Assessment of *Pennisetum purpureum* clones obtained by *in vitro* tissue culture

R.S. Herrera, M. García, Ana M. Cruz and Aida Romero

Instituto de Ciencia Animal, Apartado Postal 24, San José de las Lajas, Mayabeque, Cuba
Email: rherrera@ica.co.cu

The performance of some agronomic indicators of 12 new *Pennisetum purpureum* clones (14, 18, 19, 20, 21, 24, 30, 36, 39, 41, 42 and 43) obtained by *in vitro* tissue culture was assessed during a year and they were compared to their progenitor (P. purpuereum vc. Cuba CT-115) through a completely random design with four repetitions. Plots of 25m2, without irrigation and fertilization, were used. The samplings were conducted every 60 and 90 d in the rainy and dry seasons, respectively. There were differences ($P < 0.001$) in the clones' height, but none of them surpassed the control (67.9 cm) in the rainy season, while the 14, 18, 20, 30 and 36 highlighted in dry season ($P < 0.001$). Similar performance was recorded for the leaves' length during the rainy season. The highest ($P < 0.001$) width was for the clones 39 (3.7cm) and 43 (3.1cm) in the rainy and dry seasons, respectively. The highest ($P < 0.001$) leaves percentages were reached by the clones 41 and 43 (65.8 and 66.1 %, respectively) in the rainy season and the 39, 41 and 43 (53.5; 53.1 and 54.8 % respectively) in the dry season. The clone 20 recorded the highest ($P < 0.001$) total yield (14.9 tDM/ha/year), but only produced the 28.2 % in the dry season. This clone also had the highest population (17.6 bunch/5m) at the end of the experiment. There are new clones of *P. purpureum* with favorable productive characteristics increasing the available gene-fund of this species, mainly the clone 20 that surpassed the control in the yield, content of leaves and population. Basic studies to explain the differences found between clones are necessary, as well as the study of their responses when using maintenance fertilization.

Key words: *Pennisetum purpureum, clones, agronomic indicators, yields.*

In the middle 80's of the last century, the genetic improvement program of *Pennisetum purpureum* through different ways began in the Institute of Animal Science. This was due to this species, mainly the king grass variety, occupied 85 % of the forage areas (Herrera and Ramos 2006) for cattle feeding, and the production based in only one variety, needing fertile soils of good superficial and internal drainage, as well as fertilizers and irrigation.

Through the *in vitro* tissue culture and physical mutagenesis new clones with specific characteristics to develop technologies for their use in grazing and forage production were obtained (Martínez and Herrera 2006). Throughout the physical mutagenesis, there are clones that surpass the yield of their progenitor when exploited with irrigation or fertilization or under dry conditions (Herrera and Martínez 2006). There are also other clones that their tolerance to draught (Díaz 2009) and salinity (Álvarez 2009) has been demonstrated.

As result of this improvement program, new clones have been obtained (Herrera 2001) that, after the initial selection, their assessment was necessary for knowing the performance of some agronomic indicators.

Materials and Methods

Localization. The research was carried out in the Experimental Station of Pastures and Forages "Miguel Sistachs Naya", from the Institute of Animal Science, on a typical red ferralitic soil (Hernández *et al*. 1999). Table 1 shows the performance of rainfall and the maximum temperature during 1970-2006 and the experimental period, being the latter of lower rainfall.

Treatment and design. The treatments consisted of 12 new clones of *Pennisetum purpureum* (14, 18, 19, 20, 21, 24, 30, 36, 39, 41, 42 and 43), obtained by *in vitro* tissue culture in the Institute of Animal Science. They were compared, through a completely randomized design with four repetitions, to the progenitor (*Pennisetum purpureum* vc. Cuba CT-115).

The soil was prepared conventionally. The sowing was carried out when the rainfall was stable in the rainy season, with seeds of five months of age. They were deposited in furrows, with one meter of space between them. They were cut with a machete in three or four buds, assuring that each furrow and plot had the same amount of them, and then they were covered with a hoe. Neither irrigation nor fertilization was used.

After 135 d of the sowing, the establishment cut was conducted at 10 cm of height above the soil level, in plots of 25 m^2 of total area. From that time on, samplings were carried out every 60 and 90 d in the rainy and dry seasons, respectively.

The whole green material of the harvestable area (previously eliminating the border effect) of each plot was weighed and five samples of 500 g were randomly collected to determine: length and width of the fourth leaf completely open, numbered from the growth point; length and width of the fourth internode, counted from the soil level; content of leaves and stems, DM percentage, yields and population an height, according to Herrera (2006).

Statistical analysis. Analysis of variance was carried out according to the experimental design (SAS 2001). When necessary, Duncan's test (1955) was applied for

Table 1. Some climatic of historic indicators and from the experimental period

Months	Season 1970-2006		Experimental period	
	Rainfall, mm	Maximum temperature, °C	Rainfall, mm	Maximum temperature, °C
January	48.5	26.8	71.8	26.5
February	44.2	27.4	77.2	27.8
March	63.5	28.6	0.0	29.5
April	69.1	30.3	87.5	30.2
May	40.8	31.5	46.1	30.3
June	264.9	31.8	112.6	33.9
July	192.8	32.5	117.7	32.6
August	186.7	32.6	324.4	33.0
September	194.9	31.6	195.6	31.3
October	105.9	30.8	70.2	34.0
November	56.0	29.1	0.1	29.1
December	35.2	27.6	4.2	26.6

comparing the mean values. The number of bunches/5m was transformed according to √n.

Results and Discussion

In the rainy season, any clone surpassed the control height (CT-115), while, in the dry one, the clones 14, 18, 20, 30 and 36 had higher ($P < 0.001$) height than that of the control (table 2).

It is known that the height tends to have lower variability between the clones than between varieties and species, determined, among other aspects, by the biochemical and physiological individuality of each plant category (Herrera 2006). This is proved by the results of Díaz (2009) and Álvarez (2009), when assessing clones of *P. purpureum* tolerant to drought and salinity respectively, reported similar performance to that obtained in this study.

Clones (20, 21, 41, 42 and 43) with lower size than the control (CT-115) were also found in the rainy season. However, in the dry season, only the 41 was inferior to the control. This could suggest that they have characteristics for grazing and forage production, and these should be confirmed in further studies.

The highest height reached by the clones in the dry season could be due to the samplings every 90 d, while they were conducted every 60 in the rainy one. This makes differences in the plants growth in each season.

Differences were found ($P < 0,001$) in the length and

Table 2. Height of the clones, cm

Clones	Season	
	Rainy	Dry
CT-115	67.9^{ab}	63.6^{cd}
14	54.9^{c}	72.7^{a}
18	60.0^{bc}	70.5^{ab}
19	62.0^{bc}	62.5^{cd}
20	56.1^{c}	69.5^{ab}
21	56.1^{c}	65.2^{bcd}
24	60.1^{bc}	68.1^{abc}
30	58.6^{bc}	70.0^{ab}
36	59.4^{bc}	72.5^{a}
39	72.4^{a}	65.6^{bcd}
41	42.7^{d}	52.9^{e}
42	53.9^{c}	67.2^{abc}
43	41.5^{d}	59.9^{d}
SE ±	3.1***	1.9***

^{abcd}Values with dissimilar letters differ at $P<0.05$ (Duncan 1955)

***$P < 0.001$

width of the clones' leaves. Any clone surpassed the control for the leaves' length in both seasons. However, the width of the clone 39 was higher in the rainy season, while it occurred in the dry season in the clones 19, 20, 41, 42 and 43 (table 3).

The development of the plant foliar system is of vital importance as the photosynthesis and other metabolic processes determining the production of metabolites needed for the plant growth and development take place in it (Herrera 2006). However, from the theoretical point of view, the higher is the foliar system, the higher the possibility of capturing higher sun radiation but, studies of Herrera *et al*. (2009) showed that this is not totally true, as no defined relation between the size of the foliar system and the content of green pigments were found. This is of special interest and further researches to clear up these aspects would be appropriate.

Differences (P<0,001) were achieved in the width and length of the internode. In the first case, the clones 42 and 43 were superior to the control, while in the second, this occurred in the 14, 18, 19, 20, 24, 30, 36, 39 and 42 (table 4).

Table 3. Length and width of the clones' leaves

Clones	Leaf length, cm		Leaf width, cm	
	Rainy season	Dry season	Rainy season	Dry season
CT-115	91.8^{ab}	76.1^{ab}	2.8^{bc}	2.3^{c}
14	88.5^{abc}	44.0^{g}	2.7^{c}	1.3^{g}
18	91.5^{ab}	51.5^{fg}	3.2^{abc}	1.6^{f}
19	98.2^{a}	81.5^{a}	3.1^{abc}	2.6^{b}
20	86.2^{abcd}	53.4^{abcd}	3.2^{abc}	2.6^{b}
21	95.6^{ab}	69.5^{bcd}	3.1^{abc}	1.9^{e}
24	95.2^{ab}	53.0^{efg}	3.1^{abc}	2.3^{c}
30	88.3^{abc}	53.4^{efg}	3.2^{abc}	2.1^{d}
36	90.5^{abc}	62.6^{cde}	3.0^{bc}	1.8^{e}
39	86.5^{bcd}	71.8^{abc}	3.7^{a}	1.6^{f}
41	80.2^{cd}	60.2^{def}	3.4^{ab}	2.8^{b}
42	75.9^{d}	59.5^{def}	3.0^{bc}	2.6^{b}
43	76.6^{d}	59.5^{def}	3.2^{abc}	3.1^{a}
SE ±	3.9***	3.9***	0.2***	0.06***

^{abcdefg}Values with dissimilar letters differ at P < 0.05 (Duncan 1955)
***P < 0.001

Table 4. Length and width of the internode, mm

Clones	Dry season	
	Internode width	Internode length
CT-115	8.2^{cd}	88.0^{d}
14	7.2^{def}	117.3^{bc}
18	6.4f^{g}	130.0^{ab}
19	9.5^{abc}	112.5^{c}
20	5.1^{g}	114.8^{c}
21	9.4^{bc}	100.0^{cd}
24	6.5^{efg}	120.0^{abc}
30	7.2^{def}	119.3^{abc}
36	7.1^{def}	131.8^{a}
39	9.3^{bc}	112.5^{c}
41	9.3^{bc}	94.0d
42	11.1^{a}	109.9^{c}
43	10.8^{ab}	53.8e
SE ±	0.51***	4.47***

^{abcdefg}Values with dissimilar letters differ at P < 0.05 (Duncan 1955)
***P < 0.001

This variability of the stem indicators is very important from three points of view: a) the wider is the stem, higher is its resistance to the cut, the animal bite and the wind rate, b) may store higher amount of substances, which is favourable for the plant regrowth, c) its cell wall contents (structural carbohydrates) is increased and, as consequence, contribute to diminishing its digestibility (Herrera and Ramos 2006).

This indicator was only counted during the dry season, because when sampling in the rainy season (every 60 d), the height did not surpass 75 cm. Differentiating this indicator was extremely hard, as the leaf sheat covered it totally and its growth cycle was not completed.

The DM percentages of leaves and stems differed (P < 0.001) among clones. In the case of leaves, none of them surpassed the DM content of the control in both seasons, while in the stem this occurred in the clones 14, 18, 20, 24, 30, 36 and 42 in the dry season and in the 24, 41 and 43 in the rainy one (table 5).

Each clone had characteristic values and, in general, the interval was small. This reaffirmed the criterion that the varieties of a same species have low variation on their morphological indicators. However, these values are similar to those informed by Herrera and Ramos (2006) for different varieties of Pennisetum genus, when they were studied without nitrogen fertilization and irrigation.

Michelena (2006) stated that the varieties of Pennisetum had proper characteristics to be used in silage production due to their high yields, mean content of protein and high amount of carbohydrates in the stems. Nevertheless, the low DM content was the main convenient.

This element was presented in the clones of this study, hence their possible use for silages would be determined, first, by a previous process of pre-drying to increase the DM content and the economic analysis to show the viability of the process.

There were differences (P < 0.001) between the clones for the percentage of leaves. The highest values were for the clones 41 and 43 (65.8 and 66.1 %, respectively) in the rainy season, while this occurred in the clones 19, 21, 39, 41 and 43 during the dry stage (table 6).

The clones with lowest height (41 and 43) reached higher percentages of leaves, maybe because of the lower internode distance of the plants.

Previous studies showed that obtaining clones of this species through *in vitro* tissue culture improve, in many cases, the leaves content of the new plant (Herrera 2011). This was confirmed with the results of this study.

Herrera (2006) stated the relation between the pigments photo-synthetically active and the amount of leaves of the Pennisetum varieties. He also indicated that there might be problems when using the light efficiently due to the amount and distribution of leaves, the shadow of the upper leaves over those below and the chlorosis in the lasts. This could influence on the synthesis of substances needed for the growth and development of the plant, aspects that should be considered in further assessments.

The DM yield differed (P < 0.001) between clones in both seasons. In the rainy season, the clones 20, 21, 24, 36 and 43 surpassed the CT-115, while, in the dry season, this occurred in the 14, 18, 19, 20, 21, 24, 30 and 36. However, the clones 14, 18, 19, 20, 21, 24 and 36 had the highest total yield, with values up to 14.9 tDM/ha/year. It was encouraging that clones like 14, 18, 19 and 21 produced more than 30 % of their yield

Table 5. DM percentage of leaves and stems

Clones	Leaves DM, %		Stems DM, %	
	Rainy season	Dry season	Rainy season	Dry season
CT-115	21.4^{abc}	28.0^{a}	9.9^{cd}	16.8^{d}
14	22.4^{ab}	28.5^{a}	9.9^{cd}	21.0^{bc}
18	21.9^{abc}	25.6^{bc}	10.1^{cd}	22.4^{ab}
19	20.9^{bc}	25.2^{bc}	9.6d	15.3^{d}
20	21.7^{abc}	27.9^{a}	10.4^{abcd}	21.0^{bc}
21	21.5^{abc}	27.9^{a}	11.0^{abcd}	16.7^{d}
24	21.7^{abc}	26.8^{ab}	11.4^{ab}	20.8^{bc}
30	23.3^{a}	28.5^{a}	10.5^{abcd}	24.3^{a}
36	21.8^{abc}	28.7^{a}	11.0^{abcd}	21.6^{b}
39	19.3^{c}	23.9^{c}	11.2^{abc}	16.0^{d}
41	22.5^{ab}	24.8^{bc}	11.8^{ab}	17.1^{d}
42	20.0^{ab}	28.0^{a}	10.3^{bcd}	19.5^{c}
43	22.0^{ab}	26.2^{ab}	12.0^{a}	16.7^{d}
SE ±	0.6***	0.7***	0.5***	0.6***

^{abcd}Values with dissimilar letters differ at P < 0.05 (Duncan 1955)
***P<0.001

Table 6. Leaves percentage of the clones

Clones	Season	
	Rainy	Dry
CT-115	57.1[bc]	46.1[cd]
14	59.6[abc]	37.5[e]
18	62.9[ab]	38.8[e]
19	56.0[c]	53.2[a]
20	59.9[abc]	47.0[c]
21	60.3[abc]	52.9[a]
24	57.8[bc]	40.9[de]
30	58.8[bc]	38.8[e]
36	59.6[abc]	45.1[cd]
39	58.2[bc]	53.5[a]
41	65.8[a]	53.1[a]
42	63.0[ab]	46.7[c]
43	66.1[a]	54.8[a]
SE ±	2.2***	1.8***

[abcde] Values with dissimilar letters differ at P < 0.05 (Duncan 1955)

***P < 0.001

Table 7. Dry matter yield (t/ha)

Clones	Season		Total	% in dry basis
	Rainy	Dry		
CT-115	6.2[c]	2.1[fg]	8.3[c]	25.3
14	8.1[abc]	4.4[a]	12.5[ab]	35.2
18	7.5[bc]	4.3[ab]	11.8[ab]	36.4
19	8.8[abc]	4.0[abc]	12.8[a]	31.2
20	10.7[a]	4.2[ab]	14.9[a]	28.2
21	9.0[ab]	4.1[ab]	13.1[a]	31.3
24	9.4[ab]	3.4[bcde]	12.8[a]	26.6
30	8.1[abc]	3.1[cde]	11.2[abc]	27.7
36	9.0[ab]	3.1[cde]	12.1[ab]	25.6
39	8.6[abc]	2.1[fg]	10.7[abc]	19.6
41	6.8[bc]	2.6[efg]	9.4[bc]	27.7
42	7.2[bc]	1.7[g]	8.9[bc]	19.1
43	9.0[ab]	3.0[def]	12.0[abcd]	25.0
SE ±	0.9***	0.3***	1.1***	

[abcdef] Values with dissimilar letters differ at P<0.05 (Duncan 1955)

***P<0.001

in the dry season (table 7).

An aspect to highlight is that these yields were reached without irrigation and fertilization, and they can be considered as acceptable. Besides, in spite of the differences found in the dry season, the clones 14, 18, 19, 20, and 21 had almost the double yield than that of the CT-115. This was inspiring as the pastures yield diminishes notably in this period and there were clones that duplicate the yield. This will allow a better use of the land and of the available resources. It also increases the amount of animals to be fed, representing, therefore, economic advantages. This same thought may be applied to the total yield.

Table 8 shows the performance of the population at the beginning and end (1 year) of the experiment. There were differences between the clones and, at the beginning of the experiment, the CT-115 had the highest (P < 0.001) population. However, after a year, only the clone 20 surpassed (P < 0.01) the control.

This result could be determined for the individual germination capacity of each plant also in absence of irrigation and fertilization during the experiment. This could have caused stressing conditions. This, together with the intense cutting frequency, propitiated that the plant could not recover its regrowth and tillering capacity, diminishing the population.

Although the statistical analysis between the beginning and the end of the experiment was not conducted, the clones 20 increased its population at the end of the study and reached higher total but only produced 28.6 % of yield in the dry season. These are favorable aspects of this clone and its negative attributes could be attenuated with the proper management including the maintenance fertilization.

Table 8. Number of sprouts/5m at the beginning and end of the experiment

Clones	Beginning	End
CT-115	3.5[a] (12.3)	2.9[b] (8.4)
14	3.3[b] (10.9)	3.1[b] (9.6)
18	3.2[bc] (10.2)	3.0[b] (9.0)
19	3.1[c] (9.6)	2.9[b] (8.4)
20	3.3[b] (10.9)	4.2[a] (17.6)
21	3.3[b] (10.9)	3.1[b] (9.6)
24	3.1[c] (9.6)	2.9[b] (8.4)
30	3.3[b] (10.9)	3.0[b] (9.0)
36	3.1[c] (9.6)	3.0[b] (9.0)
39	3.3[b] (10.9)	3.0[b] (9.0)
41	3.2[bc] (10.2)	2.9[b] (8.4)
42	3.3[b] (10.9)	2.8[b] (7.8)
43	3.2[bc] (10.2)	3.0[b] (9.0)
SE ±	0.09**	0.3**

[abc] Values with dissimilar letters differ at $P < 0.05$ (Duncan 1955)
( ) Original values
** $P < 0.01$

The remarkable seasonal performance was an important aspect for all the clones. Each showed values of the indicator studied en each season. This showed that the weather is a determining factor on the plants performance and establishes the efficiency of their physiologic and metabolic processes. All seems to indicate that the system of five cuttings per year was severe for these clones, distinguished by long growth cycle, as characteristic of the *Pennisetum purpureum* varieties (Martínez and Herrera 2006). This could eliminate the reserves and diminish the regrowth capacity. Besides, the cutting height was of 10 cm above the soil level. The absence of irrigation and fertilization could also influence on the results.

The clones studied, a priori, could be grouped according to the indicators assessed: plants of lower size than the control, like 43; similar to the control, the 20 and higher than the control, the 39. This preliminary classification would indicate their possible use for different productive purposes like the direct grazing or forage production. However, these aspects should be assessed in further studies.

It is important to state that Herrera (2001), when conducting the initial assessment of these clones informed that the highest variability between them was explained through the height, leaf content, length and width, length and width of the internodes and yields. Thus, these indicators were used in this study. The DM content and that of the population were also included.

The main objective of this study was to determine the distinctive characteristics of each clone, to know its intrinsic attributes and establish the differences with its progenitor (CT-115). This was absolutely established in the indicators studied, but, in many cases, the range values were close and further studies to select the clones more precisely are needed. This would imply conducting new experiments under different edafoclimatic conditions for a longer period of time and applying strategic fertilization, whether mineral or organic.

Designing studies on the basic sciences, such as plant physiology and biochemistry to explain the differences found, as well as those related with quality and nutritive value is also needed. These researches would contribute to designing technologies for the efficient use and exploitation of these new clones.

Finally, it is unquestionable that these new clones increase the available germoplasm of *P. purpureum* and may be the basis for further studies on the genetic improvement from different ways like the classic genetics, biotechnology and physical and chemical mutagenesis.

References

Álvarez, Y. 2009. Evaluación inicial de clones de *Pennisetum purpureum* con resistencia a la salinidad. Tesis de Maestría. Instituto de Ciencia Animal, La Habana, Cuba

Díaz, D. 2009. Estudio de nuevos clones de Pennisetum con resistencia a la sequía en el Valle del Cauto. Tesis de Maestría. Instituto de Investigaciones Agropecuarias "Jorge Dimitrov". Bayamo, Cuba

Duncan, D.B. 1955. Multiple range and multiple F test. Biometrics 11:1

Hernández, A., Pérez, J. & Boch, D. 1999. Nueva versión de la clasificación genética de los suelos de Cuba. AGROINFOR-MINAG. La Habana, Cuba. p. 26

Herrera, R.S. 2001. Obtención de clones de *Pennisetum purpureum* con mejores características agronómicas y de calidad. Informe final de proyecto. ICA-CITMA. La Habana, Cuba

Herrera, R.S. 2006. Fisiología, Calidad y muestreos. In: Fisiología, producción de biomasa y sistemas silvopastoriles en pastos tropicales. Abono orgánico y biogás. Eds. Herrera, R.S., Rodríguez I. y Febles, G. Ed. Instituto de Ciencia Animal. La Habana, Cuba. p.1

Herrera, R.S. 2011. Evaluación de clones de *Pennisetum purpureum* con resistencia a la sequía y salinidad. Informe final del proyecto ICA-CITMA. La Habana, Cuba

Herrera, R.S., Fortes, D., García, M., Cruz, A.M. & Romero, A. 2009. Green pigments in *Pennisetum purpureum* varieties at different times of the year and with different regrowth ages. Cuban J. Agric. Sci. 43:67

Herrera R.S. & Martínez, R.O. 2006. Mejoramiento genético por vías no clásicas. In: *Pennisetum purpureum* para la ganadería tropical. Eds. Herrera, R. S., Febles, G. y Crespo. G. Instituto de Ciencia Animal. La Habana, Cuba. p. 15.

Herrera, R. S. & Ramos, N. 2006. Factores que influyen en la producción de biomasa y calidad. En: *Pennisetum purpureum* para la ganadería tropical. Eds. Herrera, R. S., Febles, G. y Crespo. G. Instituto de Ciencia Animal. La Habana. p. 79

Martínez, R.O. & Herrera, R.S. 2006. Empleo del Cuba CT-115 para solucionar el déficit de alimentos durante la seca. In: Producción y manejo de los recursos forrajeros tropicales. Eds. Velasco, M.E., Hernández, A., Perezgrovas, R.A. y Sánchez, B. Univ. Autónoma de Chiapas, México. p.75

Michelena, J.B. 2006. Ensilaje. In: *Pennisetum purpureum* para la ganadería tropical. Eds. Herrera, R. S., Febles, G. y Crespo, G. Ed. Instituto de Ciencia Animal. La Habana. p. 151

SAS. 2001. User´s Guide. Statistic Version 8. Ed SAS Institute Inc. NC. USA.

Received: January 4, 2012