

# Bio-methane Production from Sorghum Elite Lines under the Climatic Conditions of Pakistan

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## Abstract

Fossil foils are depleting dramatically to meet the ever blooming energy demands. Plant biomass is a best source of renewable energy which can be used for bio-fuel production in order to meet the energy demands. Therefore, this study was conducted for two consecutive years 2016 and 2017 to screen out best lines of sorghum for biomass yield, chemical composition and bio-methane yield. The results revealed that tested lines had differential responses for biomass yield, biomass quality and methane yield. Line 5018, performed remarkably and produced maximum leaf area index (LAI), leaf area duration (LAD) and crop growth rate (CGR) followed by L-6024 whereas the minimum LAI, LAD and CGR were recorded for L-5025. Maximum plant height, leaves per plant and dry matter yield ha<sup>-1</sup> was observed in L-5018, whereas the minimum plant height leaves per plant and dry matter yield ha<sup>-1</sup> was recorded in L-5025. Likewise, L-5018 also had maximum protein content, acid detergent fiber, neutral detergent fiber, lignin content and ash content whereas the L-1914 had the minimum values for these parameters amongst the tested lines. In addition, L-1914 produced maximum specific yield, however, L-5018 produced maximum methane yield ha<sup>-1</sup> owing to higher dry matter yield ha<sup>-1</sup>. The results of this study suggested that L-5018 can be used to develop high biomass cultivars with good methane yield potential.

**KeyWords:** Lines, DM yield, Biomass quality, Bio-methane,

## Introduction

The energy consumption and demands are increasing rapidly around the globe, owing, to ever blooming population and development of economies (Zhou et al, 2011). Amongst the energy sources, fossil fuels are at the top of list (Midilli et al, 2006; Kibazohi and Sangwan 2011) nonetheless, these resources are limited and major cause of greenhouse gas emissions (Petersson et al, 2007). Therefore, to conquer the challenges of depleting fossil fuel resources and threats of climate change and global warming due to burning of fossil fuels, world have decided to look for sustainable and environmental friendly energy sources (Midilli et al, 2006; Zhuang et al, 2011). Plant biomass identified as prime energy source in the scenarios of jeopardizing fossil fuels and increase in greenhouse gas emissions (Vander et al, 2011). Among plant biomass, particularly the energy crops are copious, versatile and renewable energy source which are considered to be important for reducing the greenhouse gas emissions and contributing to the safeguarding of energy supply in future (Zhou et al, 2011; Zhuang et al, 2011).

Sorghum (*Sorghum bicolor* Moench L.) is one of the prime sources explored as energy crop (Hassan et al, 2018). Sorghum produces a biomass compositionally

similar to that of maize, and is known to be highly productive for biomass in very short duration (Mahmood et al, 2013). The versatile characteristics, including low water, and nutrient requirements and drought, water logging and salinity tolerance, makes it more suitable energy crop (Vasilakoglou et al, 2011). In addition, it is short growing and requires less input, moreover, it can be grown successfully in arid and semi arid regions (Reddy and Sanjana 2003). Therefore, the sorghum is good source for bio-energy production in order to meet the energy requirements (Reddy et al, 2005).

Similarly, the suitable selection of cultivar plays a major role in final biomass yield, chemical composition and ultimately the bio-gas yield. The success of any cultivar for bio-methane purpose mainly depends on chemical composition and biomass yield. Cultivars may vary in terms of growth behavior, biomass yield and chemical composition (Ayub et al, 1998; Hassan et al, 2018). Moreover, Bertoia et al. (2006) also found the remarkable changes among the cultivars in the context of biomass yield whereas, Ashbell et al, (1999) reported that cultivars differed significantly, in chemical composition including the sugar contents, protein contents and acid and neutral detergent fiber contents. The composition of biomass and its biodegradability

play a crucial role in the final bio-methane yield (Mahmood et al, 2015). The compositional compounds including, proteins, fat, fiber contents, sugars, cellulose and hemicelluloses significantly influence the bio-fuel yield (Amon et al, 2007).

Thus, great variations can be expected among the sorghum cultivars for biomass yield and chemical composition, both of these attribute ultimately affect the bio-methane production. Therefore, in order to use the sorghum as bio-energy crop, under the prevailing conditions of Pakistan the selection of appropriate cultivar is of prime importance. In addition, no study is available regarding the use of sorghum for bio-methane purpose in Pakistan. Therefore, this study was conducted to ascertain the potential of different lines of sorghum for biomass yield, chemical composition and bio-methane yield. The results of this research will also assist the breeders in developing new cultivars, with good biomass yield and chemical composition useful in the context of bio-methane production.

## Materials and Methods

### Experimental site and climate

The study was conducted during the year 2016 and 2017 at Post Graduate Agriculture Research (PARS), University of Agriculture Faisalabad Pakistan. Before sowing of crop, composite soil samples were collected from the depth of 0-30 cm to determine the various physical and chemical characteristics by the standard procedures as described by Homer and Pratt, (1961). The soil was sandy loam and averagely contained organic matter (0.89%), pH (7.95), nitrogen (0.03%) phosphorus (6.43 ppm) and potassium (186 ppm). The experimental site falls in semiarid region, moreover, the weather conditions during the crop growth periods are given in Table 1.

### Experimental design and plant Material

The experiment was carried out in randomized complete block design with three replications. The net plot size was 8×6.9 m. The seeds of Sorghum lines

i.e., 5025, 6024, 5018, 113 and 1914 were obtained from the Fodder Research Institute, Ayub Agriculture Research Institute Faisalabad, Pakistan.

### Soil preparation and crop management

Plots were ploughed three times followed by planking to obtain the required seedbed. Seeds were sown with hand drill by maintain the row to row distance of 30 cm with the seed rate of 75 kg ha<sup>-1</sup>. In both years, nitrogen (N) was applied as urea (46% N) at the rate of 60 kg ha<sup>-1</sup>, while phosphorus was applied as single super phosphate (21% P) at the rate of 40 kg ha<sup>-1</sup>. All the P and half of N were applied at the time of sowing, whilst the remaining N was applied with first irrigation. No potassium (K) was applied based on soil test results indicating that sufficient level. In total, three irrigations were applied during the whole crop season including the soaking irrigation. All the other management practices were kept uniform in order to get the good crop stand. The crop was sown on 2<sup>nd</sup> May and 6<sup>th</sup> May during the year 2016, and 2017 respectively. Moreover, the crop was harvested at physiological maturity manually on 16<sup>th</sup> August, 2016 and 20<sup>th</sup> August, 2017.

### Observations and Measurements

Leaf area was measured by using leaf area meter (CI-202, CID Bio-Science). Furthermore, leaf area index (LAI) was determined by the standard procedures of Watson (1947), while, leaf area duration (LAD) and crop growth rate (CGR) was measured by standard procedures as detailed by (Hunt, 1978). First LAI, LAD and CGR were measured after 40 days of sowing while the subsequent measurements were taken after 10 days interval. At maturity, ten plants were selected from each plot to measure the plant height, stem diameter and leaves per plant. Before harvesting, plants from 1m<sup>2</sup> area were harvested and separated into leaves, stems and panicles. The separated leaves, stems and panicles were individually dried and weight to determine their proportions. Moreover, whole plots were harvested and dried to determine the dry matter yield, and later on mathematically converted into per hectare basis.

**Table 1: Prevailing climatic conditions for the experimental site during year 2016 and 2017**

Months	Monthly mean maximum temperature (°C)		Monthly mean minimum temperature (°C)		Monthly average temperature (°C)		Rainfall (mm)		Relative Humidity (%)	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
May	39.8	41.1	25.6	26	32.7	33.5	25	10.1	28.8	29.8
June	40.2	39.8	28.5	27.3	34.4	33.5	39.9	41.6	38.9	44.5
July	36.6	38.5	27.4	28.9	32	33.7	193.5	161.4	59.6	70
August	35.7	38.1	26.5	28.6	31.1	33.4	48.1	66	62.2	68.9

### Biomass analysis

Plant samples were collected, oven dried, grounded and later on digested to determine various parameters. Protein and ash contents were determined by standard methods of AOAC, (1990), whereas, sugar contents and acid detergent fiber, neutral detergent fiber and lignin contents were measured by standard methods as detailed by Dubois et al, (1956), and Vansoest et al, (1991). Methane measurement was taken using Bioprocess Control's AMPTS equipment. Liquid manure was used a source of bacteria in order to an-aerobically digest the sorghum sample. The 16 g of substrate was used in each digester after that the total volume of digesters were made up to 400 ml. Afterwards, digesters was perched with nitrogen gas in order to create the an-aerobic conditions. The temperature of digesters was kept constant to 37°C by standing them in water bath. The samples were allowed to digest for 28 days, in laboratory. The methane produced by each sample on each day was recorded from computer operated systems. At the end by using the amount of volatile solids the quantity of specific methane produced by each sorghum sample was calculated. Later on the specific methane produced by each sample was converted into hectare basis mathematically.

### Statistical analyses

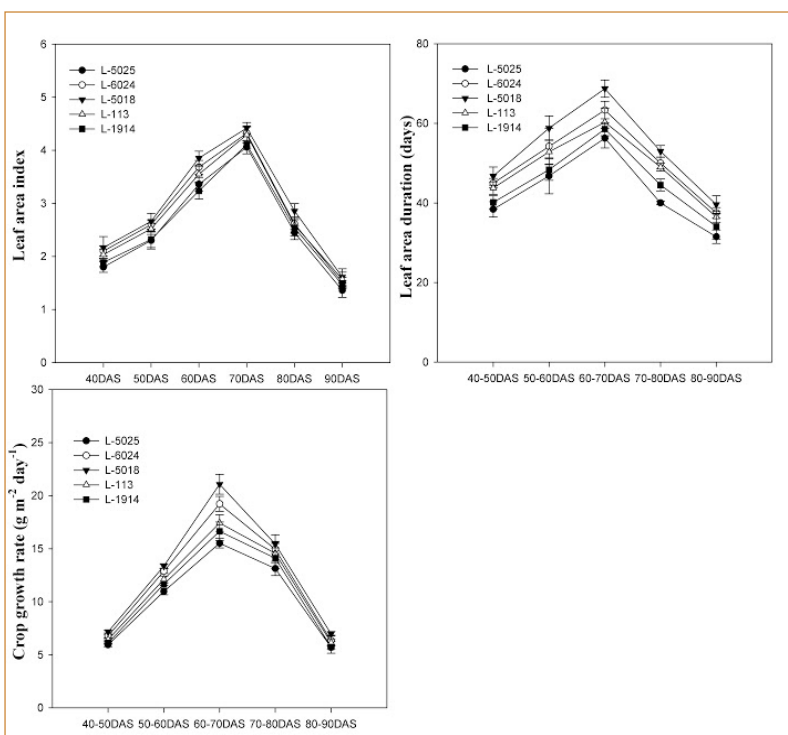
In combined analysis, year effect was found non-significant; therefore, data were pooled for both the years (2016 and 2017) and average was taken. The collected data were analyzed by computer software Statistix 8.1. The difference among the treatment means was compared by using the least significant difference test at 5% probability level (Steel et al, 1996). Graphs were made by using Sigma Plot 9.0.

## Results

### Growth attributes

Sorghum lines responded differently for growth attributes like, LAI, LAD and CGR (Fig 1 A,B,C). All the lines under investigation attained the maximum LAI 70 days after sowing (DAS), after that LAI was reduced. Line 5018 attained maximum LAI which was similar to L-6024, whereas the L-5025 produced the minimum LAI among the lines. Likewise, after 70 DAS a rapid reduction in LAI was observed in L-5025, L-113 and L-1914, as compared to the L-5018 and L-6024 (Fig 1A). Similarly, maximum LAD and CGR was recorded at 60-70 DAS, and L-5018 produced the maximum LAD and CGR, whilst, L-5025 produced the minimum LAD and CGR. Nonetheless, a decreasing trend in LAD and CGR was also observed after 60-70 DAS, however, this

reduction was greater in L-5025, L-113 and L-1914 as compared to the L-5025 and L-6024 (Fig 1B, C). Lines also had differential response for the tillers m<sup>-2</sup> plant height stem diameter and leaves per plant. L-5018 produced maximum tillers m<sup>-2</sup> followed by L-6024, whereas the minimum tillers m<sup>-2</sup> was produced by L-5025. The maximum plant height (191 cm) was recorded in L-5018 that was similar to L-6024 and L-113, while, the minimum plant height (158.3 cm) was found in L-5025. L-6024 produced thick plant and had maximum stem diameter (1.16 cm), while the minimum stem diameter (0.86 cm) was recorded in L-5025. Likewise, maximum leaves per plant (12) were recorded for L-5018, followed by L-6024 and L-113, although the minimum leaves per plant (10) were found in L-5025 (Table 2).



**Figure 1: Effect of lines on leaf area index (A), leaf area duration (B) and crop growth rate (C) of sorghum bicolor.**

**Table 2: Effect of tested lines on yield attributes and dry matter yield of sorghum**

Lines	Tillers m-2	Plant height (cm)	Stem girth (cm)	Leaves per plant	Dry matter yield t ha <sup>-1</sup>
L-5025	8.00d	158.33c	0.86c	10.17d	11.26d
L-6024	12.00b	187.50a	1.16a	11.39b	13.00b
L-5018	14.00a	191.33a	1.05ab	11.96a	14.31a
L-113	10.00c	180.50ab	1.01bc	11.18b	12.35bc
L-1914	11.00 bc	170.83bc	0.92 bc	10.80c	11.85cd
LSD (p ≤0.05)	1.51	16.23	0.149	0.361	0.93

Means sharing different letters differed significant at  $p \leq 0.05$

**Table 3: Effect of tested lines on protein, sugar, acid detergent fiber (ADF), neutral detergent fiber (NDF), lignin and ash concentrations of sorghum**

Lines	Protein (%)	Sugar (%)	ADF (%)	NDF (%)	Lignin (%)	Ash (%)
L-5025	7.58d	8.6c	33.00bc	50.91b	4.06c	6.55d
L-6024	9.29b	10.50a	36.53ab	52.43b	4.75ab	7.21b
L-5018	10.00a	10.10a	37.50a	57.62a	5.21a	7.63a
L-113	8.26c	9.90ab	34.46ab	51.35b	4.33bc	7.05bc
L-1914	8.00cd	9.20bc	30.06c	50.43b	3.91c	6.78cd
LSD (p ≤0.05)	0.45	0.78	4.23	3.54	0.48	0.30

Means sharing different letters differed significant at  $p \leq 0.05$

### Biomass parameters

The tested lines had significant effect on biomass parameters, i.e. leaf biomass proportion, stem biomass proportion, panicle biomass proportion and dry matter yield (Fig 2). The maximum leaf biomass proportion was recorded in L-5018, whereas the minimum value of leaf biomass proportion was observed in L-1914. Likewise, maximum stem biomass proportion was recorded in L-5018, followed by L-113, moreover, the minimum stem biomass proportion was found L-5025. L-5025 had highest panicle biomass proportion (24.55%), while L-5018 was characterized by significantly lower panicle biomass proportion among the tested lines (Fig 2). L-5018 produced maximum dry matter yield (14.31 t ha<sup>-1</sup>) followed by L-6024 and L-113, whereas, L-5025 produced the minimum dry matter yield (Table 2).

### Chemical composition

The lines under study differed significantly ( $P \leq 0.05$ ) in concentrations of protein, sugar, acid detergent fiber, neutral detergent fiber, lignin and ash (Table 3). L-5018 accumulated the maximum protein content (10%), followed by the L-6024 (9.29), whereas 5025 accumulated the minimum protein content in biomass (7.58%). Similarly, L-6024 was characterized by high sugar concentration (10.50%), similar to L-5018, while

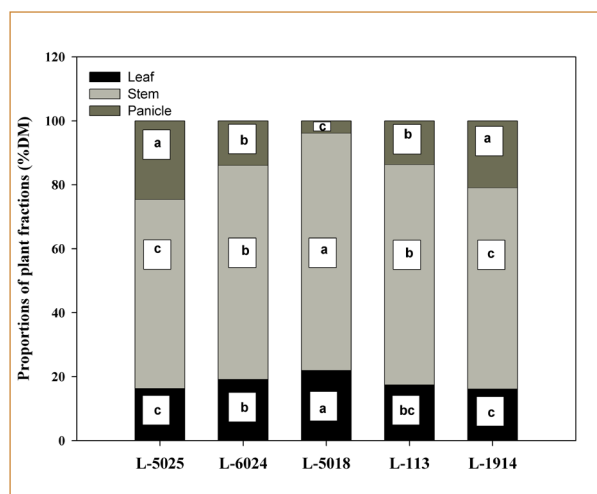
L-5025 was characterized by low sugar concentration (8.63%). Similarly, the maximum ADF (37.50%), NDF (57.62%) and lignin (5.21%) were found in L-5018, meanwhile, the lowest ADF (30.06%), NDF (50.43%) and lignin (3.91) was found in L-1914. Likewise, all tested lines also had the differential response for the ash contents. The maximum ash contents (7.63%) was found in the biomass of L-5018, followed by L-6024, meanwhile, the lowest ash contents (6.55%) was found in the biomass of L-5025 (Table 3).

### Methane yield

The results revealed that all the tested lines responded differently for specific methane yield and methane yield ha<sup>-1</sup> basis. The maximum specific methane yield was found in L-1914 that was comparable with L-5025, whereas the minimum specific methane yield was found in L-5018 amongst the tested lines (Fig 3A). Conversely, highest methane yield ha<sup>-1</sup> was recorded in L-5018, followed by L-6024, while the minimum methane yield ha<sup>-1</sup> was recorded in L-5025 (Fig 3B).

### Discussion

In this study, we found that all the tested lines had differential response for growth parameters, like, LAI, LAD and CGR (Fig 1). The higher LAI attained with L-5018 can be ascribed to more number of leaves per



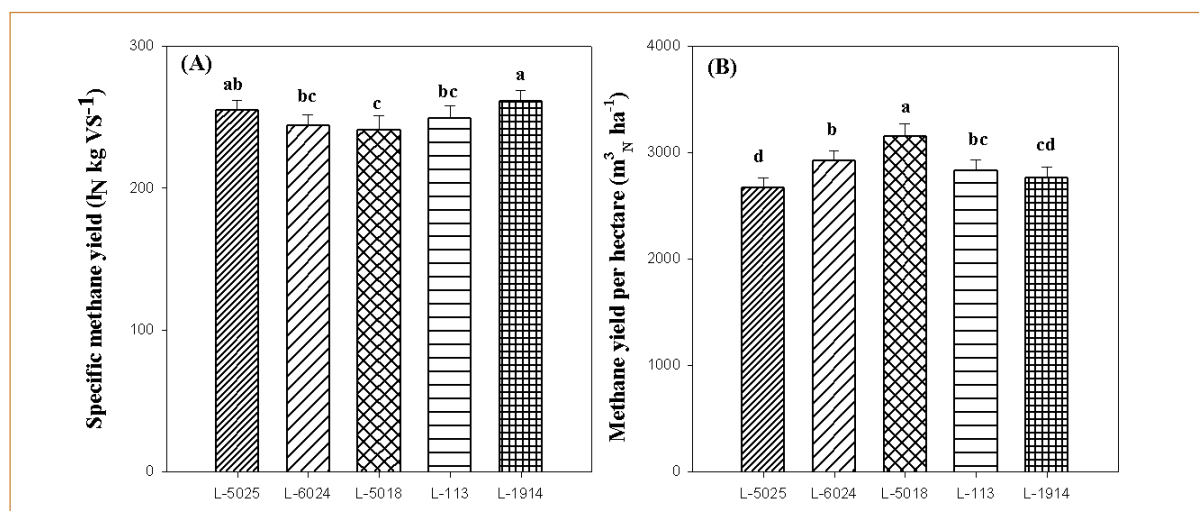
**Figure 2: Influence of lines on biomass proportion of sorghum**

plant and rapid plant establishment. These findings are supported with the previous results of Wiedenfeld and Matocha (2010) who also reported significant differences among the cultivars for LAI. We observed that lines had clear impact on the LAD and CGR, with L-5018 attained maximum LAD and CGR, while L-5025 produced the minimum LAD and CGR (Fig 1). The maximum LAD in L-5018 was due to more LAI produced by this line, likewise, the maximum CGR in L-5018 can also be attributed to variation in LAI, because plants of L-5018 attained more LAI, captured more light and produced more dry matter. Similarly, significant variations in LAD and CGR due the cultivars have been also reported by other researchers (Ahmed and Farooq 2013). Among the lines, L-5018 produced maximum tillers  $m^{-2}$  while L-5025 produced the minimum tillers  $m^{-2}$ . The difference among the cultivars for tillers  $m^{-2}$  can be ascribed to their genetic potential of tiller formation.

Plant height ranged from 158-191 cm, whereas L-5018 had maximum plant height and L-5025 had minimum plant height. Similarly, considerable differences in plant height due to cultivars have been reported by (Hussain et al, 2010; Awan et al, 2001). Leaves per plant are the important character of plant which substantially affects the LAI. Similarly, Iptas and Acar (2003) and Kusaksiz (2010) also found the considerable variations among the genotypes for number of leaves per plant.

In present study, lines also had a significant effect on biomass proportion (leaf, stem and leaves). We found that stem had highest proportion in biomass production as compared to the leaf and panicle proportion. Remarkable differences in biomass proportion have been also reported by other researchers (Dolciotti et al, 1998; Amaducci et al, 2004). L-5018 produced maximum dry matter yield which might be due to markedly higher values of LAI, CGR and plant height, as compared to other tested lines. Similarly, remarkable changes in dry matter yield due to cultivars have been also reported by many researchers (Habyarimana et al, 2004; Amaducci et al, 2004; Zhao et al, 2009).

Maximum protein concentration in this study was recorded for L-5018, while the minimum protein concentration was observed in biomass obtained from plants of L-5025. The reasons for the higher protein contents in L-5018 might be due to formation of maximum number of tillers in L-5018. Tillers are the younger plants, which have higher activities for protein synthesis than the older leaves and stem. In addition, fibrous compounds like cellulose and accumulation of carbohydrates are continuously increasing in main stems, therefore, both these factors elucidates the higher protein contents in L-5018. Likewise, previous



**Figure 3: Effect of lines on specific methane yield and methane yield hectare basis of sorghum bicolor, IN: Norm litter, VS: Volatile solid, m<sup>3</sup>N:norm cubic meter**



researchers also explained the considerable differences among the cultivars for protein concentration (Miron et al, 2005; Beck et al, 2007). Sugar concentration in this study ranged from 8.60-10.50, with L-6024 accumulated the maximum sugar, similar to L-5018, while L-5025 accumulated lower sugar concentrations. Similarly, Dolciotti et al, (1998) also reported the considerable variations among the cultivars for sugar concentration. We observed the maximum ADF, NDF and lignin in L-5018, whereas minimum was observed in L-1914 amongst the tested lines. These maximum values of ADF, NDF and lignin in L-5018 might be due to higher stem proportion (Fig 2) because stems have higher amount of structural fibers and lignin contents as compare to other plant parts. Significant differences among the cultivars for ADF, NDF and lignin contents have been also reported by various researchers (Miron et al, 2005; Beck et al, 2007). The results showed that tested lines had differential responses for the specific methane yield and methane yield  $\text{ha}^{-1}$  basis. The maximum specific methane yield in L-1914 was due to lower lignin content which remarkably increased the dry matter digestibility and consequently the specific methane yield. Tested lines also performed differently for methane yield  $\text{ha}^{-1}$  basis. The maximum methane yield  $\text{ha}^{-1}$  was recorded in L-5018 that was owing to higher dry matter yield. These results are corroborated with previous findings of many researchers who also found the considerable differences among the cultivars for specific methane yield and methane yield  $\text{ha}^{-1}$  (Tatah et al, 2007; Amon et al, 2007; Mahmood et al, 2013).

## Conclusion

In conclusion, lines under investigation responded differently in biomass yield, chemical composition and methane yield. L-5018 performed remarkably in terms of biomass yield, however, it had high concentration of structural fiber and lignin contents, whereas line 5025 produced minimum biomass yield lower in structural fiber and lignin contents. The maximum specific methane yield was found in L-1914 nonetheless, it was overcompensated by L-5018 owing higher dry matter yield  $\text{ha}^{-1}$ . Therefore, the line 5018 can be used to develop the cultivars having good biomass and methane yielding potential.

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