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Compatibility studies on sweet sorghum and legumes in sole and intercropping systems for biomass and bioethanol production

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Abstract

Adoption of multiple intercropping for increasing land productivity and biodiversity has special significance for current and future biomass and bioenergy demands for the mitigation of environmental issues. In dry seasons of 2009/10 and 2010/11, biomass and bioethanol production of eight intercropping patterns of sweet sorghum composed of two legumes (viz. soybean and mungbean), two planting patterns (viz. alternative single rows and alternate double rows), and two seeding times (viz. simultaneous and staggered seeding) were evaluated together with three sole crops. The theoretical bioethanol yield was highest in sweet sorghum-soybean intercropping established with staggered seeding (16,673 L ha⁻¹, 13,410 L ha⁻¹), that was greater by 8% and 7%, respectively, compared to sweet sorghum sole crop in both the years. The same combination gave above-ground biomass of intercropped sweet sorghum at par in the first year but was higher by 0.7 t ha⁻¹ in the second year compared to the sole crop of sweet sorghum. Cellulose, hemi-cellulose, soluble sugar, and starch contents in intercropped sweet sorghum were negligibly reduced in staggered seeding compared to its sole crop.

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Introduction

Renewable biomass sources for bioethanol production have received an escalated attention due to the threat of changing climatic conditions due to extensive use of fossil fuels. Expected depletion of world's petroleum reserves has stimulated the scientists to search for non-petroleum-based alternative sources of energy (Kerr, 1998). Plant biomass has been considered the most promising renewable source for the production of biofuel with low CO₂ emissions and cost of production (Berndes *et al.*, 2003; Antonopoulou *et al.*, 2008). Currently, biomass is providing about 14% of total global energy needs (IEA, 2011) and is helping to maintain and improve the ecological and social sustainability (Parikka, 2004; Xiong *et al.*, 2008). Sweet sorghum (*Sorghum bicolor L.*) is one of the prime energy crops, which have inherent potential to reduce dependency on petroleum fuel. In this context search for measures to increase bioethanol production from sweet sorghum would help alleviate energy crisis and rural poverty thereof (Zhao *et al.*, 2009; Arshad *et al.*, 2014).

Sweet sorghum has been recognized as a high potential crop for bioethanol production (Zhao *et al.*, 2009). The crop grows well at low soil moisture and high temperatures in tropical and sub-tropical climates (Arshad *et al.*, 2013; Dajue, 1995) and also on marginal soils (Nahar, 2011). The crop is capable of yielding around 30 t ha⁻¹ year⁻¹ of sugar containing dry stalks (Grassi, 2000). Relatively short maturity period and ability to avoid unfavorable weather conditions have favored sweet sorghum cultivation in China, India and few other countries (Griffiee, 2000). It is grown as sole crop on marginal lands where water is scarce and crops like corn, sugarcane, and cassava could become hardly successful. The soluble sugar content in the stalk of sweet sorghum ranges between 43.6 and 58.2% (Arshad, 2012; Billa *et al.*, 1997; Antonopoulou *et al.*, 2008) while insoluble carbohydrates (cellulose and hemi-cellulose) and

grain starch are in the range of 22.6–47.8% (Rattunde *et al.*, 2001; Antonopoulou *et al.*, 2008) and 39–48%, respectively (Zhao *et al.*, 2009).

Intercropping increases the vegetation diversity and biomass production of agricultural lands compared to sole cropping (Arshad and Ranamukhaarachchi, 2012; Shrestha *et al.*, 2010). Studies have also confirmed that grain and forage sorghum can be successfully grown with legumes in intercropping, especially in tropical areas of the world and produced the high fodder and grain yields (Okigbo and Greenland, 1976; Azraf *et al.*, 2006). However, its potential for intercropping with legumes for increasing food and bioethanol production has not yet been fully explored. However, little information is known about intercropping sweet sorghum with legumes for bioethanol production. Therefore the current study investigated the bioethanol production potential of sweet sorghum-legume intercropping under different agronomic practices of selected planting patterns and times of seeding.

Materials and methods

Study Site Characteristics

This study was conducted at the Agricultural Systems and Engineering Research Farm of the Asian Institute of Technology in Pathumthani Province of Thailand (13° 44' N, 100° 30' E) during the dry seasons of 2009/10 and 2010/11. The soil type was Ongkarak clay (very fine texture, mixed acid, isohyper, sulfic tropaquepts). Soil properties of the site include sand 6.5–6.7%, silt 27.1–27.6% and clay 65.9–66.2%, pH 4.9–5.0, organic matter 2.1–3.0%, and total N 0.13–0.15% and P and K 11.5–12.2 and 210.6–213.5 ppm, respectively.

Treatment Design and Layout

Eight sweet sorghum-legume intercrops composed of 2 × 2 × 2 factorial combination of two legumes (viz. mungbean and soybean), two planting patterns [viz. alternate single row (ASR) and alternate double row (ADR)], and two seeding times (viz. simultaneous and staggered) and three sole crops of sweet sorghum,

mungbean and soybean were tested in a randomized complete block design with three replications.

Experiment material and management

The cultivated crop varieties were KKU-40 for sweet sorghum, Chinat-72 for mungbean (determinate growth type) and Nakhorn Swan-1 for soybean (indeterminate growth habit) recommended by the Department of Agriculture, Thailand (Pothisoong and Jaisil, 2011; DOA, 2012). Alternate single row pattern (ASR) had single row of sweet sorghum seeded in 45 cm rows and one row of legume seeded in between two adjacent sweet sorghum rows while in the ADR pattern, paired rows of sweet sorghum spaced at 30 cm and two adjacent pairs were established in 60 cm, and two rows of legume seeded between two paired rows of sweet sorghum leaving 20 cm distance from the sweet sorghum rows. Simultaneous seeding implied seeding of both sweet sorghum and legume at the beginning of the experiment whereas, in staggered seeding sweet sorghum was seeded one month after legume. Planting density of sweet sorghum, mungbean and soybean was approximately 148,148; 222,222 and 111,111 plants ha⁻¹, respectively in intercropping of staggered pattern and in sole stands but planting density of legumes was reduced by 50% in case of simultaneous pattern by doubling the intra row spacing. Intra-row spacing of sweet sorghum, mungbean and soybean was 15, 10 and 20 cm, respectively, in sole and staggered intercropping plots.

Land preparation was adopted by ploughing once, harrowing twice and leveling the land at last. Each experimental unit was 3.6 m × 6.0 m. plots within replicates were separated by a 1.0 m wide and 0.3 m deep drains were prepared between the experimental units (3.6 m × 6 m) and replicates were separated by two-meter wide area. In intercrops with simultaneous seeding, sweet sorghum and legume were seeded on 1st December, 2009/10, and sweet sorghum was seeded one month later (1st January 2010/11) for staggered seeding.

Plots were maintained at non-water stressed conditions with four irrigations each of 5.5 cm depth were applied at 7 days after germination, 21 days after germination, 35 days after germination and at full vegetative stage, respectively. Sweet sorghum in both sole and intercropping was given N at 80 and P and K each at 30 kg ha⁻¹. Total P and K were applied to plots prior to seeding as basal dressing. The dose of N was applied as two splits of 40 kg each, the first dose as basal dressing and the second dose as top dressing at 50 % booting for intercropping established with simultaneous seeding. For intercrops established with staggered seeding N was applied as three splits: 25% each at seeding of legume and sweet sorghum, and the remainder of 50% at booting of sweet sorghum. For sole cropped mungbean and soybean 30 kg ha⁻¹ each of N, P and K were applied at the time of seeding only. Weeds were manually removed from all the plots.

Plant sampling and measurement

For measurements, above-ground portion of the plants in a four-meter row section from sole crops of sweet sorghum and legume was harvested during the physiological maturity to obtain dry matter of leaf, stalk and grains of sweet sorghum, and stubble and seeds of legumes. From intercropping, both sweet sorghum and legume plants in adjacent rows were harvested. These plant materials were oven-dried at 70 °C until a constant weight was reached, and weights were recorded. From the same plant samples, sub samples of leaf, stalk and grain were taken for the analysis of soluble sugar, starch, cellulose, and hemicellulose contents. Sample preparation and analysis were adopted as described by Sadasivam and Manickam (2005).

Bioethanol Yield Computation

Bioethanol yield (BEY) was calculated using dry weight (t ha⁻¹) of stalk, leaves and grains of sorghum, plant stubble and seed weight of legumes, and soluble sugars, starch, cellulose, and hemicellulose in dry matter obtained from chemical analysis, and using

following equations as described by others (IJE, 2006; Zhao *et al.*, 2009).

$$\text{BEY (soluble sugars)} = \text{DW} \times \text{S}_1 \times \text{F}_1 \times \text{E}_1 \times \text{S}_2 \quad \text{--- Eq. 1}$$

$$\text{BEY (starch)} = \text{DW} \times \text{S}_1 \times \text{F}_2 \times \text{F}_1 \times \text{E}_1 \times \text{S}_2 \quad \text{--- Eq. 2}$$

$$\text{BEY (cellulose \& hemicelluloses)} = \text{DW} \times \text{S}_1 \times \text{F}_3 \times \text{E}_2 \times \text{F}_1 \times \text{E}_1 \times \text{S}_2 \quad \text{--- Eq. 3}$$

Where,

BEY = Bioethanol yield, L ha⁻¹

DW = Dry weight of plant part, t ha⁻¹

S₁ = Chemical substance in percentage of dry weight of plant part

F₁ = Conversion factor from sugar to ethanol (0.51)

E₁ = Process efficiency from sugar to ethanol (0.85)

F₂ = Conversion factor from starch to sugar (1.11)

F₃ = Conversion factor from cellulose or hemicellulose to sugar (1.11)

E₂ = Process efficiency from cellulose or hemicelluloses to sugar (0.85)

S₂ = Specific gravity of ethanol (1000/0.79)

Data Analysis

Orthogonal contrast procedure was used to compare the performance between intercropping and sole crops, while the analysis of variance was adopted to determine the significance of the contribution by experimental treatments and their interactions using

the SAS program. Fisher's protected Least Significant Difference (LSD) was used for comparing the treatment means (Steel and Torrie, 1980).

Results

Climatic Conditions

Accumulated solar radiation and mean air temperature were greater in the year 2009/10 (791.0 KW m⁻² and 29.4 °C, respectively) than in the year 2010/11 (721.0 KW m⁻² and 28.7 °C, respectively) while, cumulative rainfall was greater in the year 2009/10 (1054.1 mm) than in the year (87.6 mm).

Biomass Production

Above ground biomass (AGB) of intercropped sweet sorghum was significantly ($P \leq 0.001$) greater in the 2009/10 (38.2 t ha⁻¹) than in the 2010/11 (33.3 t ha⁻¹) (Table 1). Above ground biomass of sweet sorghum decreased significantly ($P \leq 0.001$) in intercropping with mungbean (30.5–35.5 t ha⁻¹), but remained at par ($P > 0.05$) with soybean (36.0–41.0 t ha⁻¹) compared to sole cropping (36.7–41.2 t ha⁻¹) (Appendix Table 1, Table 2). In 2009/10, AGB significantly ($P \leq 0.001$) reduced in the alternate double row pattern (ADR) (36.7 t ha⁻¹) compared to the alternate single row pattern (ASR) (39.8 t ha⁻¹) and, in the simultaneous seeding (35.8 t ha⁻¹) compared to the staggered seeding (40.7 t ha⁻¹) (Appendix Table 2, Table 2).

Table 1. Year analysis of above ground biomass and chemical composition of intercropped sweet sorghum and bioethanol yield of sweet sorghum and legume intercropping.

Parameter	MS for year	2009/10	2010/11	MS for error	CV, %
Intercropped sweet sorghum					
Biomass, t ha ⁻¹	301.0*** ^{1/}	38.2	33.3	5.36	6.5
Soluble sugar, %	68.4***	27.0	24.6	2.78	6.5
Cellulose, %	115.6***	15.2	12.1	2.06	10.5
Hemicelluloses, %	1.6	13.1	12.7	2.04	11.1
Starch, %	11.2***	6.3	5.4	0.27	8.9
Intercropping Bioethanol yield, L ha ⁻¹					
Sweet sorghum + Mungbean	46685908.9***	12510.4	9720.9	644083.0	7.2
Sweet sorghum + Soybean	65644944.9***	15473.2	12165.5	1036902.2	7.4
Sweet sorghum + legume	111525104.7***	13991.8	10943.2	784792.8	7.1

^{1/}** and ***- indicate the significance of the comparison between sole and intercropping at p=0.01 and 0.001, respectively.

In 2010/11, there were significant two-way interactions on AGB of sweet sorghum (Appendix Table 2): between type of legume and time of seeding (Fig 1a) and between planting pattern and time of seeding (Fig 1b).

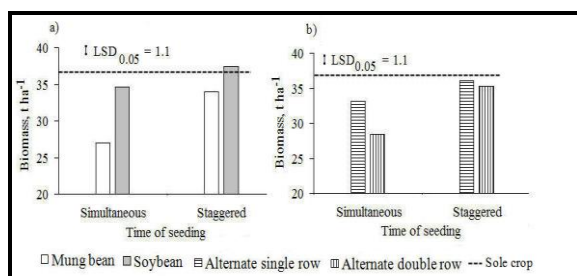


Fig. 1. Two-way interactions between intercrop legume and time of seeding (a) and between planting pattern and time of seeding (b) for biomass yield of sweet sorghum in 2010/11.

In the former interaction, soybean combined with staggered seeding produced the highest AGB of sweet sorghum (37.4 t ha⁻¹) among the rest of the combinations and was also greater than the sole crop. In contrast, mungbean and simultaneous seeding combination yielded the lowest AGB (27.0 t ha⁻¹). According to latter interaction, AGB was highest in the ASR pattern and staggered seeding combinations

(36.1 t ha⁻¹), but significantly (P = 0.05) reduced in simultaneous seeding regardless of its combination with ASR pattern (33.2 t ha⁻¹) or ADR pattern (28.4 t ha⁻¹). However, staggered seeding reduced the difference in AGB between the two planting patterns (P > 0.05).

The AGB of legume included dry weight of stubbles and grain. The AGB of mungbean significantly (P ≤ 0.01) reduced in intercropping with sweet sorghum (3.3–3.5 t ha⁻¹) compared to sole crop (4.1–4.8 t ha⁻¹) (Appendix Table 3, Table 3). The AGB significantly (P < 0.5) reduced in the ASR pattern (3.0 t ha⁻¹) compared to the ADR pattern (3.5–4.1 t ha⁻¹) and in the simultaneous seeding (2.7–2.8 t ha⁻¹) compared to the staggered seeding (3.8–4.3 t ha⁻¹) (Appendix Table 4, Table 3). Similar to mungbean, AGB of soybean was also reduced significantly (P ≤ 0.001) in intercropping (2.7–3.2 t ha⁻¹) compared to sole stand (3.6–3.9 t ha⁻¹) (Appendix Table 3, Table 3), and in intercropping, in the ASR pattern (2.5–2.9 t ha⁻¹) compared to the ADR pattern (3.0–3.6 t ha⁻¹) and in the simultaneous seeding (2.2–2.8 t ha⁻¹) compared to the staggered seeding (3.3–3.6 t ha⁻¹) (Appendix Table 3, Table 4).

Table 2. The effect of type of legume, planting pattern and time of seeding on biomass, cellulose, hemicelluloses, soluble sugars, and starch contents of intercropped sweet sorghum in 2009/10 and 2010/11.

Treatment	2009/10					2010/11				
	Biomass, t ha ⁻¹	Cellulose, %	Hemi-celluloses, %	Soluble sugars, %	Starch, %	Biomass, t ha ⁻¹	Cellulose, %	Hemi-celluloses, %	Soluble sugars, %	Starch, %
Sole cropping	41.2 ± 0.9	17.6 ± 0.5	16.3 ± 2.1	29.1 ± 1.2	6.5 ± 0.6	36.7 ± 3.2	14.7 ± 1.9	14.9 ± 1.0	27.1 ± 1.4	6.5 ± 1.1
Intercropping										
Intercrop legume										
Mungbean	35.5 ± 1.6	13.8 ± 1.1	11.9 ± 1.3	25.7 ± 1.8	6.4 ± 0.8	30.5 ± 2.5	11.0 ± 1.8	11.4 ± 1.0	23.3 ± 1.4	5.0 ± 0.4
Soybean	41.0 ± 2.0	16.7 ± 0.6	14.3 ± 1.3	28.3 ± 1.6	6.3 ± 0.7	36.0 ± 4.1	13.3 ± 1.5	14.1 ± 0.9	26.0 ± 2.1	4.5 ± 0.4
LSD (p=0.05)	1.6	0.8	1.3	1.4	ns ^{2/}	1.1	1.5	0.9	0.7	0.4
Planting pattern ^{1/}										
ASR	39.8 ± 2.1	16.2 ± 0.7	14.3 ± 1.0	27.9 ± 1.3	6.3 ± 0.8	34.6 ± 3.2	13.7 ± 1.3	13.5 ± 0.7	25.4 ± 1.6	5.6 ± 0.3
ADR	36.7 ± 1.5	14.3 ± 1.0	11.9 ± 1.6	26.2 ± 2.2	6.4 ± 0.7	31.9 ± 3.4	10.6 ± 2.0	12.0 ± 1.2	23.9 ± 1.9	3.8 ± 0.4
LSD (p=0.05)	1.6	0.8	1.3	1.4	ns	1.1	1.5	0.9	0.7	ns
Time of seeding										
Simultaneous	35.8 ± 1.7	13.6 ± 1.4	11.9 ± 1.3	26.0 ± 2.3	6.4 ± 0.8	30.8 ± 2.9	11.1 ± 1.9	11.0 ± 1.4	22.5 ± 1.8	5.2 ± 0.5
Staggered	40.7 ± 1.9	16.9 ± 0.3	14.3 ± 1.3	28.1 ± 1.1	6.3 ± 0.6	35.7 ± 3.7	13.1 ± 1.4	14.4 ± 0.5	26.7 ± 1.6	4.2 ± 0.3
LSD (p=0.05)	1.6	0.8	1.3	1.4	ns	1.1	1.5	0.9	0.7	0.4

1/ ASR – Alternate single rows; ADR – Alternate double rows

2/ ns – not significant at p=0.05

Table 3. Effect of planting pattern and time of seeding on biomass, cellulose, hemicelluloses, and starch contents of mungbean in 2009/10 and 2010/11.

Treatment	2009/10				2010/11			
	Biomass, t ha ⁻¹	Cellulose, %	Hemi-celluloses, %	Starch, %	Biomass, t ha ⁻¹	Cellulose, %	Hemi-celluloses, %	Starch, %
Sole cropping	4.8 ± 1.3	33.1 ± 5.9	27.2 ± 7.5	15.6 ± 6.0	4.1 ± 0.5	29.3 ± 0.4	20.1 ± 0.8	24.4 ± 2.7
Intercropping								
Planting pattern ^{1/}								
ASR	3.0 ± 0.3	39.4 ± 2.9	22.1 ± 2.5	06.9 ± 0.7	3.0 ± 0.4	27.4±2.9	15.4±2.8	23.2±3.4
ADR	4.1 ± 0.9	32.9 ± 3.3	19.4 ± 3.3	12.3 ± 3.5	3.5 ± 0.4	28.2±2.4	16.4±2.1	22.1±2.1
LSD (p=0.05)	1.0	3.2	ns ^{2/}	4.2	0.2	ns	ns	ns
Time of seeding								
Simultaneous	2.8 ± 0.1	37.2 ± 3.8	13.2 ± 2.0	06.4 ± 0.8	2.7 ± 0.5	25.6±3.9	13.6±3.7	21.4±4.4
Staggered	4.3 ± 1.0	35.0 ± 2.4	28.4 ± 3.8	12.8 ± 3.3	3.8 ± 0.4	29.9±1.3	18.1±1.2	23.9±1.2
LSD (p=0.05)	1.0	ns	ns	4.2	0.2	2.3	2.9	ns

1/ ASR – Alternate single rows; ADR – Alternate double rows

2/ ns – not significant at p=0.05

Table 4. Effect of planting pattern and time of seeding on biomass, cellulose, hemicelluloses, and soluble sugar contents of soybean in 2009/10 and 2010/11.

Treatment	2009/10				2010/11			
	Biomass, t ha ⁻¹	Cellulose, %	Hemi-celluloses, %	Soluble sugars, %	Biomass, t ha ⁻¹	Cellulose, %	Hemi-celluloses, %	Soluble sugars, %
Sole cropping	3.9 ± 0.8	30.1 ± 3.1	21.3 ± 2.7	9.5 ± 1.2	3.6 ± 0.7	34.0 ± 2.2	14.7 ± 2.5	6.5 ± 2.6
Intercropping								
Planting pattern ^{1/}								
ASR	2.9 ± 0.3	28.2 ± 2.8	19.8 ± 3.8	6.5 ± 1.1	2.5 ± 0.4	25.0 ± 0.8	10.4 ± 2.2	6.2 ± 1.7
ADR	3.6 ± 0.9	26.7 ± 6.0	17.5 ± 4.5	8.3 ± 3.1	3.0 ± 0.4	27.8 ± 3.1	11.2 ± 4.2	4.7 ± 1.1
LSD (p=0.05)	0.5	ns ^{2/}	ns	ns	0.4	ns	ns	ns
Time of seeding								
Simultaneous	2.8 ± 0.6	24.5 ± 4.5	16.5 ± 5.6	5.7 ± 2.0	2.2 ± 0.2	20.2 ± 1.7	8.6 ± 3.4	4.8 ± 1.2
Staggered	3.6 ± 0.6	30.4 ± 4.3	20.9 ± 2.7	9.1 ± 2.2	3.3 ± 0.6	32.6 ± 2.2	13.0 ± 2.9	6.2 ± 1.5
LSD (p=0.05)	0.5	4.8	3.5	2.6	0.4	2.9	4.3	ns

1/ ASR – Alternate single rows; ADR – Alternate double rows

2/ ns – not significant at p=0.05

Bioethanol Yielding Chemical Substances

Bioethanol yielding chemical substances, i.e. cellulose, hemi-cellulose, soluble sugar, and starch of intercropped sweet sorghum were reduced in 2010/11 compared to 2009/10 (Table 1). However, the reduction was significant ($P \leq 0.05$) for cellulose, soluble sugar and starch (12.1, 24.6 & 5.4%, respectively, in 2010/11 and 15.2, 27.0 & 6.3%, respectively, in 2009/10). Sole crop of sweet sorghum had significantly ($P \leq 0.05$) greater contents of each of the substances compared to intercropping with mungbean (Appendix Table 1, Table 2). Only the hemi-cellulose content in 2009/10 (14.3%) and soluble sugar (26.0%) and starch (5.8%) contents in 2010/11 were reduced significantly ($P < 0.05$) in sweet sorghum when intercropped with soybean compared to sole cropping.

Cellulose content was significantly ($P \leq 0.01$) reduced in the simultaneous seeding (11.1%) compared to the staggered seeding (13.1%) in 2010/11 (Appendix Table 2, Table 2). There were two-way interactions (Appendix Table 2): for cellulose between legume type and time of seeding (Fig 2a) and between planting pattern and time of seeding (Fig 2b) in 2009/10, and also between legume type and planting pattern in the 2010/11 (Fig 2c). According to the first, second and third interactions, cellulose content was highest in the combinations of soybean and staggered seeding (17.6%), ASR pattern and staggered seeding (17.3%), and soybean and ASR pattern (14.0%) while, the lowest was in the combination of mungbean and simultaneous seeding (11.3%), the ADR pattern and simultaneous seeding (12.2%) and mungbean with ADR pattern (8.6%), respectively.

Hemi-cellulose content was reduced significantly ($P \leq 0.001$) in the ADR pattern than the ASR pattern in 2009/10 and was also influenced by two-way interactions: between legume type and time of seeding in the same year (Fig 2d), and between legume type and the planting pattern (Fig 2e) and also between planting pattern and the time of seeding (Fig. 2f) in 2010/11. Compared to hemi-cellulose content in the sole cropped sweet sorghum (16.3% in the first year and 14.9% in the second year, the minimal decrease was found in the combination of soybean and the staggered seeding (14.7%), soybean and the ASR pattern (14.3%) and the ASR pattern and staggered seeding (14.6%). The maximum decrease was in combination of mungbean and simultaneous seeding (9.9%), mungbean and the ADR pattern (10.0%), and ADR pattern and simultaneous seeding (9.8%), respectively.

Soluble sugars in intercropped sweet sorghum were significantly lower ($P \leq 0.01$) in intercropping with mungbean (25.7%) than with soybean (28.3%), in the ADR pattern (26.2%) than ASR pattern (27.9%) and in the simultaneous seeding (26.0%) than staggered seeding (28.1%) in 2009/10. In 2010/11, soluble sugar content of intercropped sweet sorghum was influenced by two-way interactions between the legume type and the time of seeding (Fig 2g) and between the planting pattern and the time of seeding (Fig 2h). According to the former interaction, the highest soluble sugars were in soybean and staggered seeding (27.1%) while the lowest in the mungbean and simultaneous seeding combination (20.1%). The soybean and staggered seeding combination significantly improved the soluble sugars to the level at par with the sole cropped sweet sorghum. The latter interaction showed the improvement of soluble sugars in the staggered seeding compared to simultaneous seeding regardless of the planting pattern.

Starch content of sweet sorghum in intercropping was significantly influenced by the two-way interaction between legume type and the time of seeding in

2010/11 (Fig 2i). The highest starch content was in soybean and staggered seeding combination (5.8%) while the lowest in mungbean and simultaneous seeding combination (4.7%). However, none of the interactions did yield starch at least at par with the sole cropped sweet sorghum. In mungbean, cellulose, hemicelluloses and starch contents were reduced in intercropping compared to sole stand and the reduction was significant for cellulose in 2010/11 (26.9%), for hemicelluloses in the first (20.8%) and second (15.2%) years and for starch only in first year (9.6%) ($P < 0.05$). Similarly, cellulose content was also significantly reduced ($P \leq 0.001$) in the ADR pattern (32.9%) compared to the ASR pattern (39.4%) in 2009/10 (Appendix Table 4, Table 3).

In 2010/11, there was a two-way interaction between the planting pattern and the time of seeding for cellulose content (Appendix Table 4, Fig 3a), which showed the highest and significantly greater cellulose content in the combination of ASR pattern and staggered seeding (30.3%). The same combination had greater cellulose than mungbean sole crop. In 2010/11, hemi-cellulose content in mungbean was significantly reduced ($p \leq 0.001$) in the simultaneous seeding (14.4%) compared to staggered seeding (15.9%). In 2009/10, starch content in mungbean was significantly ($P \leq 0.05$) reduced in the ASR pattern (6.9%) compared to ADR pattern (12.3%) and in the simultaneous seeding (6.4%) compared to staggered seeding (12.8%) (Table 3). Cellulose, hemicelluloses and soluble sugar contents in soybean were decreased in intercropping compared to its sole crop in both the years (Appendix Table 3, Table 4). But significant difference was found for cellulose in 2010/11 ($P \leq 0.05$) and for hemicelluloses in both the years ($P \leq 0.05$). The simultaneous seeding significantly reduced cellulose and hemicelluloses contents in both the years and soluble sugars in 2010/11 compared to staggered seeding ($P \leq 0.05$) (Appendix Table 4, Table 4). There was a significant two-way interaction between planting pattern and time of seeding for the cellulose content in 2010/11 (Fig 3b): the highest cellulose content was in the ASR pattern and

staggered seeding combination (33.0%) followed by the ASR pattern and staggered seeding combination (32.2%) while, the lowest cellulose was in the simultaneous seeding and ASR pattern combination (17.1–23.3%).

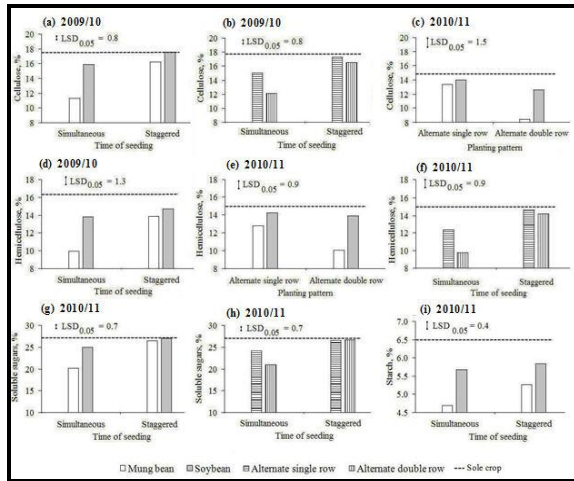


Fig. 2. Two-way interactions: between intercrop legume and planting pattern for cellulose (c) and hemicelluloses (e) in 2010/11; between intercrop legume and time of seeding for cellulose (a) and hemicelluloses (d) in 2009/10 and soluble sugar (g) and starch (i) in 2010/11; and between planting

pattern and time of seeding for cellulose in 2009/10 (b) and hemicelluloses and soluble sugar (h) in 2010/11 (Sole crop performance of sweet sorghum was shown by horizontal line).

Bioethanol Yield

Average bioethanol yield (BEY) of sweet sorghum-legume intercropping significantly ($P \leq 0.001$) decreased (10943.0 L ha⁻¹) in 2010/11 compared to 2009/10 (13992.0 L ha⁻¹) (Table 1). The bioethanol yield was significantly ($P \leq 0.001$) lower in the sweet sorghum-mungbean intercropping (12510.4 L ha⁻¹ in 2009/10 and 9721.0 L ha⁻¹ in 2010/11) than sweet sorghum sole crop (15424.6 L ha⁻¹ in 2009/10 and 12516.0 L ha⁻¹ in 2010/11) (Appendix Table 1). Sweet sorghum-soybean association produced BEY at par with the sole cropped sweet sorghum in 2009/10 (15473.2 L ha⁻¹) and in 2010/11 (12165.5 L ha⁻¹) ($P > 0.05$). Within intercropping, the BEY was significantly ($p \leq 0.001$) influenced by planting pattern in 2009/10 whereby the ADR pattern decreased BEY (13057.2 L ha⁻¹) compared to the ASR pattern (14926.4 L ha⁻¹) (Appendix Table 5, Table 5).

Table 5. The effect of type of legume, planting pattern and the time of seeding on bioethanol production of intercropping systems in 2009/10 and 2010/11.

Treatment	2009/10	2010/11
Sole cropping	15424.6 ± 789.0	12515.9 ± 1212.2
Intercropping		
Intercrop legume		
Mungbean	12510.4 ± 537.0	9720.9 ± 877.0
Soybean	15473.2 ± 930.3	12165.5 ± 1275.3
LSD (p=0.05)	700.0	441.0
Planting pattern		
ASR1/	14926.4 ± 755.8	11781.7 ± 1119.3
ADR	13057.2 ± 711.6	10104.7 ± 1033.1
LSD (p=0.05)	700.0	441.0
Time of seeding		
Simultaneous	12109.3 ± 820.8	9160.2 ± 1023.2
Staggered	15874.3 ± 646.5	12726.2 ± 1129.1
LSD (p=0.05)	700.0	441.0

1/ ASR – Alternate single rows; ADR – Alternate double rows

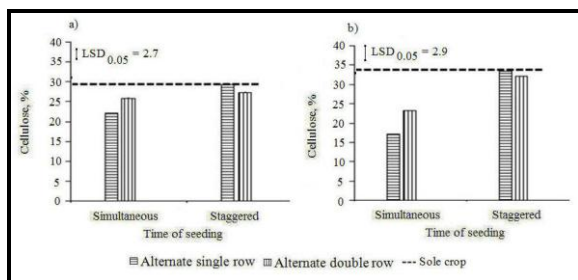


Fig. 3. Two-way interactions between planting pattern and time of seeding for cellulose contents in mungbean (a) and soybean (b) in 2010/11 (Sole crop performance of legumes is shown by horizontal line).

Moreover there were two-way interactions ($P \leq 0.001$): between legume type and time of seeding in 2009/10 (Fig 4a) and 2010/11 (Fig 4b), and between legume type and the planting pattern (Fig 4b) and between planting pattern and the time of seeing (Fig 4d) in 2010/11. According to the first, second and third interactions, BEY was highest in soybean and staggered seeding (13410.0–16673.0 L ha⁻¹), soybean and the ASR pattern (12750.0 L ha⁻¹) and ASR pattern and staggered seeding (13093.0 L ha⁻¹) combinations where as was lowest in mungbean and simultaneous seeding (7402.0–9944.0 L ha⁻¹), mungbean and the ASR pattern (8625.0 L ha⁻¹) and the ADR pattern and simultaneous seeding (7850.0 L ha⁻¹) combinations.

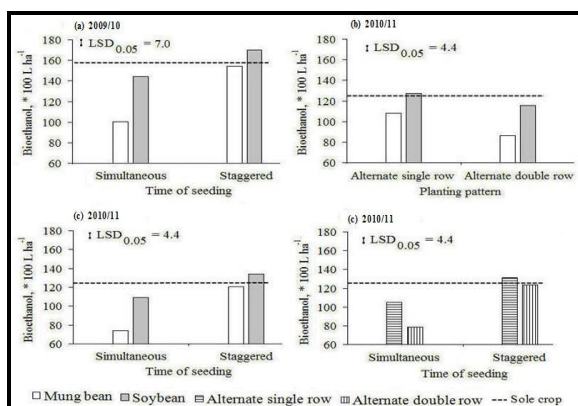


Fig. 4. Two-way interactions for bioethanol yield of intercropping system: between intercrop legume and planting pattern in the 2010/11 (b), between intercrop legume and time of seeding in 2009/10 (a) and 2010/11(c), and between planting pattern and time of seeding in 2010/11 (d). (Sole crop performance of sweet sorghum is shown by horizontal line).

Discussions

The above ground biomass (AGB) production of individual crops of sweet sorghum, mungbean and soybean was greater in sole stands than intercropping. Intercropping with mungbean reduced the AGB of sweet sorghum by 5.7 t ha⁻¹ in 2009/10 and by 6.2 t ha⁻¹ in 2010/11. But with soybean, the reduction was 0.2 and 0.7 t ha⁻¹ in 2009/10 and 2010/11, respectively ($P > 0.05$). However, these reductions were attributed to inter-specific competition from legume (Egbe and Adeyemo, 2006). Mungbean has a faster growth and rapid ground cover formation than soybean (Dhope *et al.*, 1992) and hence its more aggressive growth appeared to have affected the growth of sweet sorghum thus reducing AGB by 5.5 and 8.0 t ha⁻¹ in 2009/10 and 2010/11, respectively. This competition may have been aggravated by the high population used. As reported by Ranamukhaarachchi (1985), one-third to two-thirds of mungbean was found appropriate for corn-mungbean intercropping. Dhope *et al.* (1992), Subbian and Selvaraju (2000), and Singh and Jadhav (2003) also reported a greater yield reduction of sorghum in intercropping with mungbean or pigeon pea than with soybean. This decline in intercropping could be related to their spatial arrangements in the system leading to unfair acquisition of available soil and above ground resources (Beets, 1982; Sharma, 1994; Egbe and Bar-Anyam, 2011).

The AGB of sweet sorghum in the ADR pattern was significantly reduced during 2009/10 by 3.1 t ha⁻¹ and by 2.7 t ha⁻¹ during 2010/11, compared to ASR pattern. The reduction could be attributed to greater interference for resources (nutrients) by better growth of legume and mutual shading among sweet sorghum plants as a result of reduced spacing between its rows in the ADR pattern. However, the staggered seeding of sweet sorghum helped delay its emergence during the rapid growth period of the legume and thus separating peak resource requiring phases of the two component crops. Staggered seeding also facilitated the intercropped legume to complete most of its vegetative phase before the

linear growth phase of sweet sorghum begins and thus increasing the biomass yields of both component crops. Therefore staggered seeding gave higher AGB of sweet sorghum (by 4.9 t ha⁻¹ in 2009/10 and 4.7 t ha⁻¹ in 2010/11) than simultaneous seeding, and appreciably reducing the difference in the AGB of sweet sorghum due to legume from 7.0 t ha⁻¹ in simultaneous seeding to 2.8 t ha⁻¹ in staggered seeding and due to the planting pattern from 4.8 t ha⁻¹ in simultaneous seeding to 0.8 t ha⁻¹ in staggered seeding in 2010/11.

The interaction between legume type and time of seeding in 2010/11 showed that sweet sorghum intercropped with soybean in staggered seeding produced the highest AGB (37.4 t ha⁻¹) compared to other treatment combinations. According to the interaction between planting pattern and the time of seeding, the ASR pattern and staggered seeding combination produced the highest AGB of 36.1 t ha⁻¹ compared to other treatment combinations. As explained above, these combinations had exposed sweet sorghum for favorable conditions, and hence increasing its AGB.

The AGB of mungbean and soybean was also reduced in intercropping compared to their sole crops by 1.3 and 0.7 t ha⁻¹, respectively, during 2009/10 and 0.8 and 0.9 t ha⁻¹, respectively, during 2010/11. The AGB of legume was also influenced by planting pattern and the time of seeding. Row arrangement determines the volume of soil accessible to each component crop and hence accessibility to resources in intercropping (Baker, 1978). Wider spacing (60 cm) in the ADR pattern helped legume use sunlight and the available soil resources more than in the ASR pattern. This resulted in greater AGB of the legume in the ADR pattern. Similar findings were also reported by Rashid *et al.* (2002) where the ASR pattern reduced vegetative growth of mungbean more than in the ADR pattern. The reduction also occurred when sweet sorghum was seeded simultaneously compared to staggered seeding. The former had more shading on legume than the latter. In addition, allelopathic

effects of sweet sorghum have also been reported to be partly responsible for the reduction of AGB (Moosavi *et al.*, 2011).

The percentage of cellulose, hemi-cellulose, soluble sugars and starch decreased in the AGB of sweet sorghum when intercropped with legume, and such reductions were significant when associated with mungbean. The reduction in hemicelluloses was significant in 2009/10 and soluble sugars and starch in 2010/11 when associated with soybean. Although reasons for such reductions were not clear, shading and inter-plant competition promoted by the year effects may have altered assimilate partitioning and utilization among different processes, thus changing the composition of each vital substance. Cellulose, hemi-cellulose and soluble sugars in sweet sorghum were reduced more in intercropping with mungbean than soybean. These reductions could partly be attributed to the reduction in the AGB of sweet sorghum due to legume and partly due to competition. In intercropping with soybean, the reduction in AGB was negligible and hence the above substances were higher in concentrations.

Changes in chemical composition in the biomass of intercropped sorghum were also reported by several others (Wanjari *et al.*, 1994; Mpairwe *et al.*, 2002; Rashid *et al.*, 2002; Javanmard *et al.*, 2008). Row pattern in additive mixtures alters the physico-chemical characteristics of component crops (Tsubo *et al.*, 2003; Azraf *et al.*, 2006; Bildirici *et al.*, 2009). In the current study, the ADR pattern reduced the cellulose, hemicellulose, soluble sugars and starch compared to the ASR pattern. Reductions in cellulose, hemi-cellulose and soluble sugar contents in AGB under simultaneous seeding compared to the staggered seeding were significant. However, the difference in starch content was insignificant between the two seeding times. The staggered seeding regardless of the type of legume and the row pattern produced cellulose, hemi-cellulose and soluble sugars at par with the sole cropped sweet sorghum. The reduction in differences of such substances could be

attributed to the separation of peak growth periods of legume and sweet sorghum and providing competition free period for the two components to proceed with synthesizing such substances as also observed by Nnko and Doto (1982). According to the interaction between the legume type and the time of seeding, soybean combined with the staggered seeding decreased the difference in soluble sugars in 2009/10, and cellulose and hemi-cellulose in 2010/11 compared to sole cropping. Similarly, the interaction between the planting pattern and the time of seeding, the ASR pattern and staggered seeding combination also decreased the difference in the same substances between intercropped and sole cropped sweet sorghum, which may be due to reduced competition by ASR pattern and staggered seeding, thus providing the opportunity for normal/unaffected growth in intercropping compared to sole cropping.

Cellulose and hemi-cellulose contents of intercropped mungbean significantly decreased compared to its sole cropping during both the years, but the starch content remained unchanged. The senescence of leaves associated with competition for resources and shading by tall sweet sorghum may have led to such difference. The reduction and increment in the contents of chemical substances in intercropping patterns were attributed to the interaction between row pattern and time of seeding. The row pattern through its exposure to different degree of shading significantly decreased the cellulose contents and improved the starch contents in 2009/10. As increasing tissues will increase maintenance respiration requirement, shading appeared to have reduced excessive tissue development and increases assimilate storage as a way to reduce impact of shading.

In addition, the cellulose content of mungbean decreased while the starch content increased in the ADR pattern compared to the ASR pattern in 2009/10. In the ADR pattern, more assimilates might have been transferred from vegetative to reproductive parts to support seed quality under increased

competition, thus increasing the starch and decreasing the cellulose contents compared to the ASR pattern. Similarly, staggered seeding resulted in significant increase in the chemical substances in the AGB of mungbean in both the years compared to simultaneous seeding. Mungbean was heavily shaded by sweet sorghum in simultaneous seeding. The competition promoted assimilate partition towards developing seeds and the shading continued for a longer period thus resulting in the senescence of leaves and reducing chemical substances in AGB. Cellulose and hemi-cellulose decreased significantly in intercropped soybean compared to its sole cropping in 2010/11, but increased in the ADR pattern compared to the ASR pattern. In the staggered seeding, soybean produced greater cellulose, hemi-cellulose and soluble sugar contents than simultaneous seeding in 2009/10. According to the interaction between the planting pattern and the time of seeding in 2010/11, the ADR pattern and staggered seeding combination produced cellulose contents lower than sole cropped soybean. Therefore, the contents of chemical substances of soybean reduced in intercropping compared to the sole cropping and this agrees with the findings of Mahmoud *et al.* (1990) and Khan *et al.* (2002).

Bioethanol yield from sweet sorghum in sole cropping was greater by 18% in the year 2009/10 and 22% in the year 2010/11 compared to the total BEY from sweet sorghum-mungbean intercropping system, but at par with the sweet sorghum-soybean intercropping system. Decrease in the AGB and its chemical substances led to the reduction of BEY by 12% in the ADR pattern compared to the ASR pattern during 2009/10. The interaction between soybean and staggered seeding increased BEY by 8% (16672.6 L ha⁻¹) in 2009/10 and 7% (13410.0 L ha⁻¹) in 2010/11 compared to sole cropped sweet sorghum, and in addition the ASR pattern and staggered seeding combination in 2010/11 also produced 13093.0 L ha⁻¹ which was 5% higher than sole cropped sweet sorghum.

Conclusions

Biomass based bioethanol (BEY) production was found well suited for intercropping sweet sorghum with soybean compared to sole cropped sweet sorghum. Above ground biomass and composition of bioethanol yielding chemical substances of intercropped sweet sorghum were decreased in intercropping established with mungbean, alternate double row pattern and simultaneous seeding compared to that in intercropping established with soybean, alternate single row pattern and staggered seeding. The highest BEY was observed in sweet sorghum-soybean association established with alternate single row pattern and staggered seeding and the lowest was in sweet sorghum-mungbean intercropping established with alternate double row pattern and simultaneous seeding.

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