



## Productive and morphogenetic responses of buffel grass at different air temperatures and CO<sub>2</sub> concentrations

Roberta Machado Santos<sup>1,3</sup>, Tadeu Vinhas Voltolini<sup>2</sup>, Francislene Angelotti<sup>2</sup>, Saulo de Tarso Aidar<sup>2</sup>, Agnaldo Rodrigues de Melo Chaves<sup>2</sup>

<sup>1</sup> Programa de Pós-graduação em Recursos Genéticos Vegetais, Universidade Federal de Feira de Santana - Feira de Santana, BA, Brasil.

<sup>2</sup> Embrapa Semiárido, Petrolina, PE, Brasil.

<sup>3</sup> Graduate fellow student by FACEPE.

**ABSTRACT** - The objective of the present trial was to evaluate the productive and morphogenetic characteristics of buffel grass subjected to different air temperatures and CO<sub>2</sub> concentrations. Three cultivars of buffel grass (Biloela, Aridus and West Australian) were compared. Cultivars were grown in growth chambers at three temperatures (day/night): 26/20, 29/23, and 32/26 °C, combined with two concentrations of CO<sub>2</sub>: 370 and 550 μmol mol<sup>-1</sup>. The experimental design was completely randomized, in a 3 × 3 × 2 factorial arrangement with three replications. There were interactions between buffel grass cultivars and air temperatures on leaf elongation rate (LER), leaf appearance rate (LAR), leaf lifespan (LL) and senescence rate (SR), whereas cultivars vs. carbon dioxide concentration affected forage mass (FM), root mass (RM), shoot/root ratio, LL and SR. Leaf elongation rate and SR were higher as the air temperature was raised. Increasing air temperature also promoted an increase in LAR, except for West Australian. High CO<sub>2</sub> concentration provided greater SR of plants, except for Biloela. Cultivar West Australian had higher FM in relation to Biloela and Aridus when the CO<sub>2</sub> concentration was increased to 550 μmol mol<sup>-1</sup>. West Australian was the only cultivar that responded with more forage mass when it was exposed to higher carbon dioxide concentrations, whereas Aridus had depression in forage mass. The increase in air temperatures affects morphogenetic responses of buffel grass, accelerating its vegetative development without increasing forage mass. Elevated carbon dioxide concentration changes productive responses of buffel grass.

Key Words: carbon dioxide, *Cenchrus ciliaris*, climate change

### Introduction

Human activities have been identified as major determinants of climate changes. Among these changes are the increase in air temperature and atmospheric CO<sub>2</sub> concentration. According to IPCC (2007), the temperature of the Earth may increase from 2 to 6 °C throughout the present century, while the CO<sub>2</sub> concentration can rise from the current 360 μmol mol<sup>-1</sup> to 550 μmol mol<sup>-1</sup>.

Arid and semiarid regions, which represent large areas around the globe, may be quite vulnerable in terms of possible changes in climate, especially the agricultural and livestock systems, which are very important to those places (Barros, 2011). According to IPCC (2013), CO<sub>2</sub> levels in 2011 were 391 μmol mol<sup>-1</sup>, exceeding the pre-industrial values by about 40%.

In this region, as well as in other arid and semiarid regions worldwide, buffel grass (*Cenchrus ciliaris* L.) is

one of the most important grasses for livestock (Baig et al., 2005; Voltolini et al., 2010, 2011; Souza et al., 2013). A negative impact on buffel grass pastures may promote considerable social and economic losses. Information on physiological responses and quality of forage plants is important to foresee and mitigate the impacts of climate change and encourage the development of public policies (Santos et al., 2011).

The increase in temperature is noted as a factor that can elevate the biomass production of C<sub>4</sub> plants (Zhu et al., 2008). In a classic study evaluating several tropical legumes and grasses in relation to temperature increase, Sweeney and Hopkinson (1975) concluded that *Cenchrus ciliaris* showed no depression in productive response with increase in temperature.

Regarding carbon dioxide concentration, it is not clear whether a rise in atmospheric CO<sub>2</sub> partial pressure will also influence the productivity of the C<sub>4</sub> grasslands. The reason for this uncertainty is that relatively few CO<sub>2</sub> enrichment studies have focused on the response of C<sub>4</sub> grasses compared with the large number of studies on C<sub>3</sub> plants (Ghannoum et al., 2000).

Contrary to earlier hypotheses that the growth of C<sub>4</sub> grasses would not change in high CO<sub>2</sub>, Wand et al. (1999)

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Corresponding author: tadeu.voltolini@embrapa.br

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and Ghannoum et al. (2000) suggest that the growth responses of C4 plants to doubling the current CO<sub>2</sub> ranges from 22% to 33%. Thus, the objective of this study was to evaluate productive and morphogenetic characteristics of three buffel grass cultivars subjected to different air temperatures and CO<sub>2</sub> concentrations.

### Material and Methods

The experimental trial was carried out at Campo Experimental da Caatinga, Embrapa Semiárido – Petrolina/PE, at latitude 09°04'16,4"S, longitude 40°19'5,37"W, elevation 379 m. Two growth chambers (phytotron) measuring 3.10 × 1.90 × 2.50 m with controlled air CO<sub>2</sub> concentration, air temperature, humidity (40-60%) and light were used.

Three cultivars of buffel grass (*Cenchrus ciliaris* L.) - (Biloela, Aridus and West Australian), characterized by high (1-1.6 m), medium (0.75-1.0 m) and low height (up to 0.75 m), respectively, were evaluated. The plants were grown in plastic pots with volume capacity of 16 L containing soil classified as Vertisol, collected from Campo Experimental de Bebedouro (latitude: 09°09'S, longitude: 40°22'W, elevation 365 m), and organic fertilizer based on tanned sheep manure produced in Embrapa Semiárido, at the proportion of 2:1 (Table 1).

The experimental design was completely randomized, with three replicates, in a 3 × 3 × 2 factorial arrangement (cultivars × air temperatures × CO<sub>2</sub> concentrations).

Ten days after sowing, plant thinning was performed, leaving five plants per pot. These plants were grown at three different temperatures (day/night): 26/20, 29/23 and 32/26 °C, combined with two CO<sub>2</sub> concentrations (550 and 370 μmol mol<sup>-1</sup>), and a photoperiod of 13 hours (fixed in 400 μmol m<sup>-2</sup>s<sup>-1</sup> of photosynthetic photon flux density continuously during the light period). The temperature treatments were defined based on the average air temperature of Petrolina region (Da Silva et al., 2005; Medeiros et al., 2005), with an increase of 3 and 6 °C from this temperature, 29 and 32 °C, respectively.

The carbon dioxide concentration of 370 μmol mol<sup>-1</sup> corresponds to the current concentration, and 550 μmol mol<sup>-1</sup> represents possible future scenarios (IPCC, 2007). The CO<sub>2</sub>

concentration was monitored with the use of the SITRAD (4.8) software. The experiment was carried out in three phases, each one performed with the two CO<sub>2</sub> concentrations (370 and 500 μmol mol<sup>-1</sup>, one for each chamber) with the thermoperiods of 26/20, 29/23 and 32/26 °C (day/night), respectively.

The experiment lasted 50 days for each treatment used, which was the time necessary for completing the plant physiological cycle. During the period, plants were watered daily, applying 300 mL of water per pot three times per week. The forage mass (FM) was evaluated at the end of the experimental period by cutting all five plants in each pot, at the ground level. Forage samples collected were pre-dried in an oven at 55 °C for 72 hours, ground to 1 mm and dried again at 105 °C to obtain the dry weight.

All five plants were separated, three of which were full plants. The plant components (stem, leaves, inflorescence and dead material) were separated, constituting the samples of these components. After the removal plants from pots, roots were also collected, separated, washed to remove soil and left to dry in open air.

Thus, samples were composed of whole plant (shoot), (root, stem, leaves, inflorescence and dead material) for each replicate of their respective cultivar. All samples were weighed fresh and after drying in an oven at 55 °C for 72 hours. These data were used to estimate forage mass (FM), root mass (RM) and shoot/root (SR) ratio.

The plants were evaluated 15 days after sowing; however, to evaluate the morphogenetic characteristics, three tillers were marked in each pot, totaling 27 tillers for each treatment. The following measurements were recorded: tiller height, stem length, number of leaves, leaf blade length, leaf width and number of dead leaves.

The tiller height was estimated by measuring the tiller from the ground level up to the apex of the highest leaf. The stem length was measured from the highest point of the stem (ligule of highest expanded leaf) to the ground level. The number of leaves was obtained by direct counting expanded or expanding leaves per tiller. Leaf blade length was measured as the distance between the leaf apex and its ligule, considering expanded or expanding leaves. Leaf width was estimated by measuring the distance between

Table 1 - Results of chemical analysis of soil and organic fertilizer plus soil

Samples	pH (H <sub>2</sub> O)	P <sup>1</sup>	K	Ca	Mg	Al	SB	Fe	Zn
		mg/dm <sup>3</sup>			cmolc/dm <sup>3</sup>			mg/dm <sup>3</sup>	
Soil	4.8	4.8	0.21	2.0	1.0	0.1	3.23	22.6	3.8
Organic fertilizer + soil	7.6	182.1	4.8	3.9	1.6	0.0	10.7	39.8	18.5

SB - sum of bases (Ca, Mg, K and Na).

<sup>1</sup> Mehlich-1 extraction.

the two edges of the leaves, while the number of dead leaves was estimated by direct counting leaves presenting more than 50% of their constitution as dead tissue. From these measurements, the morphogenetic and structural characteristics below were obtained:

Leaf lifespan (LL, days): estimated as the time between the appearance of the apex and the first sign of leaf senescence ( $LL = T_{lv} - T_{sn}$ , in which  $T_{lv}$  = time (days) for the appearance of leaf vertex; and  $T_{sn}$  = time (days) for the onset the first sign of senescence). Senescence rate (SR, mm/day): the loss of dead tissue (in mm) of each tiller, calculated by dividing the value found by the number of days in the evaluation. Senescence rate (SR): total number of dead leaves/experimental growth days (50 days). Leaf elongation rate (LER, mm/leaf/day): calculated as the difference between initial and final leaf lengths; the leaf blade was measured up to its full expansion, i.e., until the appearance of the ligule ( $LER = \frac{\Sigma IL_{leaves} - FL_{leaves}}{D}$ , in which  $\Sigma$  = total sum of leaves;  $IL_{leaves}$  = initial length of leaves;  $FL_{leaves}$  = final length of leaves; and  $D$  = time (experimental period, in days)). Leaf appearance rate (LAR, leaf/tiller/day): obtained by dividing the number of emerging leaves on marked tillers by the number of days involved ( $LAR = \frac{\text{total emerged leaves per tiller}}{\text{experimental growth days}}$ ).

Statistical analyses were performed using the Assisat (2008) software version 7.5 beta, applying variance analysis and Tukey's test, considering as significant effect probability values lower than 5% ( $P < 0.05$ ).

## Results and Discussion

Forage mass (FM), root mass (RM), shoot/root ratio (SR ratio), life lifespan (LL), leaf elongation rate (LER), leaf appearance rate (LAR) and senescence rate (SR) were influenced by air temperatures. Cultivars affected LER, LAR, and SR, whereas the concentration of CO<sub>2</sub> influenced SR ratio and SR (Table 2). There were interactions between

buffel grass cultivars and air temperatures on LER, LAR, LL, and SR, whereas cultivars vs. carbon dioxide concentration affected FM, RM, SR ratio, LL, and SR (Table 2).

The FM was increased under 29/23 and 32/26 °C in comparison with 26/20 °C. At 29/23 and 32/26 °C FM were similar (Table 3). According to Fernández et al. (2014), C4 plants have higher photosynthetic and metabolic rates at high temperatures, promoting more biomass.

Lower RM was found at 32/26 °C, suggesting that the intermediate temperature (29/23 °C) was the optimum point for root growth. Possibly, at 32/26 °C plants presented high tissue synthesis rates (LAR, LER and SR) and the photoassimilates can be primarily directed to shoot in relation to root, promoting lower RM values. The lower SR ratio at 26/20 °C can be justified by the higher response of FM at 29/23 and 32/26 °C, promoted by higher tissue synthesis.

The FM, RM and SR ratio were not affected by the interaction between cultivars and air temperatures, indicating that all three buffel grass cultivars could be used in temperatures up to 32/26 °C.

Santos et al. (2013), who evaluated germinative responses of *Biloela*, *Aridus* and *West Australian*, suggested 25.5 to 31.5 °C as the optimum range for buffel grass seed germination, and reported that seeds of these cultivars presented tolerance to the temperature of 40 °C.

At 26/20 °C (day/night), *Biloela* had shorter LL in comparison with *Aridus*, whereas *West Australian* showed lower LAR than the others. Leaf elongation rates were similar for all three buffel grass cultivars evaluated. At a high temperature combination (32/26 °C), *West Australian* had lower LER, LAR and LL in comparison with *Aridus* and *Biloela*. At this same temperature (32/26 °C), the lowest SR was found for *Biloela* (Table 4).

According to Newman et al. (2001), FM for C4 plants is increased when they are exposed to a temperature above the standard. In their study, they found 11 to 26% more FM increasing the air temperature by 4.5 °C above the standard

Table 2 - P-values of main effects (cultivar, temperature and CO<sub>2</sub> concentration) and interactions between buffel grass cultivars (C) and air temperatures (T)

Effect	Characteristic						
	FM	RM	SR ratio	LER	LAR	LL	SR
C	0.1553ns	0.0874ns	0.1203ns	<0.001**	<0.001**	0.0576ns	<0.001**
T	<0.001**	<0.001**	<0.001**	<0.001**	0.001**	0.001**	<0.001**
CO <sub>2</sub>	0.245ns	>0.050ns	<0.001**	>0.050ns	>0.050ns	0.1633ns	0.0262*
C × T	>0.05ns	0.1329ns	0.2879ns	<0.001**	0.0026**	0.0021**	<0.001**
C × CO <sub>2</sub>	0.028*	0.002**	0.0297*	0.0679ns	0.1339ns	<0.001**	0.0055**

FM - forage mass (g of DM/pot); RM - root mass (g of DM/pot); SR ratio - shoot/root ratio; LER - leaf elongation rate (mm day<sup>-1</sup>); LAR - leaf appearance rate (leaf day<sup>-1</sup>); LL - leaf lifespan (days); SR - senescence rate (mm day<sup>-1</sup>).

C - cultivar; T - temperature; CO<sub>2</sub> - concentration of CO<sub>2</sub>; C × T - interaction between cultivar and temperature; C × CO<sub>2</sub> - interaction between cultivar and concentration of CO<sub>2</sub>.

temperature. In the present research, the obtained results were different because FM was not increased when the air temperature was raised from 3 to 6 °C. However, this may have occurred because this variable was measured at the end of the growth cycle; thus lost senescent leaves during growth were not computed. On the other hand, the high temperature values applied in the present research may have not promoted more FM, probably due to the high tissue turnover which may have exerted greater energy expenditure by plants, thus lowering their FM accumulation.

On the other hand, the results found in present study are in accordance with those reported by Sweeney and Hopkinson (1975), who evaluated 19 tropical and sub-tropical grasses and legumes, including *Cenchrus ciliaris*

at eight combinations of day/night temperatures rising in 3 °C steps from 15/10 to 36/31 °C, and reported no depression in growth rate for *Cenchrus ciliaris* when it was exposed to a high temperature, indicating that this forage plant could tolerate elevated temperatures without decreasing its production.

The increase in air temperature promoted significant differences in the morphogenetic characteristics of buffel grass. The combination of temperatures tested (26/20, 29/23 and 32/26 °C - day/night) probably provided changes in biosynthesis rates, accelerating the phenological development of plants, which is in line with the reports described by Fageria et al. (2006).

On the other hand, a possible stimulation for biomass synthesis at daily temperature (32/26 °C combination) could have been annulled due to the higher night temperature, which might increase the intake of photoassimilates by respiration. Thus, new combinations of temperatures can be tested, using the minimum and maximum temperatures of the region as a reference index and thus applying new increments of temperature.

The increase in LER, LAR and SR and the decrease in LL represent acceleration in the phenological development of plants, with high vegetative tissue turnovers. Thus, despite the stimulation to increase forage mass by LER and LAR, there were higher losses of forage mass due to higher SR and lower LL, which suggests a physiological steady state of plants, in which there is adjustment in turnover rates of vegetative tissues. Consequently, the life cycle of these buffel grass cultivars is expected to reduce when air temperature is increased.

The acceleration in the phenological cycle of buffel grass rising air temperature may cause a considerable impact on pasture-based production systems, affecting mainly the grazing management, because the plants will have a faster development, rapidly reducing their nutritional value and thus reducing the time for grazing, which can reduce the efficiency of use of the produced forage. This fact will imply the need to establish new grazing management strategies for the buffel grass pastures.

West Australian presented approximately 15 to 25% lower FM in comparison with Aridus and Biloela, respectively, when it was exposed to 370  $\mu\text{mol mol}^{-1}$  of  $\text{CO}_2$ . On the other hand, at 550  $\mu\text{mol mol}^{-1}$  of carbon dioxide concentration, West Australian and Biloela had similar FM and RM, but Aridus presented lower FM than with 370  $\mu\text{mol mol}^{-1}$  (Table 4).

According to Ghannoum et al. (2000), the growth stimulation of C4 plants to a doubling of the current  $\text{CO}_2$  ranges from 22 to 33%. In addition, Bhatt et al. (2007)

Table 3 - Forage mass (FM), root mass (RM) and shoot/root ratio (SR ratio) of buffel grass cultivars (Biloela, Aridus and West Australian) subjected to three air temperature combinations

Characteristic	Air temperature (day/night)			CV (%)
	26/20 °C	29/23 °C	32/26 °C	
FM, g of DM/pot	5.92b	15.31a	16.00a	40.00
RM, g of DM/pot	4.67c	9.30a	7.16b	24.90
SR ratio	1.03b	1.54a	1.53a	27.30

Means followed by same letter in the rows did not differ statistically by Tukey's test, considering 5% of probability ( $P < 0.05$ ).  
CV - coefficient of variation.

Table 4 - Leaf elongation rate, leaf appearance rate, leaf lifespan and senescence rate of buffel grass cultivars in three combinations (day/night) of air temperature

Cultivar	Air temperature (day/night)		
	26/20 °C	29/23 °C	32/26 °C
Leaf elongation rate ( $\text{mm day}^{-1}$ )			
Aridus	44.16aC	71.58aB	82.06aA
Biloela	37.78aC	45.71cB	77.74aA
West Australian	37.43aC	53.46bB	63.37bA
CV, %	9.62		
Leaf appearance rate ( $\text{leaf day}^{-1}$ )			
Aridus	0.21aB	0.22aB	0.25aA
Biloela	0.20aAB	0.18bB	0.21bA
West Australian	0.15bA	0.16cA	0.16cA
CV, %	5.78		
Leaf lifespan (days)			
Aridus	15.59aA	13.98bB	12.90aB
Biloela	14.23bB	15.38aA	12.87aC
West Australian	15.63aA	15.37aA	13.12aB
CV, %	5.32		
Senescence rate ( $\text{mm day}^{-1}$ )			
Aridus	9.36bB	16.78bB	57.51aA
Biloela	25.31aB	34.12aAB	41.06bA
West Australian	13.31bC	34.64aB	64.03aA
CV, %	19.53		

Means followed by same letter (lowercase for columns and uppercase for rows) did not differ statistically by Tukey's test, considering 5% of probability ( $P < 0.05$ ).  
CV - coefficient of variation.

reported that high carbon dioxide (600 ppm) in a long-term exposure (120 days) promoted greater FM in *Cenchrus ciliaris*. The results found in present study, especially for West Australian, are in accordance with those reported by Ghannoum et al. (2000) and Bhatt et al. (2007).

Rudmann et al. (2001) evaluated the influence of high CO<sub>2</sub> partial pressure on nitrogen use efficiency of the C4 grasses *Panicum coloratum* and *Cenchrus ciliaris* and reported that *Cenchrus ciliaris* had a consistently higher dry matter than *Panicum coloratum*, showing that buffel grass has a great capacity to produce more forage in a CO<sub>2</sub>-enriched atmosphere. Besides, dry mass partitioning varied between species and on average *Cenchrus ciliaris* allocated more dry matter to the stem plus sheath (42%) than did *Panicum coloratum* (27%). These studies support the results obtained in the present research, except for Aridus, whose productive responses of *Cenchrus ciliaris* could be increased or not impaired in an elevated CO<sub>2</sub> atmosphere.

The increase in CO<sub>2</sub> concentration (370 to 550  $\mu\text{mol mol}^{-1}$ ) decreased FM for Aridus but provided significantly greater FM for West Australian. Similar SR ratios were found for Biloela and Aridus at 370 and 550  $\mu\text{mol mol}^{-1}$  of CO<sub>2</sub>. In addition, West Australian presented higher SR ratio at 550 than 370  $\mu\text{mol mol}^{-1}$  of CO<sub>2</sub> (Table 5).

In the present research, *Cenchrus ciliaris* allocated 40% more for shoot than for root. Similar results were observed by Rudmann et al. (2001), who obtained higher shoot length at elevated CO<sub>2</sub> and moderate N supply.

The absence of elevated CO<sub>2</sub> concentration effects for FM, RM and SR ratio for Biloela is evidence that the plant has a C4 photosynthetic cycle. The photosynthetic

assimilation for C4 plants is stimulated until 300  $\mu\text{mol mol}^{-1}$  of atmospheric CO<sub>2</sub> concentration, approximately, which means that the compensation point of CO<sub>2</sub> occurs between 200 and 300  $\mu\text{mol mol}^{-1}$  (Sage and Pearcy, 2000). This occurs because the decarboxilation process of organic acids in bundle-sheath cells results in an increase of approximately 1,000 ppm CO<sub>2</sub>, characterizing C4 metabolism that concentrates CO<sub>2</sub> (Schulze et al., 2005). Thus, 550 ppm (CO<sub>2</sub> concentration applied in this study) is lower than the CO<sub>2</sub> level that can be accumulated in bundle sheath cells under atmospheric CO<sub>2</sub> concentration (Schulze et al., 2005). Therefore, the level of irradiance takes a decisive role for the modulation of photosynthetic assimilation of C4 plants.

However, in this study, although the irradiance (400  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ) was applied from a static source and by a constant way during the light period, it may have been a limiting factor to productive responses considering the highest temperature values applied. Different plant architectures can also interact differentially with non-saturating irradiance, contributing to differences in light absorption between cultivars.

In the case of West Australian, exclusively, the increase in FM was observed with high CO<sub>2</sub> concentration, corroborating Ghannoum et al. (1997) and Ghannoum and Conroy (1998). Ghannoum et al. (2000) suggest that increasing CO<sub>2</sub> can benefit the growth of C4 plants by: 1) stimulating CO<sub>2</sub> assimilation by increasing the partial pressure of intercellular spaces; 2) improving the water ratios of the shoots due to decreased transpiration, and consequently, increased leaf temperature; or 3) changes in daily patterns of CO<sub>2</sub> fixation.

The elevated CO<sub>2</sub> did not affect the productive characteristics of Biloela, decreased Aridus FM and increased FM for West Australian. These results indicate that in the current climate scenario, Biloela and Aridus are more productive than West Australian, but in the future climate scenario, considering the increase in carbon dioxide, West Australian may present similar productive performance compared with Biloela, and superior to that of Aridus.

Furthermore, other studies should be conducted addressing this issue to generate more information on the responses of forage plants, considering the prediction of future climate scenarios, aiming to strengthen strategies to reduce the impacts on the dry areas, like the Brazilian semiarid. These studies need to include the impacts of water stress in association with rising air temperature and carbon dioxide concentration, besides greater values of photosynthetic active radiation.

Table 5 - Interactions between buffel grass cultivars and carbon dioxide concentration (CO<sub>2</sub>) for forage mass, root mass and shoot/root ratio

Cultivar	CO <sub>2</sub> ( $\mu\text{mol mol}^{-1}$ )	
	370	550
Forage mass (g of DM/pot) - CV (%) = 40.26		
Aridus	5.05aA	4.86bA
Biloela	5.40aA	5.90abA
West Australian	3.44bB	6.33aA
Root mass (g of DM/pot) - CV (%) = 24.90		
Aridus	1.54aA	1.24aA
Biloela	1.24aA	1.48aA
West Australian	1.54aA	1.82aA
Shoot/root ratio - CV (%) = 27.30		
Aridus	3.29abA	3.80aA
Biloela	4.30aA	4.66aA
West Australian	2.44bB	3.73aA

Means followed by same letter (lowercase for columns and uppercase for rows) did not differ statistically by Tukey's test, considering 5% of probability (P<0.05). CV - coefficient of variation.

## Conclusions

The increase in day and night air temperatures affects the morphogenetic characteristics of buffel grass, accelerating its vegetative development without increasing its forage mass. Elevated carbon dioxide concentration changes productive responses of buffel grass.

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