Biomass and Bioenergy xxx (2016) 1–7

Contents lists available at ScienceDirect

Biomass and Bioenergy

journal homepage: http://www.elsevier.com/locate/biombioe

Research paper

# Growth, yield, fiber content and lodging resistance in eight varieties of *Cenchrus purpureus* (Schumach.) Morrone intended as energy crop

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A R T I C L E I N F O

*Article history:*
Received 6 August 2015
Received in revised form
4 March 2016
Accepted 7 March 2016
Available online xxx

*Keywords:*
*Pennisetum purpureum*
Cellulose
Biomass
Lignin
Napier grass
Cutting frequency

A B S T R A C T

Growth, biomass yield, fiber content and lodging resistance were studied, during a six month growth period, for eight varieties of *Cenchrus purpureus*, intended as energy crop, in Veracruz, Mexico. Then, only yield at day 182 was assessed for two additional years. The varieties were: CT115 (CT), African Cane (AC), Taiwan (TAI), King Grass (KG), Vruckwona (VRU), Roxo (RX), OM22 (OM) and Cameroon (CAM). Local weather is warm and sub-humid, historical data for monthly average temperature and annual rainfall were 25.8 °C and 1142 mm, respectively. Height, diameter and light interception were measured monthly from day 65–185. At day 185, biomass yield and tiller density were measured. Number of lying tillers was counted to estimate lodging resistance. Cellulose and hemicellulose content were estimated in leaf and stem. No differences were found for dry matter yield or stem yield at day 185 in the first year. Regarding the next two years, TAI yielded above CT, OM or ROX. Average dry matter yield was higher in the second year than in the establishment cycle (38.6 vs 21.1 Mg ha$^{-1}$), but decreased in the third year (32.2 Mg ha$^{-1}$). In both stem and whole plant, AC and KG showed higher hemicellulose content than RX, OM or CT; while AC and VRU had higher cellulose than RX in stem, or than CT in the whole plant. Furthermore, varieties AC, KG, VRU and TAI were resistant to lodging and had a higher fiber content, so they are recommended as energetic crops.



## 1. Introduction

*Cenchrus purpureus* (Schumach.) Morrone, formerly *Pennisetum purpureum* [1] is widely known as Napier Grass, Elephant Grass, or King Grass, but in some cases these names have been used to refer a particular variety. This giant grass has recently attracted interest of researchers due to its high biomass yield. Recent studies focus on topics such as production of second-generation bioethanol to replace gasoline [2], use of grass fiber as a wood substitute for the paper-mill industry [3], direct combustion of biomass as charcoal substitute [4] and as a fodder for animal feeding [5].

The varieties available in Mexico have been scarcely studied regarding agronomic or morphologic features. CAM was released in Kenya in 1952 [6], it has been classified as belonging to the Cameroons type among three groups: Cameroon, Dwarf and Napier types [7]. On the other hand, King Grass gave rise to CT by tissue culture and to OM by hybridization with *Cenchrus americanus*, both varieties were released in Cuba [8]. OM is hairless and tall, with wider leaves than those of pure genotypes. In contrast, CT has lower height, less wide leaves and more abundant trichomes than OM. RX is a variety from Togo (Africa) introduced to Estação Presidente Medici in São Paulo, Brazil in 1975 [9], it is easily distinguished from others by a purple color on its leaves, stems and inflorescences.

*Abbreviations:* CT, CT115; AC, African Cane; TAI, Taiwan; KG, King Grass; VRU, Vruckwona; RX, Roxo; OM, OM22; CAM, Cameroon; NDF, Neutral Detergent Fiber; ADF, Acid Detergent Fiber; ADL, Acid Detergent Lignin; INIFAP, Mexican Institute for Forestry, Agricultural and Livestock Research (for its given acronym in Spanish).

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http://dx.doi.org/10.1016/j.biombioe.2016.03.007
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Genetic distances between some of these varieties are: 0.02, 0.06 and 0.10 for AC vs VRU, CAM or KG; 0.07 and 0.11 for KG vs VRU or CAM and 0.02 between the last two [10].

The high biomass yield of *C. purpureus* stems after its exponential growth stage [11] makes it one of the most promising options both for the paper industry and for bioethanol extraction. A bioethanol yield of 144 mg $g^{-1}$ in Napier Grass has been reported, 44% of theoretical maximum according to 37 g of hexoses and 26 g of pentoses in 100 g of dry biomass [2].

Biomass yield in *C. purpureus* has been found to decrease after one to three years in the same field. In a study, using Merkeron variety at a single harvest per year [12], authors found that after a fertilization dose of 100-40-90 kg $ha^{-1}$ $y^{-1}$ (N-P-K) either with an inorganic fertilizer or with poultry litter as sources, dry mater yield was systematically reduced from 30 to 10 Mg $ha^{-1}$ in four years for the fertilization treatments and from 29 to 6 Mg $ha^{-1}$ when it was not fertilized. In the mentioned assay, authors found a strong reduction of nitrogen and potassium removal between successive years, which means that availability of such elements was limiting growth. In another long-term study from 1996 to 2001 at three sites equally managed, in Georgia, United States [13], the author reported that biomass yield in Napier Grass decreased after three or four years, from dry mater yields as high as 39 (year 1999, Athens), 41 (1996, Tifton) and 49 Mg $ha^{-1}$ (1998, Midville) until 16, 13 and 18 Mg $ha^{-1}$, respectively, for the 2001 year. A 4-years experiment with Merkeron variety proved that yield decrease sharply for a low input system [14], achieved yield were 30.4 and 30.1 through the first two years, but 11.2 and 7.1 Mg $ha^{-1}$ during the last two. In the mentioned assay, growth was limited by availability of minerals, since nitrogen and potassium removal had a clear trend to decrease from the first to the fourth year (6.1–1.8 and from 21.3 to 6.5 g $kg^{-1}$, respectively) and ash content were 60, 54, 45 and 29 g $kg^{-1}$ during the four years included.

In a study with Taiwan, Elephant, Dwarf and King Grass, biomass yield was measured every 14 days from day 70–140. The authors report that dry matter accumulation continues until day 126 in both stem and leaf, as well as in the whole plant [11]. They inform that dry matter yield did not surpass 16.6 Mg $ha^{-1}$ and leaf to stem ratio was 0.54 at day 140. Furthermore, from day 98–140 stem yield increased from 4.9 to 12.2 and leaf yield increased from 3.6 to 4.4 Mg $ha^{-1}$ (average for the mentioned varieties). On this basis, growth periods might range from 70 to 140 days according to biomass requirements. In a different study, it was concluded for *C. purpureus* cv. Maralfalfa, that harvest is feasible from 60 days and onwards, where both fiber content and yield were higher than at younger ages [15]. In this last assay, fertilization and nitrogen availability is reported to be a strong limitation due to the high mineral requirements of *C. purpureus*; nitrogen doses above 240 kg $ha^{-1}$ are recommended to preserve soil fertility.

Previous studies have recorded that longer growth periods promote persistence of *C. purpureus*. By studying the rhizome mass and root concentration of both nonstructural carbohydrates and nitrogen, in response to four defoliation heights and four defoliation frequencies (10, 22, 34 and 46 cm and 3, 6, 9 and 12 weeks), it was concluded that the lowest values, for the three variables were reached for the 10 cm and 3 weeks treatment, whereas the highest values succeed for the 46 cm and 12 weeks treatment [16]. According to the above mentioned, combining high cutting frequency with high cutting intensity depletes reserves and endangers persistence as it has been established before for other tropical species like *Paspalum notatum* [17]. These results, plus the high biomass accumulation in stems at the end of the exponential growth phase [11] and a higher fiber content as the crop ages [15], would help supporting the convenience of harvesting after long growth periods when grass is intended for industrial uses, as proposed in the present experiment.

Lodging has been reported to be a frequent problem in tropical grasses. In *C. purpureus*, some varieties tend to reduce the angle with the ground as a reproductive strategy, allowing axillary buds to reach soil, which in turn gives rise to new tillers. This particular feature has not been studied before for this specie.

Field assessment can help decision making according to the characteristics of each variety. A high lignin and cellulose content is desirable for the paper mill industry and for the fabrication of biomass briquettes as charcoal substitute, but a high lignin content can limit ethanol extraction [18] and is fairly undesirable for animal feeding purposes. On the other hand, industrial crops are kept on vast tracks of land and mechanical harvest cannot be done if they show lodging. The present experiment assesses growth, yield, fiber content and lodging resistance in eight varieties of *C. purpureus* intended as industrial crop in a warm, sub-humid climate in an attempt to help decision making in the field. In addition, a correlation test was run among morphological and yield traits.

## 2. Materials and methods

### 2.1. Location

The experiment was conducted at *Campus* Papaloapan of the Mexican Institute for Forestry, Agricultural and Livestock Research (INIFAP) in Veracruz, Mexico, from June 2012 through January 2013, but growth measurements started on August 30 (day 65 after planting) and ended on December 28 (day 185). Yield data for the next two wet seasons come from growth cycles beginning on June 15 of 2013 and June 07 of 2014. The *Campus* Papaloapan is located at 18°06′ N, 95°32′ W and 65 m above sea level. Climate is classified as an Aw, hot sub-humid [19], with 80% of seasonal rain occurring in summer, where historical data (40 years) for annual precipitation and average temperature were 1142 mm and 25.8 °C. Weather data for the term of the present assay is shown in Fig. 1. By means of a soil analysis realized at *Campus* Cotaxtla of INIFAP, for samples collected in the field during sowing, soil was identified as an orthic acrisol with a sandy-loam texture, low organic matter (0.34%), acid pH (3.5), low level of inorganic N, Ca, Mg and Cu (2,174, 48 and 0.2 mg $kg^{-1}$) but adequate levels of Fe, Zn, Mn and P (57, 1.6, 4.4 and 55 mg $kg^{-1}$).

### 2.2. Experimental conditions

Assessed varieties were: CT115 (CT), African Cane (AC), Taiwan (TAI), King Grass (KG), Vruckwona (VRU), Roxo (RX), OM22 (OM) and Cameroon (CAM). Experimental design consisted of four complete randomized blocks and eight treatments (varieties) for a

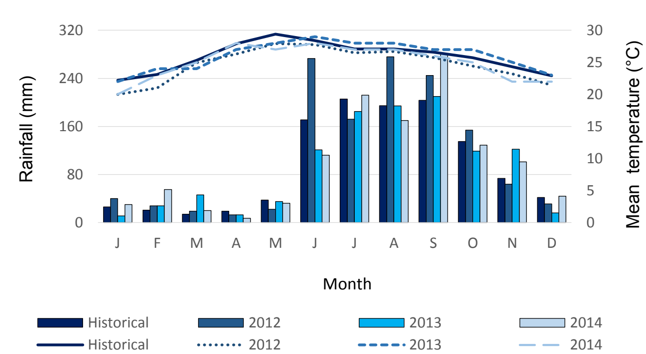


**Fig. 1.** Rainfall (bars) and mean temperature (lines) during the current assay and historical data for 40 yr.

Please cite this article in press as: J.A. Rueda, et al., Growth, yield, fiber content and lodging resistance in eight varieties of *Cenchrus purpureus* (Schumach.) Morrone intended as energy crop, Biomass and Bioenergy (2016), http://dx.doi.org/10.1016/j.biombioe.2016.03.007

total of 32 plots.

The field was plowed to 20 cm deep and then plots were furrowed with a hoe to 5 cm deep. Each plot comprised six rows 8 m long and 0.6 m apart, so useful plot was 24 linear meters, after discarding 1 row at each side and 1 m at row tips. For planting, two whole stems were placed together horizontally at bottom of rows, joining together tip and tail of each other, then culms were cut with machete in pieces containing two or three internodes, when already in the rows; then they were covered with soil. During the first growth cycle, a single fertilization dose was added by hand-broadcasting at day 45 after planting, with 60 and 40 kg ha$^{-1}$ of N and P, using 130.5 kg of di-ammonium phosphate $(NH_4)_2HPO_4$ and 35.9 kg ha$^{-1}$ of urea $CO(NH_2)_2$. For the next two years, a formula of 200-100-100 kg ha$^{-1}$ year$^{-1}$ (N-P-K) was applied half and half at days 14 and 56 after planting, using the same sources. During the whole experiment, grass development depended entirely on seasonal rain.

### 2.3. Measurements

#### 2.3.1. Growth through establishment

Plant height (cm), stem diameter (mm) and light interception (%) were monthly-measured from day 65–185, corresponding with the first growth cycle after planting. Five sub-replicates for each of the three variables were taken at each plot and average was used as repetition, representing plot. Height was measured with a graduated PVC pipe from ground level to the last folded leaf or until flag leaf in OM (only for the last two measurements), since it was at 90% flowering when all other varieties showed just sporadic inflorescences. Diameter was measured with a vernier scale around 25 cm above ground, centered between two nodes and twisting scale in oval tillers until shorter dimension. Light interception was measured with a 1-m-long ruler painted white and scaled black, containing 100 squares of 1 cm$^2$ each. Ruler was placed perpendicularly to rows between two central rows at ground level and shadowed squares were counted. Assessment started at day 65 since harvest is already feasible for industrial uses without risk for grass persistence.

At day 185, after the last measurement for growth variables was taken, herbage in the 2 edge-rows and in 1-m at each tip of the remaining four rows was cut to remove border effect. Height and number of internodes per stem were recorded in 5 tillers, then the average internode length was estimated as a ratio between the plant height and the number of internodes for each stem.

#### 2.3.2. Yield and density at day 185

To estimate tiller density in 2012 (establishment), the number of tillers was counted in six linear meters of two rows, one on each side of the plot (these rows were now exposed after cutting border effect as described in 2.3.1). The grass in those 6 m on each side was cut and weighed, taking each side as a sub-replicate, and then the average weight of these two replicates was used as the gross-sample fresh weight for each plot. For years 2013 and 2014 only one row was used to estimate yield, but no other variable was considered for these two years. The gross sample comprised all tillers which were counted to estimate density, and density was recorded as the number of tillers in a linear meter. Biomass yield was later estimated by discarding moisture from the gross-sample fresh weight and taking into account 16,666.7 linear meters in a hectare since rows were 0.6 m apart. A two tillers sub-sample from the gross sample was divided into morphological fractions: stem, leaf and dead material; then they were chopped, placed in paper bags and weighed in fresh. Fresh samples were oven dried at 60 °C until reaching constant weight. With dry and wet weights of the sub-sample fractions and the gross-sample fresh weight, the next variables were estimated: biomass yield, leaf yield, stem yield, dead material yield (all in Mg ha$^{-1}$ of dry mater) and leaf to steam ratio (in fresh and dry matter). Leaf, stem and dead material fresh proportions were used to divide the gross sample weight in morphological fractions, in order to use dry weights of such fractions to estimate dry matter yields. In addition, dry weight per tiller and dry weight per stem were calculated dividing gross sample by the number of tillers on it, then dividing tiller by morphological fraction and finally discarding moisture. Fresh weights are not reported. Density was only assessed during the first growth cycle.

#### 2.3.3. Fiber content

Dry samples of leaf and stem were ground until passing through a 1 mm mesh. Then neutral detergent fiber (NDF) and acid detergent fiber (ADF) were estimated as a fraction of the dry matter [20] using an Ankom 200® fiber analyzer. Acid detergent lignin (ADL) was obtained by sulfuric acid dilution [21]. Ash content was measured by oven combustion for 2 h at 500 °C. Hemicellulose and cellulose content were estimated by difference between NDF and ADF and between ADF and ADL, respectively. A different sample was oven dried for 4 h at 105 °C in order to express NDF, ADF and ADL in dry matter.

#### 2.3.4. Lodging resistance

The last trait taken in the field was the proportion of lying tillers. After cutting the two rows for the gross sample (as explained in 2.3.2), the number of standing and lying tillers was counted all along 6 m of the two remaining central rows now exposed. Then the number of lying tillers was expressed as a fraction of the total count of tillers. This variable was recorded two weeks after leaving only the two central rows of grass standing (January 11/2013), so tillers were even more affected by wind since this concurred with the north-wind season.

### 2.4. Analyses of data

Variables were analyzed using a GLM procedure and then a pairwise Tukey test was run. Correlation tests were run between yield and morphological variables and between pairs of morphological variables. An F test was used to compare groups of means when necessary. Analyses were performed in licensed SAS software [22] at the Colegio de Postgraduados.

## 3. Results and discussion

### 3.1. Growth

Height, stem diameter, and light interception from day 65–185 after planting, are shown in Fig. 2. At these ages, harvest is feasible without compromising persistence, while cutting intervals shorter than 60 days are not recommended in literature.

OM and CT showed contrary trends at the end of the growth period. At days 155 and 185, OM showed the greatest height ($P < 0.001$ and $P = 0.037$) because of higher internode lengths at the tip due to flowering. CT showed the lowest height at day 185, since it reduces its internode length at the tip, which is its most widely known feature and the reason why it was originally selected. In contrast, at day 65: RX, OM and CAM were shorter than CT ($P = 0.006$) and CT had a lower light interception than CAM ($P = 0.015$). Early development of CT is consistent with a higher light interception (Fig. 2). Regarding stem diameter, OM was the variety with the thickest stems from day 95 through 155 (F contrast; $P < 0.001$). At day 185 OM stem diameters were not different from RX ($P = 0.7$) or VRU ($P = 0.07$). As *C. purpureus* grows, light interception increases until day 125, when it achieves a

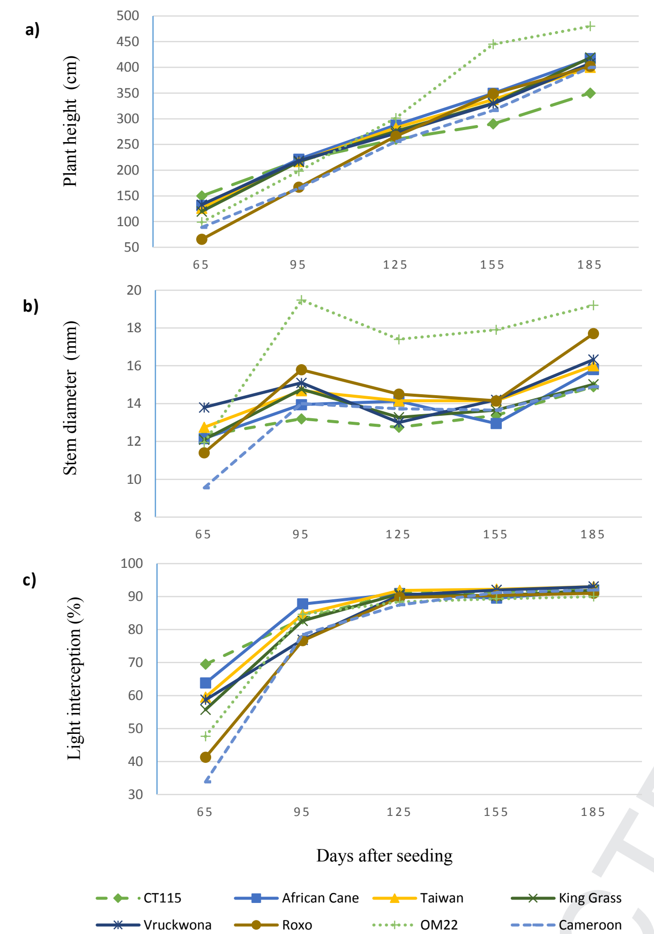


**Fig. 2.** Plant height (a), stem diameter (b) and light interception (c) in eight varieties of *Cenchrus purpureus* from day 65–185 after seeding.

maximum, but it does not reach saturation, as it has been noticed before [23]. None of the studied varieties surpassed 94% of light interception at day 185.

### 3.2. Biomass yield

Yield for each morphological fraction, and leaf to stem ratio at day 185 are shown in Table 1 for the 2012 growth cycle (establishment). Stem yield was 14.11 ± 0.63 Mg ha$^{-1}$ in dry basis, with 12.2 for RX and 16.1 for CT, but no differences were shown between varieties ($P = 0.53$). Leaf yield averaged 3.91 ± 0.21 with differences between varieties ($P < 0.004$), since CAM, CT, OM, TAI and VRU were above RX. Dead material was higher in KG and AC, clearly above CT or CAM yield, which might imply that the first varieties are drying and losing its leaves before CAM or OM. This is an important feature since leaves can give back its minerals to the soil, since they have a higher mineral content than stems [14], thus reducing extraction and keeping soil fertility.

Dry matter yield for a three years term is shown in Table 2. Average dry-matter yield increased ($P < 0.0001$) 17.5 Mg ha$^{-1}$ between 2012 and 2013, but it decreased 6.4 Mg ha$^{-1}$ between 2013 and 2014 ($P < 0.0001$). This reduction in yield in successive years has been widely reported in literature for Napier Grass [12–14]. Regarding average yield, across three years: TAI yielded above CT, OM or RX; while KG, AC, and VRU yielded above CT.

In a previous assay a dry mater yield of 30 Mg ha$^{-1}$ was reported for Maralfalfa at day 184 [23], which is consistent with the average obtained in this research for the three years and eight species (30.6 ± 1.8). In the mentioned study, they used a higher fertilization dose and a higher plant density, resulting in a more efficient use of photo-synthetically active radiation; these advantages explain such a yield in an establishment growth cycle. Furthermore, this authors found a 97% of light interception, which is above 94%, the maximum value reached in the present work.

Yields achieved in the present experiment are moderately low in comparison with those reported for Tizimin, Yucatan, Mexico, for analogous weather conditions [24], where dry matter yields of 80 Mg ha$^{-1}$ year$^{-1}$ for CT, 102 for KG and 112 for OM were found, and even increases from 15, 38 or 48 Mg ha$^{-1}$ year$^{-1}$ for those varieties, in the same order, when using 300 kg ha$^{-1}$ of pig effluent as fertilizer. These yields from Yucatan are above almost any yield reported in literature; authors used a cutting frequency of 60 days, a high fertilizing dose and continuous watering, which are

**Table 2**
Dry matter yield at day 185 during three successive years for eight varieties of *Cenchrus purpureus*.

| Variety | 2012 | 2013 | 2014 | Variety mean |
|---|---|---|---|---|
| CT115 | $24.3^{B}$ | $36.2^{Abcd}$ | $26.9^{Ab}$ | $25.4^{bc}$ |
| African cane | $21.9^{B}$ | $43.5^{Aab}$ | $32.7^{ABab}$ | $32.7^{ab}$ |
| Taiwan | $22.3^{B}$ | $45.8^{Aa}$ | $36.6^{Aa}$ | $34.8^{a}$ |
| King grass | $20.7^{B}$ | $40.7^{Aabc}$ | $34.2^{Aab}$ | $31.7^{ab}$ |
| Vruckwona | $20.7^{B}$ | $42.7^{Aabc}$ | $36.6^{Aa}$ | $33.0^{ab}$ |
| Roxo | $17.3^{B}$ | $27.6^{ABd}$ | $33.2^{Aab}$ | $25.4^{c}$ |
| OM22 | $22.7^{B}$ | $34.3^{Acd}$ | $25.8^{ABb}$ | $27.8^{bc}$ |
| Cameroon | $20.3^{B}$ | $38.4^{Aabc}$ | $31.4^{ABab}$ | $29.9^{abc}$ |
| Standard error | 2.1 | 2.1 | 2.4 | |
| Year mean | $21.1^{C}$ | $38.6^{A}$ | $32.2^{B}$ | |

a, b, c: Means with different lowercase letter in the same column are different (Tukey test, $P < 0.05$).
A, B, C: Means with different uppercase letter in the same row are different (Tukey test, $P < 0.05$).
$R^2 = 0.85$.

**Table 1**
Yield per morphological fraction (Mg DM ha$^{-1}$) and leaf to stem ratio for eight varieties of *Cenchrus purpureus* at day 185 after planting.

| Variety | Leaf yield | Stem yield | Dead material yield | Leaf to stem ratio |
|---|---|---|---|---|
| CT115 | $4.45^{a}$ | 16.11 | $3.77^{ab}$ | $0.28^{b}$ |
| African cane | $3.49^{ab}$ | 14.13 | $4.31^{a}$ | $0.26^{bc}$ |
| Taiwan | $4.35^{a}$ | 13.81 | $4.17^{ab}$ | $0.33^{ab}$ |
| King grass | $3.39^{ab}$ | 13.11 | $4.23^{a}$ | $0.26^{bc}$ |
| Vruckwona | $3.95^{a}$ | 13.02 | $3.74^{abc}$ | $0.32^{ab}$ |
| Roxo | $1.98^{b}$ | 12.24 | $3.14^{abc}$ | $0.16^{c}$ |
| OM22 | $4.12^{a}$ | 15.76 | $2.87^{bc}$ | $0.26^{bc}$ |
| Cameroon | $5.03^{a}$ | 12.73 | $2.53^{c}$ | $0.42^{a}$ |
| Standard error | 0.39 | 1.74 | 0.45 | 0.04 |
| $R^2$ | 0.68 | 0.33 | 0.51 | 0.63 |

a, b, c: Means in the same column with different letter are different (Tukey test, $P < 0.05$).



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significant advantages with respect to the conditions of the present study. In contrast, in another experiment, a maximum dry matter yield of 5.6 Mg ha$^{-1}$ for CT variety was reported [25]. In this last study, environment was strongly limiting for the growth: only 580 mm of annual rainfall and mean temperature of 22 °C, at 293 m above sea level. Nevertheless, under such conditions establishing a perennial $C_4$ grass with high temperature and water requirements, such as *C. purpureus*, is already an achievement. In a study in Brazil at a temperature of 22.5 °C and 1300 mm of rainfall CAM yielded more than 25 Mg ha$^{-1}$ (dry basis) when cut at day 100 after planting [26].

### 3.3. Leaf to stem ratio

CAM had a higher leaf to stem ratio than CT and this in turn had a higher ratio than RX, but no differences were found between CT, VRU, TAI, KG, AC or OM.

The close genetic relationship between VRU, TAI, KG and AC is consistent with the observed similarities both in yield and in leaf to stem ratio. For CAM, a leaf to stem ratio of 1.0 was reported at day 120, which is higher than the found in this experiment, but consistent with a younger fodder, as leaf to stem ratio decreases linearly with age until day 90 from 5.0 to near 1.0 in the same study [26].

### 3.4. Plant morphology and density at day 185

Morphological variables measured at day 185 after planting are shown in Table 3. Since stem comprises the higher proportion of the plant at this age, stem weight is also shown. The varieties differed for all morphological variables. However, TAI, KG, AC and VRU did not show any differences among them. In contrast, CAM, which was supposed as close related to the last four, had thinner stems than VRU and longer internodes than all four.

CT had the shorter internode length than OM, VRU and CAM; the lowest plant height and a higher tiller density than all but KG or CAM. OM showed thicker stems than all but RX or VRU and heavier tillers than CT, CAM and RX. OM had a lower tiller density than CT. Both OM and CAM had longer internodes than all other, and CAM had thinner stems than OM, RX and VRU.

### 3.5. Correlation between yield and morphological traits

Since planting was carried out by placing two whole culms horizontally in rows, tiller density was inversely related with average internode length during first growth period after planting ($r = -0.35$; $P = 0.049$). It was expected that a culm which had fewer internodes and therefore fewer axillary buds would give origin to fewer tillers in the row. So for example, OM showed longer internodes and lower density than CT at day 185. However, lower density is balanced by a greater height ($r = 0.52$; $P = 0.002$). In contrast, CT tends to have shorter internode length, and as a consequence a higher number of axillary buds, which results in a higher density given the planting method. A higher number of tillers in CT increases competition for resources and limits stem growth, in both diameter and height. One of the more important reasons for OM having the greater height is due to fast flowering, since OM was the only variety with inflorescences (more than 90%) when inflorescences were sporadic in all other varieties. In *C. purpureus*, flowering affects height because the last internodes (those at the tip) are longer and thinner than those on the rest of the plant.

In accordance with a previous study, height and density are the principal variables explaining biomass yield in *C. purpureus* [27]. In the present assay the variables which better explain yield were density ($r = 0.53$; $P = 0.001$) and plant weight ($r = 0.47$; $P = 0.006$). On the other hand, plant height was directly correlated with stem diameter ($r = 0.59$; $P = 0.003$), average internode length ($r = 0.52$; $P = 0.002$) and plant weight ($r = 0.51$; $P = 0.003$) but inversely correlated with density ($r = -0.58$; $P = 0.0005$). The inverse association between height and density has been widely discussed in literature as the self-thinning law [28].

### 3.6. Fiber and ash content

Acid detergent lignin, cellulose, hemicellulose and ashes content for stem and leaf fractions are shown in Table 4. Regarding fiber content in stems: VRU and TAI had higher lignin than CAM; AC and KG showed higher hemicellulose than OM, CT and RX; and finally VRU, AC, CAM, TAI and OM showed higher cellulose than RX. In leaves: lignin content was greater in TAI and CAM than in AC and

**Table 4**
Fiber content in eight varieties of *Cenchrus purpureus* (Schumach.) Morrone at day 185 after planting (g kg$^{-1}$).

| Variety | Lignin | | Cellulose | | Hemicellulose | | Ashes | |
|---|---|---|---|---|---|---|---|---|
| | Leaf | Stem | Leaf | Stem | Leaf | Stem | Leaf | Stem |
| CT115 | 32$^{ab}$ | 71$^{ab}$ | 269$^{ab}$ | 331$^{b}$ | 38.8$^{ab}$ | 338$^{b}$ | 83$^{ab}$ | 24$^{ab}$ |
| African cane | 27$^{b}$ | 65$^{ab}$ | 264$^{ab}$ | 377$^{a}$ | 39.3$^{ab}$ | 384$^{a}$ | 61$^{c}$ | 27$^{ab}$ |
| Taiwan | 38$^{a}$ | 77$^{a}$ | 266$^{ab}$ | 367$^{a}$ | 40.2$^{a}$ | 361$^{ab}$ | 81$^{abc}$ | 25$^{ab}$ |
| King grass | 29$^{ab}$ | 73$^{ab}$ | 251$^{b}$ | 346$^{ab}$ | 38.8$^{ab}$ | 384$^{a}$ | 68$^{bc}$ | 26$^{ab}$ |
| Vruckwona | 30$^{ab}$ | 78$^{a}$ | 278$^{a}$ | 376$^{a}$ | 39.9$^{a}$ | 368$^{ab}$ | 71$^{bc}$ | 34$^{a}$ |
| Roxo | 30$^{ab}$ | 75$^{ab}$ | 250$^{b}$ | 345$^{ab}$ | 36.5$^{b}$ | 350$^{b}$ | 92$^{a}$ | 36$^{a}$ |
| OM22 | 28$^{b}$ | 69$^{ab}$ | 251$^{b}$ | 366$^{a}$ | 36.9$^{ab}$ | 344$^{b}$ | 84$^{ab}$ | 18$^{b}$ |
| Cameroon | 37$^{a}$ | 65$^{b}$ | 260$^{ab}$ | 370$^{a}$ | 38.3$^{ab}$ | 359$^{ab}$ | 84$^{ab}$ | 23$^{ab}$ |
| Standard error | 1.5 | 1.9 | 4.2 | 5.3 | 6.1 | 5.4 | 3.6 | 2.6 |
| $R^2$ | 0.86 | 0.81 | 0.83 | 0.88 | 0.81 | 0.89 | 0.87 | 0.8 |

a, b, c: Means with different letter in the same column show statistical difference (Tukey test, $P < 0.05$).
Equivalences: hemicellulose = NDF-ADF, cellulose = ADF-ADL and lignin = ADL.

**Table 3**
Morphology of eight varieties of *Cenchrus purpureus* (Schumach.) Morrone at day 185 after planting.

| Variety | Height (cm) | Tiller density (tillers m$^{-1}$) | Stem diameter (mm) | Internode length (cm) | Tiller weight (g) | Stem weight (g) |
|---|---|---|---|---|---|---|
| CT115 | 350$^{c}$ | 11.2$^{a}$ | 14.9$^{bc}$ | 12.3$^{c}$ | 110.2$^{c}$ | 76.9$^{b}$ |
| African cane | 417$^{b}$ | 8.0$^{b}$ | 15.8$^{bc}$ | 14.3$^{bc}$ | 151.0$^{abc}$ | 107.6$^{ab}$ |
| Taiwan | 402$^{b}$ | 7.7$^{b}$ | 16.0$^{bc}$ | 14.5$^{bc}$ | 162.8$^{ab}$ | 110.3$^{ab}$ |
| King grass | 419$^{b}$ | 8.5$^{ab}$ | 15.0$^{bc}$ | 14.2$^{bc}$ | 144.6$^{abc}$ | 100.9$^{ab}$ |
| Vrukwona | 409$^{b}$ | 7.5$^{b}$ | 16.3$^{ab}$ | 14.7$^{b}$ | 138.9$^{abc}$ | 95.5$^{ab}$ |
| Roxo | 402$^{b}$ | 8.0$^{b}$ | 17.7$^{ab}$ | 14.4$^{bc}$ | 114.0$^{c}$ | 86.3$^{b}$ |
| OM22 | 479$^{a}$ | 6.2$^{b}$ | 19.2$^{a}$ | 20.5$^{a}$ | 183.5$^{a}$ | 133.9$^{a}$ |
| Cameroon | 401$^{b}$ | 9.5$^{ab}$ | 14.4$^{c}$ | 19.0$^{a}$ | 119.3$^{bc}$ | 78.8$^{b}$ |
| Standard error | 10 | 0.7 | 0.7 | 0.5 | 9.9 | 8.3 |
| $R^2$ | 0.37 | 0.63 | 0.20 | 0.57 | 0.73 | 0.69 |

a, b, c: Means in the same column with different letter show statistical difference (Tukey test, $P < 0.05$).

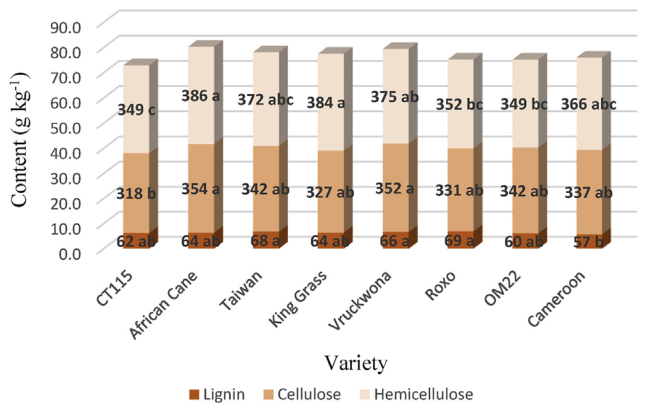


**Fig. 3.** Lignin (ADL), cellulose (ADF-ADL) and hemicellulose content (NDF-ADF) in the whole plant for eight varieties of *Cenchrus purpureus* at day 185 after seeding.

OM; VRU and TAI surpassed RX and OM in hemicellulose content; and similarly VRU had higher cellulose than RX or OM. As a general rule, stems had a higher lignin (72 vs 31 g $kg^{-1}$; $P < 0.0001$) and cellulose content (359 vs 261 g $kg^{-1}$; $P < 0.0001$) and lower hemicellulose (361 vs 386 g $kg^{-1}$; $P < 0.0001$) and ashes content (267 vs 77 mg $kg^{-1}$; $P < 0.0001$) than leaves. Cellulose to hemicellulose ratio was 0.68 for leaves and 1.0 for stems, with statistical difference ($P < 0.001$). In a previous research, ashes content was studied for six energy crops, including grasses, and always leaf surpassed stem in ashes content (86 vs 37 g $kg^{-1}$) [29], where ash content in stem was around one third of that in leaves, as it occurred in our data. In the present experiment ash content for the whole plant was $38 \pm 0.1$ g $kg^{-1}$, which according with reported data [14] could imply that growth was limited because of low mineral availability, since ash content was reported at level 60 g $kg^{-1}$ in the first year for Napier grass managed under one-harvest-a-year.

Since stem comprised around 78% of total yield at day 185 in the present assay, at this point ethanol yield would be highly dependent on fiber content in stems. Of special interest is hemicellulose content in stems, which was higher for the lodging resistant varieties, yet CAM had a high hemicellulose content as well.

Lignin, cellulose, and hemicellulose in the whole plant are shown in Fig. 3. RX, TAI and VRU had higher lignin content than CAM. AC and VRU had higher cellulose content than CT. AC and KG had higher hemicellulose than CT. CT had the lowest cellulose and hemicellulose contents.

### 3.7. Lodging resistance

Regarding lodging resistance, the varieties VRU, TAI, KG and AC (1.5, 3.75, 5.75 and 6.25% of tillers lying) were more resistant to lodging than CAM, CT, OM and RX (47, 35, 28 and 20%) ($P = 0.002$). Utilization of resistant varieties would prevent both harvest complications and weed invasion. Weeds can easily invade if tillers fall and light gains access to the ground, creating conditions for rising of herbs. In Fig. 4, a variety which shows lodging and a resistant variety are presented, this picture clearly allows noticing that harvest can be difficult under lodging conditions. In a study in Japan with *C. purpureus*, researchers found that angles between individual stems and the ground started as low as 20° and then reached 80–90° (perpendicularity with ground) when tillers achieve maturity [30], this clump architecture would maximize growth through a more efficient light utilization. As tillers were mature in the present assay, perpendicularity to the ground was expected to be the rule. Furthermore, no dense clumps were found in this first growth cycle, where tiller count ranged 2 to 4 tillers per clump, at most.

### 3.8. The best choice for growers

Growth, yield and morphology traits allowed to differentiate among OM, RX, CT and CAM, but none of these are recommended for a long growth period since they all showed lodging at certain degree. Besides that: OM, RX and CT have low hemicellulose content in stems; CT had the lowest cellulose content in stems; while CAM was the less resistant to lodging. The remaining four varieties can be used as industrial crops when managed at long growth periods, yet differences among them would help to select the most suitable choice according to utilization purpose. Notwithstanding CAM, CT and RX remain being good choices for shorter growth periods since its low fiber content would facilitate bioethanol extraction, yet the same condition confers a weakness in its stem and as a consequence, a low resistance to lodging.

A higher fiber content in stems concurred with resistance to

**Fig. 4.** African Cane (left) as an example of a variety resistant to lodging and Cameroon (right) as an example of a not resistant variety.

lodging in the same varieties (as shown in 3.6 and 3.7). For example, the two varieties with higher lignin content in stem (TAI and VRU were higher than CAM) coincided with those two with fewer lying tillers. On the other hand, the third and the fourth varieties with fewer lying tillers concurred with those with higher hemicellulose content in stems (AC and KG were higher than OM, CT and RX). KG and AC showed higher hemicellulose content than TAI and VRU (F contrast; P = 0.042) and this last two had some trend to show higher lignin content than KG and AC (F contrast; P = 0.08). Thereby AC and KG are more appropriate as energetic crops since lignin could limit ethanol extraction, while VRU and TAI can be a better choice for the paper mill industry.

## 4. Conclusions

*C. purpureus* yielded $21.1 \pm 0.74$ Mg ha$^{-1}$ of dry matter at day 185 on its first growth cycle after planting in a warm humid climate, but no differences were found among varieties. *C. purpureus* does not surpass 95% of light interception at day 185, when it reaches a 400 cm height and its stem diameter ranges from 14 to 19 mm.

Dry matter yield was higher in the second year of growth, but decreased in the third one under similar conditions.

CT and OM were the two most contrasting varieties in this study, where internode length was related to density due to the planting method.

Considering its high lodging resistance, high hemicellulose and low lignin content in stem, AC and KG are recommended as the two more important options for growers of Napier Grass in a scheme of long growth periods.

## Acknowledgments

We recognize and thank the financial support of the Colegio de Postgraduados and the Mexican Institute for Forestry, Agricultural and Livestock Research (INIFAP). Without their support this research work would not be possible.

The authors would like to thank James Patrick Killough for revising the present manuscript regarding English language style. And last but not least, to the graduate students: David Zamudio, Raciel Alvarez and Javier Cruz for their hard work in the field.

## References

[1] M.A. Chemisquy, L.M. Giussani, M.A. Scataglini, E.A. Kellogg, O. Morrone, Phylogenetic studies favour the unification of *Pennisetum*, *Cenchrus* and *Odontelytrum* (Poaceae): A combined nuclear, plastid and morphological analysis, and nomenclatural combinations in *Cenchrus*, Ann. Bot. 106 (2010) 107–130, http://dx.doi.org/10.1093/aob/mcq090.

[2] M. Yasuda, A. Miura, T. Shiragami, J. Matsumoto, I. Kamei, Y. Ishii, et al., Ethanol production from non-pretreated napiergrass through a simultaneous saccharification and fermentation process followed by a pentose fermentation with *Escherichia coli* KO11, J. Biosci. Bioeng. 114 (2012) 182–192, http://dx.doi.org/10.1016/j.jbiosc.2012.03.011.

[3] I.C. Madakadze, T.M. Masamvu, T. Radiotis, J. Li, D.L. Smith, Evaluation of pulp and paper making characteristics of elephant grass (*Pennisetum purpureum* Schum) and switchgrass (*Panicum virgatum* L.), Afr. J. Environ. Sci. Technol. 4 (2010) 465–470.

[4] R. Samson, S. Mani, R. Boddey, S. Sokhansanj, D. Quesada, S. Urquiaga, et al., Perennial grasses for developing a global BIOHEAT industry, CRC Crit. Rev. Plant Sci. 24 (2005) 461–495, http://dx.doi.org/10.1080/07352680500316508.

[5] D. Fortes, R.S. Herrera, M. Ramírez-Avilés, A.M. Cruz, A. Romero, Distribución vertical de las hojas, tallos y rendimiento de materia seca después del pastoreo del *Pennisetum purpureum* vc. Cuba CT-115 durante el período poco lluvioso, Rev. Cuba Cienc. Agric. 45 (2011) 73–77.

[6] D.S. Loch, J.E. Ferguson, Tropical and subtropical forage seed production: an overview, in: first ed., in: D.S. Loch, J.E. Ferguson (Eds.), Forage Seed Prod. Vol. II Trop. Subtrop. Species, CABI, Wallingford, UK, 1999, pp. 1–40.

[7] L.E. Passos, M.C. Vidigal, R.S. Verneque, Initial biomass production and soluble carbohydrate partitioning of contrasting elephantgrass genotypes, in: XIX Int. Grasslands Congr, International Grasslands, Sao Paulo, Brazil, 2001, pp. 12–14.

[8] R.O. Martínez, R. Tuero, V. Torres, R.S. Herrera, Modelos de acumulación de biomasa y calidad en las variedades de hierba elefante, Cuba CT-169, OM − 22 y king grass durante la estación lluviosa en el occidente de Cuba, Rev. Cuba Cienc. Agric. 44 (2010) 189–193.

[9] D.A. Gonçalez, Capim-elefante (*Pennisetum purpureum* Schumach.) cv. "Roxo de Botucatu" (I), B Ind. Anim. Nov. Odessa 42 (1985) 141–142.

[10] A. Pereira, M. Machado, A. Azevedo, C. do Nascimento, A. Campos, F. da S Ledo, Diversidade genética entre acessos de capim-elefante obtida com marcadores moleculares Genetic diversity among elephantgrass accessions estimated by molecular markers, Rev. Bras. Zootec. 37 (2008) 1216–1221.

[11] M.M. Araya, F.C. Boschini, Producción de forraje y calidad nutricional de variedades de *Pennisetum purpureum* en la meseta central de Costa Rica, Agron. Mesoam. 16 (2005) 37–43.

[12] J.E. Knoll, W.F. Anderson, R. Malik, R.K. Hubbard, T.C. Strickland, Production of napiergrass as a bioenergy feedstock under organic versus inorganic fertilization in the southeast USA, Bioenergy Res. 6 (2013) 974–983, http://dx.doi.org/10.1007/s12155-013-9328-1.

[13] J.H. Bouton, Bioenergy Crop Breeding and Production Research in the Southeast, Final Report for 1996 to 2001, USA, Tifton, GA, 2003.

[14] J.E. Knoll, W.F. Anderson, T.C. Strickland, R.K. Hubbard, R. Malik, Low-input production of biomass from perennial grasses in the coastal plain of Georgia, USA, Bioenergy Res. 5 (2012) 206–214, http://dx.doi.org/10.1007/s12155-011-9122-x.

[15] M.P. Palacios-Díaz, V. Mendoza-Grimón, J.R. Fernández-Vera, J.M. Hernández-Moreno, Effects of defoliation and nitrogen uptake on forage nutritive values of *Pennisetum* sp, J. Anim. Plant Sci. 23 (2013) 566–574.

[16] C.J. Chaparro, L.E. Sollenberger, K.H. Quesenberry, Light interception, reserve status and persistence f clipped Mott elephantgrass swards, Crop Sci. 36 (1996) 649–655, http://dx.doi.org/10.2135/cropsci1996.0011183X003600030022x.

[17] S.M. Interrante, L.E. Sollenberger, A.R. Blount, S.W. Coleman, U.R. White, K. Liu, Defoliation management of bahiagrass germplasm affects cover and persistence-related responses, Agron. J. 101 (2009) 1381–1387, http://dx.doi.org/10.2134/agronj2009.0126.

[18] W. Anderson, B. Dien, S. Brandon, J. Peterson, Assessment of bermudagrass and bunch grasses as feedstock for conversion to ethanol, Appl. Biochem. Biotechnol. 145 (2008) 13–21, http://dx.doi.org/10.1007/s12010-007-8041-y.

[19] E. García, Modificaciones al sistema de clasificación climática de Köpen, fifth ed., Instituto de Geografía de la Universidad Autónoma de México, México DF, 2004.

[20] P.J. Van Soest, J.B. Robertson, B.A. Lewis, Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition, J. Dairy Sci. 74 (1991) 3583–3597, http://dx.doi.org/10.3168/jds.S0022-0302(91)78551-2.

[21] J.B. Robertson, P.J. Van Soest, The detergent system of analysis, in: W.P.T. James, O. Theander (Eds.), Anal. Diet. Fiiber Foods, Marcel Dekker, New York, NY, 1981, pp. 123–158.

[22] SAS, SAS/STAT 9.1 User's Guide, SAS Institute Inc, Cary, NC, 2004.

[23] J.M. Calzada-Marín, J.F. Enríquez-Quiroz, A. Hernández-Garay, E. Ortega-Jiménez, S.I. Mendoza-Pedroza, Growth analysis of maralfalfa grass ( *Pennisetum* sp) in a warm humid climate, Rev. Mex. Ciencias Pecu. 5 (2014) 247–260.

[24] T. Ramos, S.J.R. Canul, V.F.J. Duarte, Producción de tres variedades de *Pennisetum purpureum* fertilizadas con dos fuentes nitrogenadas en Yucatán, México, Biociencias 2 (2012) 60–68.

[25] C.J.J. Nava, O.E. Gutiérrez, G.F. Zavala, S.E. Olivares, J.E. Treviño, B.H. Bernal, et al., Establishment of CT 115 grass (*Pennisetum purpureum*) in a semiarid area of northeast Mexico, Rev. Fitotec. Mex. 36 (2013) 239–244.

[26] R.M.B. Leite, F.J.L. de Queiroz, D.S. da Silva, Producao e valor nutritivo do capim elefante cultivar cameroon em diferentes idades, Agropeccuaria Técnica 21 (2000) 30–39.

[27] X. Zhang, H. Gu, C. Ding, X. Zhong, J. Zhang, N. Xu, Path coefficient and cluster analyses of yield and morphological traits in *Pennisetum purpureum*, Trop. Grassl. 44 (2010) 95–102.

[28] W.M. Lonsdale, The self-thinning rule: dead or alive? Ecology 71 (1990) 1373–1388, http://dx.doi.org/10.2307/1938275.

[29] A. Monti, N. Di Virgilio, G. Venturi, Mineral composition and ash content of six major energy crops, Biomass Bioenergy 32 (2008) 216–223, http://dx.doi.org/10.1016/j.biombioe.2007.09.012.

[30] F. Kubota, Y. Matsuda, W. Agata, K. Nada, The relationship between canopy structure and high productivity in napier grass, *Pennisetum purpureum* Schumach, F. Crop Res. 38 (1994) 105–110, http://dx.doi.org/10.1016/0378-4290(94)90004-3.