle fresh weight, leaf dry weight, panicle dry weight, total dry biomass and grain yield (Table 6.5). However, stem dry weight was significantly higher in Wray and Tracy when compared to other genotypes (Table 6.5).

Among the treatments, combination of drought and high temperature significantly decreased plant height, leaf area, panicle fresh weight, total fresh biomass, leaf dry weight, panicle dry weight, total dry biomass and grain yield (Table 6.6). Also, combination of drought and high

temperature significantly decreased number of leaves, number of internodes, average stem diameter, leaf fresh weight, stem fresh weight and stem dry weight and was followed by drought stress alone (Table 6.6).

Interaction effects revealed combination of drought and high temperature caused highest reduction in average stem diameter in genotype Wray (23.5%), whereas Smith recorded the lowest reduction (6.5%) when compared to their control (Fig. 6.3a). Drought stress decreased average stem diameter by 22.3% in Wray compared with control (Fig. 6.3a). Tracy showed lowest reduction in stem fresh weight (45%) on exposure to combination of drought and high temperature (Fig 6.3b).

6.3.3 Bioenergy traits

Significant differences were observed among the genotypes for brix, juice yield, sugar yield, total sugars and non-reducing sugar contents (Table 6.7). Treatments showed highly significant effects on all bioenergy traits, whereas interaction effects were found significant for all bioenergy traits except brix (Table 6.7).

Genotype Tracy recorded highest brix and was similar with Awanlek and Wray (Table 6.8). Whereas lowest brix was recorded in Smith (Table 6.8). Significantly highest juice and sugar yield was recorded in Tracy, while genotype Smith recorded significantly lowest juice and sugar yield (Table 6.8). Genotype Wray had significantly highest amount of total sugars and was followed by Tracy and Awanlek, while the lowest was observed in Smith (Table 6.8). Amount of reducing sugars was higher in Wray than other genotypes. Significantly highest amount of non-reducing sugar was observed in Tracy and Wray (Table 6.8).

The lowest brix was observed in genotypes exposed to combination of drought and high temperature and drought stress alone (Table 6.9). The combination of drought and high

temperature significantly decreased juice yield, sugar yield, total sugars, reducing sugars and non-reducing sugar contents (Table 6.9).

The interaction effects showed combination of drought and high temperature had decreased juice yield by 70.3, 69.5, 68.1 and 49.1% in genotypes Smith, Awanlek, Wray and Tracy, respectively, as compared to control (Fig. 6.3c). In the case of drought stress, a decrease in juice yield by 55.5, 51.1, 45.1 and 39.8% was observed in Wray, Awanlek, Smith and Tracy, respectively, as compared to control (Fig. 6.3c), whereas, high temperature caused a reduction in juice yield by 26.3, 21.5, 18.3 and 18.2% in Tracy, Awanlek, Wray and Smith, respectively (Fig. 6.3c).

Compared to control sugar yield was greatly decreased by 85.5, 82.3, 81.5 and 79.2% in genotypes Smith, Wray, Awanlek and Tracy, respectively, when exposed to combination of drought and high temperature (Fig. 6.3d). Drought stress had decreased sugar yield by 72.7, 71.3, 70.2 and 68.5% in Wray, Awanlek, Smith and Tracy, respectively, while high temperature stress decreased sugar yield in Smith, Awanlek, Tracy and Wray by 30.6, 28.1, 27.5 and 22.4%, respectively (Fig. 6.3d).

Drought stress decreased reducing sugars in genotypes Wray, Awanlek, Tracy and Smith by 71.9, 64.6, 38.6 and 37.5%, respectively, which was greater than the reductions under high temperature, which decreased by 53.3, 43.2, 41.7 and 25.0% in Awanlek, Smith, Wray and Tracy, respectively, as compared to control (Fig. 6.4a). Under combination of drought and high temperature stress, reducing sugar content of Awanlek, Wray, Smith and Tracy was reduced by 83.4, 79.1, 78.8 and 57.9%, respectively, compared with control (Fig. 6.4a).

Combination of drought and high temperature reduced total sugars by 76.0, 74.8, 61.8, 60.0% in genotypes Wray, Smith, Awanlek and Tracy, respectively, as compared to control (Fig. 6.4b).

Whereas, genotypes Wray, Awanlek, Tracy and Smith exposed to drought stress decreased total sugars by 60.0, 50.0, 41.4 and 35.5%, respectively (Fig. 6.4b). High temperature decreased total sugars in genotypes Wray, Awanlek, Smith and Tracy by 34.9 32.1, 29.4 and 27.3%, respectively compared with control (Fig. 6.4b).

6.4 Discussion

Our study demonstrates that sweet sorghum at early grain filling (milk) stage is sensitive to drought, high temperature and their combinations as reflected by significant changes in growth, physiology and yield attributes of different sweet sorghum genotypes. In sweet sorghum, juice and sugar yield are the most important traits for achieving higher biofuel production. We found that drought and high temperature combination caused severe reduction in juice and sugar yield. Genotype Tracy outperformed all other genotypes in terms of juice and sugar yield under different stress treatments indicating its tolerance capacity (Fig. 6.3c and Fig. 6.3d). This has indicated that Tracy possesses unique physiological and growth attributes to mitigate the impact of various stress condition to produce sustainable yield. This genotype showed maximum plant height and it appears that tallness of the plant favored production of greater amount of total biomass, as was reflected by accumulation of greater fresh biomass in the same genotype. Earlier studies involving sorghum cultivars also brought out a significant role of plant height in contributing to total dry matter (Valdes and Miller, 1982). Leaf area reflects the source size of photosynthetic system, which is also associated with leaf number and genotype Wray followed by Tracy recorded highest number of leaves and the largest leaf area. Greater leaf area provided higher assimilatory surface. Similarly, Tracy produced the highest amount of stem fresh weight and is therefore a significant factor in contributing higher juice and sugar yield. Genotype Wray, a better performer for most of the growth and yield traits as that of Tracy, recorded the highest

grain yield, total dry biomass, stem-, leaf-, and panicle-dry weight but yielded low sugar. The lower sugar yield in Wray was because of more grain production rather than sugar production. Thus, it is inferred that Tracy had the potential to increase stem fresh weight and therefore, increased sugar accumulation in stem whereas Wray mobilized carbohydrates from leaf and stem to grain. The high sugar yielding performance of Tracy was due to its greater physiological functions related to leaf chlorophyll SPAD, Fv/Fm, stomatal conductance and net photosynthetic rate under normal and different stress conditions (Fig. 6.1, Fig. 6.2b, Fig 6.2c and Fig. 6.2d). Genetic differences for growth attributes, juice and sugar yield were reported earlier in sweet sorghum (Blummel et al., 2009; Bhoyar and Thakare, 2009), but under normal growing conditions. Our study showed genotypes behaved distinctly under different environmental stresses for sugar yield and selection of suitable genotypes with inherent capabilities of stress tolerance coupled with high sugar yield would pave way for developing an effective sweet sorghum breeding program for arid and semi-arid regions.

Sweet sorghum is known to tolerate moderate environmental stresses including drought and high temperature through morphological, physiological and biochemical adjustments. Stress at reproductive growth stage had direct influence on growth, photosynthesis, dry matter accumulation and yield on sugarcane and sorghum (Ramesh, 2000; Hemaprabha et al., 2004; Su et al., 2007; Prasad et al., 2008; Prasad et al., 2009). The present results demonstrate that drought combined with high temperature greatly exacerbates the independent effect of drought and high temperature. High temperature, on the other hand, showed minimal adverse effects on growth, physiology and yield traits than drought stress alone.

The reduction in sugar yield in all stress treatments might be due to decreased chlorophyll content, net photosynthetic rate, stomatal conductance and PS II photochemistry and increased

leaf temperature (Table 6.3). Drought and high temperature caused a marked decrease in chlorophyll content and Fv/Fm and is in agreement with earlier studies (Shah and Paulsen, 2003; Wang and Huang, 2004; Ristic et al., 2007; Balla et al., 2006; Su et al., 2007). It was evident that photosynthetic rate was reduced under drought (Loreto et al., 1995; Shah and Paulsen, 2003), high temperature (Prasad et al., 2009), and combination of drought and high temperature (Shah and Paulsen, 2003) in sorghum and wheat. Combination of drought and high temperature decreased photosynthetic rate to a maximum extent compared to other stresses, and is in agreement with previous study in Kentucky blue grass, (*Poa pratensis* L.) (Wang and Huang, 2004). It might be due to photo-inhibition of PS II (Weis and Berry, 1988) and/or rapid leaf desiccation and permanent damage to PS II machinary (Jiang and Huang, 2000). Drought stress completely damaged the photosynthetic rate as evidenced by lower Fv/Fm. Similar trends were demonstrated in wheat by Balla et al. (2006). In addition, reduced photosynthetic rate was also due to reduced chlorophyll content under stress Decreasing concentration of chlorophyll due to increases in chloroplast degradation was attributed for the limitation of photosynthesis under moderate or severe drought conditions (Xiao et al., 2006). High temperature, on the other hand, did not noticeably change photosynthetic rate as compared to control. The capacity of photosynthetic process is an outcome through acclimation of high temperature rather than drought stress alone (Brigg et al., 1986; Nobel et al., 1978; Smolander and Lappi, 1984). Moreover, the increase in leaf tissue temperature under stress treatments has resulted in higher water loss which might have resulted in reduced stem juice yield.

Drought stress had decreased sugar yield and grain yield compared to high temperature stress. Results from Miller and Ottman (2010) indicated that applying drought stress did not increase sugar concentration in sweet sorghum. Drought had decreased leaf area, photosynthetic

rate and stomatal conductance (Paulsen, 1994) and inhibited sucrose accumulation in stem and also deteriorated juice quality (Ishaq and Olaoye, 2009).

The interaction effect revealed that Tracy recorded higher juice and sugar yield under all stress levels (Fig. 6.3c and Fig. 6.3d). This might be due to increased chlorophyll SPAD, Fv/Fm, stomatal conductance, Ci and photosynthetic rate associated with this genotype under various stress treatments (Fig. 6.1, Fig. 6.2b, Fig. 6.2c and Fig. 6.2d). The increases in stomatal conductance along with photosynthetic rate indicated that the sugars formed during photosynthesis were acted as an osmoticum in the stem and not in the leaf, which had resulted in higher juice yield and transpiration. This physiological adaptation in Tracy therefore, might help in maintaining higher photosynthetic rate even under independent and combined stresses.

It is generally regarded that decrease in photosynthesis under drought, high temperature and combined stress conditions could be attributed either to a decrease in stomatal conductance and/or to non-stomatal limitations (Ort et al., 1994; Shangguan et al., 1999). The relatively higher stomatal conductance of the tolerant genotypes results from mechanisms maintaining a higher leaf water status and hence more open stomata. As a consequence, CO_2 influx towards chloroplast may be longer, thus allowing greater photosynthetic rates under drought, high temperature and combined stress conditions (Hassan, 2006).

6.5 Conclusions

The effect of various stress treatments (high temperature, drought and combination of drought and high temperature) imposed for 16 d from the 10 d after complete anthesis were studied on the growth, physiology and yield components of four sweet sorghum genotypes. Genotype Tracy was found to exhibit tolerance towards combination of drought and high temperature stress, and also individual stresses by maintaining higher net photosynthetic rate,

chlorophyll SPAD and Fv/Fm compared to other genotypes. The increased photosynthetic rate has resulted in higher accumulation of sugars in juice, which is due to higher brix and juice yield. Among the various stresses, combination of drought and high temperature was found to decrease sugar and juice yield compared to drought and high temperature alone. Between individual stress effects, drought stress had higher decrease in sugar yield compared to high temperature. Significant differences were found among sweet sorghum genotypes with regards to their tolerance capacity to different abiotic stresses, which allows better selection for use of bioenergy production.

6.6 Tables and Figures

Figure 6.1 Effect of drought, high temperature and its combination on leaf chlorophyll content (SPAD) of four sweet sorghum genotypes.

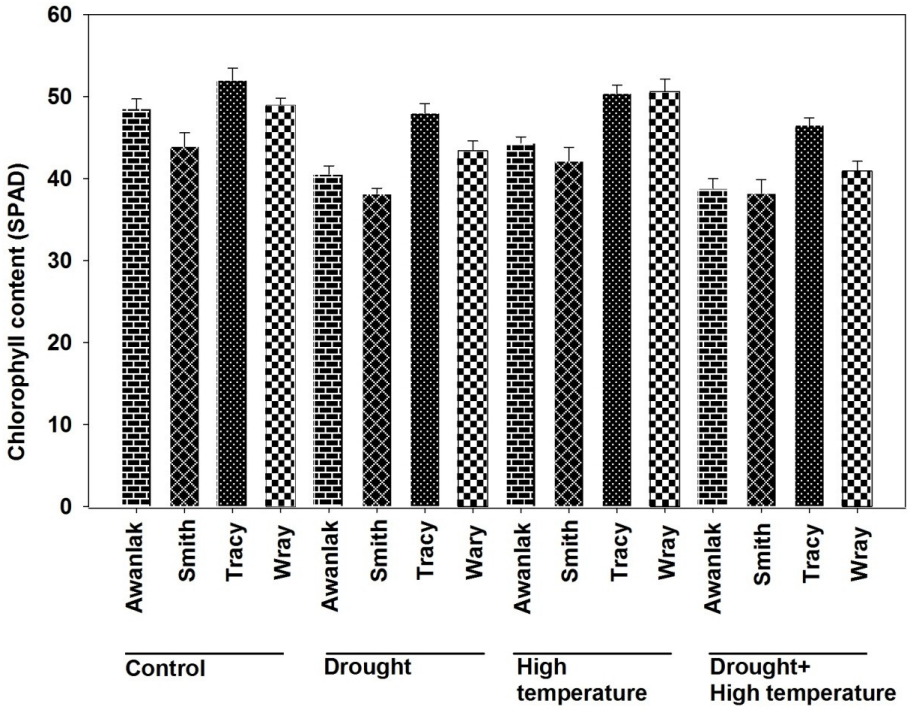

Figure 6.2 Effect of drought, high temperature and its combination on (a) leaf temperature (b) Fv/Fm (c) stomatal conductance and (d) photosynthetic rate of four sweet sorghum genotypes.

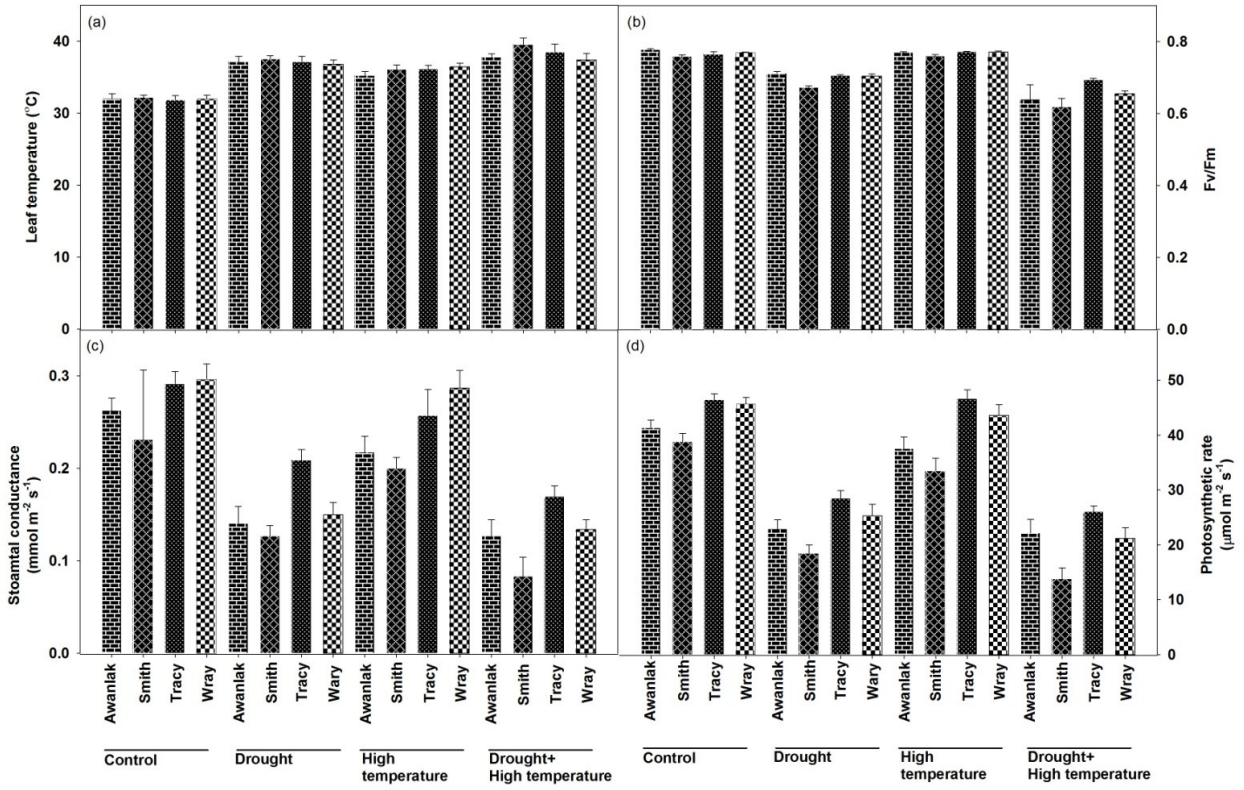

Figure 6.3 Effect of drought, high temperature and its combination on (a) stem diameter (b) stem fresh weight (c) juice yield and (d) sugar yield of four sweet sorghum genotypes. The vertical bar denotes ±SE of means (n=12).

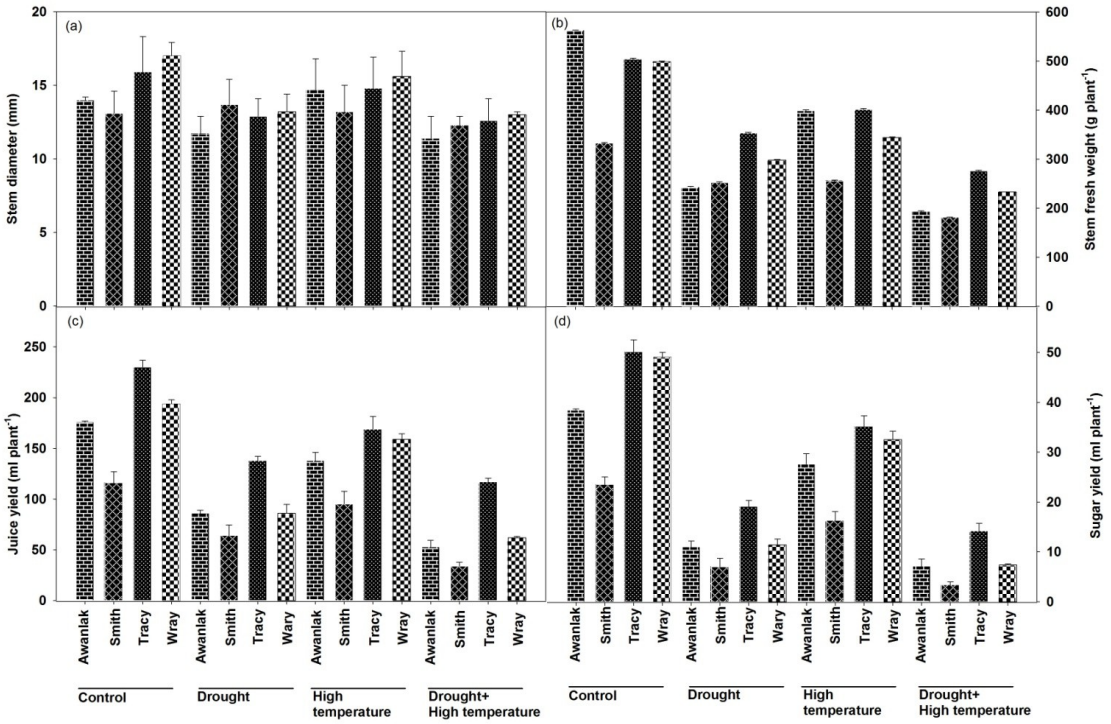

Figure 6.4 Effect of drought, high temperature and its combination on (a) reducing sugar content and (b) total sugar content of four sweet sorghum genotypes. The vertical bar denotes ±SE of means (n=12).

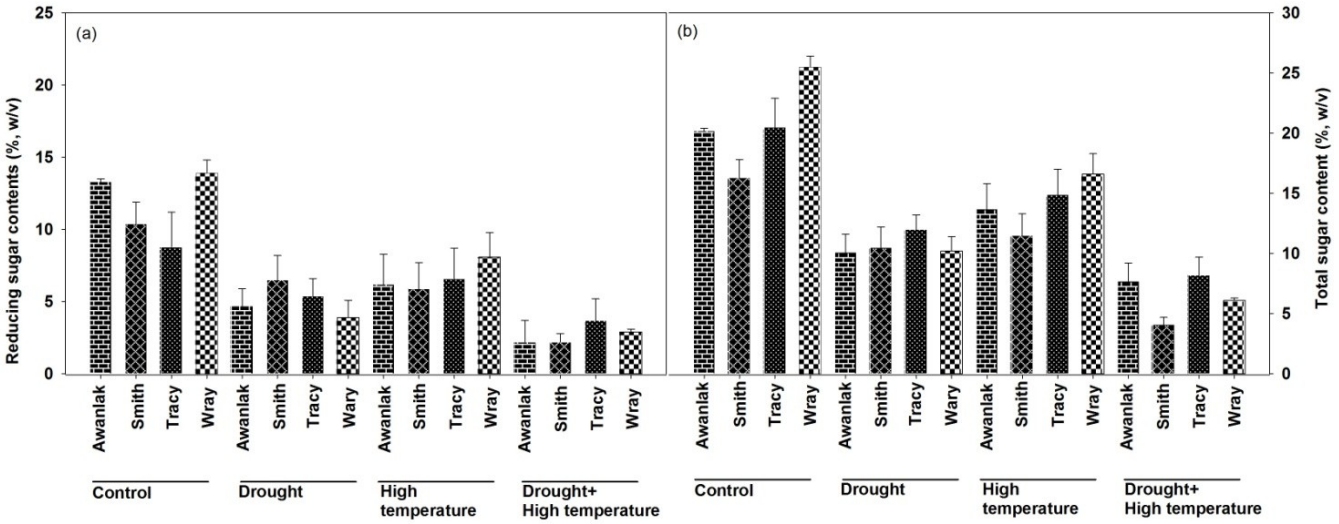

Table 6.1 Significance and P values of the effects of genotypes (G), treatments (T) and their interaction (G x T) on physiological traits in different sweet sorghum genotypes.

Physiological traits	Chlorophyll (SPAD)	Leaf temperature (°C)	Fv/Fm	Net photosynthesis (μmol CO_2 m^{-2} s^{-1})	Stomatal conductance (mmol H_2O m^{-2} s^{-1})	Intercellular CO_2 concentration (Ci) (ppm)	Transpiration rate (mmol H_2O m^{-2} s^{-1})
Genotype (G)	<0.001	<0.01	<0.001	<0.001	<0.001	NS	<0.001
Treatment (T)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.05	<0.001
Day (D)	<0.001	<0.001	<0.001	<0.001	<0.001	NS	<0.001
G*T	<0.001	<0.05	<0.05	<0.001	<0.05	NS	NS
G*D	NS	<0.05	NS	<0.001	NS	NS	<0.05
T*D	NS	<0.01	<0.001	<0.001	NS	<0.05	NS
G*T*D	NS	<0.001	NS	<0.05	NS	<0.05	NS

NS= Non-significant.

Table 6.2 Effect of various sweet sorghum genotypes on physiological traits.

Physiological traits	Genotypes				
	Awanlek	Smith	Tracy	Wray	LSD
Chlorophyll (SPAD)	43.0[c]	40.5[d]	49.1[a]	45.9[b]	0.9
Leaf temperature (°C)	35.5[b]	36.3[a]	35.9b[a]	35.6[b]	0.46
Fv/Fm	0.724[a]	0.702[b]	0.734[a]	0.724[a]	0.01
Net photosynthetic rate (μmol CO_2 m^{-2} s^{-1})	31.0[c]	26.1[d]	36.9[a]	33.9[b]	0.83
Stomatal conductance (mmol H_2O m^{-2} s^{-1})	0.186[b]	0.160[c]	0.231[a]	0.216[a]	0.01
Intercellular CO_2 concentration (Ci) (ppm)	81.9[b]	87.1[ba]	98.2[a]	89.3[ba]	14.5
Transpiration rate (mmol H_2O m^{-2} s^{-1})	5.3[b]	4.1[c]	5.8[ba]	6.2[a]	0.6

Means within the same row with different letter are significantly different at $P<0.05$. Each data is the average of four independent measurements of each genotype recorded on day 4, 8, 12, and 16.

Table 6.3 Effect of various treatments on physiological traits of sweet sorghum genotypes.

Physiological traits	Genotypes				
	Control	Drought	High temperature	Drought + High temperature	LSD
Chlorophyll (SPAD)	48.3[a]	42.5[c]	46.8[b]	41.0[d]	0.9
Leaf temperature (°C)	32.0[d]	37.1[b]	35.9[c]	38.3[a]	0.46
Fv/Fm	0.767[a]	0.699[c]	0.732[b]	0.651[d]	0.01
Net photosynthetic rate (μmol CO_2 m^{-2} s^{-1})	43.0[a]	23.7[d]	40.3[b]	20.8[d]	0.83
Stomatal conductance (mmol H_2O m^{-2} s^{-1})	0.270[a]	0.156[c]	0.240[b]	0.128[d]	0.01
Intercellular CO_2 concentration (Ci) (ppm)	77.0[b]	96.8[a]	81.7[b]	101.1[a]	14.5
Transpiration rate (mmol H_2O m^{-2} s^{-1})	6.8[a]	4.0[b]	6.9[a]	3.7[b]	0.6

Means within the same row with different letter are significantly different at $P<0.05$. Each data is the average of four independent measurements of each genotype recorded on day 4, 8, 12, and 16.

Table 6.4 Significance and P values of the effects of genotypes (G), treatments (T) and their interaction (G x T) on growth and yield traits in different sweet sorghum genotypes.

Growth and yield traits	Genotype (G)	Treatment (T)	G x T
Plant height (cm plant^{-1})	<0.001	<0.001	<0.01
Leaf area (cm^2 plant^{-1})	<0.001	<0.001	<0.05
Number of leaves plant^{-1}	<0.001	<0.001	NS
Number of internodes plant^{-1}	<0.01	<0.05	NS
Average stem diameter (mm plant^{-1})	<0.001	<0.001	<0.01
Leaf fresh weight (g plant^{-1})	<0.01	<0.001	NS
Stem fresh weight (g plant^{-1})	<0.001	<0.001	<0.01
Panicle fresh weight (g plant^{-1})	<0.001	<0.001	NS
Total fresh biomass (g plant^{-1})	<0.001	<0.001	<0.01
Leaf dry weight (g plant^{-1})	<0.001	<0.05	<0.01
Stem dry weight (g plant^{-1})	<0.001	<0.001	NS
Panicle dry weight (g plant^{-1})	<0.001	<0.001	<0.05
Total dry biomass (g plant^{-1})	<0.001	<0.001	<0.05
Grain yield (g plant^{-1})	<0.001	<0.001	<0.001

NS= Non-significant.

Table 6.5 Effect of various sweet sorghum genotypes on growth and yield traits.

Growth and yield traits	Genotypes				
	Awanlek	Smith	Tracy	Wray	LSD
Plant height (cm plant^{-1})	284.0^{b}	286.0^{b}	299.0^{a}	256.0^{c}	6.7
Leaf area (cm^2 plant^{-1})	1849.0^{c}	1834.0^{c}	2330.0^{b}	3522.0^{a}	241.1
Number of leaves plant^{-1}	9.2^{c}	9.6^{c}	10.3^{b}	11.0^{a}	0.64
Number of internodes plant^{-1}	10.1^{b}	10.0^{b}	10.5^{b}	11.5^{a}	0.72
Average stem diameter (mm plant^{-1})	12.9^{b}	13.1^{b}	14.0^{a}	14.7^{a}	0.76
Leaf fresh weight (g plant^{-1})	54.6^{bc}	50.6^{c}	67.4^{a}	64.4^{ba}	8.4
Stem fresh weight (g plant^{-1})	349.7^{b}	255.5^{c}	383.9^{a}	349.7^{b}	30.5
Panicle fresh weight (g plant^{-1})	32.3^{b}	31.2^{b}	28.3^{b}	48.6^{a}	5.7
Total fresh biomass (g plant^{-1})	427.6^{b}	312.1^{c}	479.7^{a}	435.4^{b}	35.4
Leaf dry weight (g plant^{-1})	41.4^{c}	41.3^{c}	45.3^{b}	54.8^{a}	3.7
Stem dry weight (g plant^{-1})	109.1^{a}	92.0^{b}	111.2^{a}	117.7^{a}	8.6
Panicle dry weight (g plant^{-1})	26.9^{b}	27.8^{b}	24.1^{b}	39.3^{a}	4.3
Total dry biomass (g plant^{-1})	177.5^{b}	161.2^{c}	180.7^{b}	211.9^{a}	10.5
Grain yield (g plant^{-1})	10.7^{b}	11.4^{b}	11.2^{b}	22.9^{a}	1.7

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

Table 6.6 Effect of various treatments on growth and yield traits of sweet sorghum genotypes.

Growth and yield traits	Genotypes				
	Control	Drought	High temperature	Drought + High temperature	LSD
Plant height (cm plant^{-1})	300.0^{a}	277.0^{c}	286.0^{b}	262.0^{d}	6.7
Leaf area (cm^2 plant^{-1})	3320.0^{a}	1866.0^{c}	2850.0^{b}	1501.0^{d}	241.1
Number of leaves plant^{-1}	11.0^{a}	9.7^{b}	10.4^{a}	9.1^{b}	0.64
Number of internodes plant^{-1}	11.1^{a}	10.4^{b}	10.6b^{a}	10.0^{b}	0.72
Average stem diameter (mm plant^{-1})	15.0^{a}	12.9^{b}	14.6^{a}	12.3^{b}	0.76
Leaf fresh weight (g plant^{-1})	73.7^{a}	49.1^{cb}	60.2^{b}	46.0^{c}	7.8
Stem fresh weight (g plant^{-1})	474.8^{a}	287.0^{c}	349.9^{b}	274.8^{c}	30.5
Panicle fresh weight (g plant^{-1})	43.6^{a}	33.0^{b}	38.8^{a}	25.0^{c}	5.7
Total fresh biomass (g plant^{-1})	592.2^{a}	340.6^{c}	449.0^{b}	273.1^{d}	35.4
Leaf dry weight (g plant^{-1})	49.3^{a}	46.5^{ba}	45.5^{b}	41.6^{c}	3.7
Stem dry weight (g plant^{-1})	134.8^{a}	95.6^{c}	109.6^{b}	90.1^{c}	8.6
Panicle dry weight (g plant^{-1})	37.1^{a}	28.4^{b}	31.1^{b}	21.5^{c}	4.3
Total dry biomass (g plant^{-1})	221.2^{a}	170.6^{c}	186.3^{b}	153.3^{d}	10.5
Grain yield (g plant^{-1})	18.5^{a}	13.7^{c}	15.6^{b}	8.3^{d}	1.7

Means within the same row with different letter are significantly different at $P<0.05$; NS= Non-significant.

Table 6.7 Significance and P values of the effects of genotypes (G), treatments (T) and their interaction (G x T) on bioenergy traits in different sweet sorghum genotypes.

Bioenergy traits	Genotype (G)	Treatment (T)	G x T
Brix (%)	<0.001	<0.001	NS
Juice yield (ml plant^{-1})	<0.001	<0.001	<0.01
Sugar yield (ml plant^{-1})	<0.001	<0.001	<0.001
Total sugars (%, w/v)	<0.001	<0.001	<0.001
Reducing sugars (%, w/v)	NS	<0.001	<0.001
Non-reducing sugars (%, w/v)	<0.001	<0.001	<0.001

NS= Non-significant.

Table 6.8 Effect of various sweet sorghum genotypes on bioenergy traits.

Bioenergy traits	Genotypes				
	Awanlek	Smith	Tracy	Wray	LSD
Brix (%)	16.9[a]	14.5[b]	17.1[a]	16.8[a]	1.2
Juice yield (ml plant^{-1})	113.4[c]	77.6[d]	163.6[a]	125.5[b]	10.6
Sugar yield (ml plant^{-1})	21.0[c]	12.6[d]	29.7[a]	23.3[b]	2.2
Total sugars (%, w/v)	12.9[b]	10.6[c]	13.9[ba]	14.6[a]	1.0
Reducing sugars (%, w/v)	6.6[ba]	6.2[b]	6.1[b]	7.2[a]	0.8
Non-reducing sugars (%, w/v)	6.2[b]	4.3[c]	7.7[a]	7.3[a]	0.7

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

Table 6.9 Effect of various treatments on bioenergy traits of sweet sorghum genotypes.

Bioenergy traits	Genotypes				
	Control	Drought	High temperature	Drought + High temperature	LSD
Brix (%)	21.3[a]	12.6[c]	19.6[b]	11.7[c]	1.2
Juice yield (ml plant^{-1})	179.3[a]	93.7[c]	140.4[b]	66.8[d]	10.6
Sugar yield (ml plant^{-1})	38.5[a]	12.1[c]	27.9[b]	8.0[d]	2.2
Total sugars (%, w/v)	20.6[a]	10.7[c]	14.2[b]	6.5[d]	1.0
Reducing sugars (%, w/v)	11.6[a]	5.1[c]	6.7[b]	2.8[d]	0.8
Non-reducing sugars (%, w/v)	9.0[a]	5.5[c]	7.4[b]	3.7[d]	0.7

Means within the same row with different letter are significantly different at $P<0.05$; NS= Non-significant.

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General Summary

The objectives of this study were to (1) quantify genetic variability for plant height, stem fresh weight, brix, juice yield and sugar yield in sweet sorghum germplasm; and to identify potential drought tolerant sweet sorghum genotypes, (2) determine the optimum harvest time for obtaining maximum juice yield, (3) obtain information on the various growth and physiological traits influencing sugar yield of various sweet sorghum genotypes, (4) quantify effects of water stress on brix, juice and sugar yield, and (5) quantify effects of drought, high temperature and its combination on juice and sugar yield characteristics.

The study found that there was a wide genetic variability among the sweet sorghum germplasm for plant height, stem diameter, stem fresh weight, °brix, juice yield and sugar yield. There were significant positive correlation between sugar yield and growth (plant height and stem weight), physiological (photochemical efficiency) and bio-energy traits (juice yield). Growth and physiological traits were not affected by the rain-fed condition; however, there were significant decreases on traits such as °brix, stem fresh weight, juice yield and sugar yield. Among the 78 genotypes, Wray, MN 4564 and Caxa had higher sugar yield. Genotypes Sanyagie, MN 818 and Dale_1 had lower relative sugar yield reduction (RSYR) indicating their drought tolerant potential with sustainable sugar yield.

In an effort to identify optimum stage of harvest, the study found that harvesting plants at hard dough stage gave the highest brix, total sugars, reducing sugars and non-reducing sugars in stem juice. The highest level of sugar and juice in stem was obtained from plants harvested from milky stage to hard dough stage. Hence, the optimum harvest time for maximum juice and sugar yields for the sweet sorghum variety M81E is between milk and hard dough stage.

To understand the morpho-physiological factors for sugar yield, the study observed high sugar yielders possessed higher green leaf numbers, tall plant stature, high average stem diameter, higher Fv/Fm and higher stem biomass accumulation, and low grain yield. Whereas, the low sugar yielders had more assimilates in grain. Of the thirty sweet sorghum genotypes, genotypes, Wray, Honey No. 6, Isidomba, MN 4135 and No. 5 Gambela were identified as the high sugar yielders. In addition, principal component analysis (PCA) established similar groups of genotypes, according to their sugar yielding characteristics, as well as identified stem fresh weight a major trait contributing for sugar yield.

The water stress experiment found significant differences among the genotypes for all growth, physiology and bioenergy traits. Overall, across all genotypes severe water stress significantly decreased brix, juice yield, sugar yield, sucrose content, total dry biomass and grain yield. Genotype Tracy produced significantly highest juice and sugar yields under both irrigated and water stress conditions compared to genotypes Wray, Awanlek and Smith. The water stress tolerance of Tracy could be ascertained based on significant increase in chlorophyll SPAD, net photosynthetic rate, stomatal conductance, and transpiration rate. Also, genotype Tracy accumulated relatively greater amounts of sugars (glucose, fructose and sucrose) in the juice than other genotypes.

The effect of various stress treatments (high temperature, drought and combination of drought and high temperature) revealed genotype Tracy was found to exhibit tolerance towards combination of drought and high temperature stress, and also individual stresses by maintaining higher net photosynthetic rate, chlorophyll SPAD and Fv/Fm compared to other genotypes. The increased photosynthetic rate has resulted in higher accumulation of sugars in juice, which is due to higher brix and juice yield. Among the various stresses, combination of drought and high

temperature was found to decrease sugar and juice yield compared to drought and high temperature alone. Between individual stress effects, drought stress had higher decrease in sugar yield compared to high temperature. Significant differences were found among sweet sorghum genotypes with regards to their tolerance capacity to different abiotic stresses, which allows better selection for use of bioenergy production.

Future Directions

1. We found wide genetic variability among 78 sweet sorghum cultivars for plant height, stem diameter, stem fresh weight, brix, juice yield and sugar yield. However, it is based on two years and in one location data. Hence, the study has to be conducted in multi locations, to confirm the genetic variability and identification of diverse parental lines.

2. Harvesting plants at hard dough stage have resulted in highest brix, total sugars, reducing sugars and non-reducing sugars. However, ethanol was not quantified in the present study, to optimize the stage of harvest ethanol yield is important. Hence, the above study has to be repeated in multi-location site with ethanol quantification to confirm the present result.

3. Identification of morpho-physiological traits for high sugar yield was done for one year; the traits have to be validated in multi locations along with ethanol quantification. In the present study there were some medium sugar yielders; in future high sugar yielder and low sugar yielder alone should be used for validating the traits.

4. The drought study was conducted in pot culture experiment; however it has to be verified in field conditions. The juice content was not extracted using press mill in the present study, in future; press mill has to be used. Ethanol has to be quantified for understanding the drought stress effect.

5. The abiotic stress (drought, high temperature and combination) study was conducted in pot culture experiment; however it has to be verified in field conditions. Ethanol has to be quantified for understanding the effects of drought, high temperature and combination of both.