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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 inch every 4, 6, 8,10, or 12 days) and dryland in 2015 and 2016 growing seasons. Results showed 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% and 8.22—12.50%, 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.

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1. 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 corn 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 corn. Irmak et al. (2016) evaluated the effects of deficit irrigation on corn production and developed crop yield response factors for field corn. 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 (El-Hendawy et al., 2017) as well. Zhang et al. (2016) reported rice production improved 4—8% and reduced 20.5% water consumption 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.,

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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 corn 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 corn 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, corn has become overused as a renewable source, which may impact the amount of corn used for human food and directly as animal feed consumption. If all of the corn 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 corn 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 corn 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.

2. Materials and methods

2.1. 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. The climate is semi-arid with mean annual precipitation of 450 mm.

2.1.1. 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 inches. 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.

2.1.2. 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 per hectare 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.

2.2. 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. 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".

2.4. 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.

2.5. 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}.

2.6. 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 then and at160 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.

2.7. 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).

2.8. 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).

2.9. Statistical analysis

Statistical analysis was conducted using the PROC GLIMMIX procedure in SAS (SAS Institute, Cary, NC). Correlation analysis was implemented using Pearson's correlation. All statistical analysis were conducted at a 5% level of significance.

3. Results and discussion

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

Table 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

Table 1
Physiochemical and pasting properties, fermentation efficiency, and bioethanol yield of grain sorghum samples.

	Irrigation treatments[a]											
	2015						2016					
	1	2	3	4	5	6	1	2	3	4	5	6
1000 kernel weight (g)	29.61a[b]	27.24b	26.04cd	25.52cde	24.57ef	23.47g	27.35b	26.46bc	25.49cde	25.00de	23.92 fg	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	70.91 fg	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 (%)	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 (%)	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 (%)	2.93abc	2.92abc	2.98 ab	2.94 ab	2.81cd	3.03a	2.72de	2.71de	2.63e	2.64de	2.73	2.90bc
Fiber (%)	1.42b	1.03d	1.19c	1.06cd	1.05d	1.03	0.98b	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	3101 ab	2883cd	2946c	2753e	2853d	3028b	2800d	3110a	2814de
Fermentation efficiency (%)	89.79b	89.67b	89.07b	89.29	90.04 ab	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

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

Table 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

test weight. In Table 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 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 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% with a mean value of 71.53%; protein content ranged from 8.22% to 12.50% with a mean value of 9.78%; fat content ranged from 2.61 to 3.06% with a mean value of 2.82%; and fiber content ranged from 0.83 to 1.63% with a mean value of 1.11%. Sorghum produced full irrigation produced ~3% more starch than sorghum produced 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 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 2015 than 2016 (Table 2). The Pearson correlation shown in Table 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 12.46% was found in 2016 for samples under the treatment that received least amount of

Table 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	0.96**	−0.89**	0.78**	0.83**	−0.77**	−0.01	0.39	−0.56**	0.93**
Kernel diameter	0.96**	1	−0.96**	0.89**	0.84**	−0.71**	−0.13	0.25	−0.50*	0.98**
Kernel hardness	−0.89**	−0.96**	1	−0.95**	−0.79**	0.64**	0.25	−0.20	0.55**	−0.96**
Test weight	0.78**	0.89**	−0.95**	1	0.67**	−0.45*	−0.33	0.08	−0.31	0.92**
Starch	0.83**	0.84**	−0.79**	0.67**	1	−0.94**	0.22	0.11	−0.59**	0.85**
Protein	−0.77**	−0.71**	0.64**	−0.45*	−0.94**	1	−0.36	−0.22	0.66**	−0.69**
Fat	−0.01	−0.13	0.25	−0.33	0.22	−0.36	1	−0.14	−0.08	−0.16
Fiber	0.39	0.25	−0.20	0.08	0.11	−0.22	−0.14	1	−0.24	0.21
Fermentation efficiency	−0.56**	−0.50*	0.55**	−0.31	−0.59**	0.66**	−0.08	−0.24	1	−0.51*
Bioethanol yield	0.93**	0.98**	−0.96**	0.92**	0.85**	−0.69**	−0.16	0.21	−0.51*	1

*Correlation is significant at the 0.05 level (2-tailed).
**Correlation is significant at the 0.01 level (2-tailed).

irrigation. Low irrigation yielding high protein content of grain supports the observations of previous researchers (Daniel and Triboi, 2002; Weightman et al., 2008).

3.2. 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 1, for all of the sorghum samples, the mean and range across deficit irrigation levels and years were 74.37 °C and 71.95–76.89 °C for pasting temperature (the temperature starch granule start swelling and gelatinization), 5.32 min and 5.12–5.61 min for peak time, 2781 cP and 2157–3249 cP for peak viscosity (the maximum viscosity during heating and starch gelatinization), 1713 cP and 1612–1836 cP for holding strength (an indicator of the water holding capacity of starch), 1064 cP and 501–1442 cP for breakdown (the starch granule rupturing and releasing amylose), 4613 cP and 4461–4834 cP for final viscosity, 2895 cP and 2745–3146 cP for setback. In Table 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). For this reason, RVA curves of sorghum samples with different deficit irrigation in 2015 was selected and demonstrated in Fig. 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 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. 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).

3.3. 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 (Table 1). A significant effect of irrigation capacity was observed for bioethanol yield of sorghum (Table 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 than sorghum grown under deficit irrigation using current approaches. While there was no significant correlation between final fermentation efficiency and deficit irrigation level, Fig. 1c demonstrates a clear trend that sorghum grown under low irrigation higher fermentation efficiency in the first 48 h of fermentation than sorghum grow under full irrigation sorghum. This was kindly due to sorghum grown under deficit irrigation having lower starch content and higher protein content, which would produce higher fermentation efficiency in the early stage of when inoculating the same amount of yeast per sample (i.e. a higher yeast to starch ratio), and high protein samples may have higher freer amino acid content as nutrient for yeast. 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 3 and 4). However, the Person correlation analysis showed that bioethanol yield had significant correlation with all physiochemical and pasting properties. Supplement 1 shows the relationship between starch content of sorghum and bioethanol yield and fermentation efficiency. Although starch content is the most import predictor in bioethanol

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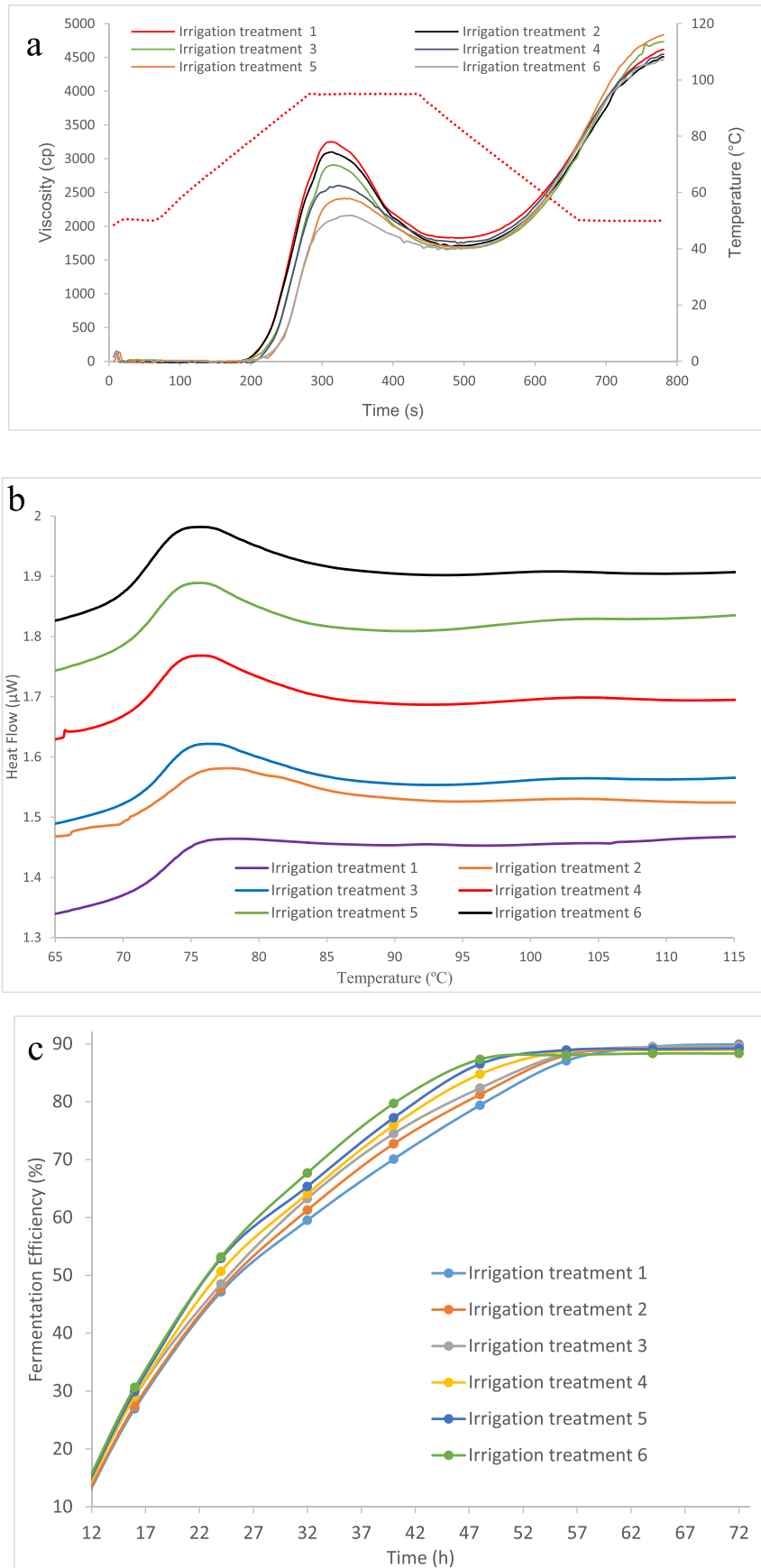**Correlation is significant at the 0.01 level (2-tailed).

Fig. 1. RVA (a), DSC (b) and fermentation efficiency (c) 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, irri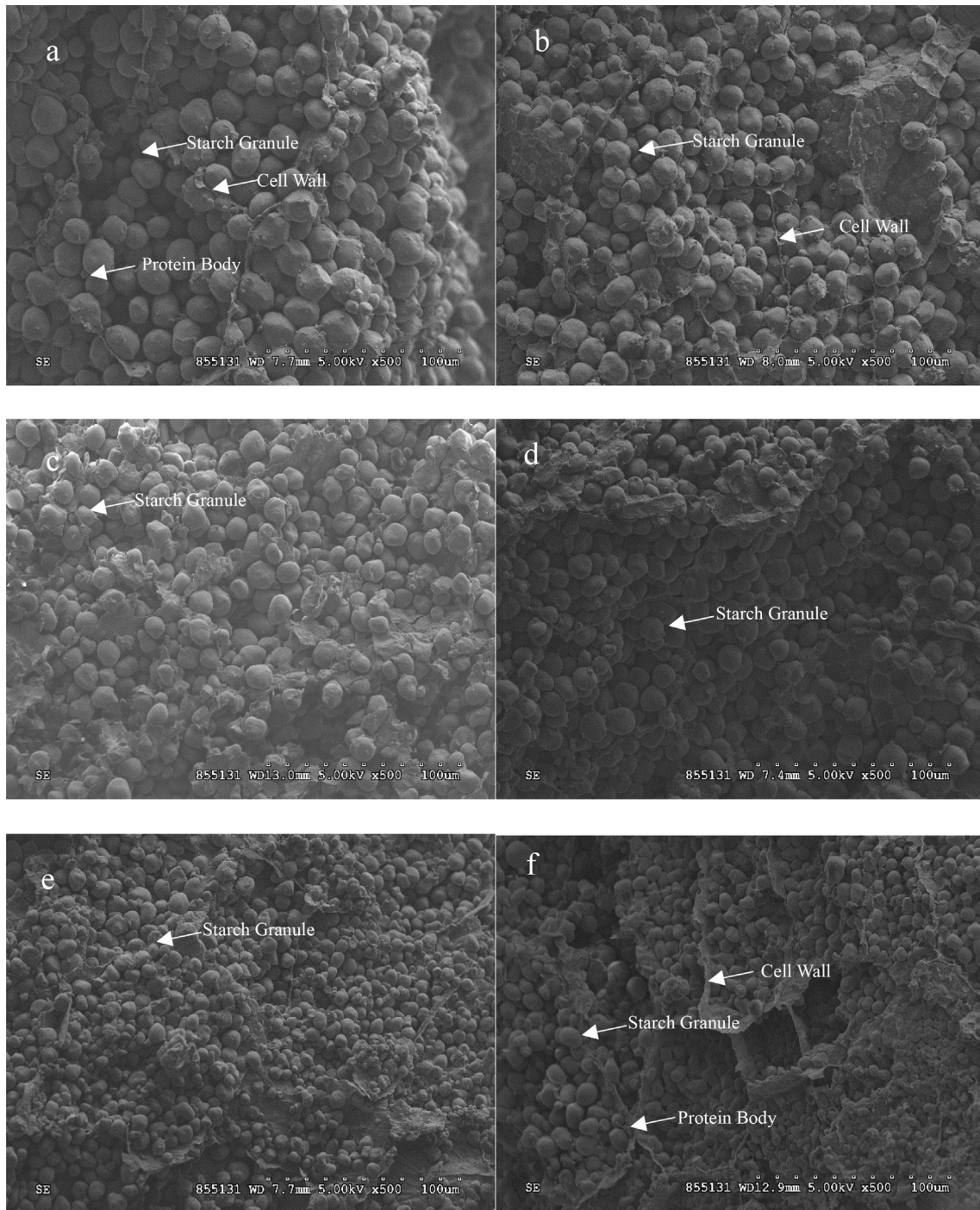gation limited to 250 mm, 3) 100% ET limited to 250 mm, 4) 50% ET prior to booting of grain sorghum and 100% ET after boot and total irrigation limited to 150 mm, 5) 100% ET limited to 150 mm, and 6) dryland.

Fig. 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).

yield ($R^2 = 0.71$), it did not correlate to fermentation efficiency. Of irrigation capacity, sorghum grown under high full irrigation produced greater starch and bioethanol than sorghum grown under deficit irrigation. Table 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.

3.4. 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. Sorghum produced under full irrigation (Fig. 2 a) had larger starch granule size than the sorghum produced under moderate (Fig. 2 b–e) and high deficit irrigation levels (Fig. 2 f). These results fit 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. (2010) 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 a). 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).

4. 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%, 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, 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.

## Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jcs.2017.09.002

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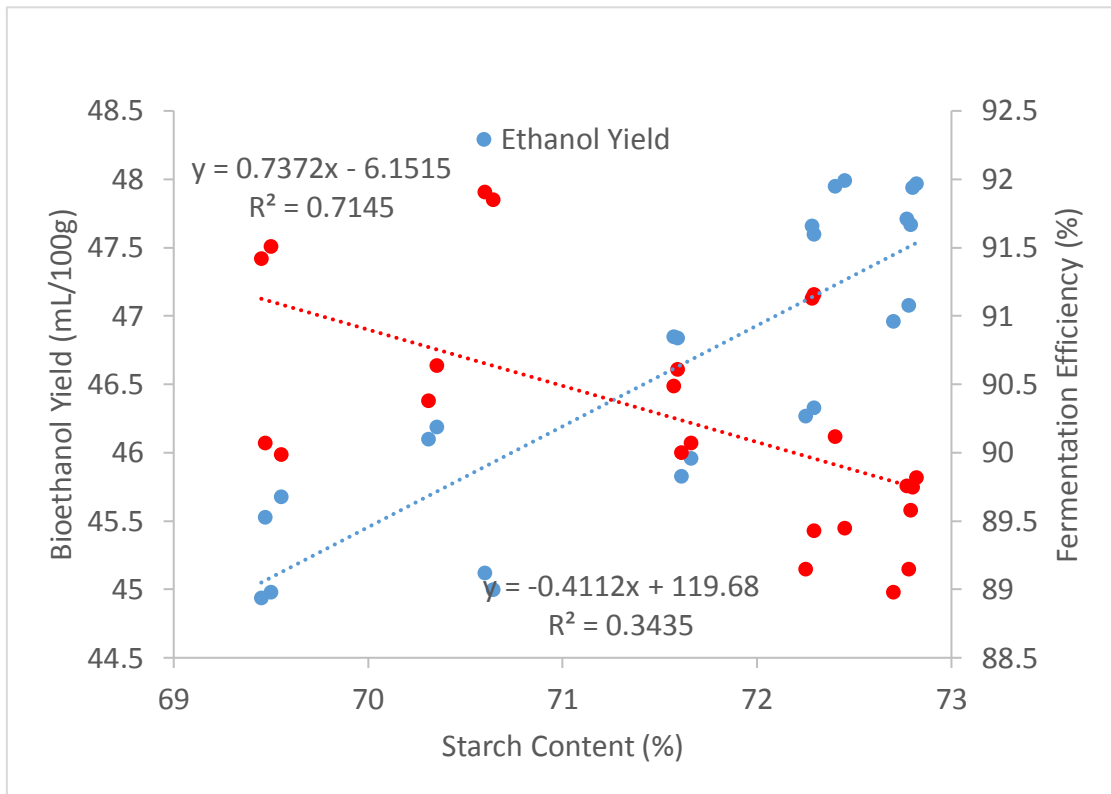
Supplement 1. Starch content of sorghum versus bioethanol yield and fermentation efficiency.