Article

Characterization of mombaza grass as raw material to produce bioethanol

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Abstract

The objective of this study, made in 2017, was to characterize mombaza grass (*Megathyrsus maximus*) to assess its potential as a raw material for the production of liquid biofuel (bioethanol). Annual biomass production, calorific power, chemical composition and theoretical bioethanol yield were determined at four cutting frequencies (30, 60, 90 and 120 d after regrowth). The data were analyzed with the GLM (SAS) procedure and the means of the treatments were compared with the Tukey test ($p\leq 0.05$). The highest biomass production, calorific power, energy production, bioethanol production, FDN, LDA and hemicellulose were obtained at the cutting frequency of 120 d with 11 Mg ha^{-1} year^{-1}; 16.1 MJ kg^{-1}; 178.4 GJ ha^{-1} year^{-1}; 238.2 L Mg^{-1} MS and 68.6, 6.5, 23.3%, respectively. However, the highest cellulose and FDA content were found at the cutting frequency of 90 d, with 41.2 and 47.4%, respectively. The highest values of humidity, PC and ash were found at the cutting frequency of 30 d with values of 8.2, 10.4 and 12.1%, respectively, while the EE content was higher at the cutting frequency of 60 d (1.6%). According to the results obtained in this study mombaza grass (*Megathyrsus maximus*) can be considered as an attractive raw material for the production of bioethanol in tropical climates.

Keywords: biofuel, biomass, calorific value, chemical composition grass.

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Introduction

Pasture production systems in the subhumid tropics of Mexico depend on weather conditions throughout the year, affecting biomass yield and modifying its chemical composition. In the coastal region of the Gulf of Mexico the growth of plants is determined by three times in the year: 1) rainy season; from June to October, when temperature and water favor the growth of plants; 2) northern times; from November to February, in which the growth of forage species decreases, because low temperatures and high cloudiness and 3) dry season; from march to May, when biomass yield is drastically reduced by almost zero precipitation (Hernández *et al*., 1990).

Forage species with high potential for use in cutting y grazing systems have been introduced to increase meat and milk production in tropical areas of Mexico. Of these, the varieties of the genus *Megathyrsus maximus* [Sin. *Panicum maximum*, (Simon and Jacobs, 2003)] stand out, which are species with the greatest potential for growth and production of dry material (MS) per hectare (Ramírez *et al*., 2009) in addition, due to their great adaptability they grow at sea level and also at more than 1 000 m of altitude.

The constitution of pastures is determined by the chemical components of the wall and its cellular content (Mertens, 1997). In the grasses the cellulose and hemicellulose represent approximately 70% of the total biomass (MS) and its lignin content varies between 10-30%. Sugars are attached to lignin through hydrophobic covalent links, which gives it high resistance to any treatment to be separated (Weijde *et al*., 2013). On the other hand, the chemical composition of the plants also varies according to the species, type of tissue, growth stage and growing conditions (Wongwatanapaiboon *et al*., 2012).

Bioethanol produced from lignocellulosic biomass can be used as a petrol oxygenator, improving air quality because reduces CO_2 emissions. The International Energy Agency (2018), in 2016 reported a production of 13760.81 MMtep of bioethanol. The biggest producers were China (17.2%), the United States of America (13.9%), Russia (10%), Saudi Arabia (4.9%) and India (4.1%), while Mexico provided only 1.3%. [https://www.iea.org/reports/world-energy- balances-2019].

The chemical composition of tropical pastures and their concentration of structural sugars can be used to obtain second generation bioethanol and other forms of energy. However, in Mexico studies on the production of ethanol from biomass and its relationship to chemical composition, biomass yields, cutting age, phenological status and plant species are very limited (Rueda *et al.*, 2016; Santiago *et al*., 2016; Ventura *et al*., 2017). The objective of this study was to evaluate the biomass yield, chemical composition, energy characterization and theoretical bioethanol yield of mombaza grass (*Megathyrsus maximus*) harvested at four cutting frequencies (FC).

Materials and methods

Biomass yield was assessed at the experimental site 'Papaloapan' of the National Institute for Forestry, Agricultural and Livestock Research (INIFAP), at 18° 06' north latitude and 95° 31' west longitude and 65 meters above sea level (msnm), in Isla City, Veracruz, Mexico, with climate A_{wo}

and average temperature of 25.7 °C (García, 2004); the soil is orthic acrisol, loam-sandy, with pH of 4 to 4.7, poor in organic material, nitrogen, calcium, potassium and medium to high phosphorus and magnesium content (Enríquez and Romero, 1999).

The variables evaluated were biomass yield, dry material, raw protein (PC), neutral detergent fiber (FDN), acid detergent fiber (FDA), cellulose, hemicellulose, lignin digestible in acid (LDA), ethereal extract (EE), ash, calorific power, humidity and bioethanol yield.

Establishment of plots

The planting took place on July 22, 2017, in grooves with a separation of 0.50 m, in experimental plots 5 m wide by 16 m in length, with three repetitions. Two fertilizations were applied at 43 and 112 d after planting, with the formula: 120-80-00 kg ha^{-1} of N and P_2O_5.

Biomass yield (Mg MS ha^{-1} $year^{-1}$)

Biomass accumulation was determined per unit surface for each FC: 30, 60, 90 and 120 d after homogenization cut (ddch) in destructive sampling for one year. On each plot it was randomly located five times a metal quadrant of (1 m^2) and the total fodder was cut at 20 cm of residual height. The biomass harvested was weighed in precision balance (Ohaus, Mod. GT-4000; 6.200 kg ±0.1 g). The subsample was weighed and dehydrated on a forced convection stove (Felisa, Mod. FE-243A), at 55 °C for 72 h and the MS was calculated.

Preparing samples for analysis

Chemical determinations and calorific power were made with ground dry samples (Wiley® mill PA, USA) and sifted with meshes number 40 (0.42-1.00 mm) and number 60 (0.25-0.42 mm).

Chemical analysis

The samples were incinerated for two hours at 600 °C to obtain the organic material and ash content (Rule ASTM D 1102-84; 2012). The PC concentration was measured by the Kjeldahl method (N x 6.25) and the EE in Soxhlet extractor (AOAC, 1990). Concentrations of FDN, FDA (Van Soest *et al*., 1991) and LDA (Goering and Van Soest, 1970) were obtained sequentially in the ANKOM$^{200®}$ fiber analyzer (Ankom Technology, Fairport, NY, USA) by using Ankom® F57 filter bags with pore size of 30 microns. Sodium sulfite ($Na_2 SO_3$) and α-amylase were used to determine FDN to remove nitrogen and starch from the sample, respectively. Hemicellulose and cellulose were calculated by the difference between FDN and FDA, and between FDA and lignin, respectively.

Calorific power

It was determined in an adiabatic pump calorimeter (Isoperibol, Parr 1266), in accordance with ASTM (E711), at 30 ±0.5 °C, with compressed pickups of 1 g maximum. The moisture content was determined on an Ohaus MB45 thermal balance®. Five determinations were made per sample and 15 repetitions per FC.

Theoretical bioethanol yield

The theoretical yield of bioethanol (RTE) was determined based on hydrolysis reactions and transformation of sugars to ethanol, taking into account its stechymetry. The formulas proposed by Badger (2002) and Dien (2010) were used.

Hemicellulose cellulose: $RTE_C = C \times C_{g/c} \times E_{CC} \times R_{et} \times E_{fg} \times D_{et}$ $RTE_H = H \times H_{x/h} \times E_{CH} \times R_{et} \times E_{fx} \times D_{et}$. Total $RTE = RTE_c + RTE_H$.

Where: RTE: $LMg^{-1}MS$; C: $kg_{cellulose}Mg^{-1}Mg^{-1}_{biomass}$; H: $kg_{hemicellulose}Mg^{-1}_{biomass}$; $C_{g/c}$: glucose concentration ($1.111\frac{kg_{glucose}}{kg_{cellulose}}$); $C_{h/x}$: xylose concentration ($1.136\ \frac{kg_{xylose}}{kg_{hemicellulose}}$);
E_{cc}:cellulose convertion efficiency (0.76);
E_{ch}:hemicellulose convertion efficiency (0.9);
RTE:stoichiometric yield of ethanol $\left(0.511\ kg_{ethanol}/kg_{glucose}; 0.511\ kg_{ethanol}/kg_{xylose}\right)$;
E_{fg}:glucose fermentative efficiency (0.75); E_{fx}:xylose fermentative efficiency (0.50);
D_{et}:ethanol density (0.78 Mg m^{-3}).

Mombaza's annual theoretical bioethanol yield per hectare was calculated for each FC multiplying the theoretical bioethanol yield per unit of biomass by the annual biomass yield.

Statistical analysis

The data were analyzed as a completely random design, where the cutting frequency of the mombaza grass was considered as treatments (30, 60, 90 and 120) with three repetitions for each treatment. A variance analysis (Anova) was performed to identify the effect of the cutting frequency on response variables by using the GLM/SAS procedure and the treatment means were compared with the Tukey test ($p \leq 0.05$) using SAS for Windows version 9.3 (SAS, 2011).

Results and discussion

Biomass yield

Biomass yield increased linearly as the plant advanced at its physiological maturity, the higher biomass production was found in FC of 120 d (11 Mg ha^{-1} year), which was different (pc 0.05) from the rest of the cutting frequencies (Table 1). The FC of 120 d exceeds 23.5, 11.1 and 6%, at frequencies of 30, 60 and 90 d, respectively. Verdecía *et al*. (2009), reported yields of 3.6 and 6.4 Mg MS ha^{-1} to 30 and 60 days, respectively, lower values than found in this study at the same harvest frequencies.

On the other hand, Ramírez *et al*. (2009) evaluated the biomass production of mombaza grass and reported variations from 9.7 to 20.6 Mg MS ha^{-1} at 3 to 7 weeks in rainy season, respectively. The biomass yield in this experiment was different in each FC, this can be attributed to the growing conditions, factors associated with plant physiology and in particular to temperature and humidity factors that have the greatest influence on biomass production throughout the year.

Table 1. Biomass yield and energy production of mombaza grass (*Megathyrsus maximus*) harvested at different cutting frequencies.

Cutting frequency (days)	Yield (Mg MS ha^{-1} $year^{-1}$)	Calorific power (MJ kg^{-1})	Energy production (GJ ha^{-1} $year^{-1}$)	Moisture (%)
30	8.9 d	15.8 b	142.1 c	8.2 a
60	9.9 c	13.7 d	136.2 d	8 b
90	10.4 b	14.5 c	151.6 b	7.7 c
120	11 a	16.1 a	178.4 a	8 ab
Average	10	15	152.1	8
EE	0.83	0.2	3.3	0.04

EE= standard error. Different letters show differences between cuts (Tukey, $p \leq 0.05$).

Calorific power, energy production and moisture content

The highest calorific power content was found in the FC of 120 d (16.1 MJ kg^{-1}), statistically different value ($p \leq 0.05$) than the other FC (Table 1). In this study the range of calorific power values was 13.7 to 16.1 MJ kg^{-1}, FC with 30 and 120 d were the highest concentration of energy.

The values obtained in this experiment are lower than reported by Santiago *et al*. (2016) and Mohammed *et al*. (2015), who reported on average 16.5 MJ kg^{-1} in Toledo grass (*Urochloa brizantha*) and Napier grass (*Pennisetum purpureum*). On the other hand, it has been reported for Switch grass and sweet sorghum bagasse 17.3 and 13.7 MJ kg^{-1}, respectively (Nhuchhen and Salam, 2012; Shankar, 2015).

On the other hand, energy production per hectare was based on dry material yield. The highest energy production per hectare (178.4 GJ ha^{-1} $year^{-1}$; $p \leq 0.05$) was found in the FC of 120d (Table 1). This value was 31, 25.5 and 17.6% higher than those found in the FC of 60, 30 and 90 d respectively. The biggest energy production was lower than published by Santiago *et al.* (2016) who report 466.6 GJ ha^{-1} $year^{-1}$ in the same FC in Toledo grass (*Urochloa brizantha*).

The highest humidity content was presented in the FC of 30 d (8.2%), which was similar ($p \geq 0.05$) to the FC of 120 d, but different ($p \leq 0.05$) to the others (Table 1). It has been reported that high moisture content per unit of mass reduces combustion efficiency, because much of the released heat is used to evaporate water instead of chemical reduction of the material (Nhuchhen and Salam, 2012).

Raw protein

Protein raw content decreased when FC increased. The highest concentration of raw protein was found at 30 d (10.4%), higher value ($p \leq 0.05$) than others FC (Table 2). The raw protein content decreased by 37.5, 41.1 and 51%, despite the lowest FC (30 d) to 60, 90 and 120 d, respectively. In this way, PC concentration in plants has been shown to be related to soil fertility. However, in the early stages the demand for this nutrient is high in the plant and decreases when aerial biomass increases (Ramírez *et al*., 2013).

Table 2. Chemical composition of the mombaza grass (*Megathyrsus maximus*) harvested at different cutting frequencies.

Cutting frequency (days)	Chemical composition (%)					
	PC	FDN	FDA	LDA	Ethereal extract	Ashes
30	10.4 a	60.4 c	43 d	4.2 d	1.2 c	12.1 a
60	6.5 b	60.2 d	44.7 c	4.7 c	1.6 a	11 b
90	5.5 c	62.5 b	47.4 a	6.2 b	1.1 d	10.6 c
120	5.1 d	68.6 a	45.2 b	6.5 a	1.5 b	10.6 c
Average	6.9	62.9	45.1	5.4	1.3	11.1
EE	2.2	3.6	1.6	1	0.2	0.6

PC= raw protein; FDN= neutral detergent fiber; FDA= acidic detergent fiber; LDA= digestible lignin in acid. EE= standard error. Different letters show differences between cuts (Tukey, $p \leq 0.05$).

For his part, Rojas *et al.* (2018) reported the reduction in PC concentration of 34 and 52%, from 35 to 49 d and from 35 to 63 d cut, respectively in Cobra grass (*Brachiaria* hybrid BR02/1794). The results obtained in this study have a trend similar to that observed by Rojas *et al*. (2018). Ventura *et al*. (2019) reported decreases of 34% from 30 to 60 d and 42% from 30 to 90 days of cutting Maralfalfa grass (*Cenchrus purpureus* Schumach.) Morrone. On the other hand, raw protein concentrations of 8.7, 9 and 8.5% are reported for the cultivar Mombaza, Privilegio and Tanzania, respectively (Ortega *et al*., 2011).

Neutral detergent fiber and acidic detergent fiber

The highest FDN and FDA content (≤ 0.05) were found in FC of 120 d (68.6%) and 90 d (47.4%), respectively (Table 2). The use of grass fibers in the bioethanol industry is not documented; however, they have significant use in the animal feed industry, pulp and paper production (Wheel *et al*., 2016). Ortega *et al*. (2011) reported in *Megathyrsus maximus* cv. Mombaza 68.9 and 47.1% and in Tanzania grass 73.6 and 46.3% for FDN and FDA, respectively.

On the other hand, Alves *et al.* (2014) reported 67.9% of FDN and 39.3% of FDA for *Brachiaria brizanta* cv. Marandu and 66.1% of FDN and 37.1% of FDA for *Brachiaria brizanta* cv. Xaraes. In this regard, Mertens (1997) mentioned that the chemical composition of pastures and other forage sources is influenced by the compounds that form the wall and cellular content in the plant at harvest time. These compounds are modified by the addition of fertilizers, rain and solar radiation.

Lignin digestible in acid

Unlike PC and ash, the highest LDA content was found in the FC of 120 d (6.5%) which was different ($p \leq 0.05$) from the rest of the CB (Table 2). The concentration of LDA at 120 d exceed in 55, 38 and 5% at 30, 60 and 90 d, respectively. The results obtained are similar to those obtained by Wongwatanapaiboon *et al*. (2012), who reported 4.4% for pangola grass (*Digitaria decumbens*), 5.6% for atratum (*Paspalum atratum*) and 4% for guineo grass (*Megathyrsus maximus*).

Coêlho *et al*. (2018) reported 1, 1.3 and 1.9% for *Megathyrsus maximus*, *Digitaria pentzii* and *Urochloa mosambicensis*, respectively. On the other hand, in coniferous woods and broad-leaved the concentration of LDA is higher than in pastures or herbaceous. Sharma *et al*. (2016) reported concentrations of 30 to 60% in fir (*Abies alba* Mill) and pine (*Pinu*s spp.), while Limayen and Ricke (2012) report 20 to 25% for hardwoods or broad-leaved.

Ethereal extract

The highest concentration of ethereal extract was found at 60 d (1.6%), statistically different value ($p\leq 0.05$) from the rest of the FS (Table 2). The ethereal extract content range was 1.1 to 1.6%, similar to the results obtained in other research. For example, Kondo *et al*. (2015) reported 2.8, 2, 2.1 and 1.8% for *Andropogun gayanus*, *Urochloa decumbens*, *Megathyrsus maximus* and *Urochloa mutica* respectively. In bioenergy studies it is important to quantify fat-soluble fats, pigments and other types of substances that may alter subsequent analyses (Sluiter *et al*., 2008).

Ashes

The highest ash content was recorded at 30 d (12.1%), which was statistically different ($p\leq 0.05$) to the others (Table 2). So, when the regrowth age increases the ashes concentration decreases. Normally the content of ash and inorganic material is high in early stages of plant development, but this compound does not offer any energy input (Mohameed *et al*., 2015).

The ash content is higher in herbaceous and agricultural residues, compared to other lignocellulosic materials (Limayen and Ricke, 2012). Santiago *et al.* (2016) reported 7% for Toledo grass (*Urochloa brizantha*) while Rueda *et al*. (2016) reported in crops of the genus (*Cenchrus purpureus* Schumach.) Morrone, 8.8, 9.4, 10.6 and 10.7% for African cane, King grass, Taiwan and CT115, respectively.

Cellulose

The highest concentration of cellulose was found at 90 d (41.2%; 4.2 Mg ha^{-1} $year^{-1}$), which was different ($p\leq 0.05$) to the others FB (Table 3). At 90 d the cellulose content was 24, 8.3 and 0.2% higher than found at 30, 60 and 120 d, respectively. The results obtained in this study are similar to those reported with other lignocellulosic materials; for example, Jahirul *et al*. (2012) reported 24% cellulose for *Miscanthus* sp., while Wongwatanapaiboon *et al.* (2012) and Santiago *et al.* (2016) recorded 34.5 and 42.1% for Ruzi grass (*Brachiaria ruziziensis*) and Toledo (*Urochloa brizantha*), respectively. The chemical composition and the structural sugars in pastures change as the plant progresses in its physiological maturity (Lima *et al*., 2014).

Table 3. Concentration of cellulose and hemicellulose from mombaza grass (*Megathyrsus maximus*) harvested at different cutting frequencies.

Cutting frequency (days)	Cellulose		Hemicellulose	
	(%)	(Mg ha^{-1} $year^{-1}$)	(%)	(Mg ha^{-1} $year^{-1}$)
30	38.7 c	3.4 d	17.4 b	1.5 b
60	40 b	3.9 c	15.5 c	1.5 b
90	41.2 a	4.2 a	15.1 d	1.5 b
120	38.7 c	4.2 a	23.3 a	2.5 a
Average	39.6	4	17.8	1.8
EE	1.1	0.07	3.5	0.09

EE= standard error. Different letters show differences between cuts (Tukey, $p\leq 0.05$).

Hemicellulose

FC 120 d had the highest content of hemicellulose (23.3%; 2.5 Mg ha^{-1} $year^{-1}$) which was statistically different ($p\leq 0.05$) from the rest of the CS (Table 3). The concentration of this component at 120 d was 54, 50 and 34% higher than that found at 90, 60 and 30 d, respectively. Hemicellulose concentrations of 22.1, 27 and 28% have been reported for Elephant grass (*Cenchrus purpureus* Schumach.) Morrone, cane bagasse (*Saccharum officinarum*) and sorghum (*Sorghum biocolor* L.; Moench), respectively (Carrolland Somerville, 2009; Weijde *et al*., 2013; Mohameed *et al*., 2015). Grass walls and herbaceous plants contain lower concentrations of hemicellulose than agricultural residues (25-50%), conifer woods (22-29%) broad-leaved (25-40%), according to Limayen and Ricke (2012).

Theoretical yield to bioethanol

The highest theoretical yield of cellulosic ethanol was found at 120 d (238.2 L Mg^{-1} MS; 2 640 L ha^{-1} $year^{-1}$). This value represented a representative statistical difference from the rest of the FC ($p\leq 0.05$) (Table 4). The yield at 120 d was higher in 35, 22 and 14.5% than that found at 30, 60 and 90 d, respectively.

Table 4. Theoretical bioethanol yield of mombaza grass (*Megathyrsus maximus*) harvested at different cutting frequencies.

Cutting frequency (days)	Bioethanol production	
	(L Mg^{-1} MS)	(L ha^{-1} $year^{-1}$)
30	218.5 c	1 954.8 d
60	217.5 d	2 156.8 c
90	221.1 b	2 304.9 b
120	238.2 a	2 640.5 a
Average	223.8	2 264.2
EE	1.7	52.1

EE= standard error. Different letters show differences between cuts (Tukey, $p\leq 0.05$).

Some of the studies known in Mexico with lignocellulosic materials for the production of bioethanol report, 272 L Mg^{-1} MS with *Urochloa brizanta* cv. Toledo (Santiago *et al*., 2016) and with maralfalfa grass (*Cenchrus purpureus* Schumach.) Morrone 3851 L ha^{-1} (Ventura *et al*., 2017). On the other hand, Lima *et al.* (2014) reported yields of 282.6, 283.3 and 285.7 L Mg^{-1} MS in cane bagasse, *Eucaliptus grandis* bark and *Megathyrsus maximus* respectively.

Annual yield estimates are similar to those published by Wongwatanapaiboon *et al*. (2012) who reported 2 561 and 2 621 L ha^{-1} for Napier and King grass (*Cenchrus purpureus* Schumach.) Morrone. In other research with first generation raw materials, Zhao *et al.* (2009) and Somerville *et al*. (2010) reported yields from 2 967 to 13 032 L ha^{-1} in sorghum (*Sorghum bicolor* L.) Moench and 3 800 L ha^{-1} for maize (*Zea mays* L.), respectively.

According to the results obtained in this study, mombaza grass represents a promising raw material for bioethanol production. However, other aspects should be taken into account to ensure the technical, economic and environmental relevance of the use of this energy crop for the production of bioethanol.

Conclusions

The increase in harvest time allowed for higher air biomass yield of mombaza grass (*Megathyrsus maximus*), this has a direct relationship to lignin content and bioethanol production. According to the results found it was not possible establish a direct relationship between growth time and calorific power, energy production, FDN, hemicellulose, moisture percentage, PC and ashes.

Moreover furthermore, a close relationship was not detected between cutting frequency and FDA, ethereal extract and cellulose concentration. However, the results of biomass yield and chemical characteristics indicate that mombaza grass (*Megathyrsus maximus*) is a technically viable option for the production of bioethanol in tropical areas.

Cited literature

Agencia Internacional de Energía. 2020. IEA world energy balance. 2019. https://www.iea.org/regions/north-america.

ASTM. 2012. American Society for Testing and Materials. Annual book of ASTM standards, biological effects and environmental fate; biotechnology. ASTM E870. West Cornshohocken, PA, USA. 11(06):96-97.

Alves, M. G.; Aparecida-Phino, C. K.; Da-Costa S. E.; Soares, E. P.; Flavio, N. J.; Goncalves, R. M.; Bezerra, F. P.; Guimarães, S. J. F. and Gomes, G. W. 2014. Yield and chemical composition of *Brachiaria* forage grasses in the offseason after corn harvest. Am. J. Plant Sci. 5(5):933-941. https://doi.org/10.4236/ajps.2014.57106.

AOAC. 1990. Association of Official Analytical Chemists. Protein (crude) determination in animal feed. Official Methods of Analysis. 15th (Ed). Helrich, K. Arlington. VA, USA. 72-74 pp.

Badger, P. C. 2002. Ethanol from cellulose: a general review. *In*: trends in new crops and new uses. Janick, J. and Whipkey, A. (Ed). 630/J33t. ASHS Press. Alexandria, Virginia. USA. 17-21 pp. http://large.stanford.edu/publications/coal/references/docs/badger.pdf.

Carroll, A. and Somerville, C. 2009. Cellulosic biofuels. Annu. Rev. Plant Biol. 60(1):165-182. https://doi.org/10.1146/annurev.arplant.043008.092125.

Coêlho, J. J.; Leão-M. A. C.; Ferreira, D. M. V.; Batista, D. J. J. C.; Vieira-Da, C. M. and Lira, M. A. 2018. Prediction of the nutritional value of grass species in the semiarid region by repeatability analysis. Pesq. Agropec. Bras. 53 (3):378-385. https://doi.org/10.1590/s0100-204x2018000300013.

Enríquez, Q. J. F. y Romero, M. J. 1999. Tasa de crecimiento estacional a diferentes edades de rebrote de 16 ecotipos de Brachiaria spp. en Isla, Veracruz. Agrociencia. 33(2):141-148. https://www.colpos.mx/agrocien/96-99/332.pdf.

Dien, B. S. 2010. Mass balances and analytical methods for biomass pre-treatment experiments. *In*: biomass to biofuels: strategies for global industries. Vertès, A. N.; Qureshi, A.; Blaschek, H. and Yukawa, H. (Ed). Wiley. Chichester, United Kingdom. 213-231 pp.

García, E. 2004. Modificación al sistema de clasificación climática de Köppen. 4ª (Ed). Instituto de Geografía. Universidad Nacional Autónoma de México (UNAM). México. 217 p.

Goering, H. K. and Van-Soest, P. J. 1970. Forage fiber analyses: apparatus, reagents, procedures, and some applications (No. 379). Agricultural Research Service, US Department of Agriculture.

Hernández, T.; Valles, B. y Castillo, E. 1990. Evaluación de gramíneas y leguminosas forrajeras en Veracruz, México. Pasturas Trop. 12(3):29-33.

Jahirul, M. I.; Rasul, M. G.; Chowdhury, A. A. and Ashwath, N. 2012. Biofuels production through biomass pyrolysis a technological review. Energies. 5(12):4952-5001. https://doi.org/10.3390/en5124952.

Kondo, M.; Yoshida, M.; Loresco, M.; Lapitan, M. R.; Herrera, V. J. R.; Del Barrio, N.; Uyeno, Y.; Matsui, H. and Fujihara, T. 2015. Nutrient contents and *in vitro* ruminal fermentation of tropical grasses harvested in wet season in the Philippines. Adv. Anim. Vet. Sci. 3(12):694-699. http://dx.doi.org/10.14737/journal.aavs/2015/3.12.694.699.

Lima, M. A.; Gomez, L. D.; Steele-King, C. G.; Simister, R.; Bernardinelli, O. D.; Carvalho, M. A.; Rezende, C. A.; Labate, C. A.; de Azevedo, E. R.; McQueen-Mason S. J. and Polikarpov, I. 2014. Evaluating the composition and processing potential of novel sources of Brazilian biomass for sustainable biorenewables production. Biotechnol. Biofuels. 7(1):1-19. https://doi.org/10.1186/1754-6834-7-10.

Limayem, A. and Ricke, S. C. 2012. Lignocellulosic biomass for bioethanol production: current perspectives, potential issues and future prospects. Prog. Energy Combust. Sci. 38(4):449-467. https://doi:10.1016/j.pecs.2012.03.002.

Mertens, R. D. 1997. Creating a system for meeting the fiber requirements of dairy cows. J. Dairy Sci. 80(7):1463-1481. https://doi:10.3168/jds.S0022-0302(97)76075-2.

Mohammed, Y. I.; Abakr, A. Y.; Kazi, K. F.; Yusup, S.; Alshareef, I. and Chin, A. S. 2015. Comprehensive characterization of napier grass as a feedstock for thermochemical conversion. Energies. 8(5):3403-3417. https://doi.org/10.3390/en8053403.

Ortega, G. R.; Castillo, G. E.; Rodríguez, J. J.; Escobar, H. R.; Ocaña, Z. E. and de la Mora, B. V. 2011. Nutritive quality of ten grasses during the rainy season in a hot -humid climate and ultisol soil. Trop. Subtrop. Agroecosys. 13(3):481-491.

Nhuchhen, D. R. and Salam, P. A. 2012. Estimation of higher heating value of biomass from proximate analysis: a new approach. Fuel. 99(8):55-63. https://doi.org/10.1016/j.fuel.2012.04.015.

Ramírez, R. O.; Hernández, G. A.; Carneiro-Da, S. S.; Pérez, P. J.; Enríquez, Q. J. F.; Quero, C. A. R.; Herrera, H. J. G. and Cervantes, N. A. 2009. Acumulación de forraje, crecimiento y características estructurales del pasto mombaza (*Panicum maximum* Jacq.) cosechado a diferentes intervalos de corte. Téc. Pec. Méx. 47(2):203-213.

Ramírez, O. S.; Domínguez, D. D.; Salmerón, Z. J. J.; Villalobos, V. G. y Ortega, G. J. A. 2013. Producción y calidad del forraje de variedades de avena en función del sistema de siembra y de la etapa de madurez al corte. Rev. Fitotec. Mex. 36(4):395-403.

Rojas, G. A. R.; Torres, S. N.; Maldonado, P. M.; Sánchez, S. P.; García, B. A.; Mendoza, P. S.; Álvarez, V. P.; Herrera, P. J. y Hernández, G. A. 2018. Curva de crecimiento y calidad del pasto Cobra (*Brachiaria* hibrido br02/1794) a dos intensidades de corte. Agroproductividad. 11(5):34-38. https://www.researchgate.net/profile/Adelaido_Rojas_Garcia/publication/326426605.

Rueda, J. A.; Ortega, J. E.; Hernández, G. A.; Enríquez, Q. J. F.; Guerrero, R. J. D. and Carrillo, A. R. Q. 2016. Growth, yield, fiber content and lodging resistance in eight varieties of *Cenchrus purpureus* (Schumach.) Morrone intended as energy crop. Bio. Bioenergy. 88(5):59-65. https://doi.org/10.1016/j.biombioe.2016.03.007.

SAS. 2011. Statistical Analysis System. Institute. User’s Guide of SAS Release 9.3. SAS Institute Inc., Cary, NC. USA. 550 p.

Santiago, O. M. A.; Honorato, S. J. A.; Quero, C. A. R.; Hernández, G. A.; López, C. C. and López, G. I. 2016. Biomasa de *Urochloa brizantha* cv. Toledo como materia prima para la producción de bioetanol. Agrociencia. 50(6):711-726.

Sharma, V.; Sharma, S. and Kuila, A. 2016. A review on current technological advancement of lignocellulosic bioethanol production. J. Appl. Biotechnol. Bioeng. 1(2):1-7. https://doi.org/10.15406/jabb.2016.01.00011.

Shankar, T. J. 2015. Comparison of chemical composition and energy property of torrefied switchgrass and corn stover. Front. Energy Res. 3(46):1-11. https://doi.org/10.3389/fenrg.2015.00046.

Somerville, C.; Youngs, H.; Taylor, C.; Davis, S. C. and Long, S. P. 2010. Feedstocks for lignocellulosic biofuels. Science. 329(5993):790-792. https://doi.org/10.1126/science.1189268.

Simon, K. B. and Jacobs, S. W. L. 2003. *Megathyrsus*, a new generic name for *Panicum* subgenus Megathyrsus. Austrobaileya. 6(3): 571-574.

Sluiter, A.; Ruiz, R.; Scarlata, C.; Sluiter, J. and Templeton, D. 2008. Determination of Extractives in Biomass. Technical Report NREL/TP-510-42619.

Van-Soest, P. J.; Robertson, J. B. and Lewis, B. A. 1991. Methods for dietary fiber, neutral detergent fiber, and non-starch polysacharides in relation to animal nutrition. J. Dairy Sci. 74(10):3583-3589. https://doi.org/10.3168/jds.S0022-0302(91)78551-2.

Weijde, T.; Kamei, A. C. L.; Torres, A. F.; Vermerris, W.; Dolstra, O.; Visser, R. G. F. and Trindade, M. L. 2013. The potential of C4 grasses for cellulosic biofuel production. Plant Sci. 4(107):1-18. https://doi.org/10.3389/fpls.2013.00107.

Verdecía, D. M.; Ramírez, J. L.; Leonard, I. y García, F. 2009. Potencialidades agroproductivas de dos cultivares de *Panicum maximum* (c.v Mombasa y Uganda) en la provincia Granma. Rev. Electron de Vet. 10(5):1-9.

Ventura, R. J.; Honorato, S. J. A.; Hernández, G. A.; Aburto, A. J. A.; Vaquera, H. H. y Enríquez, Q. J. F. 2017. Composición química y rendimiento de biomasa de maralfalfa para producción de bioetanol de segunda generación. Rev. Méx. Cienc. Agríc. 8(1):215-221. https://doi.org/10.29312/remexca.v8i1.85.

Ventura, R. J.; Reyes, V. I.; García, S. A.; Muñoz, G. C.; Muro, R. A.; Maldonado, P. M.; Rojas, G. A. y Cruz, H. A. 2019. Rendimiento, perfil nutrimental y de fermentación ruminal *in vitro* de pasto maralfalfa (*Cenchrus purpureus* Schumach.) Morrone a diferentes frecuencias de corte en clima cálido. Acta Universitaria. 29(1):1-11. https://doi.org/10.15174/au.2019.2204.

Zhao, Y. L.; Dolat, A.; Steinberger, Y.; Wang X.; Osman, A. and Xie, G. H. 2009. Biomass yield and changes in chemical composition of sweet sorghum cultivars grown for biofuel. Field Crops Res. 111(1-2):55-64. https://doi.org/10.1016/j.fcr.2008.10.006.

Wongwatanapaiboon, J.; Kangvansaichol, K.; Burapatana, V.; Inochanon, R.; Winayanuwattikun, P.; Yongvanich, T. and Chulalaksananukul, W. 2012. The potential of cellulosic ethanol production from grasses in Thailand. J. Biomed Biotechnol. 303(748):1-10. https://doi.org/10.1155/2012/303748.