

Water deficit tolerance in genotypes of Urochloa spp.

Tolerância ao déficit hídrico em genótipos de Urochloa spp.

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ABSTRACT

It is estimated that World population will increase considerably in the coming years. Thus, there is a need to maximize the production of animal protein, of which the pasture production system is the most economical way. However, abiotic factors influence animal production, with water deficit being the factor that most limits the development of forages. This research aimed to investigate the tolerance of *Urochloa* spp. genotypes under periods with water deficit conditions. The experiment was carried out in a randomized block design, under a factorial design. Twelve forage genotypes were evaluated in three periods of water stress, with three replications. There was no interaction effect between the genotype factors and time of stress due to water deficit for all variables. There was a reduction in forage production with the increase of time in water restriction, and a decrease of 23% in leaf mass and 30% in stem. In the case of leaf attributes, there was a decrease of 28% in the area and 21% in the leaf length. For the genotype factor, the G11 material stood out in relation to the others for presenting better agronomic characteristics. However, studies are needed to demonstrate the superiority of this material in production systems.

Keywords: abiotic stress, grasses, forage breeding, water restriction.

RESUMO

Estima-se que a população mundial irá aumentar consideravelmente nos próximos anos. Diante disso, existe a necessidade de maximizar a produção de proteína animal, da qual o sistema de produção a pasto é a forma mais econômica. Porém, os fatores abióticos interferem na produção animal, em que o déficit hídrico é o fator que mais limita o desenvolvimento de forrageiras. Este estudo visou avaliar a tolerância de genótipos de *Urochloa* spp. sob períodos em condição de déficit hídrico. O experimento foi realizado sob delineamento experimental de blocos casualizados, em esquema fatorial. Foram avaliados 12 genótipos forrageiros em três períodos de estresse hídrico, com três repetições. Não houve efeito de interação entre os fatores genótipos e tempo de estresse por déficit hídrica, com diminuição de 23% para massa de folhas e 30% para colmos. No caso dos atributos da folha, foi verificada redução de 28% na área e 21% no comprimento foliar. Para o fator genótipo o material G11 se destacou em relação aos demais por apresentar melhores características agronômicas. Entretanto, são necessários estudos a fim de demonstrar a superioridade desse material em sistemas de produção.

Palavras-chave: estresse abiótico, gramínea, melhoramento genético, restrição hídrica.

INTRODUCTION

It is estimated that the world's population will increase approximately 20% by 2050 (UN, 2019). Therefore, there is a need to maximize the production of animal protein, of which the production of ruminants in pastures is the most economical way. Abiotic factors have an influence on animal grazing production, with water deficit being the main limiting environmental factor for the development of forages (Batista *et al.*, 2010; Schippers *et al.*, 2015).

It is important to know the development of forages under conditions of water deficit to understand its effects on forage production. This knowledge helps management practices to optimize the use of forage and increase animal production in pastures during this period (Araújo *et al.*, 2010; Euclides *et al.*, 2016). Thus, the search for new forage cultivars tolerant to water deficit, has become the most assertive option for diversifying pastures in production systems (Euclides *et al.*, 2014).

In the search for new cultivars to diversify pastures, Embrapa Dairy Cattle started breeding Urochloa ruziziensis (syn. Brachiaria ruziziensis) in 2004. U. ruziziensis is characterized as a perennial and creeping plant, dense leaf cover that grows at 1-1.5 m in height, with a decumbent base, with spikelets of 1 or 2 lines on one rachis side. Spikelets are approximately 5 mm long, hairy at the apical part and bisected along the rachis. In addition, it has short and strong rhizomes, hairy stem, linear and lanceolate leaves and inflorescence formed by 3 to 6 racemes. This species was used in the breeding program because it is the only genus that is diploid and sexually reproductive thus, this allows for the exploration of the existing genetic variability within the genus, making it possible to identify and select genotypes with a better forage quality (Souza-Sobrinho et al., 2016).

Therefore, it is essential to evaluate the behavior of material breeding that shows possibilities to enter the forage seed market, under water deficit conditions. Thus, the aim was to evaluate the tolerance of *Urochloa* spp. under periods of water deficit.

MATERIALS AND METHODS

The experiment was conducted in a greenhouse, at Juiz de Fora, Minas Gerais, Brazil (21°46′56.4″S and 43°22′12.7″W; 882 m asl), between February and July 2018. A thermometer located in the center of the greenhouse monitored the conditions of the greenhouse daily. The average maximum and minimum temperatures during the experimental period were 27.8°C and 18.8°C.

A completely randomized block design was used in a 12x4 factorial arrangement with 12 forage materials being nine genotypes improved by the breeding program of *U. ruziziensis* at Embrapa Dairy Cattle (G1, G2, G3, G4, G6, G7, G8, G10, G11 and BRS Integra) and two commercial cultivars *U. brizantha* cv. Marandu and *U. ruziziensis* cv. Kennedy, and three periods of stress due to water deficit, plus a control (T1 - 30 days under 100% of field capacity (FC); T2 - 10 days under 30% of FC; T3 - 20 days under 30% of FC and T4 - 30 days under 30% of FC), with three replicates, totaling 144 experimental units.

The experimental unit was one plant per cylindrical polyethylene pot of 20 centimeters (cm) in height, 10 cm in radius and with a capacity of 4 cubic decimeters (dm³). The pots were arranged on benches each containing four kilograms (kg) of soil collected from the arable layer (0 to 20 cm) at the José Henrique Bruschi Experimental field (CEJHB) of Embrapa Dairy Cattle. Subsequently, the soil was removed and sifted in a two millimeter (mm) mesh sieve and then air dried.

The soil of the experimental unit showed a clayclay-sandy texture, with 29% clay, 13% silt and 58% sand (according to the soil analysis protocol, using the "pipette" method), in the 0-20 cm layer. The pots were filled with soil with the following chemical characteristics: $pH_{(H2O)}$ 5.4; organic matter 2.18 dag kg⁻¹; Ca 1.5 cmolc dm⁻³; P 2.5 mg dm⁻³; K 51 mg dm⁻³; Mg 0.8 cmolc dm⁻³; H + Al 3.14 cmolc dm⁻³; Al 0.3 cmolc dm⁻³; effective cation exchange capacity 2.5 cmolc dm⁻³; total cation exchange capacity 5.6 cmolc dm⁻³; base saturation 44%; aluminum saturation 4%; sum of bases 2.4 cmolc dm⁻³. Based on the chemical analysis of the soil, 0.496 g kg⁻¹ of lime, 0.333 g kg⁻¹ of simple superphosphate and 0.014 g kg⁻¹ of potassium chloride were added according to the recommendations for the use of correctives and fertilizers in Minas Gerais - 5th approach (Cantarutti *et al.,* 1999). The sources of phosphorus and potassium were mixed with the soil one month after adding the limes.

Seedlings were planted on 21^{st} February 2018. After plant establishment, maintenance fertilization was carried out with nitrogen (N) and potassium (K) applying 0.1125 g kg⁻¹ of N and 0.0156 g kg⁻¹ of K, supplied in diluted water. This fertilization was divided into three applications with an interval of 21 days. During the initial phase of establishment, the soil of the pots was kept close to the field capacity (according to the methodology described by Bernardo *et al.*, 2019) to guarantee the development of the plants in this phase.

The conditioning cuts were carried out on 11th June 2018 at 15 cm from the ground. The plants were submitted to treatments on the tenth day of regrowth and the pots were weighed frequently, replenishing the water when necessary, in order to meet the imposed treatments.

The genotypes were submitted to treatments for 30 days. The variables analyzed after this period were: number of tillers, number of leaves, plant height, dry mass of leaves, dry mass of stems, leaf/ stem ratio, leaf area, leaf length, average leaf width, maximum leaf width and SPAD (Soil Plant Analysis Development) index.

The height of the plants (cm) was measured with the aid of a graduated ruler, from the soil to the top of the highest leaf when the plant was fully extended. To evaluate the dry mass of the aerial part, the plants were cut at 15 cm from the soil, separating them into leaf blades and stems. Then, the samples were weighed and placed in a forced ventilation oven at $65^{\circ}C \pm 5^{\circ}C$ for 72 hours and then weighed again.

The leaf area (cm²), leaf length (cm), average leaf width (cm) and maximum leaf width (cm) were determined using the Portable Area Meter (LI-COR model LI-3000C).

The SPAD index (relative chlorophyll index determined based on the intensity of the green color of the leaves) was measured on three leaves

per experimental unit, of which the youngest leaves were fully expanded. The reading was performed with a portable chlorophyll meter based on absorbance (SPAD-502, Konica-Minolta, Osaka, Japan).

The following model was used for variance analysis (ANOVA):

$$Y_{ijk} = \mu + b_k + \alpha_i + \beta_j + (\alpha^*\beta)_{ij} + e_{ijk}$$

Where:

 Y_{ijk} = observation in kth block, in ith genotype and in jth stress time per water deficit;

 μ = general mean;

 b_k = block effect (k= 1, 2 and 3);

 α_i = fixed effect of the genotype (i = 1, 2, 3, 4...12);

 β_j = fixed effect of stress time due to water deficit (j =0, 10, 20 and 30 days);

 $(\alpha^*\beta)_{ij}$ = interaction of the effects of genotype i on stress time due to water deficit *j*;

 e_{iik} = random error associated to $Y_{iik} [e_{iik} \sim NID(0, \sigma^2)]$.

The data was subjected to an analysis of variance ($p \le 0.05$), proceeded by the Scott-Knott method of grouping averages at the level of 5% probability for the qualitative factor (genotypes) and regression analysis for the quantitative factor (time of stress due to water deficit). Statistical analyzes were performed with the aid of the R Core Team (2019) software.

RESULTS AND DISCUSSION

There was no interaction effect between the genotype factors and stress time due to water deficit (p > 0.05) for the analyzed variables.

There was a difference in the genotype factor $(p \le 0.05)$ for all response variables (Table 1). Among the evaluated genotypes, G11 stood out from the others because it had lower height, a greater number of tillers, high leaf mass, low stem mass, greater

Table 1 - Analysis by the Scott-Knott means grouping method for the qualitative factor (genotypes) in a greenhouse in Juiz de
Fora-MG, in 2018, for the variables: plant height (PH), number of tillers (NT), dry mass of leaves (DML), dry mass of
stems (DMS), leaf/stem ratio (DML/DMS), number of leaves (NL), leaf area (LA), leaf length (LL), average leaf width
(ALW) and maximum leaf width (MLW) and SPAD (Soil Plant Analysis Development) index

Genotypes	Variables										
	PH	NT	DML	DMS	DML/DMS	NL	LA	LL	ALW	MLW	SPAD
G1	73.92 c	17 c	4.18 b	1.83 b	2.08 b	54 c	1702.6 c	1590.1 c	1.02 b	1.50 b	45.31 a
G2	75.00 c	22 b	4.39 b	2.35 a	1.82 c	67 b	1871.1 c	2051.6 b	0.86 c	1.35 b	35.07 c
G3	74.92 c	9 c	4.47 b	1.26 b	3.62 a	59 c	1995.0 b	1974.8 b	0.97 b	1.51 b	37.27 b
G4	86.00 b	15 c	4.46 b	2.23 a	1.95 c	56 c	1879.8 c	1977.0 b	0.90 c	1.42 b	38.67b
G6	89.46 b	19 c	5.25 a	2.57 a	1.95 c	55 c	2141.5 b	2126.6 b	0.96 b	1.49 b	37.94 b
G7	87.50 b	14 c	4.49 b	1.95 b	2.19 b	44 c	1679.4 c	1566.0 с	1.02 b	1.53 b	43.57 a
G8	86.42 b	17 c	3.30 c	2.21 a	1.41 c	54 c	1569.8 c	1579.7 с	0.93 c	1.37 b	36.19 c
G10	94.25 b	14 c	5.77 a	3.21 a	1.66 c	56 c	2657.5 a	1949.8 b	1.30 a	1.83 a	43.24 a
G11	76.50 c	29 a	5.54 a	1.94 b	2.61 b	95 a	2753.0 a	3391.0 a	0.76 c	1.19 b	34.76 c
BRS Integra	71.33 с	24 b	5.95 a	1.54 b	3.18 a	72 b	2020.4 b	1895.7 b	0.92 c	1.39 b	35.06 c
Kennedy	73.17 c	23 b	5.95 a	2.10 a	2.36 b	79 b	2261.1 b	2227.8 b	0.89 c	1.37 b	33.63 c
Marandu	106.25 a	14 c	5.46 a	2.38 a	2.16 b	46 c	2099.4 b	1933.2 b	0.99 b	1.48 b	43.66 a
C.V.%	10.88	30.10	19.81	44.60	28.18	24.18	25.36	28.20	18.14	16.78	8.49

*Means followed by the same letter in the column do not differ by the method of grouping Scott-Knott averages at 5% of probability. C.V.%- coefficient of variation.

number of leaves, greater area, and leaf length. These characteristics suggest that this genotype has the potential to integrate animal production systems. The small size ensures the material is easy to handle (Valle *et al.*, 2017). The largest number of tillers ensures forage greater mass production (Lima *et al.*, 2019), however, it cannot be considered in itself as an indicator of greater plant persistency (Sbrissia *et al.*, 2010). The greater amount of leaf allows animals greater selectivity, since they have better nutritional value, consequently, result in superior animal performance (Fonseca *et al.*, 2012).

The time of water deficit did not influence the number of tillers and the number of leaves (p > 0.05). The period when the largest tiller emission occurs during the establishment of the forage (Santos *et al.*, 2012; Oliveira *et al.*, 2015), when water availability was being maintained in field capacity, before the period of stress. Beloni *et al.* (2017) observed similar results, but evaluating morphological and physiological behavior of accessions of *Paspalum* sp. and *U. brizantha* cv. Marandu.

According to Silva *et al.* (2008), the action of water stress on productivity varies according to the stage of plant development. For this reason, water stress was submitted only after the plant establishment phase. Santos *et al.* (2012) evaluating wheat cultivars submitted to water deficit at the

beginning of flowering, found a reduction in the number of tillers per plant under water stress conditions. According to the authors, even if the tillering phase had already occurred before the application of water stress, the reduction was caused by the need for plants to reduce their leaf area through the death of tillers in search of their persistence.

The number of leaves showed a divergent response from the results found by Bonfim-Silva *et al.* (2011), where they observed a reduction in the number of leaves in corn and sorghum crops among water availability subjected to water stress.

When evaluating the morphogenic responses of forage grasses under different water conditions, Silva *et al.* (2005) observed a decrease in the number of leaves expanded per tiller, mainly in Angola grass and grass in more severe levels of water deficit. A similar situation was also observed in *U. brizantha* cv. Xaraés, *U. ruziziensis*, *P. glaucum* cv. ADR 7010, *P. glaucum* cv. ADR 300 and *M. aterrima* when subjected to water stress (Petter *et al.*, 2013). It is believed that the water level at 30% of the field capacity used in this work and the characteristics of the climate during the experimental period were not sufficient to cause a reduction in the number of leaves regardless of the time of water deficit used in the study.



Figure 1 - Plant height (cm) as a function of water deficit stress time (0, 10, 20 and 30 days).

For the plant height, the 1st -degree regression model was adapted (p = 0.013) (Figure 1), where it was observed the occurrence of height reduction between the times when the plants were submitted to water deficit, leading to a reduction of approximately 8% between the conditions of field capacity and the situation of longer time in water deficit. Corroborating with the data found by Silva *et al.* (2020), who observed a 60% reduction in the height of cultivars of *U. brizantha* (Piatã, BRS Paiaguás, and MG13 Braúna) when subjected to water stress. However, Beloni *et al.* (2018), found no difference in plant height between the three cultivars of *Urochloa* spp. (*U. brizantha* cv. Marandu and Paiaguás and *U. decumbens* cv. Basilisk) and cultivar Medly (*Dactylis glomerata* L.) under water deficit conditions.

The decrease in the height of plants subjected to water deficit is due to the reduction of the turgidity pressure in the cells since for the occurrence of cell growth, minimum levels of this pressure are required (Kramer and Boyer, 1995).

The biomass production of the aerial part is probably one of the most important particularities in relation to the potential yield of forage plants (Cezário *et al.*, 2015). The production of leaves (p = 0.001) and stems (p = 0.008) dry mass, adapted to the 1st degree regression model (Figure 2A). It was observed a reduction in the accumulation of leaves and stems dry mass due to the increase in the period under stress due to water deficit of 23% and 30%, respectively. The decrease in the dry mass of forage leaves of the *Urochloa* genus when subjected to water deficit was observed by Kroth *et al.* (2015), Mattos *et al.* (2005), Pezzopane *et al.* (2015) and Beloni *et al.* (2017) for accessions of the genus *Paspalum*.

Regarding the dry mass of stem in the study by Kroth *et al.* (2015), Pezzopane *et al.* (2015) and



Figure 2 - A) Production of dry mass of leaves (DML) (g) and stems (DMS) (g); B) Leaf/Stem ratio (DML/DMS) as a function of time of stress due to water deficit (0, 10, 20 and 30 days).



Figure 3 - A) Leaf area (LA) (cm²) and leaf length (LL) (cm); B) average leaf width (ALW) (cm) and maximum leaf width (MLW) (cm) as a function of water deficit stress time (0, 10, 20 and 30 days).

Poustini *et al.* (2007) also was observed a decrease in water deficit conditions. However, Mattos *et al.* (2005) observed a reduction in the dry mass production of stems in *U. decumbens* and *U. mutica,* however, the same effect was not observed in *U. brizantha* and *U. humidicula*.

Roots play an important role in regulating water intake and maintaining the plant's water balance (Rouached *et al.*, 2013; Santos *et al.* 2013; Cardoso *et al.*, 2015; Beloni *et al.*, 2017, 2018; Silva *et al.*, 2020). It is believed that the negative effects of water deficit in the portion of leaves and stems, may primarily affect the root system, which results in a decline in water assimilation, reduces the water potential of the aerial part and photosynthesis presents a more severe reduction than breathing (Pande and Singh, 1981). However, during this study, it was not possible to carry out this evaluation, which does not allow us to conclude that this occurred with the forages under study.

The leaf/stem ratio (L/S), adjusted to the 1st-degree regression model (p = 0.043) (Figure 2B), where a linear increase was observed as a function of the imposed water deficit period. This variable is essential to determine the supply of nutrients and to establish management strategies for forage grasses (Wilson, 1982), the high L/S ratio gives the forage plant a higher nutritional quality in addition to greater acceptability by animals (Mezzalira *et al.*, 2017).

Regardless of the experimental conditions imposed, the observed L/S ratio was higher than the critical one (1), described by Pinto *et al.* (1994). Kroth *et al.* (2015) also found the highest L/S ratio under water deficit conditions in cultivars of *U. brizantha*. The period of development of the plant affects these results since a larger portion of leaves was observed in the early stages of development (Rodrigues *et al.*, 2008).

The measures of leaf area (p = 0.001) and leaf length (p = 0.001) adjusted to the linear regression model (Figure 3A). It was found that when subjected to the longest time of water deficit, the measures were negatively influenced. Water stress reduced the leaf area variable by 28%, between treatment in field capacity and 30 days under stress. For the leaf length variable, this reduction was 21%.

The reduction in leaf area and length was observed in a study with several forage species (Barreto *et al.*, 2001; Guenni *et al.*, 2002; Santos *et al.*, 2013; Cardoso *et al.*, 2015; Beloni *et al.*, 2017; Silva *et al.*, 2020). The authors refer to this decline to an increase in the proportion of dead tissue, a reduction in the number and size of cells, and an increase in the allocation of resources for root development.

The quadratic regression model (Figure 3B) was applied for the variables average leaf width (p = 0.001) and maximum leaf width (p = 0.001).

The maximum point of the regression curve for both the average leaf width and maximum leaf width variable occurred at 11 days of stress due to water deficit with an average width of 1.04 cm and a maximum width of 1.55 cm. Water stress promoted a reduction of approximately 20% in the average leaf width and 17% in the maximum leaf width of the maximum points of the curves until the conditions of greater stress time due to water deficit.

The stress, submitted to genotypes, increased the width of the leaves in the early stages. According to Bonfim-Silva *et al.* (2014), when submitted to water deficit, forages tend to have their maximum leaf development, due to their compensatory growth (Páez and González, 1995). There was a reduction in leaf width with increasing time in water deficit, a strategy used by the plant, where the leaves are curled in an attempt to reduce water loss to the environment (Taiz and Zeiger, 2013). Cardoso *et al.* (2015) when evaluating *Pennisetum purpureum* cv. Napier and xUrochloa cv. Mulatto II in situations of water deficit also observed leaf curling.

The SPAD reading (p = 0.002) adjusted to the quadratic regression model (Figure 4). The maximum point of the regression curve occurred at 19 days of stress due to water deficit with an index of approximately 40 SPAD units. It is possible to verify lower rates when the plants were subjected to shorter water deficit conditions, confirming the effect of diluting the chlorophyll content (Ashraf and Chishti, 1993).

The effect of diluting the chlorophyll content in conditions of greater water availability was also reported in the work by Bonfim-Silva *et al.* (2014), where they evaluated the production and development of grass convert HD364. The reduction in the SPAD index in a long time of water deficit is mainly due to the process of premature leaf senescence in an attempt to guarantee the survival of the plants with the reduction of water loss by the leaves. This effect was also observed in



Figure 4 - SPAD index as a function of water deficit stress time (0, 10, 20 and 30 days).

the study by Beloni *et al.* (2017), which evaluated accessions of *Paspalum sp.* under water deficit.

CONCLUSION

Among the evaluated genotypes, G11 stood out as prominent. However, further studies are needed in order to demonstrate its superiority in production systems.

The water deficit affected the development of forage, with a reduction in forage production with increasing time under stress.

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