Comparison of biomass production of *Pennisetum purpureum* clones N fertilized

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In a random block design with four replications, was compared the biomass production of seven *Pennisetum purpureum* clones obtained by tissue culture (CT 21, CT 14, CT 36, CT 30, CT 115, CT 43 and CT 18) and fertilized with 60 kg/ha/cut of N (as urea). Three cuttings were practiced with 120 d of regrowth each. In the poor rainy months 30 mm of water was applied by aspersion. In each cutting fresh matter (FM) and DM yields, height and number of tillers/clump were determined. At the end of the experiment population of clumps were established. Clone 21 surpassed the rest in FM (142.2 g/ha) and DM (45.7 t/ha) yields, although in this latter case differences were not significant regarding clones 18, 36, 14 and 30. In the three cuttings carried out, clone 21 showed higher height (188 to 198 cm). In the third, clone 21 showed greater tiller values per clumps (> 17.6), but without differing significantly from clones 18, 36, 14 and 30. The lowest population value at the end of the experiment was shown by CT-115 (1.36 clumpss/m), without significant differences regarding the rest. Results cannot deny the variability between clones and allow recommending clone 21 for forage production.

Key words: *Pennisetum purpureum cv. king grass, somaclones, performance*

For many years king grass substituted Napier grass as forage plant in Cuba (both varieties came from the same *Pennisetum purpureum* genre. Therefore planting of this variety came from the same genetic material, representing an inconvenient due to the absence of genetic variability. This evidenced the need of searching for other varieties in order of improving the genetic diversity of this species and extending the regionalization program of this forage (Herrera 2004).

For this purpose, in the Institute of Animal Science of Cuba it has been successfully used the *in vitro* tissue culture technique consisting of making grow leaf parts, inflorescences and apical tissue, among others, in culture media and under controlled conditions. Later, the cells obtained (calluses) start differentiating until plant formation (Herrera and Martínez 2006).

As result of this improving program, new clones have been obtained (Herrera *et al*. 2012), that after the initial selection were evaluated for knowing the performance under dry conditions and without fertilization. In this investigation is recommended the need of continuing this experiment under fertilization and irrigation conditions with the six persisting clones. It is also suggested their comparison to the progenitor Cuba CT-115.

The objective of this study was the comparison of the biomass production and various yield indicators of these clones (including CT-115) under fertilization and irrigation conditions.

Materials and Methods

Experimental design and treatments. In a completely randomized block design with four replications, was compared forage and DM production, as well as the number of tillers/clump, population of clumpss/m and clone height of *P. purpureum* (CT 21, CT 18, CT 36, CT 14, CT 43, CT 115 and CT 30).

The experiment was carried out in a typical red ferrallitic soil (Hernández *et al*. 1999). Performance of the climatic indicators during the experimental period is shown in table 1. Annual rainfall was of 1453.4 mm, occurring from this 85.7 % in the rainy period. This performance, the same as that of temperatures, is not much different from the historical average registered in the studied zone (Herrera *et al*. 2012), though in this case the beginning of heavy rainfall was not so much moved until June, but started to be abundant in May.

Experimental procedure. The study was carried out in the plots used for an experiment previously developed in the Experimental Station "Miguel Sistachs Naya" of the Institute of Animal Science. In this case, during two years thirteen clones from the tissue culture of apical cells of king grass were evaluated under drylot conditions without fertilization. From the clones evaluated, persisted with good population seven of them. These were again evaluated in this experiment, but under fertilization and irrigation conditions. Initially, it was confirmed that clumps population/plot did not present significant differences between clones and varied between 4.1-4.9/m.

In May 2010, a uniformity cutting was made to all plots. In each one, 60 kg/ha of N (as urea) was applied. This same N dosage was used after the September 2010 and January 2011 cuttings. Regrowth age in each cutting was of 120 d.

Aspersion irrigation was applied every two weeks (each time with a water layer of 30 mm) in December 2010 and from January to April 2011.

Cuttings were made with lateral sable harvester, 15 cm from the soil drawn by a 65 HP tractor. In each cutting, height in cm, fresh forage and DM in t/ha yields, clumps population/m and number of tillers/clump, were

Table 1. Performance of climatic factors during the experimental period

Indicators	Year 2010											
	E	F	M	A	M	J	J	A	S	O	N	D
Maximum temperature, º C					31.4	31.4	33.2	31.1	28.8	25.8	25.8	26.0
Minimum temperature, º C					21.9	23.1	25.7	23.5	21.3	18.0	13.7	9.2
Rainfall, mm					206.0	134.0	244.0	253.0	251.0	14.0	4.0	2.0
Year2011												
Maximum temperature, º C	26.0	27.9	28.6	27.9	29.8							
Minimum temperature, º C	13.0	13.8	13.2	16.6	18.3							
Rainfall, mm	64.5	0.0	82.4	57.5	141.0							

determined.

Five forage heights per plot were taken one day before realizing each cutting, while the same day was determined the number of clumps/m and the number of tillers/clump, in the three central rows of the plot. For establishing FM yield, the area of each plot was cut, aside from one row of each side which was rejected as border effect according to Herrera (2006).

From each plot, 200 g of FM were randomly taken in each cutting and dried in an air circulation oven at 70º C for 72 h. The value of the DM percentage obtained, was multiplied by the FM yield of each plot, for expressing DM yield data.

Data were submitted to analysis of variance, according to a random block design with four replications. Duncan's (1955) multiple range test was used in the necessary cases. The average number of tillers/clump and clumps/m were transformed according to $\sqrt{n}$.

Results and Discussion

FM yield was higher ($P < 0.001$) in clone 21, with 142.2 t/ha, followed by 18, with 129.6 t/ha (table 2). These same clones produced higher DM yields, although without differing significantly from 36 and 30. Clones 43 and 115 produced the lowest yields.

Yields in this study practically doubled those previously found for these clones by Herrera *et al*. (2012) in one year of evaluation. This difference is due, in part, to the favorable effect of fertilization and irrigation applied in this study, allowing doubling the yield in the best performing clones. Although not presented in the table, results allow estimating that between 241 and 253 kg of DM per each kg N applied was obtained, even though N supplied by the soil was not considered. This information is not known, since it was not possible to establish plot without N for each clone.

Table 2. Accumulated fresh matter and dry matter yields of the clones

Clones	Accumulated yield, t/ha	
	Fresh matter	Dry matter
CT-21	142.2[e]	45.7[c]
CT-18	129.6[d]	44.4[cd]
CT-36	113.3[c]	43.0[c]
CT-14	99.6[b]	35.5[b]
CT-43	80.1[a]	24.5[a]
CT-115	88.3[ab]	33.4[b]
CT-30	113.3[c]	42.4[cd]
SE±	4.2***	0.9**

[a,b,c]Means with different letters in each column differ significantly at $P < 0.05$ (Duncan 1955)
**$P < 0.01$ ***$P < 0.001$

Another factor that could have influenced on higher yields was the higher cutting interval applied. Forage was left to grow until 120 d of regrowth in each cutting allowing the clones expressing practically their yield potential, together with the fertilization and irrigation. According to Herrera *et al*. (2012) the causes of the low yield of these clones in his experiment was the high cutting frequency (5/year) and the absence of fertilization and irrigation.

The most recent comparison of these selected mutants were carried out by Mocho and Fabián (2012) who concluded that clones 21 and 19 are good alternatives for forage production in Cuba. These authors recommended the realization of new studies for evaluating clones of greater biomass productivity in Ecuador regions. They also found lower yield of CT-115 respecting the rest of the clones.

In figure 1 is shown that DM yield of clones decreased markedly in the last cutting, with greater intensity in clones 43, 115, 36 and 30. The first cutting corresponded to the June-September period, the second to October-January and the third to February-May. The noticeable yield fall of the clones in the last cutting could be attributed to the lower temperatures occurring from January to April (minimum temperature ranged from 6 to 18 ºC), since water was not the limiting factor, because irrigation was applied every two weeks during that period. Suárez and Hernández (1977) had found poor growth in an important group of grasses, in spite of applying irrigation during that stage. In this period, stand out the best performance of clones 21, 18 and 14 producing also higher yields, besides the 36.

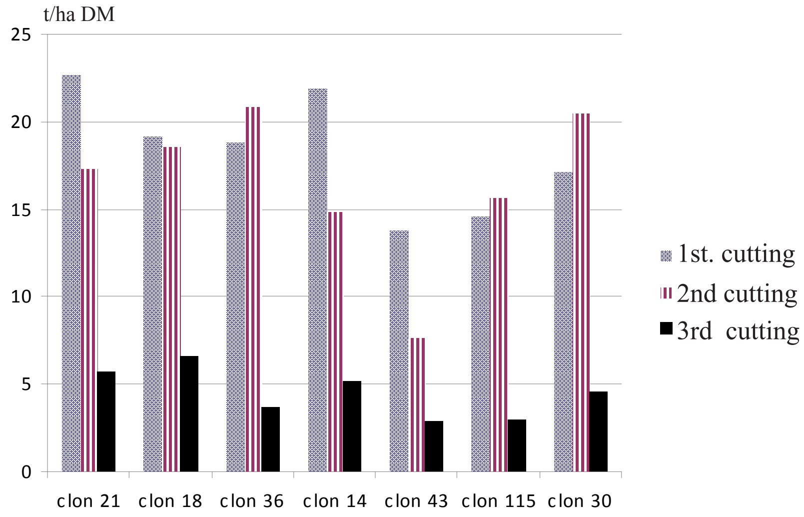

Figure 1. DM yield of the clones in the three cuttings practiced throughout the year

There were no differences in height between clones. However, the 21 showed higher value of this indicator in the first and third cutting (table 3). According to Herrera (2006), height tends to present lower variability between clones than between varieties and species, which is attributed to the biochemical and physiological individuality of each plant. This criterion was also stated by Díaz (2009) and Álvarez (2009) on assessing *Pennisetum purpureum* clones with drought and salinity resistance in Cuba.

Although in the last cutting there was the lowest clone yield, height did not presented similar correspondence. This suggests that this indicator seems to be of poor value in these studies, although it was certainly manifested the lowest height attained by CT-115 in this cutting.

The tillering/clump performance of the clones is shown in table 4. This indicator evidenced lower value in CT-115 in the first and third cuttings, while the remaining clones did not show clear differences. Even though a statistical analysis of data of this indicator was not carried out, it was shown that except for CT-115, the remaining clones exhibited higher presence of tillers/clump in the last cutting. This performance is difficult to explain, since it was precisely produced in the least favorable climatic season for plant development. Thus, further investigations are necessary for explaining more this physiological performance.

The persistence of the clone population of the clumps, measured in the last cutting (table 5), indicated that, excepting CT-115, the remaining maintained similar population regarding the beginning of the experiment. There was marked decrease of this indicator in CT-115. Also the investigation of Herrera *et al*. (2012) exhibited this performance. However, neither the application of fertilizer nor the irrigation could vary this performance in the present study.

Results demonstrated poorer performance of CT-115, regarding the indicators of yield, tillers/clump and population of clumps. This seems indicating the susceptibility of this clone to frequent cuttings, since under grazing conditions Lok *et al* (2008) found increase of these same indicators with time, when this clone was maintained in continuous grazing.

Criteria of Herrera *et al*. (2012) are shared, in regard to the need of conducting studies on physiological and biochemical sciences that help explaining the differences

Table 3. Average height of the clones at each cutting

Clone	First cutting	Second cutting	Third cutting
21	188.5[d]	198.5[c]	192.0[c]
18	164.0[a]	198.5[c]	182.6[bc]
36	169.05[bc]	192.5[c]	178.2[b]
14	171.5[c]	187.3[c]	177.3[b]
43	172.7[c]	142.7[a]	177.7[b]
115	165.2[ac]	163.1[b]	151.2[a]
30	172.0[c]	192.0[c]	178.2[b]
SE±	1.65**	4.4***	3.4***

[a,b,c]Means with different letters within each column differ significantly at $P < 0.05$ (Duncan 1955)

***$P < 0.001$

Table 4. Performance of the number of tillers/clump in the evaluated clones

Clone	First cutting	Second cutting	Third cutting
21	3.43[ab] (11.8)	3.86 (14.92)	4.19b (17.58)[c]
18	3.67[bc] (13.47)	3.82 (14.58)	3.18[a] (16.50)
36	3.64[bc] (13.27)	3.54 (12.58)	4.29[b] (18.42)
14	3.75[cd] (14.07)	3.63 (13.17)	4.19[b] (17.58)
43	3.25[a] (10.60)	3.50 (12.25)	3.80[a] (14.50)
115	3.14[a] (9.87)	3.64 (13.25)	3.45[a] (11.92)
30	3.75[cd] (14.07)	3.69 (13.67)	4.12[bc] (17.0)
SE±	0.11***	0.21	0.20***

[a,b,c]Means with different letters within each column differ significantly at P < 0.05 (Duncan 1955)
***P < 0.001 ( ) True values

Table 5. Performance of the clumps population of the clones

Clon	Clumpss/m
21	2.50[b] (4.21)
18	2.33[b] (4.05)
36	2.58[b] (4.91)
14	2.50[b] (4.55)
43	2.42[b] (4.32)
115	1.34[a] (1.36)
30	2.50[b] (4.55)
SE±	0.30***

[a,b]Means with different letters within each column differ significantly to P < 0.05 (Duncan 1955)
***P < 0.001 ( ) True values

found, as well as those related to the quality and nutritive value. This will help designing adequate technologies.

The marked effect of fertilization and irrigation on the notable increase of biomass production of the evaluated clones was demonstrated, although it will be necessary to study the effect of these agricultural inputs, at long-term, on the economic and environmental sustainability of the systems.

It was confirmed that there is an important degree of diversity between the clones studied, which is a favorable aspect for the genetic background of Pennisetum in Cuba (Barreto *et al*. 2001). Results allow recommending clone 21 as a new species for forage production.

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