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Morphological and physiological responses and the recovery ability of *Paspalum* accessions to water deficit and waterlogging

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Abstract

Limited knowledge about the agronomic potential of *Paspalum* species from the Americas hinders their use in pastures. The response mechanisms to water deficit and waterlogging were studied in five accessions of *Paspalum* sp. (*P. regnellii* cv. BRS Guará and BGP 397, *P. conspersum* BGP 402 and *P. malacophyllum* BGP 289 and BGP 293) and *Urochloa brizantha* cv. Marandu. Morphological and physiological traits at the end of the stress and after the recovery period were measured. All *Paspalum* accessions, especially BGP 289, BGP 402 and BGP 397, and also cv. Marandu, showed survival mechanisms to water deficit. BRS Guará showed adaptive features to waterlogging, although had with a low survival rate to water deficit. Under waterlogging, BGP 289 and cv. Marandu showed reduced growth, and after the recovery period, they presented low plant survival and root recovery. Although growth of BGP 293 was reduced during waterlogging, it presented high tiller survival rate. BGP 293, BGP 402 and BGP 397 showed adaptive potential strategies for waterlogging and water deficit. There is genetic variability among the evaluated accessions for water deficit and waterlogging stresses, suggesting that they may be used *per se* or as progenitors in crosses with other genotypes in breeding programmes.

KEYWORDS
Abiotic stress, *Brachiaria brizantha*, tiller survival, water stress

1 | INTRODUCTION

Agriculture faces many risks, including those associated with periodic deficits and excess of rainfall, and to extreme events like major floods and droughts. In Brazil, water deficit stress is considered the most critical risk for agricultural production and, although risks of rainfall excess and major floods are considered low (World Bank, 2015), the degradation of many pasture areas has been attributed to waterlogging (Teixeira Neto et al., 2000; Valentim, Amaral, & Melo, 2000; Dias-Filho, 2005; Dias-Filho & Andrade, 2005). In addition, the effects of future climate change are predicted to impact on meat and milk production in Latin America, particularly in Brazil, with a predicted increased incidence of droughts and waterlogged areas, and of risks associated with weather conditions (Andrade, Santos, Pezzopane, Bettiol, & Evangelista, 2014; Cardoso et al., 2013; Pezzopane et al., 2017; Trenberth et al., 2007).

High temperatures and availability of moderate-to-high light levels are the principal determinants of the distribution of C4 species (Pau, Edwards, & Still, 2013; Still, Berry, Collatz, & DeFRIES, 2003). Plants with a C4 photosynthesis pathway are favoured over C3 plants when the concentration of atmospheric carbon dioxide is below 500 ppmV (Cerling et al., 1997; Ehleringer et al., 2002). Water

availability has not been clearly reported as a key factor affecting the distribution of C4 species, although most native C4 species are located in tropical and subtropical areas with high rainfall and only few occur in arid areas (Still et al., 2003). There has been little investigation of plant responses and survival of C4 species to severe stress, although these responses could affect their future adaptation to extreme drought events under climate change (IPCC, 2013).

The use of water deficit and waterlogging-tolerant cultivars may enable the mitigation of climate risks and reduce the vulnerability of pasture-based animal productions systems in the tropics. Pasture degradation is considered one of the main problems of animal production systems in tropical areas, where it is often associated with abiotic stress (Dias-Filho, 2005; Dias-Filho & Andrade, 2005; Teixeira Neto et al., 2000; Valentim et al., 2000). Persistence of pasture swards under water deficit and waterlogging conditions depends on plant survival mechanisms and their capacity to recover after cessation of stress. Although there have been previous studies of the response of tropical forages to water deficit stress (Araújo, Santos, Mendonça, & Mourão, 2011; Guenni, Marín, & Baruch, 2002; Mattos, Gomide, & Huaman, 2005; Melo, Korndörfer, Korndörfer, Lana, & Santana, 2003), the recovery of plants after a period of water deficit has been poorly characterized.

Strategies of perennial herbaceous plants to cope with water deficit stress have been described by Volaire, Barkaoui, and Norton (2014). They include resistance to moderate water deficit (i.e. when annual accumulated precipitation less evapotranspiration is lower than −300 mm) with growth maintenance, and growth cessation with survival of plants under severe stress (i.e. when annual accumulated precipitation less evapotranspiration is between −300 and −600 mm). Resistance to moderate water deficit stress with growth maintenance depends on mechanisms that allow dehydration avoidance through increased water uptake or reduced water loss (Garwood & Sinclair, 1979; Volaire, Thomas, & Lelièvre, 1998). Water deficit survival mechanisms include the promotion of a faster leaf osmotic adjustment, which maintains the integrity of membranes and meristematic tissues (Kramer, 1980; Versules, Agarwal, Katiyar-Agarwal, Zhu, & Zhu, 2006), allowing plants to tolerate dehydration under severe drought and recover after rehydration (Bewley, 1995; Volaire & Lelièvre, 2001).

For coping with waterlogging and its effects on root system development and functioning, plants use adaptive strategies to improve gas exchange and maintain energy production (Armstrong, Brandle, & Jackson, 1994), such as the formation of aerenchyma and adventitious roots, thereby increasing oxygen supply to the roots (Dias-Filho, 2013). Cardoso et al. (2013) also observed that genotypes with improved waterlogging tolerance showed higher SPAD index values and a higher proportion of green leaf biomass. In addition, leaf elongation rate is an important mechanism to detect quickly tolerance to stress in grasses, either by waterlogging (Dias-Filho & Carvalho, 2000) or by water deficit (Ludlow & Ng, 1976; Mattos et al., 2005).

Species of Paspalum have been used for grazing, and cultivars of Paspalum atratum, Paspalum notatum, Paspalum vaginatum and Paspalum regnelli are registered or protected in Brazil and other countries (MAPA, 2016). North and South America are the centres of origin of most Paspalum species, and they are found from the USA to Uruguay and Argentina. The Paspalum genus has about 330 species (Zuloaga & Morrone, 2005) which have been distributed morphologically into informal groups by Chase (1929). Paspalum species may be found in many distinct environments, including tropical or subtropical regions, savannahs, wetlands, beaches, forests, roadsides, and at high and low altitudes (Zuloaga & Morrone, 2005). Therefore, there is high adaptation of species to many types of environment. Accessions conserved in the Germplasm Bank of Embrapa Pecuária Sudeste (located in São Carlos, SP) were collected under very different climate and soil water conditions, including semiarid and wet areas (Alelo, 2016). The characterization and classification of these accessions to drought resistance could contribute to the development of new cultivars better adapted to current and future climate scenarios (Scheffer-Basso, Jacques, & Agnol, 2002). Paspalum regnellii is a species of potential interest for use in the forage breeding programme. Accessions of P. regnellii were collected in natural areas with heavy and humid soil, with high percentage of clay (Zuloaga & Morrone, 2005), and this species may be adapted naturally to places with waterlogging. Paspalum conspersum is very close to P. regnellii, and both were included in the informal group "Virgata" by Barreto (1954) and Zuloaga and Morrone (2005). Paspalum malacophyllum is also of interest in breeding programmes because of its quality forage. All of these species have been used per se or as progenitors in crosses with other genotypes.

In this study, evaluations were made of the effects of waterlogging and water deficit stress on the morphological and physiological characteristics of Paspalum accessions and Urochloa brizantha cv. Marandu, and their ability to recover after a period under appropriate soil water conditions. The aim was to characterize the response mechanisms and to verify whether there is variability among these genotypes in their ability to tolerate waterlogging and to resist water deficit stresses.

2 | MATERIALS AND METHODS

2.1 | Plant material

Five accessions of Paspalum were used as follows: P. regnellii (BRS Guará and BGP 397), P. conspersum (BGP 402), P. malacophyllum (BGP 289 and BGP 293) and one cultivar of U. brizantha (cv. Marandu). Paspalum regnellii and P. conspersum belong to the Virgata group and P. malacophyllum to the Malacophylla group. Seeds of Paspalum accessions were obtained from the Paspalum germplasm bank at Embrapa Pecuária Sudeste. These Paspalum genotypes were selected for their contrasting responses to stress by waterlogging and water deficit, observed in preliminary experiments. Marandu presents resistance to short-term water deficit (Santos et al., 2013;.), but does not tolerate waterlogging (Dias-Filho, 2005; Dias-Filho & Carvalho, 2000), and was used for comparisons because of its better characterization and wide use in pastures throughout Brazil.

2.2 | Experimental design and plant measurements

The experiment was conducted in a greenhouse, at Embrapa Pecuária Sudeste in São Carlos, São Paulo State, Brazil (21°55′S and 47°48′W). The experimental design was a complete randomized block with a 6 × 3 × 2 factorial arrangement (six genotypes, three water conditions and two harvest dates) with three replications. The water conditions were as follows: (i) control—water-sufficient (irrigated plants were maintained at 80% of field capacity; this condition was ensured by the use of Piché Evaporimeter), (ii) water deficit (irrigation was stopped on day 0) and waterlogging (waterlogging was kept by applying water to soil until reached saturation and (iii) a water lamina of 3 cm above soil from day 0; this condition was ensured by use of pots without holes and replacement of water lost by evapotranspiration).

Seeds were planted in trays filled with commercial substrate. When the first tillers appeared (about 30 days after planting), plants were transplanted to pots (two plants per pot), and after an additional 30 days, the water conditions were imposed. Pots of 8 L capacity were filled with 6.5 kg per pot of sifted soil with the following physicochemical properties: pH_{CaCl2} 6.6, OM 24 g/dm^3, $P_{ressyne}$ 67 mg/dm^3, $S.SO_4$ 5 mg/dm^3, K 4.5 mmolc/dm^3, Ca 50 mmolc/dm^3, Mg 23 mmolc/dm^3, H+Al 19 mmolc/dm^3, Al 0 mmolc/dm^3, CTC 97 mmolc/dm^3, V 80%, sand 604 g/kg, 93 silt g/kg and 303 clay g/kg. Each pot was fertilized with 2.33 g of urea (45% N), 7.78 g of single superphosphate (18% P), 0.88 g of potassium chloride (60% K), following the recommendations of Malavolta (1980). Three times a week pots under water restriction were weighed to monitor the water content in the soil by the gravimetric method (Figure 1).

At the end of the stress period, half of the pots were harvested for evaluation of plant characteristics and production. The remaining pots were returned to close to field capacity. The plants in these remaining pots were harvested after 10 days, in order to evaluate their ability to recover from moisture stress. The experiment was carried out from 21 January to 27 February 2013, totalling 28 days of water stress and 10 days of recovery period.

One tiller per pot was used for analysis of leaf elongation rate (LER). This evaluation was performed three times a week, during the stress and the recovery periods, with a total of 15 measurements. The LER was calculated as the increase in leaf length per tiller between two consecutive measurements divided by the number of days (cm tiller^{-1} day^{-1}).

The number of tillers per plant (tillers per pot) and the physiological variables: leaf water potential—ψ_w (MPa), leaf osmotic potential —ψ_s (MPa) and SPAD index were measured once a week (days 0, 7, 14, 21, 28 and after 10 days recovery), with a total of six evaluations. Tiller survival rate after the recovery period was estimated as the percentage of live tillers over the total number of tillers per pot.

The SPAD index (relative chlorophyll index determined based on the green colour intensity of leaves) was measured in the newest fully expanded leaf of a tiller per pot, using a portable absorbance-based chlorophyll meter (SPAD-502, Konica-Minolta, Osaka, Japan). The leaf was then removed, and leaf discs of 25 mm were collected for determining mid-day ψ_w (between 9 and 13 h). The discs were placed in psychrometric chambers (Wescor model C52), and the ψ_w was determined using a microvoltmeter (Wescor model Psypro). The extract from the same portion of the leaf was used for the determination of ψ_s. The cellular content was collected with filter paper using a leaf press (Wescor Markhart Leaf Press model LP-27). The filter paper was then placed on the psychrometer for determination of ψ_s (Taiz & Zeiger, 2004).

Plants were harvested at the end of the water deficit and waterlogging periods, or after the recovery period. The biomass was separated and dried in a forced circulation oven (at 65°C for 72 hr) and then weighed to determine the leaf, stem, roots and dead material dry biomass (g per pot). Roots were washed free of soil using tap water and sieves. Before drying, leaf area (cm^2) was determined in a leaf area integrating unit (LI-COR Model LI-3100), and specific leaf area (cm^2/g) was calculated by dividing the area by the leaf dry biomass (Radford, 1967). The leaf: stem ratio (leaf dry biomass/stem dry biomass) and root: shoot ratio (root dry biomass / shoot dry biomass) were calculated. In addition, a root recovery index was calculated after the recovery period (root dry biomass after recovery/root dry biomass at the end of the stress period).

Grouped data were analysed through randomized complete block with a factorial design arrangement of 6 × 3 × 2 and repeated measures in time. ANOVA was performed with the PROC MIXED statistical package of SAS (SAS Institute, 2014) and means compared by Tukey test (5%).

FIGURE 1 Soil water content (%) of pots containing plants of *Urochloa brizantha* cv. Marandu and *Paspalum* genotypes (cv. BRS Guará, BGP 397, BGP 402, BGP 289 and BGP 293) under water deficit conditions. Values are the means of three replicates
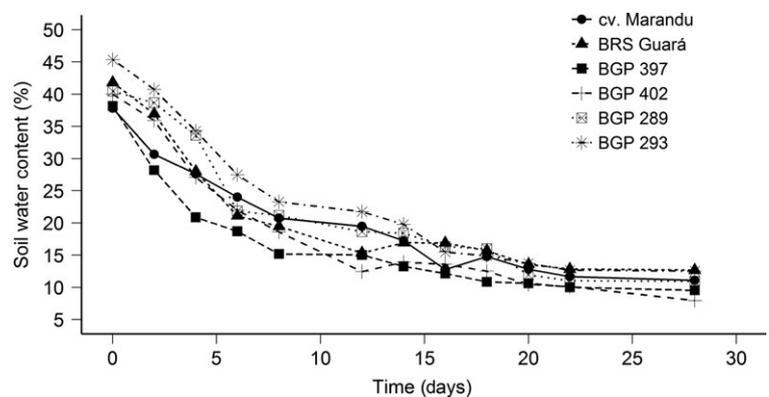
3 | RESULTS

3.1 | Morphological and structural characteristics

Specific leaf area was affected ($p < .01$) by water conditions and dates of harvest. Specific leaf area was lower in water deficit (69.1 cm²/g) than in waterlogged (178 cm²/g) or water-sufficient (176 cm²/g) plants. The specific leaf area was lower in plants at the end of the stress period (harvest 1: 120 cm²/g) than after the period of recovery (harvest 2: 163 cm²/g).

Leaf area showed a significant interaction ($p = .0409$) between genotype and water condition (Figure 2). For all genotypes, leaf area was lower in plants under water deficit. On the other hand, two patterns of response to waterlogging were observed: (i) leaf area was reduced by waterlogging in *U. brizantha* cv. Marandu ($p = .0269$) and in *P. malacophyllum* accessions (BGP 289 ($p = .0121$) and BGP 293 ($p = .0387$)), and (ii) waterlogging did not result in any change in leaf area in *P. regnellii* "BRS Guará" ($p = .9541$), *P. conspersum* BGP 402 ($p = .1520$) and *P. regnellii* BGP 397 ($p = .6677$). It is important to highlight that accessions of *P. malacophyllum* (BGP 289 and BGP 393) had the same response. The same was observed for *P. regnellii* ("BRS Guará" and BGP 397) and *P. conspersum* (BGP 402), both species from Virgata group.

Interactions between genotype and water condition ($p = .0345$) and between water condition and measurement period ($p = .055$) were significant for LER (Figure 3). Water deficit decreased LER in all genotypes (Figure 3a). Under waterlogging, LER was reduced in cv. Marandu ($p < .01$), BGP 293 ($p = .0011$) and BGP 397 ($p = .0354$), while in "BRS Guará" ($p = .0960$), BGP 289 ($p = .6268$) and BGP 402 ($p = .1294$), LER was not affected. Throughout the experiment, a decrease in LER was observed for all the conditions evaluated. The LER of plants under waterlogging was lower than that of water-sufficient plants until the fourth evaluation; subsequently, no significant differences were detected between water-sufficient and waterlogged plants until the end of the stress period, although during the recovery period LER of water-sufficient plants was slightly higher than that of waterlogged plants. On the other hand, under progressive water deficit LER decreased from the beginning of the stress period and became almost nill after the sixth period (Figure 3b). During this period of 14 days of drought stress, the soil water content was 20% for BGP 293, 18% for BGP 289, 17% for cv. Marandu and "BRS Guará," 14% BGP 402 and 13% BGP 397.
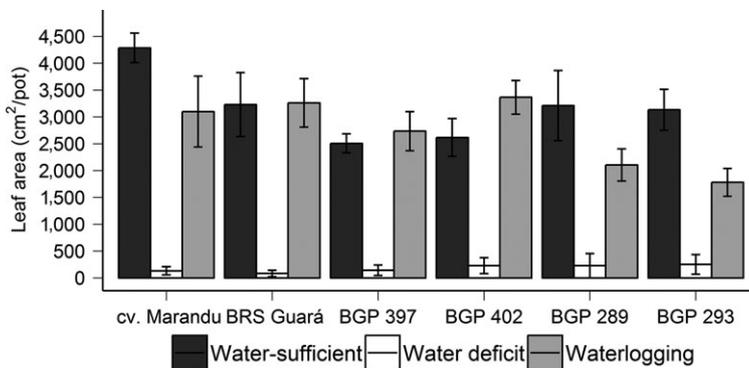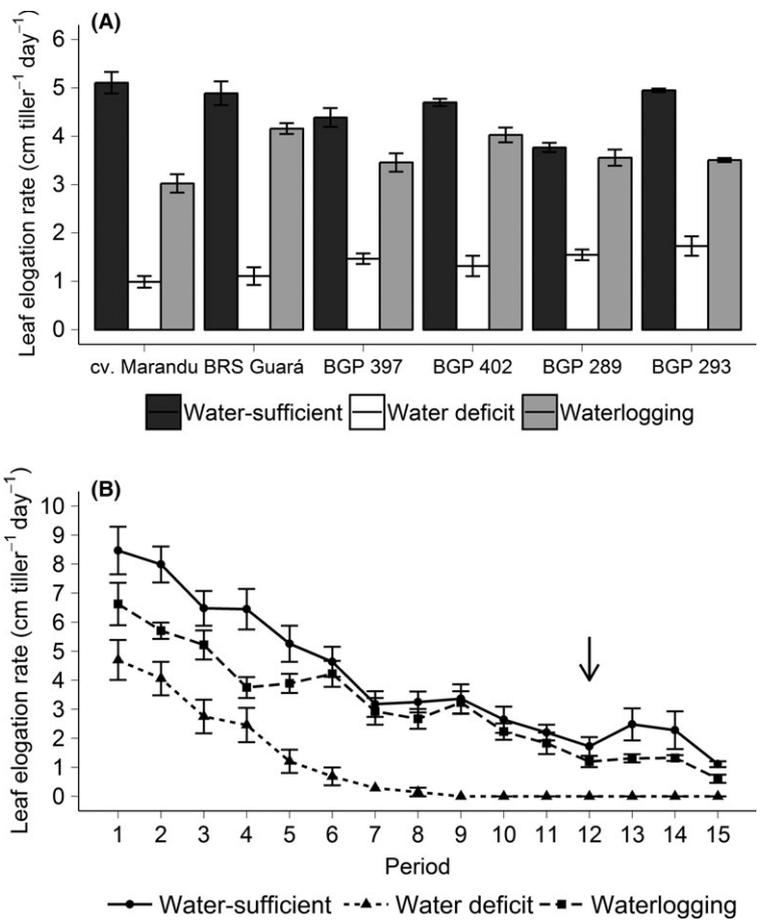
The number of tillers ($p = .04$) was lower in BGP 289 under waterlogging, after 14 days of stress. Even after 10 days under draining conditions (fifth week), BGP 289 plants were not able to recover, further reducing the number of tillers ($p = .012$), remaining lower than that of control plants (Figure 4). In BGP 293 and genotypes of Virgata Group ("BRS Guará," BGP 397 and BGP 402), no effect of waterlogging on the number of tillers was observed during the 6 weeks of evaluation. Water deficit reduced the number of tillers of all genotypes after 14 (BGP 289, BGP 293 and BGP 402) or 21 days ("BRS Guará," BGP 397 and cv. Marandu) of stress. However, after 10 days of rehydration (fifth week), the emergence of new tillers was observed in all genotypes, but "BRS Guará."

3.2 | Physiological characteristics

The SPAD index was higher ($p = .0465$) in BGP 397 (35.21) than in cv. Marandu (29.51), but these values did not differ from others accessions of *Paspalum* that presented intermediate values ("BRS Guará": 34.16, BGP 289: 33.51; BGP 293: 34.48; BGP 402: 34.66). There was a significant interaction between water condition and measurement period for SPAD index ($p < .01$). In general, the SPAD index decreased in all treatments throughout time, suggesting an overall reduction in the chlorophyll content. No difference between water-sufficient and waterlogging treatments was observed during the experiment, but plants under water deficit stress presented a lower SPAD index during the fourth and fifth weeks, pointing to a decrease in chlorophyll content due to water restriction, which recovered just after rehydration (Figure 5).

Both water potential and osmotic potential showed a significant interaction between water conditions and measurement period ($p < .01$). The response of these variables was very similar to that of the SPAD index, except that values did not decrease along the experiment for water-sufficient (mean water and osmotic potentials were -2.32 ± 0.25 and -1.67 ± 0.07 MPa, respectively) and waterlogging (mean water and osmotic potential were -2.53 ± 0.28 and -1.68 ± 0.04 MPa, respectively) plants. Water and osmotic potential of plants under water deficit were lower than those under water-sufficient and waterlogging conditions between day 14 day 28 (mean water and osmotic potential was less than -5.0 MPa and outside the range measured by the method used in this experiment).

FIGURE 2 Leaf area (cm² per pot) of *Urochloa brizantha* cv. Marandu and *Paspalum* genotypes (cv. BRS Guará, BGP 397, BGP 402, BGP 289 and BGP 293) under three water conditions (water-sufficient—dark grey bar, water deficit—white bar and waterlogging—light grey bar). Average of two harvests and three replicates, ±standard error of the means

FIGURE 3 Leaf elongation rate (LER cm tiller^{-1} day^{-1}) in (A) *Urochloa brizantha* cv. Marandu and *Paspalum* genotypes (cv. BRS Guará, BGP 397, BGP 402, BGP 289 and BGP 293) under three water conditions (water-sufficient—dark grey bar, water deficit—white bar and waterlogging —light grey bar) and (B) plants under three water conditions during period of stress and recovery. (A) Values are the means of six genotypes during 15 evaluations (three times per week) and three replicates ± standard error of the means, and (B) Values are the means of six genotypes and three replicates ± standard error of the means. The arrow indicates the beginning of the recovery period

After rehydration, water and osmotic potential recovered and no difference could be observed among treatments.

3.3 | Productive characteristics

Significant interactions were observed for leaf biomass: genotype × water condition ($p < .01$) and water condition × harvest date ($p = .0466$) (Figure 6).

Water deficit stress reduced the leaf biomass of all genotypes. Waterlogging, however, reduced the leaf biomass only of cv. Marandu ($p = .008$), BGP 293 ($p = .006$) and BGP 289 ($p = .008$) (*P. malacophyllum*) (Figure 6a). Leaf biomass was lower in the second harvest only for plants under waterlogging (Figure 6b).

Root: shoot ratio was higher ($p = .0185$) after the recovery period (2.99) than at the end of the stress period (2.39). The other productive characteristics were affected by genotype and water condition ($p < .01$) (Table 1).

Stem biomass and dead biomass were higher in *U. brizantha* cv. Marandu than in *Paspalum* accessions (Table 1). There was no difference between water-sufficient and waterlogging treatments; however, under water deficit, stem biomass was lower, and dead material dry biomass was higher than under the other water conditions (Table 1).

Root biomass and root: shoot ratio were higher for BGP 397 and BGP 402 and lower for BGP 289. "BRS Guará," BGP 293 and cv. Marandu showed intermediate values (Table 1). The nature of the stress affected root dry biomass differently. The highest values were

TABLE 1 Productive traits (SDB: stem dry biomass (g per pot), DMDB: dead material dry biomass (g per pot), RDB: root dry biomass (g per pot) and root: shoot ratio (R:S) of six grass genotypes: *Urochloa brizantha* cv. Marandu and *Paspalum* genotypes (cv. BRS Guará, BGP 397, BGP 402, BGP 289 and BGP 293) under different soil water conditions

	Genotypes						Condition		
Traits	cv. Marandu	BRS Guará	BGP 397	BGP 402	BGP 289	BGP 293	Water-sufficient	Waterlogging	Water deficit
SDB	30.37[a]	17.47[b]	18.72[b]	19.58[b]	14.88[b]	14.41[b]	25.31[A]	28.69[A]	3.71[B]
DMDB	20.91[a]	11.04[b]	12.09[b]	12.46[b]	8.43[b]	8.14[b]	8.87[B]	9.86[B]	18.00[A]
RDB	126.23[abc]	141.6[ab]	173.1[a]	160.0[a]	66.55[c]	74.58[bc]	238.99[A]	92.91[B]	39.23[C]
R:S	1.88[b]	3.29[a]	3.92[a]	3.34[a]	1.72[b]	2.00[b]	4.60[A]	1.79[B]	1.68[B]

Different letters in row indicate difference by Tukey test at $p < .05$.
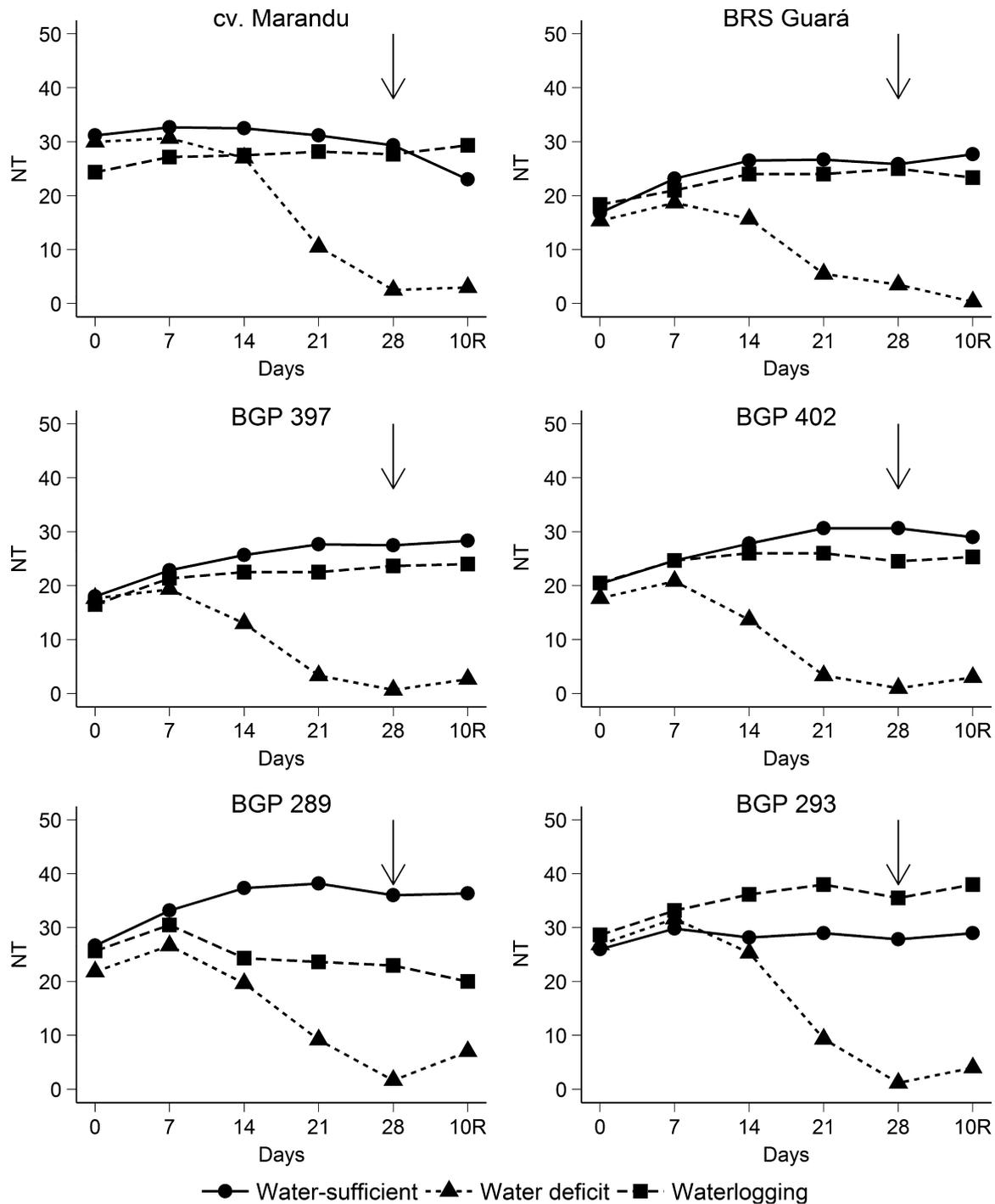
FIGURE 4 Number of green tillers (NT per pot) of *Urochloa brizantha* cv. Marandu and *Paspalum* genotypes (cv. BRS Guará, BGP 397, BGP 402, BGP 289 and BGP 293) under three water conditions (water-sufficient, water deficit and waterlogging) during 6 weeks after stress imposition. Arrows indicate the beginning of the recovery period. Values are means of three replicates

found in water-sufficient plants; intermediate values under waterlogging and the lowest root biomass values were recorded for plants under water deficit (Table 1).

After the recovery period, the L:S ratio was lower under waterlogging (Figure 7). Under this condition, increased values of root and decreased values of leaves suggested that in the post-stress period, there was a change in the pattern of partitioning and carbon

allocation in these plants. The interaction between water condition and harvest ($p < .01$) can be observed in Figure 7.

3.4 | Tiller survival and root recovery

Tiller survival rate under water deficit was higher in BGP 289 and lower in "BRS Guará" (Table 2). Differences between accessions of
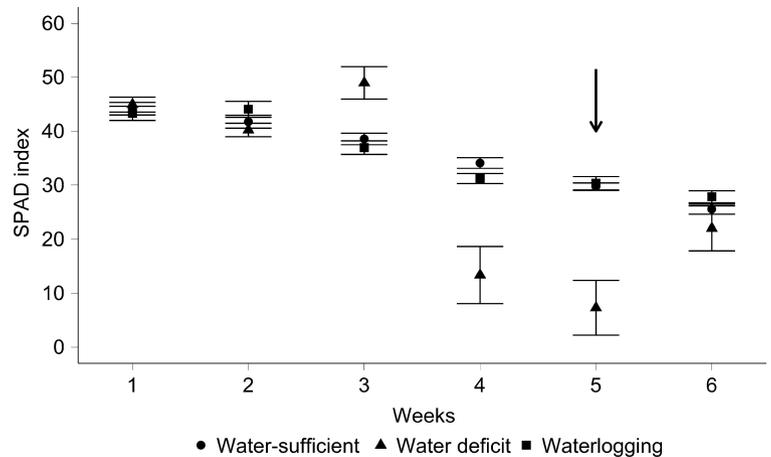
TABLE 2 Tiller survival rate (TSR, as %) and root recovery index (RRI) after recovery of *Urochloa brizantha* cv. Marandu and *Paspalum* genotypes (cv. BRS Guará, BGP 397, BGP 402, BGP 289 and BGP 293) submitted to waterlogging stress and water deficit

Genotypes	Traits			
	TSR—waterlogging	TSR—water deficit	RRI—waterlogging	RRI—water deficit
cv. Marandu	55.00[b] ± 25.00	10.00 [cd] ± 4.00	0.92[b] ± 0.25	0.81[bc] ± 0.29
BRS Guará	101.00[a] ± 9.00	1.33[d] ± 1.00	1.72[ab] ± 0.11	0.81[bc] ± 0.29
BGP 397	101.00[a] ± 12.00	26.00[ab] ± 3.00	2.49[a] ± 0.09	2.14[a] ± 0.01
BGP 402	100.00[a] ± 9.00	22.00[abc] ± 4.00	1.51[ab] ± 0.09	0.77[bc] ± 0.13
BGP 289	59.00[ab] ± 19.00	37.00[a] ± 5.00	0.92[b] ± 0.22	0.34[c] ± 0.15
BGP 293	99.00[a] ± 9.00	19.00[bc] ± 4.00	2.30[a] ± 0.26	1.23[b] ± 0.06
p-value	.0077	.0014	.0021	.0004

Different letters in column indicate difference by Tukey test at $p < .05$.

FIGURE 5 The SPAD index of *Urochloa brizantha* cv. Marandu and *Paspalum* genotypes (cv. BRS Guará, BGP 397, BGP 402, BGP 289 and BGP 293) under three water conditions (water-sufficient, water deficit and waterlogging) during weeks after stress imposition. Values are the means of three replicates, ±standard error of the means. The arrow indicates the beginning of the recovery period

FIGURE 6 (A) Leaf dry biomass (g per pot) of *Urochloa brizantha* cv. Marandu and *Paspalum* genotypes (cv. BRS Guará, BGP 397, BGP 402, BGP 289 and BGP 293) under three water conditions (water-sufficient—dark grey bar, water deficit—white bar and waterlogging—light grey bar), and (B) leaf dry biomass (g per pot) under three water conditions (water-sufficient, water deficit and waterlogging) in two harvest periods (1—final stress, and 2—after recovery). Mean values are shown (A: $n = 6$ and B: $n = 3$). Vertical bars indicate standard-error values. Different letters indicate differences among water conditions ($p < .05$, Tukey test)
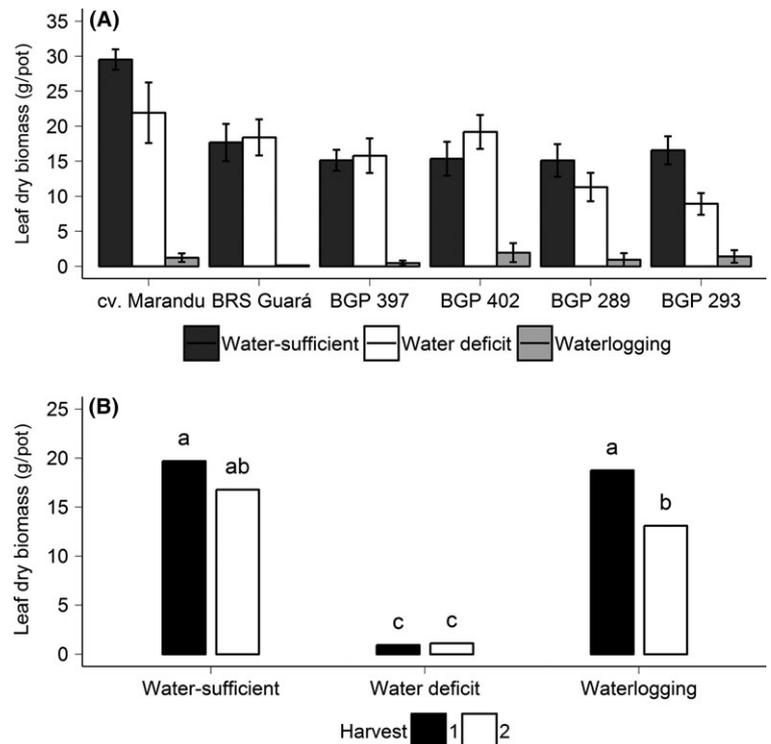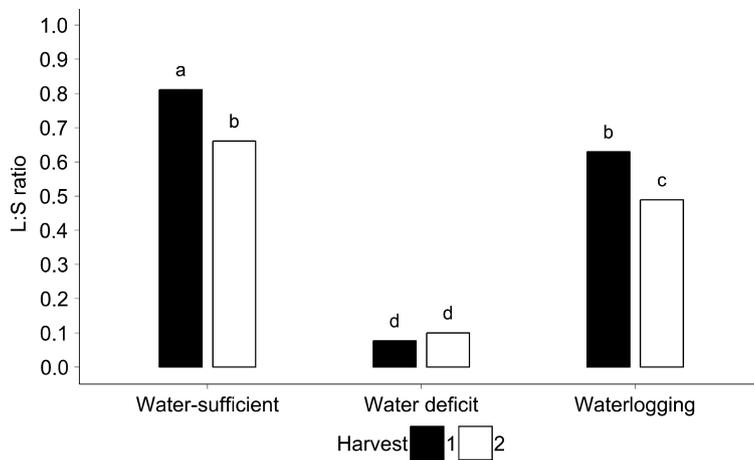
FIGURE 7 Leaf: stem ratio under distinct water treatments (water-sufficient, water deficit and waterlogging) by two harvest dates (1. at the end of the stress period and 2. after 10 days recovery). Different letters indicate differences between two harvest ($p < .05$, Tukey test)

the same species can be observed, as tiller survival rate was lower in "BRS Guará" than in BGP 397, both *P. regnellii*, and lower in BGP 293 than in BGP 289, both *P. malacophyllum*. Under waterlogging, the highest tiller survival rates were observed in "BRS Guará," BGP 293, BGP 402, BGP 397, and the lowest were in cv. Marandu, while BGP 289 did not differ from the other genotypes (Table 2).

Root recovery index was higher in BGP 397 and BRA 293, regardless of the stress nature (Table 2). Under water deficit (Table 2), roots of cv. Marandu, "BRS Guará," BGP 289 and BGP 402 did not recover (index < 1).

4 | DISCUSSION

4.1 | Water deficit survival

All genotypes under water deficit had reduced shoot development (Figures 3 and 4) and leaf biomass production (Figure 6). The lowest values of SPAD index, as well as water and osmotic potential (Figure 5), were observed after three weeks of water restriction, when soil water content reached less than 15%.

Plants resistant to moderate water deficit depend on mechanisms of dehydration tolerance to maintain growth. The accumulation of metabolites, which provide a decrease in osmotic potential through a net increase in intracellular solutes, helps with the maintenance of cell turgor (Chaves, 1991), and, consequently, favours cell expansion and leaf elongation under moderate water deficit conditions. Despite the reduction in the osmotic potential of plants from all genotypes under water deficit, this mechanism was not enough to maintain leaf growth rates (Figure 3) and prevent senescence (Table 1).

On the other hand, osmotic adjustment may have helped to protect the growth regions (Premachandra, Saneoka, Fujita, & Ogata, 1992; Spollen & Nelson, 1994), contributing to the emergence of new tillers after rehydration and plant recovery, except in *P. regnellii* "BRS Guará" (Figure 4). Dehydration-avoidant species lose their leaves with increasing water deficit, as a possible mechanism for reducing transpiration and, consequently, reducing the rate of water loss from meristems (Munne-Bosch & Alegre, 2004; Volaire &

Lelièvre, 2001; Volaire et al., 1998). Under severe water deficit, senescence of leaves helps to protect meristematic tissues and allows recovery of plants after water deficit cessation (Bewley, 1995; Volaire & Lelièvre, 2001; West, Oosterhuis, & Wullshleger, 1990), once meristematic tissues are protected from rapid evaporative water loss by their location enclosed within the mature leaf sheaths (Barlow, Munns, & Brady, 1980), and are strong sinks within the plants resulting in carbohydrate accumulation (Schnyder and Nelson, 1989; Volaire et al., 1998). In addition, meristems also exhibit the greatest osmotic adjustment relative to other tissues during dehydration stress (Matsuda & Riazi, 1981; Munns, Brady, & Barlow, 1979; West et al., 1990).

Water deficit survival, assessed by tiller survival rate (Table 2), is a characteristic of tolerance to dehydration. Although "BRS Guará" also presented high dead biomass under water deficit conditions, no new tillers appeared after the recovery period. In addition, "BRS Guará" also had low root recovery rate, indicating that damage caused by water deficit stress could not be reversed. The root recovery index (Table 2) measures the degree of preservation of the meristematic tissue and of membrane integrity in the root system. Therefore, the increase in senescence under severe water deficit in "BRS Guará" seems to be a prelude of plant death instead of a strategy to save water and guarantee plant survival.

The highest root recovery index was recorded in BGP 397 of *P. regnellii* (Table 2) suggesting a high water deficit tolerance of this genotype. The protection of root meristems allowed a quick recovery of the root system and favoured new tiller appearance and shoot growth in the recovery period (Figures 4 and 8a).

The high tiller survival rate and low root recovery rate under water deficit stress shown by *P. malacophyllum* BGP 289 (Figures 8a and 9a), suggests there was preferential remobilization of reserves to shoots in order to restore photosynthesis quickly. *Paspalum malacophyllum* BGP 289 also presented low root and shoot dry biomass (Table 1). A reduced root system cannot be interpreted as a negative feature and may be related to a conservation-resource-use strategy, as a deep root system requires higher energy cost for synthesis,

maintenance and absorption of the roots (Adiku, Rose, Braddock, & Ozier-Lafontaine, 2000).

4.2 | Mechanisms of tolerance to excess of water

Higher plants are unable to survive for long periods without accessing molecular oxygen (Dias-Filho, 2005). In pasture grasses, it is assumed that the most tolerant plants are the ones able to develop adaptive processes to transport air oxygen to the water-logged tissues (Dias-Filho, 2013). Morphological and physiological changes may occur among species or within the same species, according to acclimation ability and to the stress intensity (Dias-Filho, 2013), thereby altering the degree of tolerance to adverse conditions.

In general, the presence of adventitious roots was detected in all genotypes above soil level, which may have promoted an improved absorption of water, oxygen, and nutrients (Dias-Filho, 2006). The production of adventitious roots is a common response in plants that are tolerant of waterlogging (Armstrong et al., 1994; Liao & Lin, 2001). The increase in height of the stem and the formation of aerenchyma in the roots and the base of the stem, modifying vessel diameter, may also help the maintenance of water, oxygen and nutrient uptake under waterlogging (Armstrong et al., 1994; Pereira, Castro, Souza, & Magalhães, 2008; Vasellati, Oesterheld, Medan, & Loreti, 2001). These morpho-anatomical changes considerably increase the weight and size of the stem. The length of plant stems was not evaluated in this study; however, stem dry biomass did not differ between control and water-logged plants (Table 1), suggesting that this phenomenon did not occur.

The responses of *Paspalum* genotypes to waterlogging were similar to those observed by Cardoso et al. (2013) in *Urochloa* accessions considered tolerant to waterlogging. In that study, genotypes with improved waterlogging tolerance showed higher SPAD index values, and a higher proportion of green leaf biomass. Cardoso et al. (2013) also found that interspecific hybids of *Urochloa* (*U. ruziziensis* × *U. brizantha* × *U. decumbens*) were susceptible to waterlogging. Several studies have shown that waterlogging generally reduces photosynthetic capacity in non-tolerant plants (Liao & Lin, 2001), the main cause of which is the reduction in stomatal aperture (Dias-Filho, 2002; Dias-Filho & Carvalho, 2000).

In this study, *Paspalum* accessions under waterlogging presented greater SPAD index, larger leaf area and no reduction in green leaf biomass, although lower values for biomass of dead material were observed in these plants, which may indicate low tissue renewal and resource-conservation strategy.

LER is an early detection mechanism for assessing the sensitivity to water stress, either by waterlogging (Dias-Filho & Carvalho, 2000) or by water deficit (Ludlow & Ng, 1976; Mattos et al., 2005). A similar leaf elongation rate of plants under water-sufficient and water-logging treatments after the fourth evaluation suggests the existence of adaptive mechanisms. Tolerant plants promote metabolic, morphological and anatomical adjustment that allows survival and

production for longer periods in unfavourable condition (Alves et al., 2002).

The larger leaf area in *P. regnellii* ("BRS Guará" and BGP 397) and *P. conspersum* (BGP 402) during the waterlogging period may have favoured the partitioning of photoassimilates to the root system and the accumulation of organic reserves, increasing the resilience of plants after cessation of stress. It is interesting to note that *P. regnellii* and *P. conspersum* are very close species, both being from the informal Virgata group. According Zuloaga and Morrone (2005), *P. regnellii* is found in humid areas with clay soils, on forest borders, occupying sites that have been modified. The same characteristics can be found for *P. conspersum*. The increased number of tillers and tiller survival rate confirm this effect after the recovery period of the same genotypes (Figures 4 and 5).

In all genotypes, the root: shoot ratio was also higher after recovery. Reports in the literature indicate that under stress there is reallocation of biomass between the root system and the shoot (Dias-Filho & Carvalho, 2000; Yamamoto, Sakata, & Terazawa, 1995), modifying the root: shoot ratio. In the post-stress period, based on results of the root recovery rate, it seems that, in general, *Paspalum* accessions are damaged less by waterlogging than by water deficit (Table 2). suggesting that there was accumulation of reserves and protection of meristems (Table 2).

4.3 | Genetic variability of *Paspalum* sp. accessions in response to water stress

According to the morphophysiological responses, "BRS Guará" is a genotype with adaptive features to water excess and low survival rate after water deficit. In contrast, BGP 289 showed a resource-conservation strategy when subjected to water deficit. This accession belongs to *P. malacophyllum* species, which according to Zuloaga and Morrone (2005) can be found in Mexico, Brazil, Paraguay, Bolivia and Argentina, in hills, on rocky slopes and near roads or forests. In such environments, biomass allocation pattern is an important survival strategy (Scheffer-Basso et al., 2002). Under water deficit, BGP 289 had interrupted growth and senescence; however, it also presented high tiller survival rate after rehydration. Moreover, under waterlogging, it reduced development, and after the recovery period, it presented a tiller survival rate of 59% (Table 2).

Similarly to the BGP 289, under waterlogging BGP 293 (also from *P. malacophyllum*) conserved resources during the stress period by reducing LER, number of tillers and leaf biomass (Figures 3, 4, 6); however, it presented high tiller survival rates (Table 2). In general, BGP 293, BGP 402 and, especially, BGP 397 showed adaptive potential strategies for both types of stress, which can be related to high root recovery rate (Table 2). The high root: shoot ratio in BGP 397 may have contributed to the higher relative performance, and its extensive root system may possibly be more efficient (Buttery & Bizzell, 1972). The results of the post-stress in cv. Marandu, regardless of stress, show that the damage to the root system could not be reversed in the short term (10 days).

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The results indicate that the tiller survival rate and the recovery rate of the root system (Figures 8 and 9) can contribute for the distinction of tolerant genotypes. Knowing the growth pattern of a species allows greater understanding of its production potential in adverse conditions (Scheffer-Basso et al., 2002). The monitoring of responses to water deficit and waterlogging, from various types of forage material, during plant development is important for screening for tolerance and resistance mechanisms that allow adequate discrimination and genotypic characterization. This experiment was carried in greenhouse conditions, and the results obtained were useful for selection of the best genotypes. It will be necessary to carry out further experimentation under field conditions, of at least 2 years duration, to confirm the morphological and physiological responses of Paspalum accessions to water deficit and waterlogging.

5 | CONCLUSIONS

There is variability among the evaluated accessions in terms of their response to water deficit and waterlogging. The genotypes of *Paspalum* sp., especially BGP 289, BGP 402 and BGP 397, and the *Urochloa brizantha* cv. Marandu, present survival mechanisms under water deficit. *Urochloa brizantha* cv. Marandu and BGP 289 are not tolerant to waterlogging. Waterlogging reduces growth of BGP 293 accession; however, it is able to survive periods of up to 28 days under waterlogging. Waterlogging does not affect growth of root and shoots of the genotypes *P. regnellii* ("BRS Guará" and BGP 397) and *P. conspersum* (BGP 402) both species from Virgata group.

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