



Adaptive leaf anatomical characteristics of *Brachiaria ruziziensis* (Poaceae) genotypes in different environments

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ABSTRACT. Studies on morphological plasticity are extremely important to plant breeding seeking to optimize genotype interactions with their environments. The present study evaluated 38 genotypes of *Brachiaria ruziziensis* cultivated during the rainy (October to March) and dry (April to September) seasons and examined variations in their anatomical characteristics. Fully expanded leaves were collected and fixed in FAA₇₀ (formaldehyde: acetic acid: ethyl alcohol) for 72h and subsequently stored in 70% alcohol. Median sections of leaf blades were examined and evaluated according to traditional plant anatomical procedures. Genotypes 15, 27, 40, 53, and 90 demonstrated interactions with the environment in five of the anatomical characteristics evaluated; genotypes 1 and 33 presented no plasticity in any of the characteristics examined. Except for the thickness of abaxial (ABT) and adaxial (ADT) epidermal surfaces, the genotypes showed different behaviors for all the other characteristics evaluated: mesophyll thickness (MT), xylem diameter (XD), distance between bundles (DB), number of bundles (NB), number of bulliform cells (NBC), sclerenchyma tissue area (%L), and bulliform cell area (ABC).

Keywords: morphological plasticity, plant anatomy, plant breeding, forage.

Características anatômicas foliares adaptativas de genótipos de *Brachiaria ruziziensis* (Poaceae) em diferentes ambientes

RESUMO. O estudo da plasticidade morfológica é de grande importância para os programas de melhoramento que desejam obter materiais promissores quando apresentam a interação genótipo com ambiente. O objetivo deste trabalho foi avaliar 38 genótipos de *Brachiaria ruziziensis* cultivados na época das águas (outubro a março) e da seca (abril a setembro) e observar variações nas características anatômicas nas diferentes estações. Folhas completamente expandidas foram coletadas e fixadas em FAA₇₀ por 72h e armazenadas em álcool 70%. Amostras foliares na parte mediana foram avaliadas de acordo com os procedimentos usuais de anatomia vegetal. Os genótipos 15, 27, 40, 53, e o 90 apresentaram interação com o ambiente em cinco características avaliadas. Os genótipos 1 e 33 não apresentaram plasticidade para nenhuma mensuração. Exceto para espessura das faces abaxial (EBA) e abaxial (EDA) da epiderme, os genótipos apresentaram comportamento diferente para as demais características avaliadas: espessura do mesofilo (EM), diâmetro do xilema (DX), distância entre feixes (DF), número de feixes (NF) e de células buliformes (NCB), área do tecido esclerenquimático (%L) e das células buliforme (ACB).

Palavras-chave: plasticidade morfológica, anatomia vegetal, melhoramento genético, forrageiras.

Introduction

Leaves are the major source of green material of forage plants and one of the plant organs most commonly used to determine animal feed and forage productivity and quality. Leaves also show high morphological plasticity – the capacity to alter their genetic expression in response to distinct environmental conditions (BRADSHAW, 1965; CHEN; WANG, 2009; MATESANZ et al., 2010; SULTAN, 2004; WHATLEY; WHATLEY, 1982).

Plasticity can be expressed by morphological, physiological, or biochemical changes, and can affect

structural characteristics as well as the proportions of different plant tissues (notably in leaves), including: mesophyll and cell wall thicknesses, number of vascular bundles and bulliform cells, distance between vascular bundles, thickness of abaxial and adaxial epidermal surfaces, and stomatal density.

Numerous authors have examined the relationship between anatomical characteristics and environmental factors (MELO et al., 2007; NERY et al., 2007; PACIULLO et al., 2001; PACIULLO, 2002; PINTO et al., 2007), and several have noted the influence of light on tissue organization (SMITH et al., 1998; VIEIRA, 1995).

Grasses have especially high capacities to adapt their tissues to environmental conditions and to maximize their functions under extreme soil and climatic conditions. *Brachiaria* (Poaceae) is an economically important species group in Brazil as these grasses readily grow on acidic and low-fertility Cerrado soils, and thus have aided the colonization and development of central Brazilian plains (VALLE et al., 2008). Studies on *Brachiaria brizantha* and *Brachiaria humidicola* (DIAS FILHO, 2000, 2002) have demonstrated reductions in photosynthetic capacity and morphological plasticity under shade conditions as well as tolerance to reduced light levels. Both species were able to quickly develop phenotypic adjustments in response to low light. Specific leaf area and leaf area ratio were higher for low-light plants during the entire experimental period. However, detailed information about anatomical characteristics of these grasses and about the behavior of different genotypes under different habitat conditions are currently insufficient to drive any rapid advances in genetic improvement.

Carvalho et al. (2002) studied six forage grasses under direct light and shaded conditions and found that only one species could not tolerate shade conditions (and demonstrated lower production rates).

Information concerning degrees of morphological and/or anatomical plasticity in forage plants is of extreme importance to scientists working with plant breeding due to the diverse conditions of soil and climate in Brazil and the fact that anatomical characteristics can influence forage quality (ALVES DE BRITO et al., 1999, 2004; PACIULLO et al., 2001, PACIULLO, 2002; BATISTOTI et al., 2012). Studies of this type are relatively recent, however, especially those comparatively analyzing forage plants during different seasons of the year; fewer workers yet have analyzed leaf anatomy in differing climates (PACIULLO, 2002).

The present study evaluated variations in anatomical characteristics of 38 different genotypes of *Brachiaria ruziziensis* during the rainy (October to March) and dry (April to September) seasons to identify genotypes that could be useful in plant breeding.

Material and methods

Thirty-eight genotypes of *Brachiaria ruziziensis* grown at Coronel Pacheco Experimental Fields (Minas Gerais State) were studied as part of breeding programs at Embrapa Gado de Leite – Juiz de Fora, Minas Gerais State, Brazil, in conjunction with the Federal University of Lavras – UFLA, Lavras, Minas Gerais State, Brazil.

The average values of climatic data (luminosity, temperature, insolation, humidity, and rainfall) collected at the Embrapa Gado de Leite Weather Station, located in the Experimental Fields, are presented in Table 1.

Table 1. Average climatic variables during the experimental period. Data provided by Embrapa Gado de Leite Weather Station – Juiz de Fora (Minas Gerais State, Brazil).

Month/year	Relative humidity (%)	Temperature (°C)	Insolation (hours month ⁻¹)	Luminosity (watt m ⁻²)	Rainfall (mm month ⁻¹)
Jan/09	92.50	24.08	183.75	621.25	38
Feb/09	89.61	24.15	207.68	642.14	118
Mar/09	88.00	24.69	215.75	645.00	42.4
Apr/09	87.67	20.20	214.67	621.67	0.2
May/09	91.29	19.18	149.94	578.71	3.8
Jun/09	90.13	17.22	153.77	553.33	37.6
Jul/09	88.97	18.92	158.23	561.13	3.2
Aug/09	86.16	18.64	164.90	589.19	4.2
Sep/09	84.33	22.08	192.63	609.83	113.4
Oct/09	89.26	22.23	159.21	600.00	58

Samples were collected from each forage genotypes in two different seasons during 2009: on March 12th, representing the rainy season (October to March), and on August 31st, representing the dry season (April to September), characterized according to the rainfall of southeastern Brazil (ALVES et al., 2005; ROLDÃO; ASSUNÇÃO, 2012). Additionally, a total of eight harvest cuts were made for assessing forage production. The closest grass productivity and forage quality harvesting dates were: January 27th, 2009, March 16th, 2009, April 27th, 2009, and October 19th, 2009.

Three completely expanded leaves from the second stem node were collected from each genotype. These leaves were fixed in F.A.A.₇₀ (formaldehyde: acetic acid: ethyl alcohol) for 72 hours (KRAUS; ARDUIM, 1997) and subsequently preserved in 70% ethyl alcohol until analysis. Cross sections of the median third of leaf blades were prepared using a manual microtome. Sections were cleared in 50% sodium hypochlorite, washed in distilled water, neutralized in 1% acetic acid, stained with astra blue-safranin (KRAUS; ARDUIM, 1997), and mounted on slides with 50% glycerin.

Cross sections of leaf blades were photographed using a digital camera coupled to a microscope, and the following characteristics were evaluated: mesophyll thickness (MT), abaxial epidermal thickness (ABT), adaxial epidermal thickness (ADT), xylem diameter (XD), distance between vascular bundles (DB), number of bundles (NB), number of bulliform cells (NBC), area of bulliform cells (ABC), sclerenchyma tissue area (AES).

Anatomical measurements were made in areas between the first and second vascular bundles

(using the central bundle as reference) in all genotypes. Tissue thickness was measured using three leaves from each plant. Photomicrographs were analyzed using UTHSCA-Image tool® image analysis software, with nine repetitions per leaf.

Comparisons of mean values of the different genotypes were performed using the Scott-Knott grouping test, at a 5% probability level.

Results and discussion

The individual analysis of variance (per season) indicated that only AES had not varied significantly in any of the different genotypes during the dry season ($p > 0.05$) (Table 2). All other characteristics had significant variations of genotypes in each season. The behavior of genotypes was significant ($p < 0.01$) in the analysis of variance (“rainy” and “dry”) for all the characteristics evaluated (Table 3), confirming that genotypes showed genetic variability in terms of anatomical characteristics of leaves – which could presumably be taken advantage of by plant breeding.

Table 2. Results of the individual ANOVA per season (rainy and dry) for the variables: mesophyll thickness (MT), abaxial epidermal thickness (ABT), adaxial epidermal thickness (ADT), xylem diameter (XD), distance between vascular bundles (DB), number of bundles (NB), number of bulliform cells (NBC), area of bulliform cells (ABC), sclerenchyma tissue area (AES). All measurements in μm . MS=means square; DF = degrees of freedom.

		ABT	ADT	MT	XD	ABC
Season	DF*	MS**	MS	MS	MS	MS
Rainy	37	1.93 ⁽¹⁾	1.5 ⁽¹⁾	527.76 ⁽³⁾	28.86 ⁽¹⁾	613076 ⁽³⁾
Dry	37	1.51 ⁽¹⁾	1.04 ⁽¹⁾	756.24 ⁽¹⁾	31.58 ⁽¹⁾	1595476 ⁽¹⁾
		DB	NB	NBC	AES	
Season	DF*	MS**	MS	MS	MS	
Rainy	37	29.75 ⁽²⁾	0.2 ⁽³⁾	0.26 ⁽¹⁾	3435778 ⁽¹⁾	
Dry	37	64.72 ⁽¹⁾	0.22 ⁽³⁾	0.29 ⁽¹⁾	1164809 ⁽⁴⁾	

⁽¹⁾ $p < 0.001$; ⁽²⁾ $p > 0.001$; ⁽³⁾ $p < 0.05$; ⁽⁴⁾ $p > 0.05$.

In terms of epidermal thickness (ABT and ADT), no significant differences were observed between the two seasons in the joint analysis ($p > 0.05$), although genotypic interactions were observed with the environment ($p < 0.05$) (Table 3).

Significant differences in ABT ($p < 0.01$) were observed in the different seasons in genotypes 2, 27, 29, 40, 53, 54, 64, 71 and 97, with genotypes 29 and 71 showing the highest mean values during the dry season (5.38 μm and 7.13 μm respectively); mean values for the other genotypes of this group were higher during the rainy season. In relation to ADT, genotypes 2, 27, 54 and 97 presented significant differences between seasons, with higher values during the rainy season (7.60, 7.55, 8.08 and 6.75 μm respectively) ($p < 0.05$).

The results for ABT and ADT (which were higher during the rainy season) agreed with many previously published results. Levels of insolation (hours day⁻¹) and luminosity during March (rainy season) were higher

than observed in August (dry season). The epidermal thickness of the two leaf surfaces are known to vary and have been found to either diminish (DICKISON, 2000; GRATANI et al., 2006; PANDEY; KUSHWAHA, 2005) or increase (MORAIS et al., 2004; VOLTAN et al., 1992) in response to light intensity.

Table 3. Results of the pooled ANOVA for mesophyll thickness (MT), abaxial epidermal thickness (ABT), adaxial epidermal thickness (ADT), xylem diameter (XD), distance between vascular bundles (DB), number of bundles (NB), number of bulliform cells (NBC), area of bulliform cells (ABC), sclerenchyma tissue area (AES) in rainy and dry seasons among the 38 genotypes of *Brachiaria ruziziensis* in 2009. All measurements in μm . VS = Variation source; MS = means square; DF = degrees of freedom.

		ABT	ADT	MT	XD	ABC
VS	DF	MS	MS	MS	MS	MS
G*	37	1.86 ⁽¹⁾	1.64 ⁽¹⁾	873.01 ⁽¹⁾	45.59 ⁽¹⁾	1309206.73 ⁽¹⁾
E**	1	1.75 ⁽³⁾	1.46 ⁽³⁾	14706.99 ⁽¹⁾	2582.43 ⁽¹⁾	29609052.68 ⁽¹⁾
G*E	37	1.57 ⁽¹⁾	0.88 ⁽¹⁾	410.99 ⁽²⁾	14.84 ⁽³⁾	899345.1 ⁽¹⁾
		DB	NB	NBC	AES	
FV	DF	MS	MS	MS	MS	
G*	37	69.25 ⁽¹⁾	0.25 ⁽¹⁾	0.32 ⁽¹⁾	2464259.91 ⁽¹⁾	
E**	1	80.85 ⁽²⁾	4.58 ⁽¹⁾	3.86 ⁽¹⁾	47010000.3 ⁽¹⁾	
G*E***	37	25.21 ⁽¹⁾	0.16 ⁽³⁾	0.22 ⁽²⁾	2136327.06 ⁽¹⁾	

G* = effect of genotypes; E** = effect of seasons (rainy and dry); G***E* = genotype vs. environment interaction ⁽¹⁾ $p < 0.01$; ⁽²⁾ $p > 0.01$; ⁽³⁾ $p < 0.05$.

In terms of MT, the joint analysis evidenced differences between seasons ($p < 0.05$) with higher mean thickness during the dry season, and genotype vs. environment interaction was observed (Tables 3 and 4). Genotypes 4, 6, 15, 29, 35, 40, 41, 44, 90, 93 and 97 also showed higher mean mesophyll thickness values during the dry season, with an average thickness of 170.68 μm .

Mesophyll thickness appears to be influenced ($p < 0.05$) by luminosity and insolation condition, showing higher values during the rainy season. The results obtained in the present study for this characteristic were different from previously reported in literature in terms of temperature, insolation, and luminosity conditions during the months of August and March (Table 1). The mesophyll absorbs the light incident on the leaves, and plants grown in full sunlight will have very thick leaves (due to the development of more palisade and spongy parenchyma tissue) (ABRAMS et al., 1994; BOARDMAN, 1977; CUI et al., 1991; LEE et al., 2000).

Regarding the sclerenchyma tissue (AES), the joint analysis indicated higher mean value in the rainy season, as well as genotype vs. environment interactions (Tables 3 and 4). Nine genotypes (3, 41, 44, 51, 58, 64, 66, 99 and 100) demonstrated distinct behavior in the different seasons as for this characteristic, with higher values during the rainy season ($p < 0.05$). Temperature, insolation, and

luminosity were greater in the rainy season (Table 1) and, according to the literature plants accelerated their development, with concomitant increases in the lignification of cell walls (ALVES DE BRITO et al., 1999; JUNG, 1989; PACIULLO, 2002; WILKINS, 1972). Lignin is the major factor limiting forage digestibility, and according to Akin and Burdick (1973), lignification sites may be more important than the actual amount of lignin present in the plant.

The number of bulliform cells (NBC) presented significant differences between the seasons ($p < 0.05$) in the joint analysis, being one order of magnitude greater during the rainy season; genotype vs. environment interactions was also significant ($p < 0.05$) (Tables 3 and 4).

Table 4. Averages of the ANOVA for the variables: abaxial epidermal thickness (ABT) - μm , adaxial epidermal thickness (ADT) - μm , mesophyll thickness (MT) - μm , distance between vascular bundles (DB), xylem diameter (XD), number of vascular bundles (NB), number of bulliform cells (NCB), sclerenchyma tissue area (AES) - μm^2 , and area of bulliform cells (ABC) - μm^2 in dry and rainy seasons in 38 genotypes of *Brachiaria ruziziensis* in 2009.

Season	ABT	ADT	MT	XD	DB	NB	NCB	AES	ABC
Dry	6.37a*	5.90a	157.99a	22.09b	25.17a	4.87b	4.77b	3437.13b	4254.75a
Rainy	6.54a	6.06a	141.92b	28.82a	23.98b	5.15a	5.03a	4345.28a	3534.01b

*Means followed by the same letter in the column are not significantly different by F-test.

In relation to NBC, genotype 27 had an average of 5.33 cells (during the dry season), and genotypes 15, 53, 70, 90, 93 and 96 had an average value of 5.35 (during the rainy season), thus demonstrating significant differences between seasons ($p < 0.05$). The average areas of bulliform cells (ABC) were greater during the dry season in 13 genotypes (4, 6, 15, 21, 29, 40, 44, 48, 51, 54, 64, 90 and 93) ($p < 0.05$).

Bulliform cells were only observed on the adaxial surface of leaves – a common characteristic in grasses (ELLIS, 1976). Genotypes 8, 43, 44 and 58 had the smallest average number of bulliform cells (4.38) ($p < 0.05$). Two groups were formed in terms of ABC, with the group with the highest average being composed of 14 genotypes (2, 6, 7, 8, 21, 35, 40, 43, 51, 54, 56, 58, 64 and 97). Bulliform cell area can be used as indicator of adaptability to cultivation in environments with water deficits (or high environmental temperatures) because these cells are involved in leaf curling that diminishes the leaf surface area (and thus water loss through transpiration) – and the larger the area occupied by bulliform cells, the better the response of the genotype to dry/hot environmental conditions. Melo et al. (2007) subjected *Paspalum* plants to water stress conditions (water deficit) and verified that this

experimental group had bulliform cells with higher length than control plants. These results corroborate the present study, in which bulliform cells were observed to occupy larger areas during the dry season (when greater water saving is necessary) (Table 1).

Considering the diameter of metaxylem vessels (XD), significantly greater mean diameters were observed during the rainy season in the joint analysis (Tables 3 and 4). Approximately 65% of the genotypes showed significant differences between rainy and dry seasons in terms of the character XD, all with higher mean diameters in the rainy season. No interactions were observed between different genotypes and environment for this character ($p > 0.05$) (Table 4).

Metaxylem vessel diameter is an important factor in water flow under stress conditions, as larger diameter vessels are more susceptible to cavitation than smaller diameter vessels (VASELLATI et al., 2001). Dickison (2000) studied a number of species and determined that high frequencies of vessels per square millimeter provided security against embolism in plants growing in dry environments. The Scott Knott test resulted in three groupings, with genotypes 33, 48, 66 and 93 forming a group with small mean XD values – which might be useful for evaluating during the dry season.

Reduction in conducting vessel diameter can ensure more security to the water transport when plants are subjected to some types of water stress, and factors such as drought, flood, altitude, latitude, soil composition and fertility, successional stage of the vegetation, and pollution are known to significantly alter plant anatomy (BAAS; SCHWEINGRUBER, 1987; BAAS et al., 1983; CARLQUIST; HOEKMAN, 1985). This type of behavior was apparently observed among genotypes evaluated during the dry season, as they demonstrated smaller xylem tube diameters, probably due to water stress, as the mean daily rainfall in August 2009 was only 0.14 mm (Table 1). According to Baas et al. (1983), large diameter vessels are more efficient but present a greater risk of embolism.

The average distance between vascular bundles in the joint analysis was greater during the dry season, as was the interaction of genotype with environment (Tables 3 and 4). While genotype 13 demonstrated greater vascular bundle distances in the rainy season (average of $26.18 \mu\text{m}$) ($p < 0.05$), genotypes 7, 14, 29, 53 and 70 demonstrated greater bundle distances in the dry season, varying from $30.99 \mu\text{m}$ to $39.3 \mu\text{m}$. Climatic data collected during the experimental period (Table 1) indicated lower

insolation and luminosity levels during the dry period.

The distance between the vascular bundles is apparently related to the efficient transport and distribution of carbohydrates and water to mesophyll cells. Smaller distances between the bundles will facilitate the translocation of photosynthetic products and water in plants growing in water-stressed environments at high temperatures. As *B. ruziziensis* grows only slowly during the dry season, genotypes 2, 9, 13, 15, 16, 86, 95 and 97 (which show smaller distances between vascular bundles) may be useful in breeding program as the capacity to internally distribute water and mineral salts should be more efficient and could result in higher production. In the same way, genotypes 2, 8, 13, 14, 15, 35, 43, 44, 58, 96 and 100 have high numbers of vascular bundles and should promote higher production levels under dry and hot conditions.

In the joint analysis on the number of vascular bundles we observed high mean values as well as significant genotypes vs. environment interactions (Tables 3 and 4). In evaluating the number of bundles (NB) in the different seasons, the highest mean was observed with genotype 27 (5.44) during the dry season (when the luminosity and insolation levels were lower than in the rainy season) (Table 1). Genotypes 15, 40, 48, 53, 90 and 96, however, had higher mean number of bundles during the rainy season.

Conclusion

Breeding programs are designed to select the most productive genotypes, and these must be evaluated in diverse environments and during different seasons of the year. If the interaction is significant, the genotypes can be recommended for specific environments. Otherwise, if not significant, the recommendation is for use in various environments.

There were a number of promising genotypes demonstrating significant variability in terms of anatomical characteristics examined (except for the characters ABT and ADT), as well as variations in these characters during different seasons of the year – and these genotypes proved to be useful in genetic improvement programs.

Morphological plasticity was especially evident in genotypes 15, 27, 29, 40, 44, 53 and 90, as they demonstrated interactions with the environment in at least four of the nine characteristics evaluated. Genotypes 1 and 33, on the other hand, have not

presented interactions with the environment for any of the characteristics examined in this study.

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