Effect of irrigation on grain sorghum ethanol yield and sorghum mutants on biomass composition

by

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B.S., Kansas State University, 2015

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Biological and Agricultural Engineering
College of Engineering

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2017

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Abstract

Bioprocessing is widely involved in our daily life and significantly relative to the general public because bio-products are widely used in eating, clothing, and living as well as transportation. Due to the public concern of the environmental deterioration, limited fossil fuel resources, and energy price volatility, biofuel as a clean, safe and sustainable energy needs to be developed in response to this growing concern. Sorghum, an important dryland crop, represents a renewable resource currently grown on 8 million acres throughout the United States. Due to climate variability and the continuous decline of water resources, utilization of dryland to grow sorghum and forage sorghum is critically important in order to ensure available energy resources and sustainable economic development. The objectives of this research were 1) to study the impact of deficit irrigation strategies on sorghum grain attributes and bioethanol production, and 2) to evaluate the potential fermentable sugar yield of pedigreed sorghum mutants. Results showed that average kernel weight and test weight of grain sorghum increased as irrigation capacity increased, whereas kernel hardness index decreased as irrigation capacity increased. Starch content increased as irrigation level increased and protein contents decreased as irrigation level increased. Irrigation also had a significant effect on starch properties and bioethanol yield. Sorghum mutants had a significant effect on chemical composition and physical properties such as glucan content, glucan mass yield, ash content, and high heating value, and also had a significant effect on fermentable sugars yield and enzymatic conversion efficiency.

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Acknowledgements

I would like to thank everyone who has helped me over the course of this thesis study. Especially, I would like to thank my major professor, Dr. Donghai Wang for all his excellent guidance, support, caring patience, and continuous encouragement. With his help, I improved my research skills and prepared myself better for the challenges that I will face in the future. Hardly can I imagine without his tremendous support this research would not be possible. Thanks Dr. Wang for allowing me to conduct research in the field of biofuel and bioenergy, for which I am passionate about. I would also like to thank my committee members, Dr. Xiuzhi Sun and Dr. Lisa Wilken for their expertise and guidance. Thanks for their patience and support throughout this process. I appreciated their comments and insights to help me produce a product I am proud of.

I would like to appreciate Dr. Ke Zhang, the postdoc in Dr. Wang's group, for all his advice and assistance. I would also like to thank Dr. Renyong Zhao and Dr. Ningbo Li for their precious help.

I would like to thank Dr. Joseph Harner, Department Head, Dr. Naiqian Zhang, Graduate Program Director, Ms. Barb Moore, Mr. Randy Erickson, and all other faculty members and staff in the Department of Biological and Agricultural Engineering at Kansas State University for their generous support and assistance.

I would also like to thank Dr. Isaya Kisekka for providing raw material. I am grateful for Michael Moore from Department of Grain Science and Industry for using his instrument.

Finally, I want to express my gratitude to my beloved parents. Thank you for enveloping me in love and kindness through my entire life.

Dedication

To my dear parents, my first and only heroes

Chenjiu Pang & Yan Li.

Chapter 1 - Introduction

1.1 Problem Statement

As reported by the United Nations, the world population in 2017 is nearly 7.6 billion and is projected to increase to 9.8 billion by 2050 (UN, 2017). The increase in world population brings out the growing concern about the available energy and energy sustainability for the general public (Meneguzzo et al., 2016). In addition, the increased fossil energy consumption causes environmental issues. According to Intergovernmental Panel on Climate Change (IPCC) report (2014), energy production is the biggest source of greenhouse gases emission. Currently, the challenge is to reduce our dependence on fossil fuels and develop a sustainable, renewable area, environmental-friendly energy supply (Shankar et al., 2017). The development of renewable energy could make a significant contribution towards a more sustainable future and would also contribute to reduce CO_2 emissions (Gelfand et al., 2013; Fytili and Zabaniotou, 2017). The U.S. Department of Energy (DOE) and the U.S. Department of Agriculture (USDA) are making significant efforts to develop bioenergy and are strongly committed to expanding the role of bioenergy (Perlack et al., 2005). Experimental investigations of flow patterns in bioenergy conversion have been presented in numerous publications (Daniell et al., 2012; French and Czernik, 2010; Mohan et al., 2006; Tilman et al., 2006; Wang et al., 2008; Zhang et al., 2016). They conducted research on production of syngas, electricity, biodiesel, bioethanol, bio-oil, and hydrocarbons from biomass, respectively. Biomass based biorefinery system has capability to produce multiple bio-base products which can be find in Fig. 1.1 (Naik et al., 2010).

The demand of low-carbon fuels is becoming a high priority due to the carbon dioxide emissions from fossil fuels (Fargione et al., 2008). In 2015, there was about 19Mb/d of liquid fuel consumed for cars ranked a fifth of global demand (Simões-Filho, 2017). According to BP

Energy Outlook (2017), global liquids fuel demand (oil, biofuels, and other liquid fuels) increases by around 15 Mb/d, and will reach to 110 Mb/d by 2035 (Fig. 1.2). Fossil fuels need millions of years to form and cause greenhouse gas emissions that stored in plant biomass and soil millions of years ago, comparing with fossil fuels, biofuels are produced from the short cycle of growing plants and generate less or no carbon dioxide (Kumar et al., 2009).

Sorghum is one of five most important crop in the world, defined by the Food and Agriculture Organization of the United Nations, used for food, feed, the production of alcoholic beverages, and biofuels (Rooney et al., 2007; Smith and Frederiksen, 2000.). Sorghum is the second commonly used grain in ethanol production following the maize grain in the United States (Murray et al., 2008). As a major raw materials preparing for fermentation to produce bioethanol, sorghum seed is comprised of 60 to 75% starch, 7 to 15% protein and 2 to 5% fat (Dicko et al., 2006). High starch and protein content makes the sorghum the primary source to produce biofuel. In addition to grain sorghum, sorghum biomass is also excellent raw material for biofuel product. In general, sorghum biomass contains cellulose, hemicellulose and lignin. Sweet sorghum, for example, mainly consist of sucrose (55%) and of glucose (3.2%), cellulose (12.4%) and hemicellulose (10.2%). And because of the rich amount of fermentable sugars, sorghum biomass can be considered as an excellent raw material for fermentative hydrogen production (Antonopoulou et al., 2008). Overall, compare with many cultivated crops, sorghum seems to be a promising raw material for biofuel production (Kresovich and Henderlong, 1984; Rooney et al., 2007).

1.2 Objectives

The goal of this study was to evaluating the sorghum performance in bioethanol production. More specifically, there were two objectives: 1) to study the impact of deficit

irrigation strategies on sorghum grain attributes and bioethanol production, and 2) to evaluate the chemical composition and potential fermentable sugars yield of pedigreed sorghum mutant biomass.

1.3 Significance of Work

Among the biofuels, ethanol is one of the most attractive product. In USA and Brazil, it is already produced on a large scale and can easily be blended with gasoline to operate in spark ignition (SI) engines. Bioethanol is most commonly used with gasoline in the proportions of about 24% to operate in gasoline engines or in any proportion in flexible-fuel vehicles (FFV) (Macedo et al., 2008). According to Renewable Fuel Association's (RFA) data, U.S. fuel ethanol production was 14.7 billion gallons in 2015.

1.4 Literature Review

1.4.1 Starch

Starch is a type of storage polysaccharide that most abundant in plants and a major dietary source of carbohydrates (Sajilata et al, 2006). According to Zhan et al. (2006) research, sorghum is a starch-rich grain and has similar starch content to maize. Biofuels derived from sugar or starch through fermentation are called first generation biofuels (Naik et al., 2010). Followed by cellulose, starch is the second most abundant biomass found in the world (Katopo et al., 2002). As primary metabolites from photosynthetic plants, starch containing crops are include wheat, rice, sorghum, corn grains and root plants like potato and cassava (Naik et al., 2010). Composed by simple fermentable sugar, starch contains two different glucose polymers which called amylose and amylopectin (Fig. 1.3) (Vu and Marletta, 2016). Amylose is linked by glucose units with α, 1-4 linkages in a linear fashion (Torney et al., 2007). In starch, amylose

ranges from 0 to 80% depending upon the species and the genetic variations within a species (Rooney and Pflugfelder, 1986). Different with that, amylopectin, the most abundant component of normal starches, is more branched linked by glucose units with α, 1-6 linkages and α, 1-4 linkages (Rooney and Pflugfelder, 1986; Torney et al., 2007). Rooney and Pflugfelder (1986) point out that amylopectin make up 70 to 80% of most cereal starches and is the only starch in waxy genotypes of corn and sorghum.

In starch, amylose and amylopectin molecules are highly organized together by hydrogen binds to form starch granules (Rooney and Pflugfelder, 1986).The particle size of starch is one of the important properties that affect their processing. According to the particle size distribution and characteristics of the particles within them, the stability, thermal and rheological properties of polymers may be changed (Mali et al., 2004; Matzinos et al., 2002; Morris, 1990). Particle size affects dispersibility and division of the starch (Thomas and Atwell, 1999). According to Wang et al. (2008) research, the finely ground samples had approximately 5% higher fermentation efficiencies than the uneven samples. Large particles would have 5-10˚C gelatinization temperature higher than the smaller particles.

When starch molecules processed in excess water with heat, starch gelatinization happened with irreversible loss of the crystalline regions (Cooke and Gidley, 1992). Gelatinization of starch is stimulated at the liquefaction process to convert semi-crystalline starch granule to amorphous conformation which is an enzyme susceptible form (Srichuwong et al., 2012). In this process, a lot of physical and chemical reaction happened. Starch native structure is destroyed, allowing water to enter the granule and the granule swells, which changes the starch particle size, swelling and water uptake results in system viscosity increase (Hegenbart, 1996). Gelatinization properties of starch mainly depend on concentration of starch, the ratio of

amylose/amylopectin, granule size and interactions among the close-packed granules and their rigidity during the heating process (Li et al., 2011). Amylose content in the starch has a significantly impact on starch gelling behavior. Generally, the higher levels of amylose, the greater tendency to form a gel after cooking (Thomas and Atwell. 1999). The property of amylopectin that contributes to the formation of the crystalline part in the granules affects the gelatinization (Zhu, 2014). The rich of short unit chains (DP 6-12) of amylopectin result in a deficient crystallinity structure, thus a lower gelatinization temperature and a smaller gelatinization enthalpy change (Ai et al., 2011). Previous research (Wu et al., 2007; Zhan et al., 2006) indicated that phenolic compounds, such as tannins, and low starch, protein digestibility had negative impacts on biofuel production, whereas high starch content and low viscosity during liquefaction were favorable characteristics. Tannin in the fermentation broth can inhibit yeast growth and starch enzymatic hydrolysis, thus, slow ethanol production (Ai et al., 2011). Starch granules may be fasten by a protein matrix and led to the low digestibility of starch (Zhan et al., 2006). Complete gelatinization subsequently helped hydrolytic enzyme access to the starch molecules, resulting in better conversion to glucose sugar (Wang et al., 2008).

1.4.2 Lignocellulosic Biomass

Lignocellulosic plants are the most abundant and sustainable biomass with substantial worldwide production, including agricultural residues, forestry wastes, dedicated energy crops, and organic municipal solid waste; making it an indispensable feedstock for the production of commercialized biofuels and renewable chemicals (Saini et al., 2015). As a cheap and abundant nonfood material from plant biomass, large amount of lignocellulosic materials can be used for production of second-generation biofuels (Naik et al., 2010) The predominant compounds in lignocellulosic biomass are mainly cellulose, hemicellulose, and lignin, and cellulose is meanly

structural carbohydrates (Demirbas, 2007; Naik et al., 2010; Rooney et al., 2007). In the biomass, cellulose is generally the largest fraction, representing about 30 to 50% of the total biomass by weight; hemicellulose portion represents 20 to 40% of the material by weight, and lignin accounts about 15 to 30% (McKendry, 2002).

The structure of cellulose has been studied since two centuries ago (Cosgrove, 2014). Payen (1839) first found out that wood majorly consists of a fibrous and stiff material with the empirical formula of $C_6H_{10}O_5$, which was named as cellulose. In 1922, Staudinger found the cellulose structure of repeated-linked glucose units. Cellulose is a polymer of anhydroglucose units (AGU) with β-1, 4 linkage, which can be considered as combination of linear chains of (1, 4)-D -glucopyranose units, and is found in both the crystalline and noncrystalline structure (Klemm et al., 2005; McKendry, 2002). Properties of cellulose are highly linked to its degree of polymerization (DP), which represents the number of AGU that make up one polymer molecule (Rinaldi and Schüth, 2009; Varshney and Naithani, 2011). Cellulose is insoluble in water and dilute acid solutions at room temperature, but in alkaline solutions, swelling of cellulose occurs and cellulose with low molecular weight (DP < 200) was dissolved (Harmsen et al., 2010).

Hemicellulose has a branched structure instead of linear and substituted as glucans, xylans, mannans, and anionic components such as the galacturonic acid-containing pectic polysaccharides (Pauly and Keegstra, 2008). As the second profuse polysaccharide in the plant cell wall (cellulose is the most abundant), hemicellulose consists of various monosaccharides include pentose(β-D-xylose, α-L-arabinose), hexoses (β-D-mannose, β-D-glucose, α-D-galactose), uronic acids (β-D-glucuronic acid, α-D-4-O-methylglucuronic acid and α-D-galacturonic acid) (Aro et al., 2005; Pereira, 2011). The polysaccharides are bond tightly, but noncovalently, to the surface of each cellulose microfibril (McKendry, 2002).

In biomass processing to produce biofuel, the main issue is the low accessibility of cellulose caused by the lignin protection (Mood et al., 2013; Zeng et al., 2014). The structure of lignin building blocks is believed to be a three carbon chain attached to rings of six carbon atoms, called phenyl-propanes (Goyal et al., 2008). Cellulose and hemicellulose are tightly bound to lignin mainly by hydrogen bonds but also by some covalent bonds (Lin and Tanaka, 2006). The rigid association of cellulose with lignin caused difficulties within conversion process and breaking down lignin block is one of the main aim of pretreatment (Mood et al., 2013). There are three types of lignin: G lignin, S lignin and H lignin and the major structure of the lignin are the hydroxycinnamyl alcohols (or monolignols) coniferyl alcohol and sinapyl alcohol, with typically minor amounts of p-coumaryl alcohol (Vanholme et al., 2010).

Hexoses such as glucose, galactose and mannose are fermentable to ethanol by many naturally occurring organisms. However, pentoses such as xylose and arabinose are not readily fermented, the ketose of xylose, xylulose, is converted to ethanol by *S. pombe*, *S. cerevisiae*, *S. amucae*, and *Kluveromyces lactis* (Mosier et al., 2005). Parallel cellulose fibers are cross-linked by hemicellulose, protein, and lignin to form a 3D structure which can be seen in Fig. 1.4 (Rubin, 2008). This structure would have a high mechanical and chemical strength (Ilnicka and Lukaszewicz, 2015). Potential energy sources of cellulose are including woody crops and grass plants. Woody plants are usually defined as slow growth and are composed of tightly bound fibers, giving a hard external surface. Different from that, grasses or herbaceous plants are usually perennial, with more loosely bound fibers, indicating a lower proportion of lignin, which binds together the cellulosic fibers. The relative proportion of cellulose and lignin is one of the determining factors in identifying the suitability of plant species for subsequent processing as energy crops (McKendry, 2002).

1.4.3 Biomass Pretreatment

Pretreatment is an important step for practical cellulose conversion process. It is mainly used to break down the tight structure and make the cellulose more accessible to the enzyme, so that carbohydrate polymers can easily be converted to sugar in the fermentation step. Cellulose in native biomass is difficult to digest by enzymes and its sugar yield is usually lower than 20% (Mosier et al., 2005). Pretreatment of biomass feedstocks is used to breakdown barrier and open cellulose more accessible for further saccharification conversion. Numerous pretreatment methods have been developed to overcome the recalcitrant structure, such size reduction, steam explosion, liquid hot water, dilute acid, lime, ammonia, organic solvent, and ionic liquid pretreatments. Challenges in the current pretreatment processes include incomplete separation of cellulose and lignin, which could reduce the subsequent enzymatic hydrolysis efficiency; formation of inhibitors that affect ethanol fermentation, such as acetic acid from hemicellulose, furans from sugar degradation and phenolic compounds from lignin composition; high usage of chemicals and energy-intensive processes and also high cost of waste disposal.

1.4.4 Hydrolysis

Hydrolysis of starch is the first key step for converting starch to bioethanol. During this process, two major polymers, amylose and amylopectin, are converted to fermentable sugars and subsequently converted to ethanol by yeast or bacteria. Two key enzymes are involved in this "cold process" (Baras et al., 2002). A-amylsaes, which is starch liquefying enzymes with an optimum temperature of 85-110 ˚C, can interact with internal α-d-(1–4)-glucosidic linkages in starch and catalyze the hydrolysis consequently(Aggarwal et al., 2001; Mojović et al., 2006). Glucoamylases are used as the starch saccharifying enzymes at a temperature of 60-70 ˚C to

catalyze the hydrolysis of α-d-(1–4) and α-d-(1–6)-glucosidic bonds of starch (Mojović et al., 2006; Ruiz et al., 2011).

Different from starch, cellulose is more difficult to convert to glucose. During ethanol production, the unique structure of lignocellulosic requires material hydrolysis of the carbohydrate polymers to monomeric sugars for the fermentation step (Mosier et al., 2005). Lignocellulosic biomass need pretreatment to separate cellulose, hemicellulose, and lignin, followed by enzyme-catalyzed hydrolysis can break down the complex carbohydrate molecules to simple sugars (Nigam and Singh, 2011). Enzymatic hydrolysis of cellulose requires cellulase enzymes, which is a mixture of various enzymes including xylanase, β-xylosidase, glucuronidase, acetylesterase, galactomannanase and glucomannanase, β-glucosidase, endoglucanases, exoglucanase or cellobiohydrolase. These enzymes can either breakdown hemicellulose or hydrolyzes cellobiose to produce glucose or attack regions of low crystallinity in the cellulose fiber, creating free chain ends or degrade the molecule further by removing cellobiose units from the free chain ends (Bisaria, 1991; Duff and Murray, 1996; Nigam and Singh, 2011). According to Wu et al., (2010), enzyme source, concentration, and enzyme combinations affect cellulase activity. The temperature for enzymatic hydrolysis is around 50 ˚C and with a 75–95% glucose yield after several days hydrolysis, consequently. Except enzymatic hydrolysis, dilute acid hydrolysis (<1% H_2SO_4, 215˚C, 3 min with 50–70% glucose yield) and concentrated acid (30–70% H_2SO_4, 40 ˚C, a few hours, >90% glucose yield) are other possible hydrolysis methods (Wu et al., 2010).

Accessible surface area, cellulose fiber crystallinity and lignin and hemicellulose content would all affect hydrolysis of cellulose (Nigam and Singh, 2011).Hydrolysis process may result in a production of inhibitors affecting the following fermentation step. And there are mainly

three groups: furan derivatives, weak acids and phenolic compounds. These groups would reduce microorganism activity and ethanol yield in the following step. Thus, we need find inhibitor-tolerant microorganisms in the fermentation (Almeida et al., 2007).

1.4.5 Fermentation

Fermentation is an essential process that can convert any material that contains sugar to ethanol. In general, there are three types of raw materials that can be convert to ethanol via fermentation: sugar, starch and cellulose. Sugar from sugarcane, sugar beets, molasses and fruits can be used directly to produce ethanol. Starch can be easily hydrolyzed using α-amylase, saccharafied by glucoamlase, and fermented into ethanol by *Saccharomyces cerevisiae*.

However, cellulose in the plants is not easy be hydrolyzed to fermentable sugars by enzymes or mineral acids (Lin and Tanaka, 2006). Cellulose and hemicellulose are polysaccharides, they could be degraded to simple sugars via different cellulolytic enzymes. After that, pentose and hexose that come from the polysaccharides can be fermented by yeast to produce ethanol. Lignocellulosic biomass processing with enzymatic hydrolysis typically involve four critical transformations: 1) the production of saccharolytic enzymes (cellulases and hemicellulases); 2) the hydrolysis of carbohydrate components present in pretreated biomass to sugars (saccharification); 3) hexose sugars (glucose, mannose and galactose) fermentation; and 4) pentose sugars (xylose and arabinose) fermentation (Wang, 2015). All four transformations separated is called separate hydrolysis and fermentation (SHF); second and third transformation are combined together plus first transformation and fourth transformation is simultaneous saccharification and fermentation (SSF); second, third and fourth transformation are combined together plus first transformation is simultaneous saccharification and co-fermentation (SSCF); combination of all four transformation in a single step process is Consolidated Bioprocessing

(CBP). Due to the simpler process, CBP is considered more economic with a higher efficiency compare to SSF, SSCF (Lynd et al., 2005). And the critical difference between CBP and other biomass processing strategies is that a single microbial community is employed for both cellulase production and fermentation. Combination of substrate-utilization and product formation for microorganisms was always a big issue. However, in recent years study (Wang, 2015), yeast with recombinant strain can make that happen.

Cellulases is a group of important enzymes that can hydrolyze cellulose. However, cellulases are inhibited by their hydrolysis products cellobiose and glucose. The advantages of SSF or SSCF are that it can impede invasion by unwanted organisms because of the present of ethanol and also save the processing cost because of hydrolysis and fermentation in the same reactor (Brethauer and Wyman, 2010). CBP is considered to offer higher efficient and low costs for biofuel production because it combined cellulase production, substrate hydrolysis, and fermentation in a single process step by microorganisms that express cellulolytic and hemicellulolytic enzymes (Carere et al., 2008). Once simple sugars are formed, enzymes from microorganisms can readily ferment them to ethanol. Different from starch, lignocellulose is more complex in structure due to the mixture of carbohydrate polymers (cellulose and hemicellulose) and lignin.

Saccharomyces cerevisiae is the most popular microorganisms used in ethanol fermentation process with high tolerance to the end-product (e.g., ethanol) and to other compounds presenting in hydrolysates, which it innate tolerance to furan and phenolics (Almeida et al., 2007). Wild type *Saccharomyces cerevisiae* can efficiently ferment glucose to ethanol. However, in lignocellulosic biomass, the pentose sugar xylose also referred as “wood sugar” is the major sugar that cannot be fermented by wild-type strains of *Saccharomyces cerevisiae*. To

overcome this, genetically modified yeast was developed and used for cellulosic biomass ethanol production. Some eukaryotic genes could be functionally expressed in *Saccharomyces cerevisiae*, enabling this yeast to metabolize xylose and recombinant yeasts can directly use multiple sugars for fermentation (van Maris et al., 2006). The genome editing such as CRISPR-Cas9 system has been used to allow yeast utilize a set of genes or integrate new pathway genes faster than before (Wang, 2015). During fermentation, there are several stress factors affect the process. First of all is the temperature. *Saccharomyces cerevisiae* can ferment glucose to ethanol under anaerobic conditions in a production rate of 30 mmol g biomass^{-1} h $^{-1}$ at 30℃ in defined media (van Maris et al., 2006). Contamination is other factor that can greatly affect ethanol fermentation. During biomass transportation, *Lactobacilli* bacteria may be involved in the biomass, and this bacteria consumes glucose to produce by-product such as lactic acid during fermentation and results in reduced ethanol production. In addition, ethanol level in fermentation broth would have an influence on yeast activity and affect final ethanol yield. High ethanol level will slow down the fermentation speed until totally stop. Yeast activity will be reduced after 13% ethanol concentration. In industry, different fermentation methods such as batch fermentation and continuous fermentation were used to reduce the ethanol level effect. According to a study of the advantages and disadvantages of continuous and batch fermentation processes, batch process with yeast recycle showed less susceptible to bacterial contamination and the corresponding loss in productivity (Brethauer and Wyman, 2010). Distillation is the final stage of industrial ethanol production. During distillation, an ascending vapor stream contacting a counter-current descending liquid stream, ethanol is separated from the fermentation broth.

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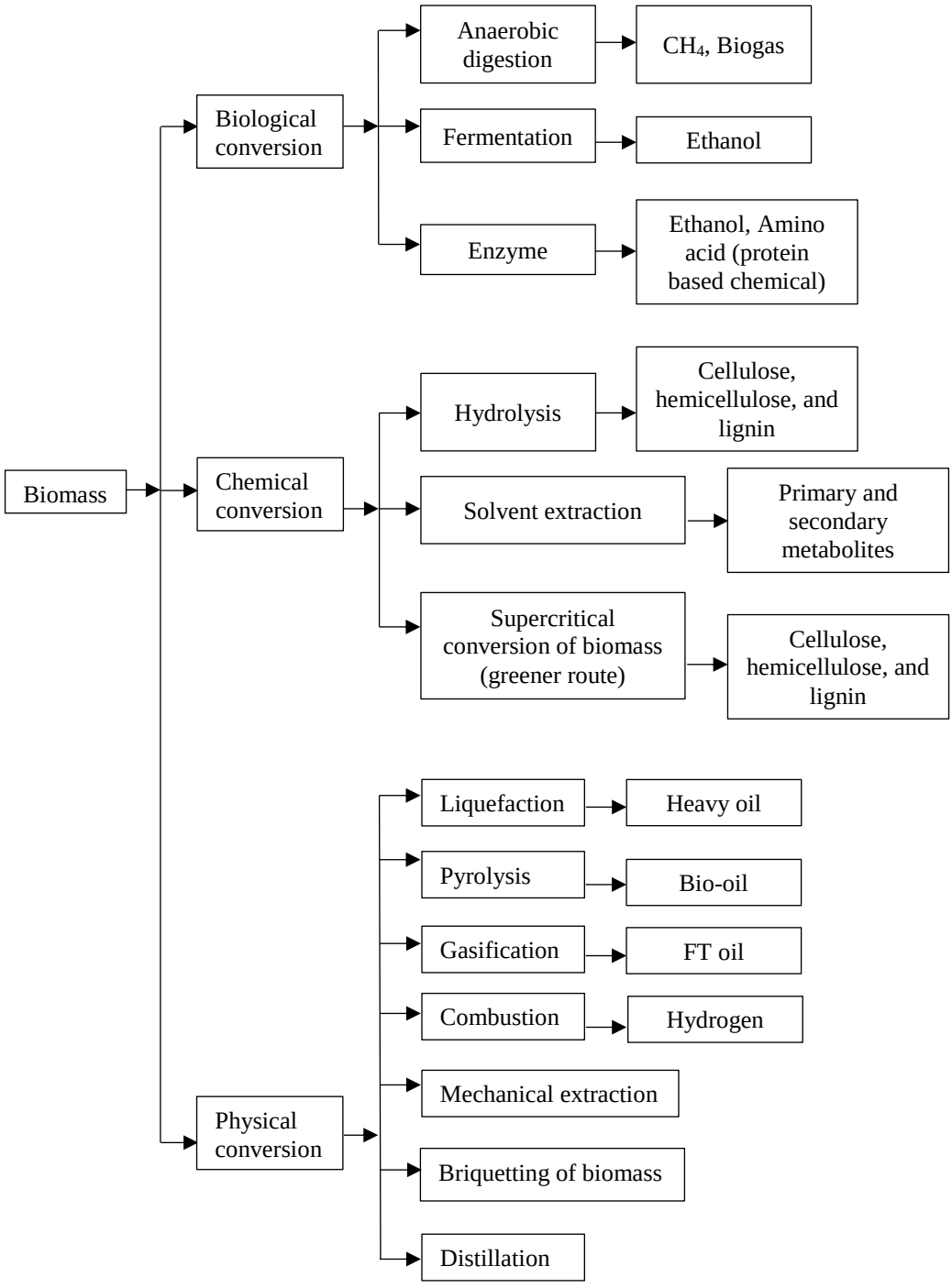

Figure.1.1 Biomass conversion processes (Adapted from Naik, et al., 2010).

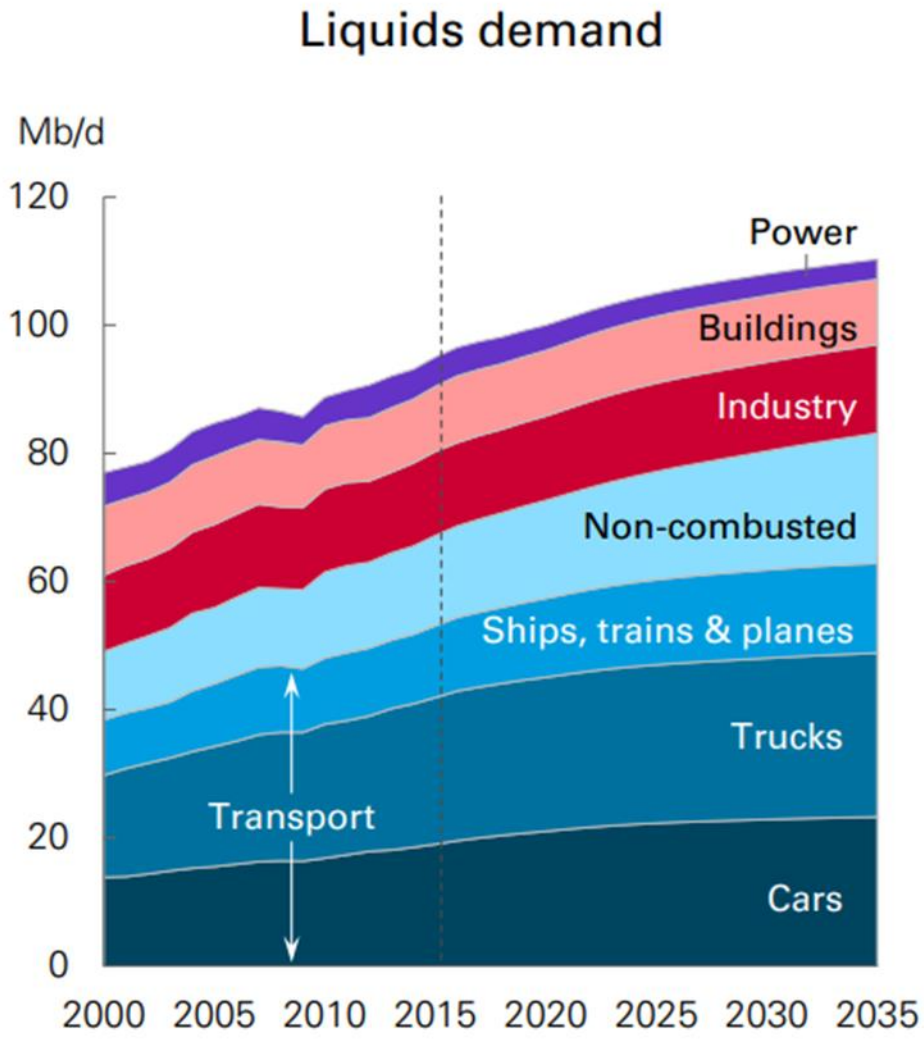

Figure 1.2 BP 2017 Energy Outlook (Simões-Filho, 2017).

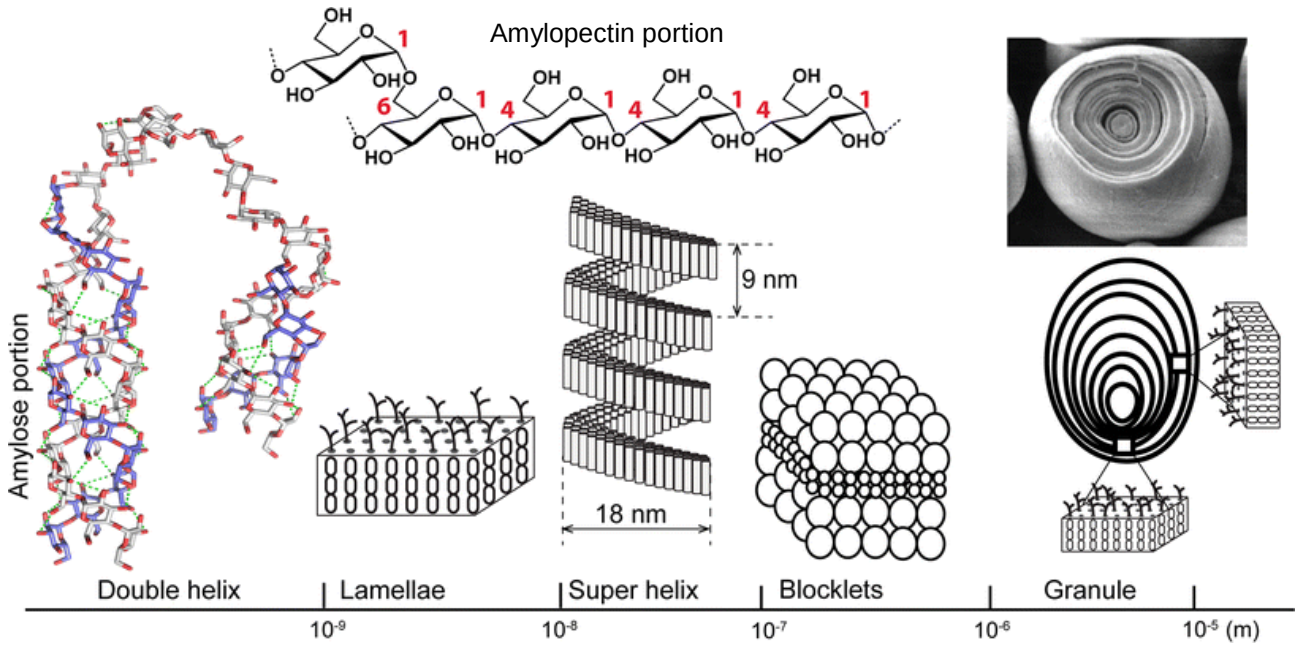

Figure 1.3 Primary structure of amylopectin, and higher order structures of starch on different size-scales (Vu and Marletta, 2016).

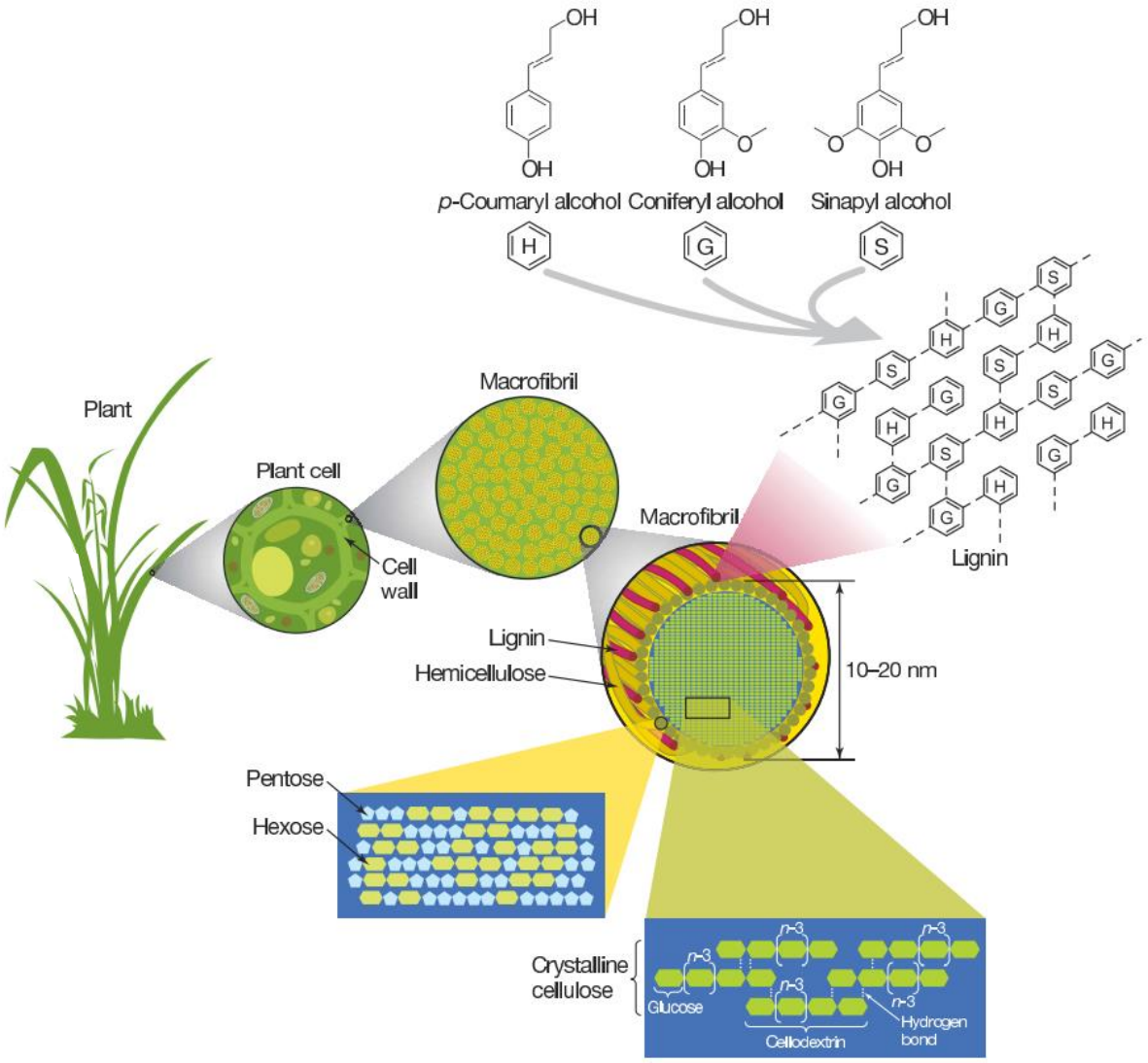

Figure 1.4 Structure of lignocellulose (Rubin, 2008).

Chapter 2 - Evaluating Effects of Deficit Irrigation Strategies on Grain Sorghum Attributes and Biofuel Production [1]

2.1 Abstract

With reduced water resources available for agriculture, scientists and engineers have developed innovative technologies and management strategies aimed at increasing efficient use of irrigation water. The objective of this research was to study the impact of deficit irrigation strategies on sorghum grain attributes and bioethanol production. Grain sorghum was planted at Southwest Research-Extension Center near Garden City, KS, under five different irrigation capacities (1 in. every 4, 6, 8,10, or 12 days) and dryland in 2015 and 2016 growing seasons. Results showed that the average kernel weight, kernel diameter and test weight of grain sorghum increased as irrigation capacity increased, whereas kernel hardness index decreased as irrigation capacity increased. Starch and protein contents of sorghum ranged from 69.45 to 72.82 g/100g and 8.22 to 12.50 g/100g, respectively. Starch pasting temperature and peak time decreased as irrigation capacity increased. Irrigation capacity had a positive impact on bioethanol yield, whereas both year and interaction between irrigation capacity and year did not show significant effect on bioethanol yield resulting from above normal rainfall received during the growing seasons.

Keywords: Deficit irrigation; Grain sorghum; Starch content; Bioethanol yield

[1] Results have been published. Pang, B., Zhang, K., Kisekka, I., Bean, S., Zhang, M. and Wang, D., 2018. Evaluating effects of deficit irrigation strategies on grain sorghum attributes and biofuel production. Journal of Cereal Science 79, 13-20

2.2. Introduction

In the United States, ~80% of the nation's consumptive water use is used for agriculture and more than 90% of the nation's water in many semi-arid and arid areas (USDA-ERS, 2016). Irrigation is an essential technology as it supplements inadequate rainfall to enhance crop yield. However, the availability of water for irrigation has been decreased due to the depletion of the Ogallala Aquifer (McGuire, 2012) in areas such as the southern High Plains. With reduced water resources for agriculture, scientists and engineers have developed innovative technologies and management strategies aimed at increasing the efficient use of irrigation water including deficit irrigation strategies.

Researchers have studied the effects of limited or deficit irrigation on crop yield. Van Donk et al. (2010) studied yield response of maize to deficit irrigation in west-central Nebraska. Their research showed that it takes 65–100 mm of water for an extra yield of 1.6 Mg ha^{-1} of maize. Irmak et al. (2016) evaluated the effects of deficit irrigation on maize production and developed crop yield response factors for field maize. Wheat yield, biomass, and water productivity response to deficit irrigation was studied in western KS (Berhe et al., 2017). El-Hendawy et al. (2017) also studied the effects of full and limited irrigation on wheat growth as well. Zhang et al. (2016) reported rice production improved 4–8% and reduced water consumption 20.5% using regulated deficit irrigation and fuzzy control in Heilongjiang province, China. Chai et al. (2016) reviewed the influence of regulated deficit irrigation on crop production under drought stress in terms of growth stage-based deficit irrigation, partial root-zone irrigation and subsurface dripper irrigation.

Grain sorghum response to water and deficit irrigation management has been studied extensively in Kansas by several investigators (Araya et al., 2016; Kisekka et al., 2016; Klocke

et al., 2012; Stone and Schlegel, 2006). These studies in Kansas show that grain sorghum is a good crop under water limited scenarios and has potential to reduce income risk compared to maize over time. In addition to crop yield, deficit irrigation can also significantly impact crop quality and other non-food application, such as bioethanol production.

In the United States, 200 operating ethanol biorefineries in 28 states produced a record 15.25 billion gallons of bioethanol in 2016, along with 42 million metric tons of high-protein animal feed as by-products (RFA, 2016). The majority of bioethanol was produced from maize with only ~4% produced from grain sorghum. While an overall minor component of total bioethanol production, the portion of bioethanol made from sorghum represents ~45% of the grain sorghum produced in the United States, primarily in plants located in the High Plains regions (RFA, 2016). With the increase in bioethanol production, maize has become overused as a renewable source, which may impact the amount of maize used for human food and directly as animal feed consumption. If all of the maize in the United States was converted into bioethanol, it would only meet 25% of that needed to replace gasoline (Conca, 2014).

Grain sorghum has good potential as a bioethanol crop due to its fit as a more cost effective crop for semiarid regions in the United States (Yan et al., 2011). In 2015, grain sorghum production increased by 38% compared with 2014, while maize production decreased by 4% (USDA-NASS, 2016). This shift in production demonstrates there is a possibility for grain sorghum to be incorporated at greater rates in bioethanol production and to move towards less dependence on maize alone.

Previous research has been carried out to evaluate grain sorghum for bioethanol production. Wu et al. (2007) reported that high starch content and low viscosity during liquefaction were favorable characteristics for the conversion of grain sorghum to bioethanol,

whereas tannin content and low protein digestibility had negative impacts. Yan et al. (2011) evaluated the fermentation performance of waxy grain sorghum for ethanol production and reported that the advantages of using waxy sorghums for ethanol production include easier gelatinization and low viscosity during liquefaction, higher starch, and protein digestibility, higher free amino nitrogen (FAN) content, and shorter fermentation times.

Our previous research reported effect of irrigation levels on sorghum physical and chemical properties and ethanol yield (Liu et al., 2013). In this study, we focus on the impact of deficit irrigation strategies (detailed in Irrigation Management) on sorghum grain attributes and bioethanol production. Knowledge transferred from the current study fulfills the literature gap between deficit irrigation research and grain quality and grain end-use quality, especially using sorghum grain as feedstock for bioethanol production; and, therefore, provides insight into improving water utilization in terms of bioenergy production.

2.3 Materials and methods

2.3.1. Field experimental

The experiment was conducted at the Kansas State University Southwest Research-Extension Center Finnup farm near Garden City, KS, with latitude and longitude of 38°01′20.87″N, 100°49″26.95W and elevation of 887 m above mean sea level. The soil at the experimental site is characterized as a deep well drained Ulysses silt loam with organic matter content of 1.5% and pH of 8.1 (Klocke et al., 2011). The climate is semi-arid with mean annual precipitation of 450 mm.

2.3.1.1. Irrigation management

The study was conducted under a lateral move sprinkler irrigation system modified to apply irrigation water in any desired treatment combination. The experimental design was a

randomized complete block design with four replications and six treatments: 1) full irrigation,100% evapotranspiration (ET); 2) 50% ET irrigation prior to booting of grain sorghum, 100% ET after boot and total irrigation limited to 250 mm; 3) 100% ET irrigation (total irrigation limited to 250 mm); 4) 50% ET irrigation prior to booting of grain sorghum, and 100% ET after boot, and total irrigation limited to 150 mm; 5) 100% ET irrigation (total irrigation limited to 150 mm); and 6) dryland.

As a case study, two limitations on total irrigation were compared to full irrigation as described in Kisekka et al. (2016). The limitations were 150 and 250 mm. The fully irrigated treatment was managed as a non-water limiting crop with 100% ET replenishment. Soil water in the 2.4 m soil profile was measured as a check for adequacy of the ET-based irrigation schedule and also for determination of crop water use. Soil water measurements were made using neutron scattering technique (neutron probe). In-season irrigation events were adjusted to account for rainfall amounts received during the growing season. Total irrigation applications in 2015 were 194, 169, 169, 169, 169, and 44 mm for treatments 1 through 6, respectively. Total irrigation applications in 2016 were 244, 194, 244, 169, 194, and 16 mm for treatments 1 through 6 respectively.

2.3.1.2. Agronomic management

The hybrid used was Pioneer 84G62, because it is full season and well adapted under both irrigated and dryland environments. Grain sorghum was planted at a seeding rate of 40,485 seeds/ha on June 4, 2015 and on May 23, 2016. Best management practices for fertilizer and weed control for high yielding grain sorghum were followed. For example, at planting 10:34:0 fertilizer was applied at a rate of 15 l/ha and at least 179 kg N/ha was applied. Some of the

herbicides used for weed control included atrazine 4 L at rate of 383 mL/ha and Lumax EZ at a rate of 958 mL/ha. Grain sorghum was harvested on October 20, 2015, and October 13, 2016.

2.3.2. Sample preparation and grinding

Sorghum was cleaned using a Gamet sieve shaker (Dean Gamet Manufacturing, Minneapolis, MN) with a 6.35 mm screen to remove broken kernel and small foreign material. Large broken kernels and foreign materials were manually pick removed. An UDY sample cyclone mill (UDY Corporation, Fort Collins, CO) equipped with a 0.5 mm screen was used to grind clean samples into flour. Afterward, ground sorghum was sealed in plastic bags and stored in a sealed plastic box at a laboratory with stable environmental conditions of 25 °C and 30% humidity.

2.3.3. Physical properties of sorghum

Sorghum 1000 kernel weight, single kernel diameter, and hardness were analyzed using a SKCS 4100 (Perten Instruments, Huddinge, Sweden) as previously reported (Bean et al., 2006). Test weights of sorghum samples were determined according to the AACC International Method 55–10.01 “Test Weigh per Bushel”. Moisture contents of sorghum samples were determined according to the AACC International 44–15.02 “Moisture Air-Oven Methods”.

2.3.4. Chemical composition of grain sorghum

Total starch contents of grain sorghum samples were determined according to the AACC (Method 76.13.01) using a Megazyme starch assay kits (Megazyme International Limited Company, Ireland). Megazyme Mega-Calc™ software (Megazyme International Limited, Ireland) was used to calculate the total starch content from the absorbance data and the moisture content based on a dry weight basis. Protein, fat, and fiber contents of grain sorghum samples

were determined according to AOAC official methods 990.03–2002, 920.39–1920 and 962.09–2010, respectively.

2.3.5. Thermal property of grain sorghum by differential scanning calorimetry (DSC) analysis

Thermal properties of sorghum samples including onset temperature, peak temperature and gelatinization enthalpy were determined using a differential scanning calorimetry (DSC) (DSC-Q200, TA Instruments Incorporation, New Castle, DE). The method has been described previously by Zhang et al. (2017). ~8 mg sorghum flour was mixed with ~24 μL of distilled water in a stainless steel pan before placing in a 4 °C freezer overnight. The method of analysis included for that sample was isothermal at 25 °C for 2 min and then heated to 180 °C at the speed of 10 °C min^{-1}.

2.3.6. Pasting properties of grain sorghum using rapid viscosity analysis (RVA)

Pasting properties of sorghum samples including pasting temperature, peak time, peak viscosity, breakdown, final viscosity, and setback, were determined using a rapid viscosity analyzer (RVA) (RVA-3c, Newport Scientific Limited Company, Warriewood, Australia). The AACC method (76–21.01) was applied as the analysis method: 3.5 g sorghum sample with 14% moisture content was mixed with ~25 g distilled water in a canister. The slurry was dispersed by stirring at 960 rpm for 10 s and at 160 rpm for the rest analysis. The slurry was held at 50 °C for 1 min prior to heating up to 95 °C. The slurry was held at 95 °C for 2.5 min before decreasing back to 50 °C, where the slurry was held for 2 min.

2.3.7. Bioethanol fermentation

The bioethanol fermentation process including liquefaction and saccharification, fermentation, and distillation were as described previously (Zhang et al., 2017). Briefly, 30 g of

dry sorghum flour mixed with 100 mL distilled water plus fermentation media in 250-mL Erlenmeyer flask. Next, 20 μL Liquozyme SC DC (240 KNU/g, ~1.26 g/mL, Novozymes, New York, NY) and 100 μL Spirizyme Achieve (750 AGU/g, ~1.15 g/mL, Novozymes, New York, NY) were added for starch hydrolysis and saccharification, respectively. One mL activated yeast pre-culture (Red Star Ethanol Red, Lasaffre, France) was added after adjusting the pH of slurry to 4.2–4.3 with 2N HCl. The fermentation was conducted at 30 °C in an incubator shaker (Model I2400, New Brunswick Scientific, Edison, NJ) operating at 150 rpm for 72 h. After the fermentation was completed, the finished beer was entirely transferred to a 500-mL distillation flask. Each Erlenmeyer flask was washed with a total volume of 100 mL distilled water. Distillates were collected into a 100-mL volumetric flask until approaching the 100 mL mark (~99 mL). Bioethanol was determined using a high performance liquid chromatograph (Agilent, Santa Clare, CA) equipped with a Rezex RCM column (Phenomenex, Torrance, CA) and refractive index detector (Zhang et al., 2017). Fermentation efficiency was calculated as equation (2-1):

$$\frac{Actual\ weight\ of\ bioethanol\ yield}{Theoretical\ weight\ of\ bioethanol\ yield\ from\ grain} \times 100\% \quad (2\text{-}1)$$

2.3.8. Scanning electron microscope (SEM) images

A scanning electron microscope (SEM) with an accelerating voltage of 5.0 kV (Hitachi S-3500N, Hitachi Science Systems, Limitation Company, Tokyo, Japan) was used to determine the morphological structure of sorghum grain. In preparation, some sorghum grain was cracked with a hammer to obtain small and flat fragments. A Desk II combined sputter coater covered the samples with a mixture of 60% gold and 40% palladium under vacuum conditions (Denton Vacuum, Moorestown, NJ).

2.3.9. Statistical analysis

Statistical analysis was conducted using the PROC GLIMMIX procedure in SAS (SAS Institute, Cary, NC). A two-way ANOVA with fixed factorial irrigation level and year was employed for analyze the difference of physicochemical attributes and bioethanol production. Correlation analysis was implemented using Pearson's correlation. All statistical analysis were conducted at a 5% level of significance.

2.4 Results and discussion

2.4.1. Effect of deficit irrigation on physicochemical properties of grain sorghum

Table 2.1 summarizes physiochemical properties of sorghum under six deficit irrigation management strategies in 2015 and 2016. The mean and range across irrigation capacities and years were 25.56 g and 21.96–29.67 g for 1000 kernel weight, 2.4 mm and 2.19–2.62 mm for kernel diameter, 73.09 and 66.29–78.74 for kernel hardness index, and 79.35 kg/hl and 78.84–80.18 kg/hl for test weight. In Table 2.2, statistical analysis shows that not only deficit irrigation had a significant effect on 1000 kernel weight, kernel diameter, kernel hardness index, and test weight of sorghum, but also year showed a significant effect on 1000 kernel weight and kernel diameter, whereas the interaction between deficit irrigation and year was not a significant factor. As shown in Table 2.1, both 1000 kernel weight and kernel diameter of sorghum samples increased as the level of deficit irrigation decreased. These results were consistent with previous research on wheat where drought decreased both kernel weight and kernel diameter of winter wheat kernel (Weightman et al., 2008). Weightman et al. (2008) also reported that wheat grown under low irrigation capacity produced a higher kernel hardness index than wheat under high irrigation capacity, which was similar to the results in the current study. Sorghum grown under low levels of deficit irrigation produced higher test weight than sorghum grown under high levels

of deficit irrigation. The highest test weight of 80.18 kg/hl was achieved under full irrigation capacity or 100% ET in 2016. Test weight of grain is an important indication of soundness and flour yield. Moreover, positive relationships were observed between 1000 kernel weight and kernel diameter ($R = 0.96$ and $P < 0.01$), 1000 kernel weight and test weight ($R = 0.78$ and $P < 0.01$), kernel diameter and test weight ($R = 0.89$ and $P < 0.01$) as shown in Table 2.3. However, kernel hardness negatively correlated with 1000 kernel weight, kernel diameter and test weight ($P < 0.01$). Therefore, sorghum produced under full irrigation may be better feedstock for milling. All the sorghum test weight would fall in a "high test weight" category based on the criterion described by Paulsen and Hill (1985).

For all sorghum samples across the six irrigation treatments in 2015 and 2016, starch content of sorghum samples ranged from 69.45 to 72.81 g/100g with a mean value of 71.53 g/100g; protein content ranged from 8.22 to 12.50 g/100g with a mean value of 9.78 g/100g; fat content ranged from 2.61 to 3.06 g/100g with a mean value of 2.82 g/100g; and fiber content ranged from 0.83 to 1.63 g/100g with a mean value of 1.11 g/100g. Sorghum with full irrigation produced ~3 g/100g more starch than sorghum under high deficit irrigation. The highest starch content was achieved in 2015 under 100% ET irrigation treatment. An increasing trend of starch content was observed as level of irrigation capacity increased (Table 2.1). A significant effect of irrigation was observed for all the chemical components of sorghum. Nevertheless, year and interaction between deficit irrigation and year only had significant effects on starch and protein content, which was significantly higher in 2016 than 2015 (Table 2.2). A possible explanation is that a less rainfall received during the 2016 compared with 2015 growing season, which neutralized the influence of year and interaction between year and irrigation level. The Pearson correlation shown in Table 2.3 revealed that starch content positively correlated to 1000 kernel

weight, kernel diameter and test weight ($P < 0.01$), but negatively correlated to kernel hardness index ($R = -0.79$, $P < 0.01$) and protein content ($R = -0.94$, $P < 0.01$). The highest protein content of 12.46g/100g was found in 2016 for samples under the treatment that received least amount of irrigation. Low irrigation yielding high protein content of grain supports the observations of previous researchers (Daniel and Triboi, 2002; Weightman et al., 2008). A possible explanation is that low irrigation is favor for nitrogen translocating to grain from other parts of crops for adapting adverse growing condition. For this reason, sorghum is a cereal to provide highly efficient use of water and superior drought tolerance, it will become an increasingly important bioenergy crop to varied agro-climatic conditions.

2.4.2. Effect of deficit irrigation on pasting properties and thermal properties of grain sorghum

The rheological characteristics of starch in sorghum samples were analyzed using RVA to evaluate the effect of deficit irrigation on pasting properties of sorghum. As shown in Table 2.1, for all of the sorghum samples, the mean and range across deficit irrigation levels and years were 74.37 °C and 71.95 to 76.89 °C for pasting temperature (the temperature starch granule start swelling and gelatinization), 5.32 min and 5.12 to 5.61 min for peak time, 2781 cP and 2157 to 3249 cP for peak viscosity (the maximum viscosity during heating and starch gelatinization), 1713 cP and 1612 to 1836 cP for holding strength (an indicator of the water holding capacity of starch), 1064 cP and 501 to 1442 cP for breakdown (the starch granule rupturing and releasing amylose), 4613 cP and 4461 to 4834 cP for final viscosity, 2895 cP and 2745 to 3146 cP for setback. In Table 2.4, a Pearson correlation shows that pasting temperature positively correlated to peak time and setback but negatively correlated to peak viscosity, holding strength, and breakdown ($P < 0.05$). These results are in good agreement with previous studies (Liu et al.,

2013; Wu et al., 2008). A significant effect of deficit irrigation was observed for all RVA parameters, whereas either year or interaction between deficit irrigation and year did not show significant influence (Table 2.2). For this reason, RVA curves of sorghum samples with different deficit irrigation in 2015 was selected and demonstrated in Fig. 2.1a. Sorghum under high irrigation had lower pasting temperature and peak time, higher peak viscosity, holding strength, and breakdown than that under low irrigation sorghum. The lower pasting temperature and peak time was related to the fact that the starch granules were more susceptible to swelling and gelatinizing (Saunders et al., 2011). Higher peak viscosity, holding strength, and breakdown associated with the increased accessibility of starch to enzymes, thereby enhancing the liquefaction process during bioethanol production (Wu et al., 2007). The low level of deficit irrigation being favorable for sorghum targeted to bioethanol production was confirmed by the Pearson correlation coefficient between pasting properties and bioethanol yield (Table 2.4). Bioethanol yield significantly correlated to pasting temperature ($R = -0.98$ $P < 0.01$), peak time ($R = -0.97$ $P < 0.01$), peak viscosity ($R = 0.98$ $P < 0.01$), and breakdown ($R = 0.97$ $P < 0.01$), respectively.

The DSC curves of sorghum under different deficit irrigation levels planted in 2015 are shown in Fig. 2.1b. Despite that no clear trend was observed between years, deficit irrigation showed a significant influence on onset temperature (the temperature starch start gelatinization) and peak temperature (the temperature starch completely gelatinization). Sorghum grown under full irrigation had lower onset and peak temperature than sorghum grown under high levels of deficit irrigation. Low onset and peak temperature had a positive impact on bioethanol production because as less energy is needed to initiate the starch gelatinization (Barichello et al., 1990). In addition to the main starch gelatinization peak located at 75–78 °C, there was a second

peak around 102–104 °C except under full irrigation capacity. Amylose-lipid complexes were likely responsible for this peak, which are an unfavorable factor during bioethanol production as these complexes reduced the access of enzymes to starch. Similar conclusions were drawn for sorghum. Similar conclusions were drawn from previous studies where sorghum grain produced under high irrigation produced high bioethanol yield (Liu et al., 2013; Wu et al., 2008).

2.4.3. Effect of deficit irrigation on fermentation efficiency and bioethanol yield of grain sorghum

The fermentation efficiency and bioethanol yield of all sorghum samples across different deficit irrigation levels and year ranged from 88.98 to 91.91% and 44.94–47.99 mL/100g dry sorghum grain (Table 2.1). A significant effect of irrigation capacity was observed for bioethanol yield of sorghum (Table 2.2). Bioethanol yield of sorghum increased as deficit irrigation level with the highest bioethanol yield achieved in 2015 under full irrigation. Bioethanol yield of sorghum increased as irrigation level increased. Sorghum grown under full irrigation yielded 3 mL bioethanol per 100 g feedstock higher than sorghum grown under deficit irrigation using current approaches. While there was no significant correlation between final fermentation efficiency and deficit irrigation level, Fig. 2.1c demonstrates a clear trend that sorghum grown under low irrigation had a higher fermentation efficiency in the first 48 h of fermentation than sorghum grow under full irrigation. This is probably due to the low irrigation sorghum has higher free amino acid than the sorghum with high level irrigation. This result is in agreement with previous finding by Liu et al (2013). This result was confirmed by Person correlation analysis between grain physiochemical traits/pasting properties and fermentation efficiency. Starch and protein content of sorghum and peak time and final viscosity of starch pasting did not have a significant relationship ($P > 0.05$) to fermentation efficiency (Tables 2.3 and 2.4). However, the

Person correlation analysis showed that bioethanol yield had significant correlation with all physiochemical and pasting properties. Fig. 2.3 shows the relationship between starch content of sorghum and bioethanol yield and fermentation efficiency. Although starch content is the most import predictor in bioethanol yield ($R^2 = 0.71$), it did not correlate to fermentation efficiency. Sorghum grown under high full irrigation produced greater starch and bioethanol than sorghum grown under deficit irrigation. Table 2.2 shows that both year and interaction between deficit irrigation level and year did not have significant effects on fermentation efficiency and bioethanol yield of sorghum. This is probably due to above normal rainfall received during the growing seasons.

2.4.4. Effect deficit irrigation on morphological properties of grain sorghum

Scanning electron microscope images of sorghum samples under different deficit irrigation levels is shown in Fig. 2.2. Sorghum produced under full irrigation (Fig. 2.2 a) had larger starch granule size than the sorghum produced under moderate (Fig. 2.2 b–e) and high deficit irrigation levels (Fig. 2.2 f). A possible explanation for this result is that activities of glucose pyrophosphorylase and soluble starch synthase were reduced to a low level under water stress conditions leading to smaller starch granule size and reduced amylose, amylopectin contents in dry matter (Dai et al., 2016). These results supported the fact that sorghum under full irrigation had higher starch content and bioethanol yield than sorghum under high level of deficit irrigation. In addition, the sorghum grown under full irrigation capacity had more protein bodies surrounding the starch granule than those grown under deficit irrigation. Wu et al. (2008) reported the similar findings in that sorghum from irrigated land had various degrees of association with protein bodies compared with sorghum from dry land. A greater number of protein bodies surrounding starch granules may result in a stronger protein matrix, or at least

reduced access of enzymes to starch, which has negative effect on fermentation efficiency. This may be another reason why the sorghum grown under full irrigation has a lower fermentation efficiency than that those grown under deficit irrigation in the first 48 h of fermentation.

Another factor affecting fermentation efficacy is field-sprouted grain. Yan et al. (2009) reported that more holes were found on the surface of starch granules from field-sprouted grain, which also had higher fermentation efficacy than non-sprouted samples. There were some pinholes seen on the surface of starch granules from sorghum grown under full irrigation (Fig. 2.2 a). A possible reason for the pinholes is that pre-germination in the field occurred. Amylases developing during the pre-germination attacked the starch granules. Sorghum grown under full irrigation showed weaker cell wall than that these grown under deficit irrigation, indicating that grain sorghum under full irrigation may be a better feedstock for starch yield during wet milling (Perez-Carrillo and Serna-Saldivar, 2006).

2.5. Conclusions

Grain sorghum with good adaption for production under both irrigated and dryland environments was planted under six different deficit irrigation management in 2015 and 2016. The starch content of all sorghum samples ranged from 69.45 to 72.82 g/100g, protein content ranged from 8.22 to 12.50 g/100g, bioethanol yield ranged from 44.94 to 47.99 mL/100g. The level of deficit irrigation had a significant effect on all physiochemical properties, pasting properties, and bioethanol yield. Starch content and bioethanol yield increased as irrigation level increased across two growing seasons. However, no significant effect of year and interaction between deficit irrigation and year on various properties and bioethanol yield was overserved except starch and protein contents, which was significantly higher in 2015 than that in 2016.

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Table 2.1 Physiochemical and pasting properties, fermentation efficiency, and bioethanol yield of grain sorghum samples(based on 100g dry grain).

	Irrigation treatments [1]											
	2015						2016					
	1	2	3	4	5	6	1	2	3	4	5	6
1000 kernel weight (g)	29.61a[2]	27.24b	26.04cd	25.52cde	24.57ef	23.47g	27.35b	26.46bc	25.49cde	25.00de	23.92fg	22.10h
Kernel diameter (mm)	2.60a	2.49b	2.45bc	2.36d	2.33de	2.26f	2.58a	2.49b	2.44c	2.34d	2.29ef	2.20g
Kernel hardness	69.62g	70.91fg	72.11ef	73.46cd	74.73bc	78.00a	66.98h	70.56g	72.85de	73.98bcd	75.27b	78.60a
Test weight (kg/hl)	79.63c	79.54c	79.32de	79.21e	79.00f	78.86g	80.18a	79.86b	79.41d	79.25e	79.04f	78.87g
Starch (g/100g)	72.81a	72.78a	72.74a	72.27c	71.63d	70.62e	72.43b	72.29c	71.58d	70.33f	69.51g	69.48g
Protein (g/100g)	8.22e	8.34e	8.49e	8.67e	9.22d	10.27b	9.55d	9.80d	10.14c	10.91b	11.42a	12.46a
Fat (g/100g)	2.93abc	2.92abc	2.98ab	2.94ab	2.81cd	3.03a	2.72de	2.71de	2.63e	2.64de	2.73	2.90bc
Fiber (g/100g)	1.42b	1.03d	1.19c	1.06cd	1.05d	1.03	0.98de	1.02d	1.02d	1.59a	1.03d	0.87e
Pasting temperature (°C)	72.04g2	73.18f	73.81e	74.88c	75.29c	76.22a	72.66g	73.13f	73.94e	74.82d	75.71b	76.83a
Peak time (min)	5.13e	5.17de	5.24d	5.35bc	5.41b	5.56a	5.15de	5.23d	5.26cd	5.37b	5.43b	5.60a
Peak viscosity (cP)	3245a	3089b	3082b	2636d	2608d	2275f	3210a	3094b	2912c	2630d	2425e	2171g
Holding strength (cP)	1831a	1717cd	1718cd	1731cd	1765b	1618f	1755b	1689de	1663ef	1740bc	1693de	1645ef
Breakdown (cP)	1433a	1415a	1359a	963c	845d	653e	1419a	1404a	1189b	850d	718e	531f
Final viscosity (cP)	4603cd	4520e	4566cd	4814a	4636c	4549d	4528de	4533de	4720b	4568cd	4828a	4497e

Setback (cP)	2792de	2816de	2848d	3101ab	2883cd	2946c	2753e	2853d	3028b	2800de	3110a	2814de
Fermentation efficiency (%)	89.79b	89.67b	89.07b	89.29	90.04ab	91.88a	89.79b	91.15a	90.55a	90.51a	90.03a	91.47a
Bioethanol yield (ml/100g)	47.96a	47.69b	47.02c	46.30bc	45.89d	45.06d	47.97a	47.63b	46.85bc	46.14c	45.61d	44.96d

[1] 1) Full irrigation 100% evapotranspiration (ET), 2) 50% ET prior to booting of grain sorghum and 100% ET after boot and total irrigation limited to 250 mm, 3) 100% ET limited to 250 mm, 4) 50% ET prior to booting of grain sorghum and 100% ET after boot and total irrigation limited to 150 mm, 5) 100% ET limited to 150 mm, and 6) Dryland.

[2] Different letters indicate that the means in the same row are statistically significant at 5% level.

Table 2.2 Effects of deficit irrigation management, year and their interaction on physiochemical properties, pasting properties, fermentation efficiency, and bioethanol yield of sorghum samples.

Chemical composition/ physical properties	Irrigation treatment	Year	Irrigation × year
	P > *F*		
1000 kernel weight	< 0.05	< 0.05	0.58
Kernel diameter	< 0.05	< 0.05	0.15
Kernel hardness	< 0.05	0.57	0.75
Test Weight	< 0.05	0.66	0.77
Starch	< 0.05	< 0.05	< 0.05
Protein	< 0.05	< 0.05	< 0.05
Fat	< 0.05	0.31	0.45
Fiber	< 0.05	0.07	0.09
Pasting temperature	< 0.05	0.49	0.89
Peak time	< 0.05	0.70	0.62
Peak viscosity	< 0.05	0.58	0.21
Holding strength	< 0.05	0.11	0.56
Breakdown	< 0.05	0.69	0.34
Final viscosity	< 0.05	0.78	0.65
Setback	< 0.05	0.65	0.51
Fermentation efficiency	< 0.05	0.23	0.61
Ethanol yield	< 0.05	0.12	0.51

Table 2.3 Pearson correlation coefficient between Physiochemical properties, fermentation efficiency, and ethanol yield of sorghum samples.

	1000 kernel weight	Kernel diameter	Kernel hardness	Test weight	Starch	Protein	Fat	Fiber	Fermentation efficiency	Bioethanol yield
1000 kernel weight	1	0. 956**	-0. 882**	0. 777**	0.833**	-0.774**	-0.007	0.392	-0.556**	0.931**
Kernel diameter	0.956**	1	-0.955**	0.894**	0.839**	-0.709**	-0.132	0.249	-0.503*	0.977**
Kernel hardness	-0.882**	-0.955**	1	-0.949**	-0.787**	0.642**	0.245	-0.200	0.552**	-0.962**
Test weight	0.777**	0.894**	-0.949**	1	0.671**	-0.447*	-0.333	0.076	-0.314	0.916**
Starch	0.833**	0.839**	-0.788**	0.671**	1	-0.935**	0.220	0.111	-0.586**	0.845**
Protein	-0.774**	-0.709**	0.642**	-0.447*	-0.935**	1	-0.363	-0.222	0.664**	-0.692**
Fat	-0.007	-0.132	0.245	-0.333	0.220	-0.363	1	-0.143	-0.084	-0.161
Fiber	0.392	0.249	-0.200	0.076	0.111	-0.222	-0.143	1	-0.242	0.207
Fermentation efficiency	-0.556**	-0.503*	0.552**	-0.314	-0.586**	0.664**	-0.084	-0.242	1	-0.509*
Bioethanol yield	0.931**	0.977**	-0.962**	0.916**	0.845**	-0.692**	-0.161	0.207	-0.509*	1

* Correlation is significant at the 0.05 level (2-tailed).

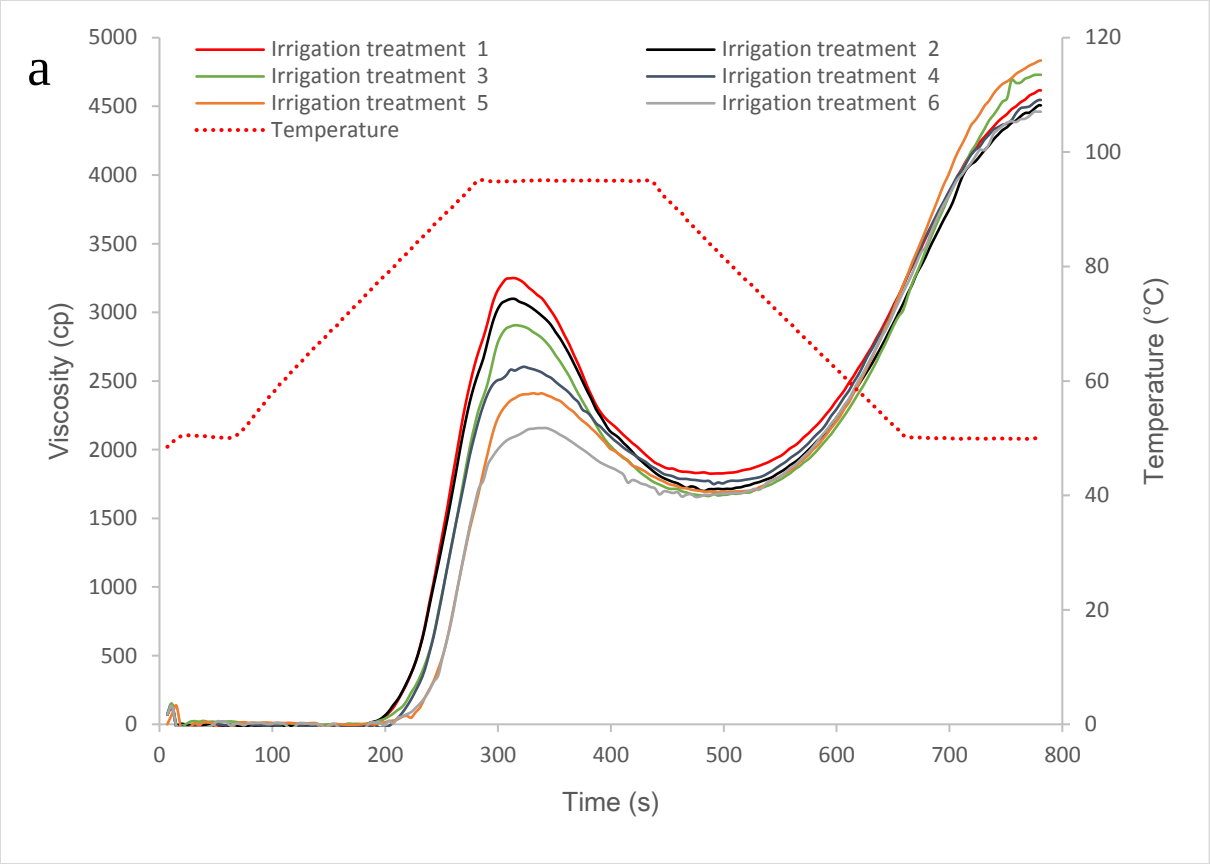
** Correlation is significant at the 0.01 level (2-tailed).

Table 2.4 Pearson correlation coefficient between pasting properties, fermentation efficiency, and bioethanol yield of sorghum samples.

	Pasting temperature	Peak time	Peak viscosity	Holding strength	Breakdown	Final viscosity	Setback	Fermentation efficiency	Bioethanol yield
Pasting temperature	1	0.97**	-0.98**	-0.62**	-0.96**	0.18	0.41*	0.49*	-0.98**
Peak time	0.97**	1	-0.97**	-0.63**	-0.95**	0.08	0.32	0.61**	-0.97**
Peak viscosity	-0.98**	-0.97**	1	0.58**	0.98**	-0.19	-0.41*	-0.55**	0.98**
Holding strength	-0.62**	-0.63**	0.58**	1	0.50*	0.05	-0.34	-0.66**	0.58**
Breakdown	-0.96**	-0.95**	0.98**	0.50*	1	-0.21	-0.37	-0.51**	0.97**
Final viscosity	0.18	0.08	-0.19	0.05	-0.21	1	0.88**	-0.35	-0.20
Setback	0.41*	0.32	-0.41*	-0.34	-0.37	0.88**	1	-0.05	-0.42*
Fermentation efficiency	0.49*	0.61**	-0.55**	-0.66**	-0.51**	-0.35	-0.05	1	-0.50*
Bioethanol yield	-0.98**	-0.97**	0.98**	0.58**	0.97**	-0.20	-0.42*	-0.50*	1

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).


a
Irrigation treatment 1
Irrigation treatment 2
Irrigation treatment 3
Irrigation treatment 4
Irrigation treatment 5
Irrigation treatment 6
Temperature
Viscosity (cp)
Temperature (°C)
Time (s)
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
120
100
80
60
40
20
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100
200
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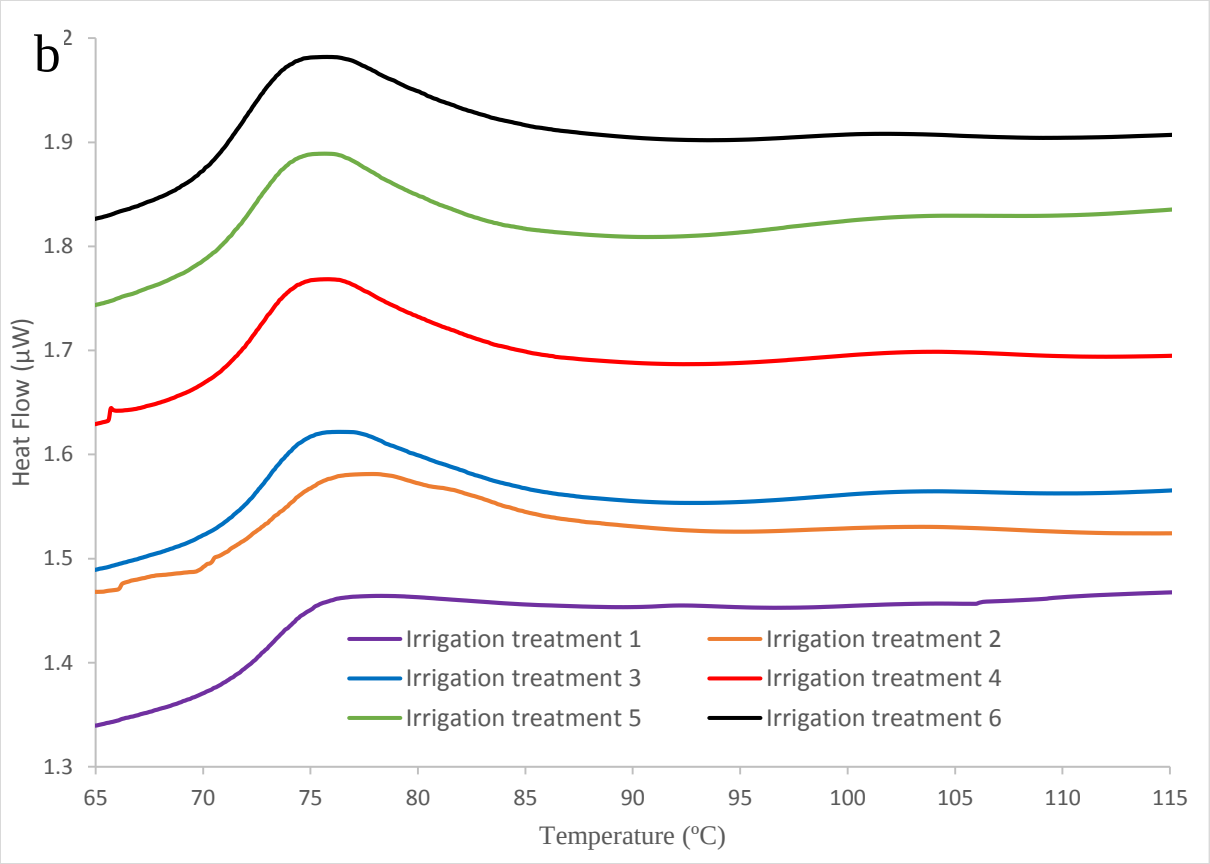
600
700
800


b2
1.9
1.8
1.7
1.6
1.5
1.4
1.3
Heat Flow (µW)
65
70
75
80
85
90
95
100
105
110
115
Temperature (°C)
Irrigation treatment 1
Irrigation treatment 2
Irrigation treatment 3
Irrigation treatment 4
Irrigation treatment 5
Irrigation treatment 6

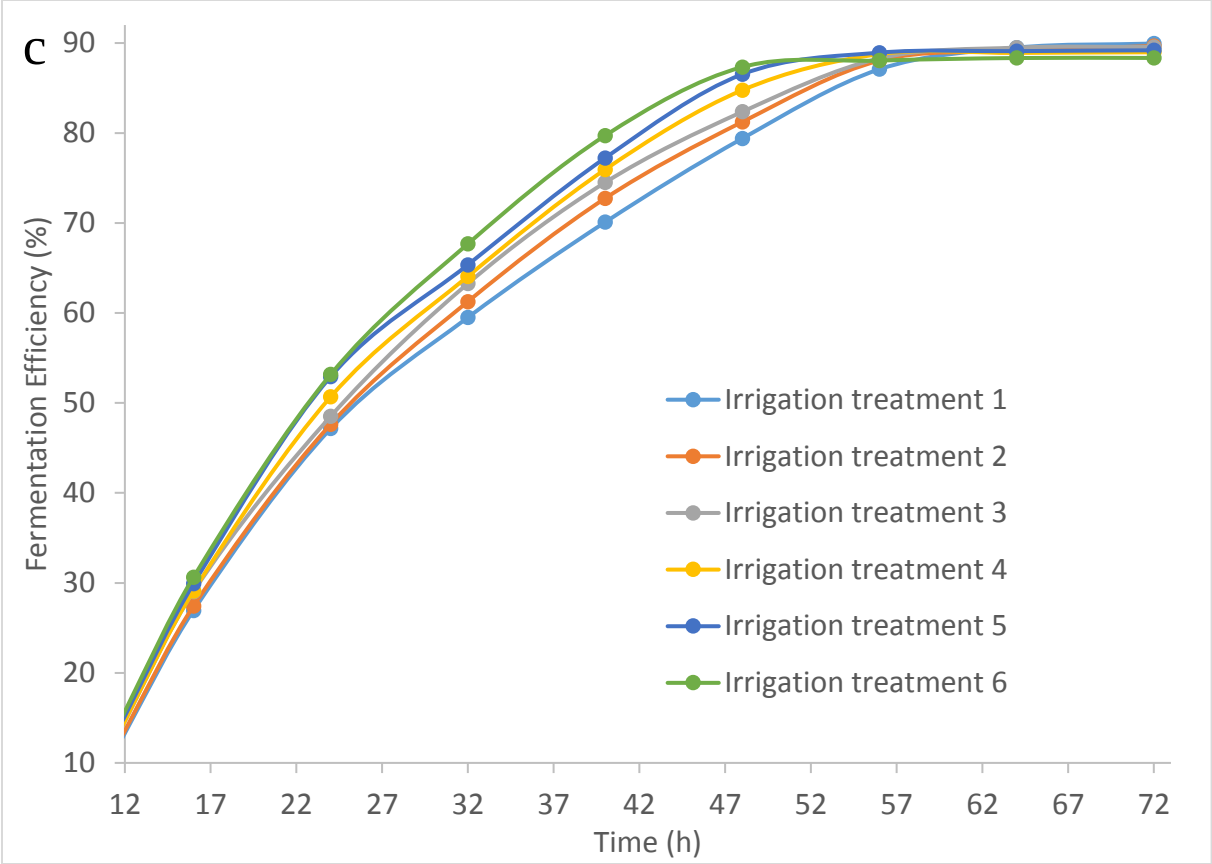


Figure 2.1 (a) RVA, (b) DSC and fermentation (c) efficiency curves of grain sorghum samples grow under different deficit irrigation management in 2015. Irrigation treatments: 1) Full irrigation 100% evapotranspiration (ET), 2) 50% ET prior to booting of grain sorghum and 100% ET after boot and total, irrigation limited to 250 mm, 3) 100% ET limited to 250 mm, 4) 50% ET prior to booting of grain sorghum and 100% ET after boot and total irrigation limited to 150 mm, 5) 100% ET limited to 150 mm, and 6) dryland.

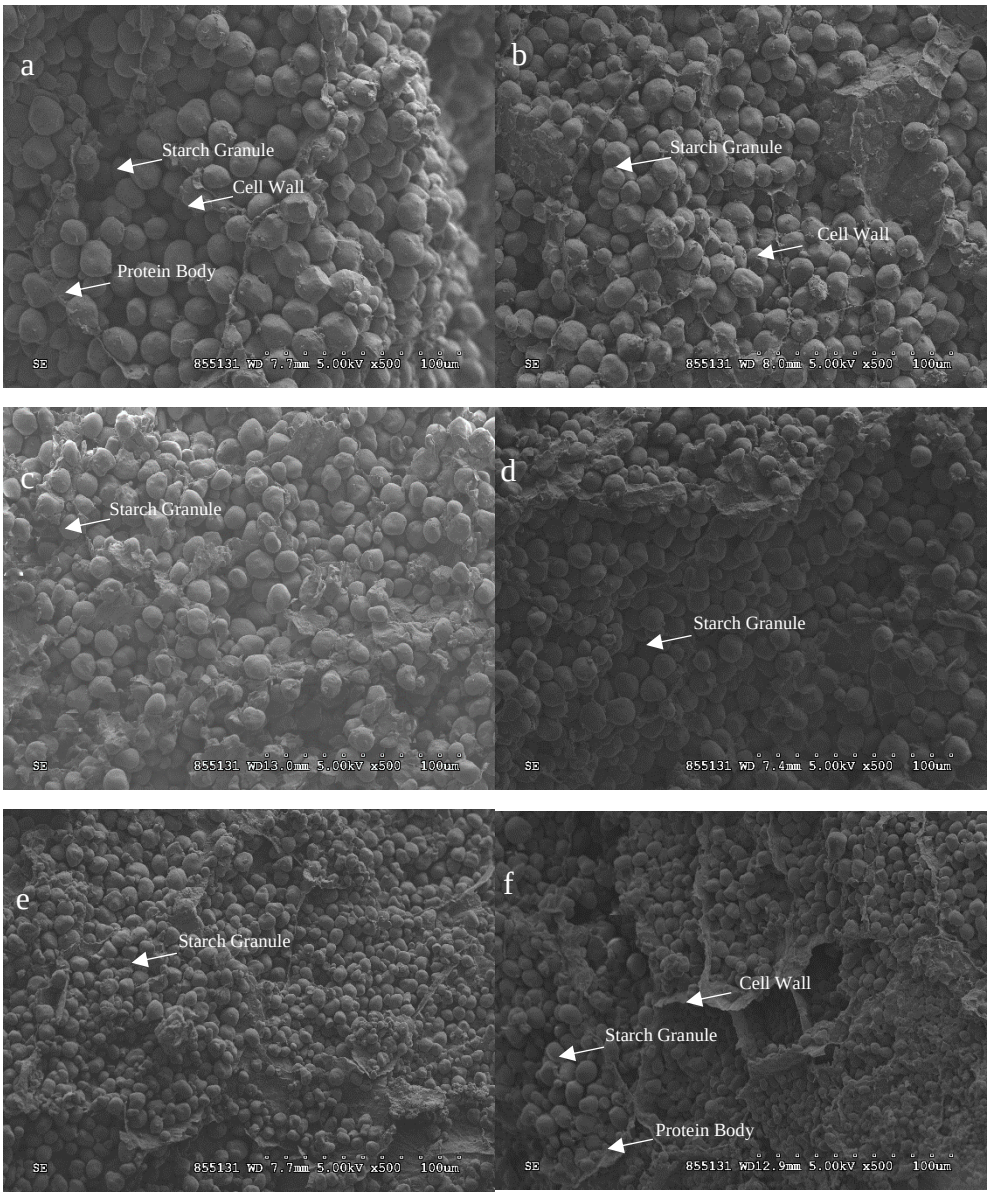


Figure 2.2 Scanning electron microscope (SEM) images of grain sorghum under different irrigation management in 2015: a is grain sorghum sample under full irrigation; b is sorghum sample under treatment 2 (50% evapotranspiration (ET) prior to booting of grain sorghum and 100% ET after boot and total, irrigation limited to 250 mm); c is grain sorghum under treatment 3 (100% ET limited to 250 mm); d is grain sorghum under treatment 4 (50% ET prior to booting of grain sorghum and 100% ET after boot and total irrigation limited to 150 mm); e is grain sorghum sample under treatment 5 (100% ET limited to 150 mm); and f is grain sorghum under treatment 6 (dryland).

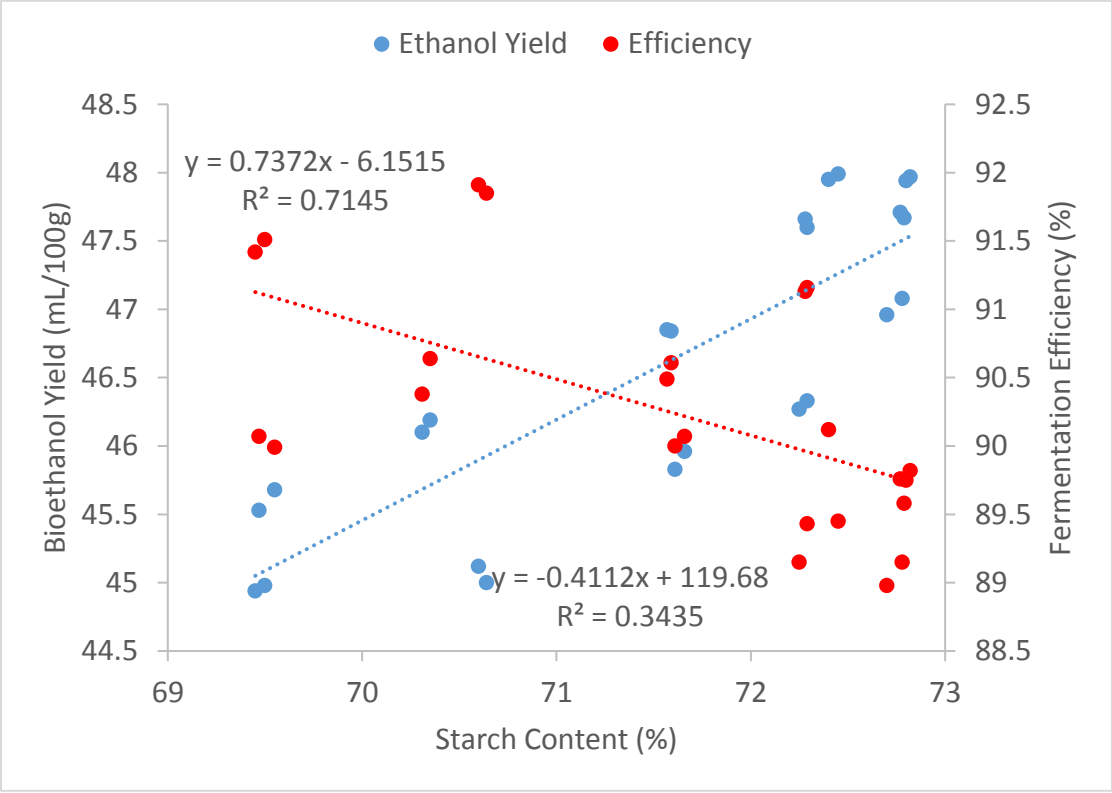


Figure 2.3 Starch content of sorghum versus bioethanol yield and fermentation efficiency.

Chapter 3 - Evaluation of the Chemical Composition and Potential Fermentable Sugars Yield of Pedigreed Sorghum Mutant Biomass

3.1 Abstract

Sorghum, an important dryland crop, is currently grown on approximately 8 million acres in the United States. Due to climate variability and continuous decline of water resources, utilization of dryland to plant sorghum is critically important for sustainable economic consideration. The objective of this research was to evaluate the chemical composition and potential fermentable sugars yield of pedigreed sorghum mutant biomass. In this study, 50 sorghum mutants from the gene-discovery panel (256 lines) were selected and evaluated. The chemical composition of the sorghum biomass were analyzed using NREL standard procedure. Dilute acid pretreatment was applied before enzymatic hydrolysis for evaluation of the fermentable sugars yield. The results showed that sorghum mutants had a significant effect on the biomass composition and final fermentable sugars yield. The contents of the sorghum mutant biomass ranged from 22.82 to 35.71% (glucan), 18.54 to 25.50% (xylan), 1.92 to 3.63% (arabinan), 10.24 to 15.02% (lignin), 0.20 to 5.57% (ash), and 20.69 to 33.94% (extractives), respectively. The mass recovery after acid pretreatment varied from 43.13 to 52.32%, and efficiency of enzymatic hydrolysis ranged from 76.48 to 91.66%. Heat content of the sorghum mutant biomass ranged from 15.44 to 16.91MJ/kg.

3.2 Introduction

As a renewable and clean energy, bioenergy is essential to national security and environment protection due to that it reduces the dependence on fossil-based fuels and decreases greenhouse gas emissions (Balat and Balat, 2009; Muktham et al., 2016; Zhang et al., 2015).

Fluctuation of the energy price especially fossil fuel price significantly affects the world economic development and civil living quality (Foster et al., 2017). Research have shown that biofuel derived from lignocellulosic biomass can substitute 30% of the US petroleum fuel requirement and have a high net energy output with reducing net carbon dioxide release (Farrell et al., 2006; Perlack et al., 2005). Among all of US bioethanol (a main biofuel) manufacturers, corn grain is the most popular raw materials (Dien et al., 2009). However, taking into consideration of the competition with the human food and animal feed, additional feedstocks need to be developed. Lignocellulosic biomass is an ideal candidate due to that it is abundant resources, relatively inexpensive and without competing with food. Besides early studies of grass, wheat strew, and woody biomass, corn stover and sorghum stalk are favorable biomass to produce ethanol due to their high glucan content (Dien et al., 2009; Theerarattananoon et al., 2012)

Sorghum, an important dryland crop, is currently grown on approximately 8 million acres in the United States (USDA, 2016). Sorghum offers high yield efficiency and high tolerance to drought which made it become a favorable feedstock to produce bioenergy (Vermerris et al., 2007; Xin et al., 2009; Zhan et al., 2006). Due to climate variability and continuous decline of water resources, utilization of dryland to plant sorghum is critically important for sustainable economic consideration. The key factors for an excellent potential bioenergy crop usually include but not limited to several features: high biomass yield, drought and heat tolerance, high thermos-chemical energy, bio-chemical energy content, and biomass composition as well as processability for biofuels or bio-products (McKinley et al., 2016). Sorghum is a potential and attractive bioenergy crop meeting all above features. As a tropical grass, sorghum grown primarily in semi-arid, dry regions, especially areas too dry for corn, in the meantime, sorghum produces 33% more dry mass than corn in dryland which mean higher ethanol yield potential (Corredor et al.,2009).

Sorghum mutants have been studied as a potential biomass for biofuel production and sorghum mutants have several advantages comparing with regular sorghum biomass. Porter et al., (1978) introduced Brown midrib (bmr) mutations into sorghum. From this original population, Fritz et al. (1981) selected three BMR mutants that were agronomically acceptable; those selections became the source of three genes: bmr-6, bmr-12, and bmr-18. These bmr genotypes have reduced amounts of caffeic acid O-methyltransferase (COMT), an enzyme responsible for lignin production. These bmr with high cellulose content and low lignin content (about 20% percent less lignin than regular sorghum silage), make the sorghum biomass highly digestible for ethanol production. Sattler et al. (2014) isolated many new alleles of bmr-6, bmr-12, and four new bmr-loci which provided new genetic resources in order to improve sorghum biomass quality. Recently, the Plant Stress and Germplasm Development Unit of USDA-Agricultural Research Services developed a pedigreed sorghum mutant library consisting of 6000 individually mutagenized M4 seed pools. In order to accelerate discovery of genes or gene mutations that underlie significant beneficial traits in sorghum, a gene-discovery panel of 256 lines was selected and the mutations annotated from the 256 lines covered more than 94% of the genes in the sorghum genome. It is believed that these mutants can be used to improve sorghum biofuel properties and to discover genes responsible for improved traits in order to design perfect molecular markers that accelerate introgression of these traits to elite biomass sorghum lines.

The objective of this research was to evaluate the potential of pedigreed sorghum mutants for biofuel production with focus on chemical composition and fermentable sugars yield.

3.3 Materials and methods

3.3.1 Materials

Fifty sorghum mutant lines were selected from gene-discovery panel of 256 lines developed by the plant Stress and Germplasm Development Unit of USDA-ARS (Lubbock, TX). The mutations annotated from 256 lines covered over 94% of the genes in the sorghum genome. The raw material of sorghum mutants stalks for present work were provided by the Plant Stress and Germplasm Development Unit of USDA-ARS. The raw sorghum biomass samples were milled using the Retsch cutting Mill (Haan, Germany) to particle size less than 1 mm, then the samples were stored at room temperature for future use. All chemicals used for this study were purchased from Sigma Chemical Company (St. Louis, MO).

3.3.2 High heating value

A calorimeter (IKA-Calorimeter C200, IKA-Werke GmbH and Co. KG, Staufen, Germany) with a benzoic acid standard was used to determine the high heating value (HHV) of the sorghum mutants. Approximately 0.7 g of ground sorghum biomass with 1 mm particle size was made as pelleted sample then put into an adiabatic bomb colorimeter and burned to ash. Ground sorghum biomass samples were compacted into pellets for measurement in order to reduce error caused by incomplete combustion resulting from dry, loose samples blown away during sudden release of volatiles (Zhang et al., 2016). In addition, HHV were also calculated based on the elemental composition of biomass samples. The following equation (3-1) was used for HHV calculation (Klass, 1998; Nunes and Catalão, 2014):

$$High\ heating\ value = 0.4571 \times \%C(db) - 2.70(MJ/dry \cdot kg) \qquad (3\text{-}1)$$

3.3.3 Chemical composition analysis

The moisture content of sorghum biomass was determined following NREL standard method (Sluiter et al., 2008a). A approximately 2 g of each ground sample was dried in a forced-air oven at 105 ℃ for 4 h. NREL laboratory analytical procedures were also used for determination of ash, extractives, glucan, xylan, and arabinan contents in sorghum mutants, as well as lignin content (Sluiter et al., 2008a, b). Followed by enzymatic hydrolysis, liquid portion was processed through a high-performance liquid chromatography (HPLC) (Shimadzu, Kyoto, Japan) to measure glucan, xylan, and arabinan contents. The HPLC was equipped with an RCM monosaccharide column (300× 7.8 mm; Phenomenex, Torrance, Calif.) and a refractive index detector (RID10A, Shimadzu, Kyoto, Japan) with a 0.6 mL min^{-1} of double-distilled water mobile phase, and the oven temperature was 80 °C (Zhang et al., 2014).

3.3.4 Elemental composition analysis

Elemental composition analysis was conducted using CHNS/O Elemental Analyzer (PerkinElmer 2400 Series II, PerkinElmer Inc., Waltham, MA). About 2-2.5 mg (accurate to 0.001 mg) of the ground sorghum biomass sample with fine uniform particle size (less than 0.5mm) was weighed into tin capsules using a PerkinElmer AD-6 Autobalance (PerkinElmer Inc., Waltham, MA). The sample was folded with foil, dropped into the combustion chamber through a funnel, and burned under a pure oxygen condition. The gases (CO_2, N_2, SO_2, and H_2O) from combustion were separated in a quartz column containing copper wires detected by a thermosconductometer detector. Elemental compositions are reported as a percentage of initial dry weight (w/w, db).

3.3.5 Sulfuric acid pretreatment

Pretreatment was conducted in a reactor (Swagelok, Kansas City Valve and Fitting Co., KS) made from 316 L stainless steel with a measured internal volume of 150 mL(outside diameter

of 50.8 mm, length of 133.4 mm, and wall thickness of 2.3 mm). The ground sorghum biomass was mixed with water and 1.5% (w/v) diluted sulfuric acid to load 8.0 % solid content (w/v, 8 g dry mass in 100 mL solution). A sand bath (Techne, Inc., Princeton, NJ) with a temperature controller was used as the heating source. After the sand temperature was increased to 160 °C, the reactor was submerged in boiling sand for 40 min, then immediately transferred to room-temperature water to decrease the internal temperature to below 50 °C in 2 min. All slurry removed from the reactor was washed with distilled water and separated by filtration. The supernatant was collected into a 500mL volumetric flask. Part of the supernatant was analyzed by HPLC, as described above. The solid residual was separated in to two parts. One part was used for moisture content measurement. The second part was used for enzymatic hydrolysis to evaluate glucan recovery. The glucan recovered as solids in pretreated biomass is called glucan recovery and calculated by equation (3-2):

$$Glucan\ recovery(\%) = \frac{m_{pretreatment}}{m_{original}} \times 100\% \quad (3\text{-}2)$$

where $m_{pretreatment}$ is the weight of glucan after acid pretreatment and $m_{original}$ is the weight of glucan in the original biomass.

3.3.6 Enzymatic hydrolysis

Enzymatic hydrolysis was conducted with the solid sample after pretreatment, at 8 % of solids concentration (grams dry weight per 100 mL) in 50 mM of sodium acetate buffer solution (pH 5.00) and 0.02 % (w/v) of sodium azide to prevent microbial growth. The enzyme loading (Accellerase 1500, containing glucan and β-glucosidase, generously provided by Dupont Genencor Science, Wilmington, DL) was 1 mL/g biomass. Flasks with mixture of pretreated biomass sample, buffer solution, and enzyme were incubated in an incubator at a constant

temperature of 50 °C and agitation of 140 rpm. Total sugar analysis was tested after 72 h enzymatic hydrolysis with supernatants by HPLC, as previously described. Efficiency of the enzymatic hydrolysis (EEH) was defined by equation (3-3):

$$EEH(\%) = \frac{c \times V \times 0.9}{m_{EH}} \times 100\% \quad (3\text{-}3)$$

where c means the concentration (g/L) of glucan in the hydrolysis liquid after 72 h enzymatic processing, V is the total volume of liquid, and m_{EH} represent the weight glucan before enzymatic hydrolysis (g).

In order to better measure the effect of the acid pretreatment during the process, glucan yield is used to express the conversion rate of glucan in the solid residual after pretreatment and EEH, and defined by equation (3-4):

$$Glucan\ yield(\%) = \frac{EEH \times glucan\ recovery}{100\%} \quad (3\text{-}4)$$

Glucan mass yield (%) was used to show the glucan yield in the raw sorghum mutants and is defined by equation (3-5):

$$Glucan\ mass\ yield(\%) = glucan\ content \times glucan\ yield \times 100\% \quad (3\text{-}5)$$

3.3.7 Statistical Analysis

Analysis of variance (ANOVA) and coefficient correlation were analyzed using SAS (SAS Institute, Inc., Cary, N.C.). In general, fully balanced ANOVA tests were performed following the general linear models (GLM) procedure.

3.4 Results and Discussion

3.4.1 Heat content and elemental composition of sorghum mutants

The HHV of the sorghum mutant biomass ranged from 15.44 to 16.91 MJ/kg with mean value of 16.23 MJ/kg and standard deviation of 0.32 MJ/kg (Table 3.1). The range is similar to the previous research reported by Pawlowski et al., (2014) and Zhang et al., (2016). The mutant samples 25m2-1974 and 25m2-1465 had the highest HHV among 50 sorghum mutants about 4.2% higher than average HHV of the sorghum mutants, and about 9.5% higher than the lowest one. Elemental composition of sorghum mutant biomass in terms of C%, H%, N%, S%, and O% is also shown in Table 3.1. The sorghum biomass samples consisted of 39.64 to 43.03% (average of 41.26±0.79%) carbon, 5.10 to 6.84% (average of 6.27±0.34%) hydrogen, 0.54 to 1.30% (average of 0.86±0.18%) nitrogen, 1.09 to 1.47% (average of 1.36±0.06%) sulfur, and 48.15 to 51.99% (average 50.24±0.89%) oxygen.

Comparing the calculated HHV with measured HHV, the standard deviations are all below 0.43 MJ/kg, and there is no significant different between calculated HHV and measured HHV at 5% statistical level. Fig. 3.1a shows a linear relationship between measured HHV and calculated HHV with coefficient of determination of 0.6259. Solid line is linear regression. Central pair of solid area show 95% confidence limits for regression, and outer pair of dotted lines show 95% prediction limits for individual samples. Scatterplots of the residuals did not reveal the significant heteroscedasticity (Fig.3.1b), which could be consider as homoscedastic (followed the assumptions of a regression model). The result indicates that the both methods can be used for determination of biomass heating value, and suggested that the HHV of the sorghum mutant biomass can be predicted by carbon content. Mutant sorghum biomass also showed an average H/C mass ratio of 1.83 and a range of 1.52 to 1.95, which indicate the sorghum mutants have a

less smoke and water steam formation as well as less energy loss if used gasification processes (Huang et al., 2013).

Table 3.1 also compared the HHV of sorghum mutants with biomass in general and coal. The HHV of sorghum mutants (15.44 to 16.91 MJ/kg) in the general range of biomass HHV (12 to 18 MJ/kg). It also compared with coal and indicated that sorghum mutants are significant lower that the HHV of coal (26 to 30 MJ/kg) (Demirbas, 2004, Nunes and Catalão, 2014). The results showed that some of sorghum mutants, such as 25M2-0493, 25m2-1974 and 25M2-1465, may be more suitable to be an energy crop with better elemental compositions and HHV compared with other sorghum mutants.

3.4.2 Effects of ash content on extractives

Biomass can be converted to biofuel through chemical breakdown by two processes, bio-chemical or thermo-chemical, left with a solid residue. When the solid is produced through high temperature with air, it is called "ash" (McKendry, 2002). Biomass would generally preferred with a low ash content due to the inverse relationship with HHV. The more ash content, the less available portion for biofuel production. Thus, the determination of ash content is meaningful in evaluating both sorghum thermal energy content and bioethanol productivity.

According to the NREL method, grounded sample need to go through water extraction and ethanol extraction to remove contaminates before compositional analysis (Ruiz et al., 2005). Similar with Vassilev's result (Vassilev et al., 2013), Fig. 3.2a shows there is a clear positive linear relationship between extractives and ash content (R^2=0.7314) and most of the data are within 95% prediction limits. Scatterplots of the residuals (Fig. 3.2b) did reveal the significant heteroscedasticity, which could be consider as homoscedastic (as expected). In general, ash is the inorganic material that is bound in the physical structure of the biomass (Sluiter et al., 2010). As

the major components in plants, cellulose, hemicellulose and lignin are bound together holding for the biomass structure. Obviously, for certain amount of biomass, the higher ash content the less portion of cellulous, hemicellulos and lignin. The result in Fig. 3.2a showed that the higher ash content, the much more extractives come out during extraction process, which could infer that higher ash content may indicate the structure stability of biomass is less stable and more extractive would be extracted during water and ethanol extraction.

3.4.3 Effects of sorghum mutants on chemical composition

The glucan content of the mutant sorghum lines showed significant differences among the 50 samples (Table 3.2). The sorghum mutant with highest glucan content was sample 25M2-1137, contents 35.71% glucan, which is 56% higher than the sample with the lowest glucan content. The glucan content ranged from 22.82 to 35.71%, which is close to the results (21.7 to 37.7%) reported by Stefaniak et al., (2012). However, variation reported by Stefaniak et al. (2012) was due to a combination of multi-factors including genetic, environment, and genotype and environment interactions, which was impossible to define the corresponding effect on each source of variation (Stefaniak et al., 2012). Data collected in this study provides insight into the effect of genotype on sorghum biomass composition which may help to fill the gaps that previous researchers left. Xylan, arabinan, lignin, ash, and extractives contents of the sorghum mutants ranged from 18.54 to 25.50%, 1.92 to 3.63%, 10.24 to 15.02%, 0.20 to 5.57%, and 20.69 to 33.94%, respectively. The previous reported range of glucan and lignin content (21.7 to 37.7% and 8.9 to 20.6%, respectively) by Stefaniak et al. (2012) is a slightly wider than sorghum mutants, which may be due to the interactions between environment and genotype. Ash content of sorghum mutants (0.20 to 5.57%) is significantly lower than that (2.3 to 9.9%) reported by Stefaniak et al. (2012). The low ash

indicates mutant sorghum contains more chemical energy and could be good candidate for biofuel production.

3.4.4 Efficiency of enzymatic hydrolysis

Diluted acid pretreatment is the most effective pretreatment method in the current studies. The diluted sulfuric acid (1.5% v/v) was used as a standard pretreatment condition for biomass treatment before enzymatic hydrolysis for analysis of the total glucan mass yield and efficiency of enzymatic hydrolysis. The glucan yield was defined as the fraction of glucan released as glucose (Wolfrum et al., 2013). As the main fermentable sugar, the higher glucan content trends to yield the higher ethanol. The 10 highest and 10 lowest glucan content samples selected from the 50 preselected sorghum mutants were used to evaluate the glucan yield and enzymatic hydrolysis efficiency.

Table 3.3 summarized the chemical composition after pretreatment, glucan yield, and enzymatic hydrolysis efficiency of sorghum mutants. The chemical composition varied significantly among sorghum mutants, as well as efficiency of enzymatic hydrolysis and glucan mass yield. Mass and glucan recovery ranged from 42.72 to 52.32% and 75.67 to 94.92%, respectively, across the 20 sorghum mutants. EEH and glucan mass yield were range from 76.48 to 91.66% and 17.57 to 24.79%, respectively. Mutant sorghum samples 25M2-1137 and 15M2-0214 showed higher glucan mass yield (24.79% and 24.58%, respectively) than other samples (Table 3.3). The glucan mass yield are also higher than the previously reported data for forage sorghum (19.5%), sweet sorghum (15.7%), grain sorghum biomass (17.3%), brown midrib sorghum (16.8%), and photosensitive (PS) sorghum (12.8%) (Theerarattananoon et al., 2011, 2012; Zhang et al., 2014), suggesting that selected sorghum sample 25M2-1137 has a great potential to serve as a bioenergy crop.

A correlation between total glucan mass yield and glucan content in the biomass was shown in Figure 3. Total glucan mass yield increased as the glucan content increased (R^2=0.714), this result indicates that glucan content is the most important factor of glucan mass yield, which highly related to ethanol yield. The effect of glucan content and mass recovery on EEH was also discussed with ANOVA table (Table 3.4). Table 3.4 shows that EEH has a strong correlation with glucan content and mass recovery with a *P*-value less than 0.05. Overall, the mutant sorghum samples 25M2-1137 and 15M2-0214 are the best sorghum mutants with high glucan mass yield (24.79% and 24.58%) and high efficiency of enzymatic hydrolysis.

3.5 Conclusions

Sorghum biomass varied in glucan content, glucan mass yield, ash content, and HHV among mutants. Gene type had a significant effects on chemical composition, HHV, glucan recovery, EEH, and glucan mass yield of sorghum mutants. Sorghum mutants 25m2-1974, 25M2-0493 and 25M2-1465 had higher thermo-chemical content and 25M2-1137, 15M2-0214 demonstrated higher ethanol yield potential compared with other sorghum mutants. These sorghum mutants may be better choice for future biofuel applications.

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Table 3.1 Elemental analysis and high heating value (HHV).

Sample	Elemental Composition (%, db)						HHV (MJ/kg)		
	Carbon	Hydrogen	Nitrogen	Sulfur	Oxygen	H/C	Calculated	Measured	SD[1]
25m2-1974	42.73	6.8	0.86	1.47	48.15	1.91	16.83	16.91	0.04
25M2-0493	42.65	6.65	0.73	1.44	48.55	1.87	16.79	16.8	0
MUT581	41.75	6.61	1.05	1.41	49.19	1.9	16.38	16.74	0.18
25M2-1465	43.03	6.29	0.77	1.37	48.56	1.75	16.97	16.7	0.13
M2P0630	41.99	6.08	0.82	1.32	49.8	1.74	16.49	16.69	0.1
MUT841	42.36	6.28	0.67	1.42	49.28	1.78	16.66	16.68	0.01
25m2-1983	42.23	6.84	1.04	1.41	48.5	1.94	16.6	16.66	0.03
25M2-1402	42.46	6.12	0.96	1.37	49.11	1.73	16.71	16.65	0.03
MUT436	42.52	6.65	0.59	1.41	48.84	1.88	16.73	16.57	0.08
25M2-1684	41.35	6.51	1.02	1.36	49.77	1.89	16.2	16.47	0.13
25M2-1603	41.87	6.23	0.73	1.36	49.82	1.79	16.44	16.43	0
25M2-0390	42.2	5.34	0.85	1.09	50.53	1.52	16.59	16.42	0.09
25M2-1517	42.27	5.93	0.97	1.3	49.54	1.68	16.62	16.4	0.11
BTx623	41.28	6.23	0.77	1.37	50.36	1.81	16.17	16.35	0.09
25M2-0475	41.03	6.45	0.87	1.42	50.25	1.89	16.05	16.35	0.15
25M2-0095	41.54	5.89	0.74	1.3	50.53	1.7	16.29	16.34	0.03

25M2-1192	40.77	6.33	0.82	1.37	50.73	1.86	15.93	16.33	0.2
25M2-1038	41.17	6.65	0.73	1.37	50.09	1.94	16.12	16.32	0.1
25M2-1100	41.06	5.92	0.96	1.31	50.76	1.73	16.07	16.32	0.13
25M2-1303	41.26	6.48	0.83	1.4	50.04	1.88	16.16	16.3	0.07
25M2-1399	41.38	6.23	0.77	1.35	50.28	1.81	16.21	16.27	0.03
25M2-1137	41.71	6.26	0.64	1.37	50.03	1.8	16.36	16.27	0.05
25M2-0549	39.64	6.26	1.05	1.38	51.69	1.89	15.42	16.27	0.43
25M2-1528	41.43	6.41	0.89	1.45	49.82	1.86	16.24	16.27	0.01
25M2-0909	41.26	6.48	0.83	1.4	50.04	1.88	16.16	16.22	0.03
25M2-0514	40.43	6.33	1.03	1.4	50.82	1.88	15.78	16.22	0.22
25M2-0782	41.13	6.54	0.95	1.43	49.96	1.91	16.1	16.21	0.06
25M2-0473	40.81	6.56	0.99	1.4	50.25	1.93	15.95	16.19	0.12
25M2-0592	40.6	6.32	1.11	1.34	50.64	1.87	15.86	16.19	0.16
15M2-0629	40.64	6.38	0.71	1.36	50.92	1.88	15.88	16.18	0.15
25M2-0850	41	6.67	0.91	1.41	50.03	1.95	16.04	16.17	0.06
25M2-0593	41.64	6.61	0.96	1.38	49.42	1.9	16.33	16.16	0.09
BTx623	41.61	6.71	0.64	1.39	49.66	1.94	16.32	16.15	0.08
15M2-0214	41.98	6.17	0.71	1.35	49.8	1.76	16.49	16.13	0.18
25M2-0188	40.71	6.21	0.79	1.34	50.97	1.83	15.91	16.12	0.11

25M2-0192	40.1	5.8	1.21	1.28	51.62	1.73	15.63	16.08	0.23
25M2-0687	40.47	5.81	1.14	1.31	51.29	1.72	15.8	16.08	0.14
15M2-0012	41.78	6.52	0.69	1.4	49.62	1.87	16.4	16.07	0.16
25M2-0635	40.46	6.57	0.98	1.36	50.64	1.95	15.79	16.06	0.13
25M2-0126	41.08	5.85	0.6	1.26	51.22	1.71	16.08	15.98	0.05
25M2-0371	40.32	6.3	1.01	1.43	50.95	1.88	15.73	15.98	0.13
10M2-0342	41.52	5.99	0.68	1.34	50.48	1.73	16.28	15.98	0.15
25M2-0216	40.74	6.46	0.92	1.45	50.45	1.9	15.92	15.95	0.01
25M2-0111	40.74	6.39	0.63	1.41	50.84	1.88	15.92	15.85	0.03
25M2-0927	40.52	6.2	0.8	1.34	51.15	1.84	15.82	15.76	0.03
25M2-0567	40.39	5.1	1.3	1.23	51.99	1.52	15.76	15.74	0.01
10M2-0304	41.01	5.81	0.88	1.31	51	1.7	16.05	15.64	0.2
25M2-0041	40.5	5.93	1.29	1.34	50.95	1.76	15.81	15.6	0.1
10M2-1060	40.03	6.35	0.54	1.36	51.73	1.9	15.6	15.6	0
25M2-0148	40.07	6.29	0.88	1.34	51.43	1.88	15.61	15.44	0.09
SD[1]	0.79	0.34	0.18	0.06	0.89	0.1	0.36	0.32	
Mean	41.26	6.27	0.86	1.36	50.24	1.82	16.16	16.23	
Range	39.64-43.03	5.10-6.84	0.54-1.30	1.09-1.47	48.15-51.99	1.52-1.95	15.42-16.97	15.44-16.91	

[1]SD =Standard Deviation.

Table 3.2 Chemical Composition of Sorghum Mutants.

Sample	Chemical Composition (%,db)					Extractives
	Glucan	Xylan	Arabinan	Ash	Ligin	(%)
25M2-0111	27.92	20.69	3.57	3.17	10.24	33.14
25M2-0475	29.46	21.61	2.70	3.15	11.81	28.42
MUT581	29.80	25.50	2.89	1.49	13.60	23.68
25M2-0473	28.08	20.63	2.81	3.55	11.79	28.80
25M2-0782	28.79	23.93	2.27	2.16	11.92	28.74
MUT436	30.67	25.41	2.47	1.35	12.91	24.38
25M2-1303	29.79	24.07	2.47	2.62	12.78	25.27
25M2-0095	30.14	24.77	3.63	1.92	12.48	25.85
10M2-1060	29.12	23.82	2.38	2.79	11.67	26.76
25M2-0493	28.48	24.60	2.27	2.27	13.56	24.55
25M2-0390	31.67	21.97	2.37	2.80	12.46	28.35
10M2-0342	31.03	21.44	3.41	2.60	11.87	28.25
25M2-0371	28.95	20.57	2.60	3.92	11.31	31.47
25M2-1465	34.01	24.67	2.33	0.88	14.37	22.87

25M2-0909	30.14	22.03	2.66	3.39	12.35	27.89
25M2-0850	28.87	19.53	2.49	5.24	12.11	29.09
25M2-0188	28.82	19.70	2.05	4.42	10.73	32.15
25M2-0567	30.52	21.22	2.48	3.13	12.54	28.21
25M2-1137	35.71	22.69	2.04	0.65	12.30	24.34
25M2-0148	30.70	20.73	2.88	1.50	12.16	28.91
25M2-0927	30.84	19.49	2.15	1.92	12.10	31.74
M2P0630	34.03	24.43	2.12	0.58	14.32	22.65
25M2-1402	32.68	23.05	2.13	0.20	14.04	23.87
15M2-0214	34.96	24.18	2.21	1.27	14.06	21.91
25M2-1603	33.90	22.65	2.03	0.25	13.78	23.36
25M2-0593	28.11	20.77	2.36	2.63	12.00	31.96
25M2-0687	28.85	20.11	2.57	4.31	11.82	28.49
25M2-0592	29.33	19.53	2.21	2.98	11.88	30.06
25M2-1684	28.01	22.19	2.47	2.70	12.02	27.78
25M2-1038	29.73	23.56	2.21	1.85	12.66	24.55

25M2-0041	26.99	23.56	2.57	1.45	10.84	29.23
MUT841	31.44	25.11	1.96	0.48	14.24	22.32
25M2-1192	28.24	21.73	2.16	2.15	12.11	27.95
25M2-1528	27.49	22.33	2.20	1.87	13.12	27.54
25m2-1974	29.26	23.99	2.13	0.73	15.02	20.69
BTx623	30.41	22.24	2.08	1.16	13.83	21.86
25M2-1100	27.70	20.32	2.30	3.05	12.64	25.48
10M2-0304	26.59	21.76	2.46	1.38	12.30	26.68
25M2-0126	27.43	21.27	2.12	0.60	12.45	28.39
15M2-0012	27.64	20.79	2.48	1.32	12.38	26.72
25M2-0514	25.54	19.77	2.43	3.97	11.01	30.70
BTx623	29.83	24.23	2.42	0.52	13.45	22.78
25M2-1399	30.02	23.37	2.07	0.63	13.16	23.97
25m2-1983	29.00	23.66	2.03	0.47	14.00	23.68
25M2-0549	22.82	18.54	2.39	5.57	10.77	33.94
25M2-0216	25.45	19.87	2.44	3.53	11.06	31.41

25M2-1517	26.68	22.42	2.20	1.05	13.32	25.32
15M2-0629	28.21	22.36	1.92	1.18	12.86	24.74
25M2-0635	24.81	19.37	2.34	2.32	12.17	30.01
25M2-0192	27.53	21.57	2.39	3.35	12.74	27.86
Mean	29.32	22.16	2.41	2.17	12.54	26.98
SD[1]	2.50	1.83	0.37	1.32	1.06	3.25
Range	22.82-35.71	18.54-25.50	1.92-3.63	0.20-5.57	10.24-15.02	20.69-33.94

[1]SD =Standard Deviation.

Table 3.3 Biomass Sugar Yield.

	Composition after Pretreatment (%, db)				Hydrolysis yield/unit mass (%, db)		Efficiency (%, db)	
Sample	Glucan	Xylan+ Arabinan	Mass recovery	Glucan recovery	Glucose	Xylose+Arabinose	EEH[1]	Glucan Mass Yield
25M2-1137	57.96	2.79	50.24	81.55	49.35	1.94	85.15	24.79
15M2-0214	56.30	2.09	49.51	79.72	49.64	0.91	88.17	24.58
M2P0630	52.67	2.03	50.80	78.64	46.02	1.44	87.38	23.38
25M2-1465	53.19	2.14	48.60	76.02	47.11	1.57	88.57	22.90
25M2-1603	54.80	1.90	52.06	84.15	45.33	1.54	82.73	23.60
25M2-1402	53.06	2.08	51.31	83.31	44.87	1.52	84.57	23.02
25M2-0390	50.28	1.54	48.55	77.08	41.57	0.73	82.67	20.18
MUT841	54.35	1.81	51.15	88.40	44.39	0.76	81.69	22.71
10M2-0342	54.09	2.47	45.76	79.78	45.91	1.77	84.86	21.01
25M2-0927	48.94	3.50	47.69	75.67	38.84	1.36	79.37	18.52
25M2-0192	50.30	1.83	51.13	93.40	38.47	0.82	76.48	19.67
25M2-1528	50.26	2.14	48.32	88.35	41.51	1.51	82.58	20.06
25M2-0126	56.06	2.43	52.32	94.48	45.29	1.74	80.79	20.94
25M2-0041	55.18	2.27	44.95	91.92	45.03	0.90	81.59	20.24
25M2-1517	55.66	2.59	42.72	91.58	49.27	0.83	88.52	21.63
10M2-0304	53.81	2.11	46.90	94.92	42.24	0.95	78.49	19.81
25M2-0514	47.22	1.30	45.22	83.61	41.72	0.60	88.34	18.86

25M2-0216	47.36	1.41	47.87	89.09	41.22	0.61	87.03	19.73
25M2-0635	48.80	1.94	48.20	94.81	41.31	1.35	84.65	19.91
25M2-0549	44.45	0.40	43.13	83.99	40.74	0.50	91.66	17.57

[1]EEH= Efficiency of enzyme hydrolysis

Table 3.4 ANOVA table for the effects of Glucan content and Mass recovery on EEH.

Analysis of Variance					
Source	DF	Sum of Squares	Mean Square	*F* Value	*Pr > F*
Model	3	13.00265	45.33422	4.61	0.0166
Error	16	157.42224	9.83889		
Corrected Total	19	293.42490			

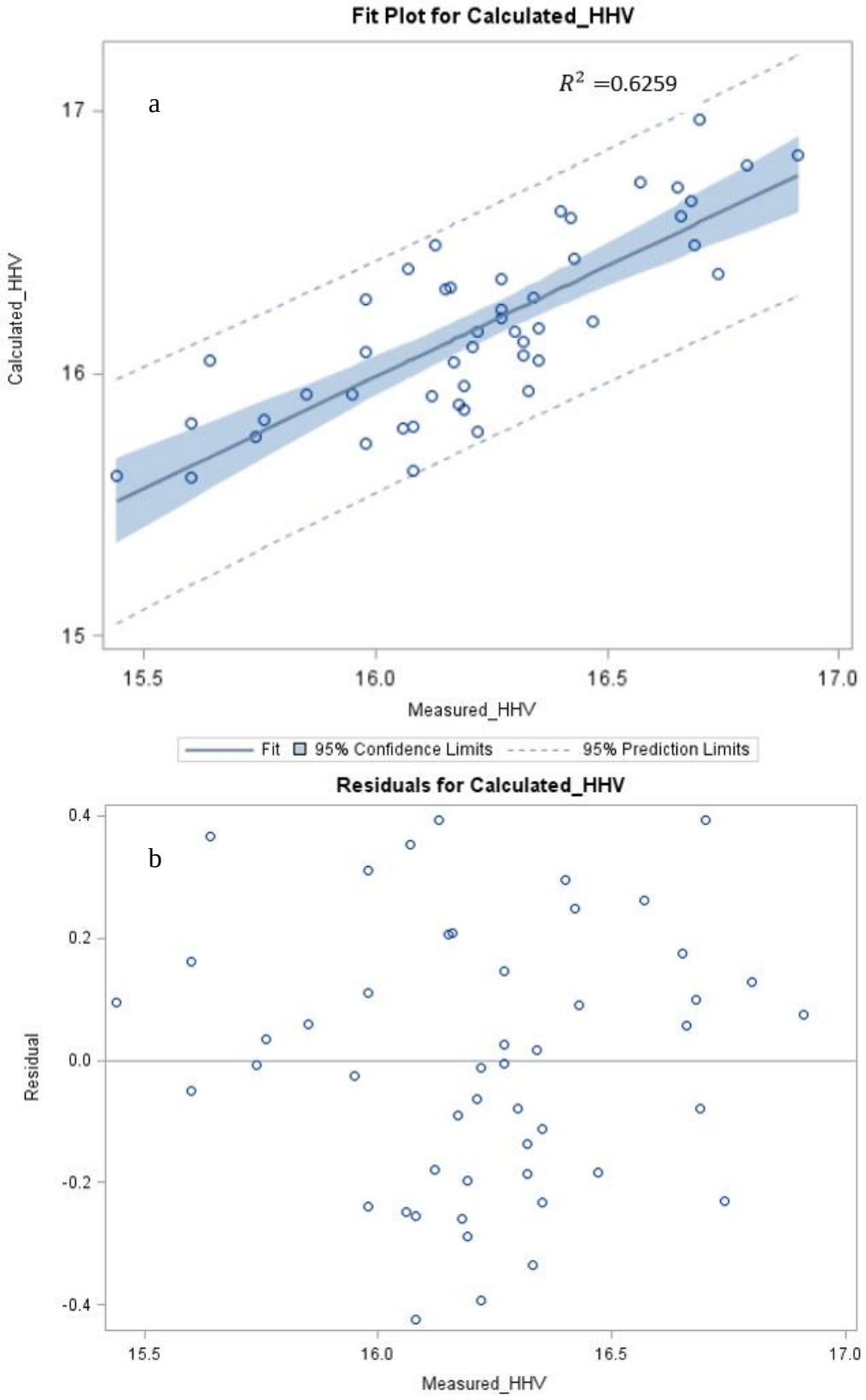

Figure 3.1 Scatterplot of calculated HHV vs. measured HHV tested by calorimeter.

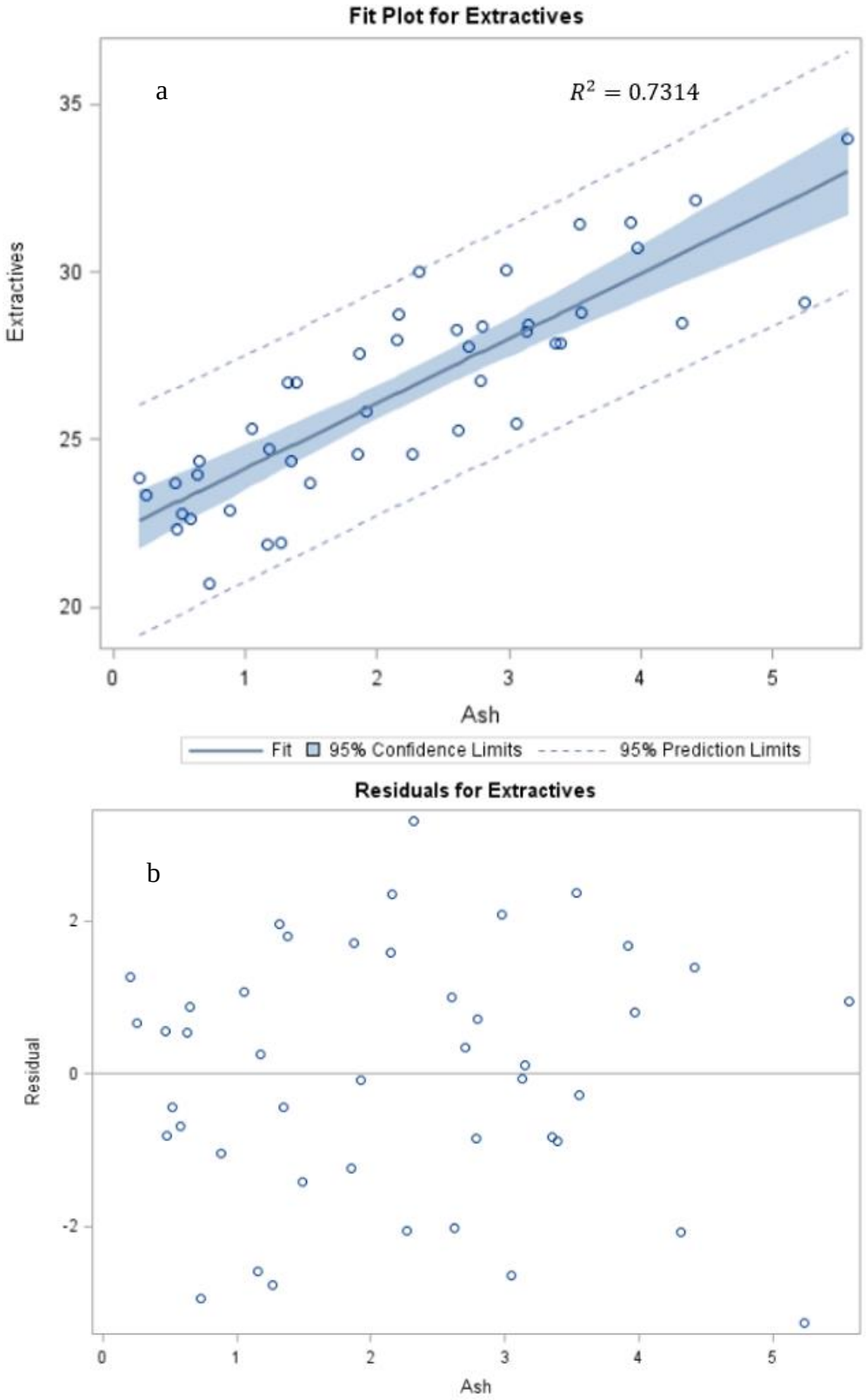

Figure 3.2 Extractives as function of ash content.

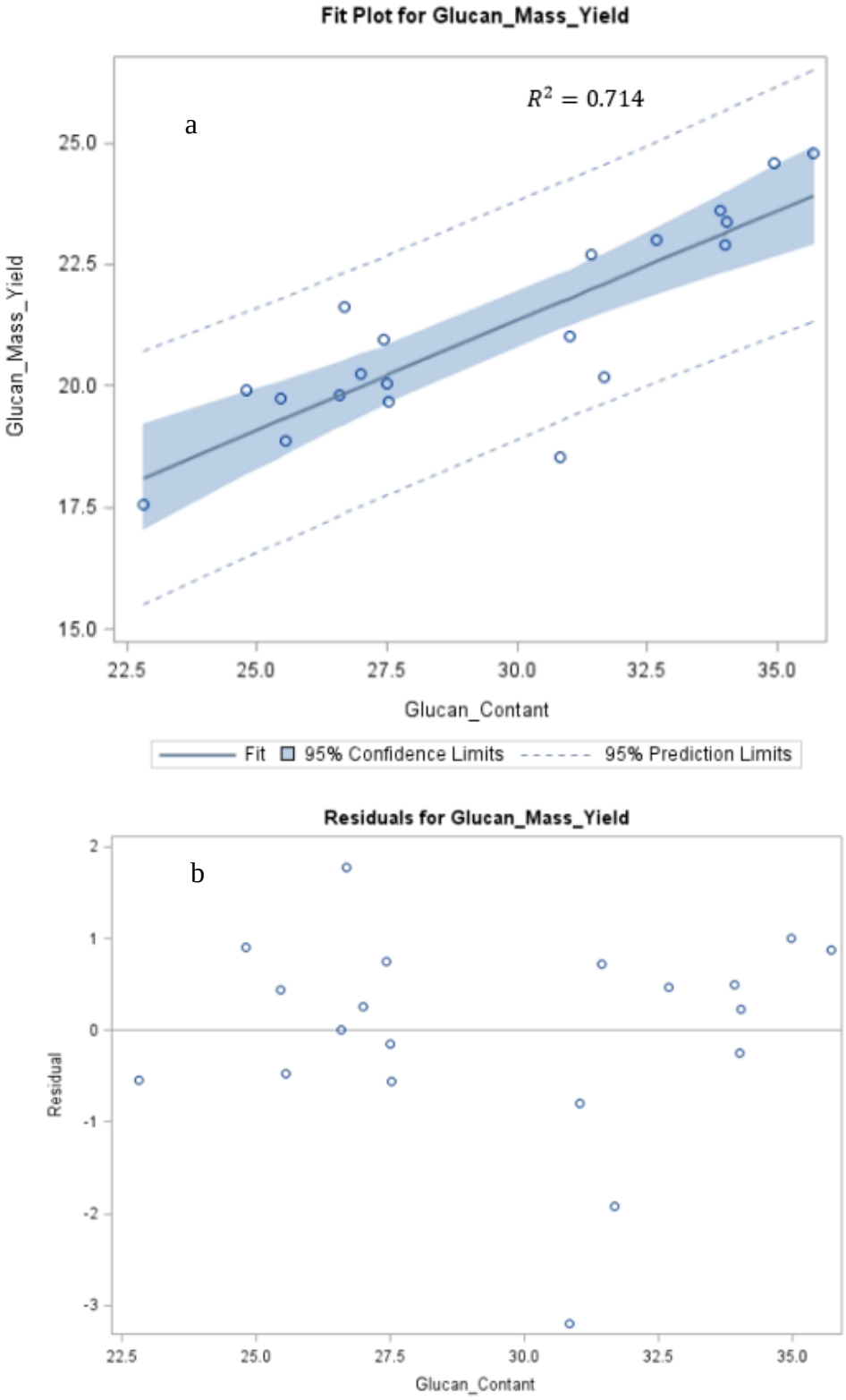

Figure 3.3 Glucan mass yield as function of Glucan.

Chapter 4 - Conclusions

4.1 Conclusion

The impact of deficit irrigation on grain sorghum physical and chemical properties as well as final ethanol yield, and the potential of sorghum mutant biomass as feedstocks for bio-ethanol production were studied. Irrigation has a significant effect on both physical and chemical properties of grain sorghum as well as bioconversion efficiency and final ethanol yield. The starch content of all sorghum samples ranged from 69.45 to 72.82%, protein content ranged from 8.22 to 12.50%, bioethanol yield ranged from 44.94 to 47.99 mL/100g. The level of deficit irrigation had a significant effect on all physiochemical properties, pasting properties, thermal properties, and bioethanol yield. Starch content and bioethanol yield increased as irrigation level increased across two growing seasons in 2015 and 2016.

Sorghum mutant biomass varied in glucan content, glucan mass yield, ash content, and HHV. Gene type had a significant effects on chemical composition, HHV, glucan recovery, EEH, and glucan mass yield of sorghum mutants. Results from 50 sorghum mutant populations selected from a gene-discovery panel of 256 lines revealed a large variation in glucan (22.82 to 35.71%), xylan (18.54 to 25.50%), arabinan (1.92 to 3.63%), lignin (10.24 to 15.02%), carbon (39.64 to 43.03%), and hydrogen (5.10 to 6.84%). Based on the higher thermos-chemical content and higher ethanol yield potential, sorghum mutants 25m2-1974, 25M2-049, 25M2-1465, 25M2-1137, and 15M2-0214 may have a great potential for biofuel production.

4.2 Future Research

Although 50 sorghum mutant was selected to test their properties as potential feedstock for biofuel production. The only limited sorghum mutants were evaluated. The gene-discovery panel contains 256 lines selected from mutant library covering about 94% of genes in the

sorghum genome. For future work, the total of 256 lines should be evaluated to find best sorghum mutants for biofuel production.

As continued decline in water resources, future research should continue to study the impact of deficit irrigation and soil water availability on grain yield, quality and end use. In addition, the impact of deficit irrigation and irrigation management on lignocellulosic biomass physical and chemical attributes, processing quality, and final product yield should be studied.