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SHORT COMMUNICATION

Sorghum Biomethane Potential Varies with the Genotype and the Cultivation Site

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Abstract With its high biomass yield potential and its adaptability to a large spectrum of crop management schemes (dedicated, double cropping), sorghum is a relevant candidate crop for anaerobic digestion. Moreover, the large genotypic variability of its biochemical composition offers opportunities to cultivate specific varieties that fit the expectations of different end-users. Within this context, the need to evaluate the variability of biomethane potential (BMP) among different genotypes cultivated at various geographical sites has become crucial. In this study, four sorghum genotypes were grown at three different sites and harvested at the same maturity stage (dough grain stage). Consistent BMP obtained from different assays enabled genotype comparisons. The methane potentials observed between genotypes and production sites ranged between 200 ± 5 NmL_{CH4}/g_{TS} and 259 ± 12 NmL_{CH4}/g_{TS}. Evaluation of the genotypic and cultivation site effects produced highly significant results, thus accounting for 36 and 34%, respectively, of the phenotypic variability.

Keywords Sorghum · Anaerobic digestion · Genotype · Multi-site trials · Biogas

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Introduction

The challenges rising from the depletion of fossil resources and global warming in the near future imply an urgent need for the development of additional renewable energies. In this context, biomethane production from renewable feedstocks is expected to play a significant role in the energy portfolio. Among the potential feedstocks for biomethane production, sorghum (*Sorghum bicolor L. Moench*) has proved to be a relevant candidate [1]. Firstly, it can be cultivated under low-input agro-systems [2] and in dry and marginal environments [1, 3]. Secondly, it is particularly adapted to temperate climates [4]. Indeed, a biomass production greater than 30 t ha^{-1} has been reported under temperate climates [5, 6]. At a worldwide level, sorghum is grown under greatly diverse crop management schemes (i.e. dedicated, double and relay cropping, intercropping) that enable the development of innovative production strategies [7, 8]. These not only optimize sorghum production per-se but also optimize ecosystemic services (multiple products on a given field, reduction of inputs for weed control, …). In 2014, sorghum covered an area of 62 863 harvested hectares in France while it covered 389 556 ha at the level of the European Union (EU) (http://faostat3.fao.org/download/Q/QC/E). Sorghum is a C4 annual plant presenting a high genotypic and phenotypic variability. A high variability in the sorghum stem composition, biomass and cell wall digestibility and hydrolysis yield potential has been highlighted [6, 9]. Benefitting from sorghum assets, breeding programs at the EU level presently aim at developing varieties that maximize methane production [4]. However, there is a crucial need to evaluate the stability of methane production for a given genotype across varying environmental conditions and to assess the differences between genotypes in order to anticipate the potential benefits of such dedicated breeding strategies. In addition, as it is not possible to simultaneously analyze the biomethane potential assessment for different sites and different years, it is of key importance to evaluate the stability of BMP estimations over different experimental series. While studies focusing on the impact of different cultivars or genotypes, planting density, row spacing and sowing time on the sorghum methane potential have been published [10], the impact related to varying production sites has not been investigated. In addition, although genotype effects on BMP have already been explored, there is limited available information regarding the extent of BMP variability for diversity panels which have been specifically selected to maximize the variability of biomass biochemical composition. In this context, in order to evaluate the range of BMP variability, four genotypes capable of maximizing the variability of biomass composition of the main stem (the main contributor to the biomass yield in "biomass type" varieties) have been selected. The objectives of this study are therefore to determine the relative importance of the genotype, production site and inoculum effects on methane production using four sorghum genotypes grown at three different sites.

Materials and Methods

Sorghum Genotypes

In this study, four sorghum accessions (IS 12804, IS 19,453, IS 20,351 and IS 30,405) were used. They were from the CIRAD sorghum core collection [11]. Based on a previous characterization of the variability of this collection for the stem biochemical composition and digestibility traits [6], the selection of these four accessions was made to maximize their properties in order to assess the range of BMP variability that can be obtained from highly contrasting genotypes. These four accessions were grown during field experimental trials during the 2013 summer cropping season, at three locations situated in the Occitanie region of France. The soil characteristics and crop management conditions are described in Table 1. The orthodromic distances between sites are 63 km (Rivières-Mondonville), 154 km (Montpellier-Rivières) and 208 km (Montpellier-Mondonville). Whole aboveground biomass samples were harvested at the dough grain stage, which corresponds to the usual harvesting stage for sorghum silage. The total solids contents at harvest time were measured for Montpellier samples and are reported in Table 2. All the samples were dried in an oven at 60 °C for 72 h and then grinded using a knife grinder (SM100, Retsch, Haan, Germany) equipped with a 1 mm sieve. At the Montpellier site, the aboveground biomass yield was roughly estimated by measuring the dry matter weight of a few sorghum plants (between 27 and 77 depending on the considered genotype).

Analytical Determinations

The different samples were analysed after they were dried and grinded. Total Solids or dry matter (TS) and Volatile Solids or organic matter (VS) were measured in accordance with APHA standard methods [12]. Fibre composition was assessed by Near Infrared Spectroscopy (NIRS) as described in Trouche et al. [6] and calibrated with the sequential method of Van Soest et al. [13] which measures total fibre (NDF, neutral detergent fibre), lignocellulose (ADF, acid detergent fibre) and lignin (ADL, acid detergent lignin) contents. Based on these results, the hemicellulose (NDF-ADF) and cellulose (ADF-ADL) contents were calculated. The NIRS calibrations were based on a CIRAD calibration database which included a total of 640 samples analysed by the reference laboratory method. Statistical tests R^2, standard error of calibration (SEC) and standard error of cross-validation (SECV) were used to verify the accuracy of the prediction models, with respectively 0.98, 1.70, 1.99 for NDF, 0.97, 1.38, 1.52 for ADF, 0.94, 0.59, 0.65 for ADL.

Methane Potential

The biochemical methane potential (BMP) is the measurement of the maximum methane volume per unit of total or volatile solids that a substrate can produce. All the samples were digested in batch in 500 mL anaerobic flasks with a working volume of 400 mL. The flask contained an anaerobic sludge

Table 1 Characteristics of the 3 experimental field sites

	1 (CIRAD)	2 (EUROSORGO)	3 (RAGT)
Coordinates	Montpellier N43°39′02″E 3°52′35″	Mondonville N43°40′41″E1° 17′12″	Rivières N 43°55′16″E 1° 59′ 34.87″
Size of plots (m^2)	5.12	16.8	14.4
Sowing density (plants/ha)	200,000	255,000	260,000
Soil type	Highly calcareous clay-loam	Calcareous clay-loam	Calcareous clay-loam
Total precipitation and water	381.5 mm	386 mm	313.7 mm

Table 2 Fibre composition and biomass methane potentials of 4 sorghum genotypes produced at 3 separate sites

Genotype	Racial classification	Site	TS[a] (% Fresh matter)	VS (%TS)	Cellulose (% TS)	Hemicelluloses (%TS)	Lignin (%TS)	BMP1 (NmL_{CH4}/g_{TS})	BMP2 (NmL_{CH4}/g_{TS})
IS 12804	Bicolor	Mondonville	nd	95	24.5	22	3.9	210 ± 2	223 ± 12
		Rivières	nd	94.9	30.7	26.2	4.9	196 ± 9	200 ± 5
		Montpellier	39.7	95.2	27.3	26.6	4.4	nd	223 ± 14
IS 19453	Durra	Mondonville	nd	95.4	21.7	23.2	2.4	251 ± 5	243 ± 7
		Rivières	nd	94	22.4	21.4	2.6	222 ± 14	226 ± 6
		Montpellier	26.3	95	20.9	23.1	2.1	nd	259 ± 12
IS 20351	Durra	Mondonville	nd	96	23.6	22.9	3.2	200 ± 21	238 ± 5
		Rivières	nd	95.6	29.7	24.9	4.7	212 ± 14	210 ± 17
		Montpellier	32.5	95.8	25.4	26.4	3.3	nd	234 ± 9
IS 30405	Bicolor-caudatum	Mondonville	nd	94.9	22.6	21.3	4.2	245 ± 10	231 ± 5
		Rivières	nd	94.4	23.9	22.7	3.7	212 ± 7	233 ± 2
		Montpellier	47.5	96	21	23.5	4.4	236 ± 7	251 ± 8

BMP1 and BMP2 correspond to the two test series

nd not determinated

[a]TS content at harvest time

at 5 g VS/L and sorghum at 5 g TS/L, a bicarbonate buffer ($NaHCO_3$,50 g/L) and macro- and microelement solutions whose compositions have been provided by Monlau et al. [14]. Once the flasks were prepared, nitrogen degasification was carried out to obtain anaerobic conditions. Triplicate bottles for each sample were incubated at 35 °C during 47 days. The volume of methane was monitored by AMPTS (Automatic Methane Potential Test System) (Bioprocess Control AB, Lund, Sweden) and was expressed in standard conditions (0 °C and 101.3 kPa). Two BMP test series were performed at an interval of 1 year, using two inoculum samples from the same source (UASB digester processing sugar factory wastewater), but collected at two different times. In the first series, BMP analyses were performed on the samples from the three sites in order to assess the genotype and cultivation site effects. In the second series, samples from two sites (Mondonville and Rivières) were used in order to evaluate the inoculum impact and the stability of the genotype effect on samples stored during 1 year.

Statistical Analysis

Statistical analysis were performed using the R software (version 3.2, R Development Core Team, R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Austria, Vienna, 2004, ISBN 3-900051-07-0. http://www.R-project.org).

The sources of measurement variation of the biomethane potential were analysed using the following ANOVA model:

$$Y_{ijk} = \mu + G_i + E_j + L_k + e_{ijk} \quad (1)$$

where Y is the biomethane potential measured for Genotype i from growing location j based on the lab experimental measurement k, μ is the trial methane potential mean, G_i is the genotype effect of the i genotype, E_j is the growing location effect of the experiment j, L_k is the BMP series effect of the k measurement and e_{ijk} is the residual error including the technical replicates.

The sources of variability of the biochemical components were analysed using the same ANOVA model as Eq. 1 but with one factor less, thus considering only genotype and growing location effects:

$$Y_{ij} = \mu + G_i + E_j + e_{ij} \quad (2)$$

Effects were considered significant in all statistical calculations for p-values lower than 0.05. Comparisons of means between sites and genotypes were performed using the Tukey's honest significant difference test [15].

Results and Discussion

The composition and the BMP obtained from the different samples analysed are presented in Table 2. Lignin, cellulose and hemicellulose contents ranged from 2.1 to 4.9% TS, 21–30.7% TS and 21.3–26.2% TS, respectively. Although these results are based on the analysis of whole aboveground biomass composition, they vary within the same range as those obtained from a large panel of sorghum genotypes (i.e. lignin content between 1.2 and 8.7% TS, cellulose content between 18 and 42% TS and hemicellulose content between 15 and 41% TS) which were obtained from stem samples

[6]. Firdous et al. [16] demonstrated that for three different cultivars of sorghum, the maximum NDF, ADF, cellulose and lignin contents could be observed in the stem fraction, followed by whole plant. This global convergence underlines the fact that it is the stem that contributes to the major portion of the whole aboveground biomass.

The methane potential ranged between 200 ± 5 NmL_{CH4}/g_{TS} and 259 ± 12 NmL_{CH4}/g_{TS} (corresponding to the IS 12804 genotype at Rivières and IS 19453 at Montpellier, respectively). Sambusiti et al. [17] obtained methane potentials in the upper part of this range for two biomass/fibre sorghums (Biomass 133 and Trudent headless) and the sweet sorghum Sucro 506 (248 ± 12, 249 ± 10 and 252 ± 20 NmL_{CH4}/g_{TS}, respectively) and a slightly higher methane potential for two sweet sorghum cultivars, Sucro 405 and BMR Sisco, (274 ± 8, and 277 ± 9 NmL_{CH4}/g_{TS}, respectively). To some extent, cultivars developed for bioethanol production and high soluble sugar yields, generally present higher methane potentials than sorghum biomass [17]. Using Goliath, Bovital, Aron, Rona 1 and Akklimat cultivars, Mahmood et al. [10] obtained a larger BMP variation from 213 to 363 NmL_{CH4}/g_{TS} (Rona 1 leading to the highest specific methane yield and Akklimat to the lowest).

As insignificant differences (p-value = 0.137) were observed between the BMP obtained from the two inocula, the use of two inoculum samples did not have any impact on this study. As a consequence, a reduced model including exclusively the effects of genotype and site was applied (Eq. 2). p-values are presented in Table 3. Both genotype and production site effects on the methane potential are significant (Table 3; Figs. 1, 2) explaining 36% and 34% of the variability, respectively. These results are consistent with the results from Mahmood et al. [10], who showed how methane yields can vary between sorghum cultivars but are not affected by different row spacing at a unique site. The impact of the genotype on BMP has also been observed for different varieties of maize by Gao et al. [18] who demonstrated that this variability is related to their biochemical compositions.

Table 3 p-Values obtained in ANOVA analysis (Eq. 2) for genotype and growing location effects on BMP values and biochemical composition

	Effect	
	Genotype	Growing location
BMP	0.00002[a]	0.00001[a]
VS	0.13	0.11
Lignin	0.013[b]	0.358
Cellulose	0.010[b]	0.036[b]
Hemicelluloses	0.116	0.089

[a]Significant impact at 0.005%

[b]Significant impact at 5%

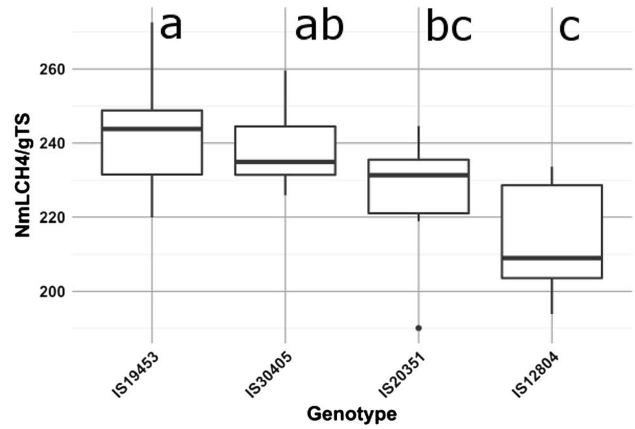

Fig. 1 Variability of BMP among genotypes. Different letters differ significantly for $p < 0.05$

Significant genotype effects have been observed on cellulose and lignin contents whereas only the cellulose content was found to be impacted by the geographical location (Table 3). The genotypes and production sites produced insignificant effects on the organic matter (VS) and hemicellulose content (Table 3). Using various fruit and vegetable solid wastes, Buffière et al. [19] observed a negative correlation between BMP values and the sum of lignin and cellulose content. This negative correlation ($r = -0.86$, p-value = 2.9×10^{-4}) has also been observed in the present study, as illustrated in Fig. 3. It can be explained by the recalcitrance of lignin to anaerobic digestion as well as the low accessibility of cellulose within the lignocellulosic matrix.

Assuming a plant density of 150,000 plants per hectare for the different genotypes, a rough estimate of the dry biomass yields per hectare for the Montpellier site ranged between 10 (for IS 12804) and 32 tTS/ha (for IS 20351). Although these yield estimations are still inaccurate

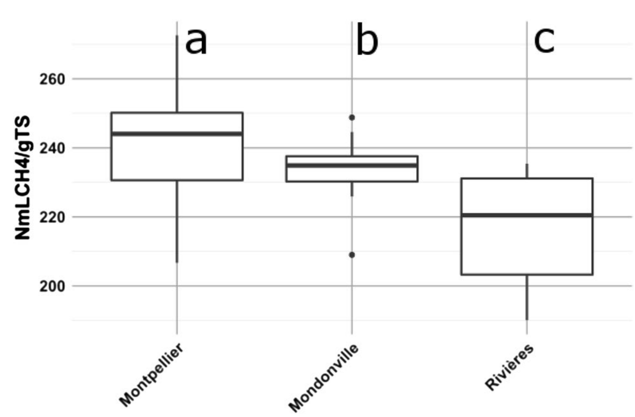

Fig. 2 Variability of BMP across cultivation sites. Different letters differ significantly for $p < 0.05$

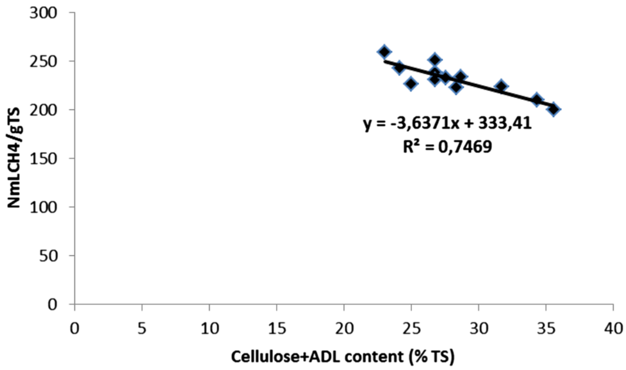

Fig. 3 Correlation between BMP and cellulose + ADL content

(replicates from various sites would be required to reach a more relevant yield estimation) and likely overestimated due to the small number of harvested plants, they illustrate the occurrence of large differences in terms of biomass yield per hectare. In accordance with the literature, these rough yield estimations clearly point to the dry biomass yield as the main component of methane yield per hectare. However, significant achievements can also be reached by improving the BMP per unit of dry matter. The observed variability corresponds to a three-fold variation in biomass production whereas the variability of the biomethane potential can reach 1.3-fold for a set of identical genotypes. This high variability in the biomass yield was expected, as the genotypes were not selected according to their ability to produce biomass but rather to cover a large biochemical diversity. It is clear that the first selection criterion for methane production has to be biomass yield. However, the variability observed for the BMP in the present study and in the literature underlines the fact that significant genetic improvements can be expected by involving the BMP criterion with the breeding objectives. The proposal of this strategy can also be backed by the fact that the BMP genotype ranking across production sites is maintained.

Conclusion

The high biomethane potentials observed for a small diversity panel of sorghum genotypes confirm the relevance of this species for biogas production by anaerobic digestion. This study also emphasized that BMP is not only strongly dependent upon the genotype, as previously reported in the literature, but also on the production sites. However, as the ranking of the genotypes over the different sites was maintained, there may be limited genotype versus cultivation site interactions. In addition, results suggested that although the methane production per hectare primarily depends on the genotype biomass yield, significant improvements can nevertheless be achieved by enhancing the BMP of high yielding genotypes.

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