

**University of São Paulo
“Luiz de Queiroz” College of Agriculture**

**Ecophysiological and agronomic responses of perennial grass
species under water stress**

Tatiane Beloni

Thesis presented to obtain the degree of Doctor in
Science. Area: Animal Science and Pastures

**Piracicaba
2015**

**Tatiane Beloni
Animal Scientist**

**Ecophysiological and agronomic responses of perennial grass species under
water stress**

versão revisada de acordo com a resolução CoPGr 6018 de 2011

Advisor:
Prof. Dr. **PATRICIA MENEZES SANTOS**

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**Piracicaba
2015**

**Dados Internacionais de Catalogação na Publicação
DIVISÃO DE BIBLIOTECA - DIBD/ESALQ/USP**

Beloni, Tatiane

Ecophysiological and agronomic responses of perennial grass species under water stress / Tatiane Beloni. - - versão revisada de acordo com a resolução CoPGr 6018 de 2011. - - Piracicaba, 2015.

90 p. : il.

Tese (Doutorado) - - Escola Superior de Agricultura "Luiz de Queiroz".

1. Alagamento 2. Atraso a desidratação 3. *Brachiaria* 4. Estresse abiótico 5. Plantas forrageiras 6. Sistema radicular 7. Tolerância a desidratação I. Título

CDD 633.2
B452e

“Permitida a cópia total ou parcial deste documento, desde que citada a fonte – O autor”

To GOD who loves us all.

*To my mother Laudelina Beloni, my father Pedro Ribeiro and my
father Celso de Oliveira.*

To my brothers Aline, Rafael, Renan and Rian,

To my niece Laura,

To second mother and friend Cida Silvestre,

For their love, education and suport,

DEDICATE

ACKNOWLEDGMENTS

To my advisor Dr. Patricia Menezes Santos, professional example, for the exemplary guidance, professionalism and dedication.

To Dr. Florence Volaire, my supervisor at Centre National de la Recherche Scientifique and Centre d'Ecologie Fonctionnelle et Evolutive (Montpellier, France), for the opportunity of training and all guidance and friendship. Also to Ecopar Team, for allowing me to use the infrastructure for the development of my experiment there. To Pascal Chapon for support, patience, help and training along the exchange period.

To “Luiz de Queiroz” College of Agriculture (ESALQ), University of São Paulo, and to the Animal Science and Pastures Graduate Program for training and support provided by faculty and staff.

To EMBRAPA – Southeast Livestock, (São Carlos, SP, BR) for infrastructure and technical support.

To Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes) and Conselho Nacional de Pesquisa (CNPq) for their financial and technical support.

To Dr. Sila Carneiro Da Silva, in the Department of Animal Science of ESALQ, for his support, trust and guidance.

To Dr. Bianca Bacilli Zanotto Vigna, Dr. Cristiana de Gaspari Pezzopane and Dr. Pedro Gomes da Cruz at EMBRAPA for their support, suggestions and help since the conception of my project.

To Gregori Rovadoscki, in the Department of Animal Science of ESALQ, for his help in the statistical analysis.

To my boyfriend Marcell Alonso for all the love, support and fellowship.

My deep gratitude goes to my family. I express my special thank you to my mother Laudelina Beloni, my father Celso de Oliveira, my father Pedro Ribeiro, Laura Beloni, Cida de Oliveira, Renan de Oliveira, Rian de Oliveira, Rafael Beloni, Aline Beloni, Ana Paula Davanso, Sofia Davanso de Oliveira, Alzira Silvestre, Marli de Oliveira, Heloneida Beloni, Janice Beloni and Clayton Beloni.

To my friends and colleagues from the Animal Science Department who I had opportunity to meet and were very important during this time: Marcell Alonso, Daiane Fausto, Jessica Drum, André Santana, Grégori Rovadoscki, Lucas Carvalho, Cleo

Fialho, Eliana Geremia, Fábio Pértile, Jacqueline Lima, Liliâne da Silva, Pedro Leopoldo, Giancarlo Moura, Pedro Cruz, Júnior Yasuoka, Guilherme Silva, Letícia Custódio, Sandra Augusto, Thaline Pachelli, Marcelo Coutinho, Maximiliano Pasetti, Valdson Silva, Fagner Gomes and Alexandre Pedroso.

To my friends from Piracicaba: Elisa Matos, Layanne Souza, Glaucia Lacerda, Luciano França, Diógenes Coriguazi, Flávia Bento, Danielle Scotton, Juliana Deganello and Juliana Eschholz. To friends of São Carlos: Lidiane Silva, Fernanda Massaro, Denise Okumura, Raquel Moreira, Renata Martins, Cristiana de Gaspari Pezzopane, Monica Ruscito and Suelen Melo.

To my friends in Montpellier: Joana Nogueira, Timo Brockmeyer, Ana Carolina Moreira, Lilian Teixeira and Federico Manna.

To support and love from my friends: Miriã Melise, Ariane Gonçalves, Vanessa Piotto, Camila Galana, Carolina Venturine, Gracielle Mari, Ana Lígia Vieira, Mariana Faria, Nilma Araújo, Otávio Lago, Carolina Boschini, Carolina Bepalhok, Jaqueline Cortês, Caroline Stanquevis, Patrícia Pereira, Lissandra Forte, Maicon Borile, Túlio Lins, Milene Martins, Paula Cristina, Ulysses Cecato and Lívia Pian.

To all those who directly or indirectly contributed to this work and supported me somehow.

“Para cada esforço disciplinado há uma retribuição múltipla.”
Jim Rohn

CONTENTS

RESUMO	13
ABSTRACT	15
1 INTRODUCTION	17
References	18
2 LITERATURE REVIEW	21
2.1 <i>Paspalum</i> genus	21
2.2 <i>Urochloa</i> genus.....	21
2.3 Water stress.....	22
2.3.1 Stress by water deficit.....	23
2.3.2 Stress by oxygen deficiency	26
2.4 Considerations in ecophysiological studies of forage plants under water stress. 28	
2.7 Hypothesis	30
2.8 Objectives	30
2.8.1 Specific objectives	30
References	31
3 MORPHOLOGICAL AND PHYSIOLOGICAL RESPONSES OF <i>PASPALUM</i> SP. ACCESSIONS UNDER WATER STRESS	39
Abstract.....	39
3.1 Introduction	39
3.2 Material and methods	41
3.2.1 Plant Material.....	41
3.2.2 Experimental design and plant measurements.....	41
3.3 Results.....	43
3.3.1 Morphological characteristics.....	43
3.3.2 Physiological characteristics	47
3.3.4 Tiller survival and root recovery	50
3.4 Discussion	51
3.4.1 Drought survival.....	51
3.4.2 Mechanisms of tolerance to excess of water	52
3.4.3 Genetic variability in response to <i>Paspalum</i> sp. access to water stress ...	54
3.5 Conclusions	55
References	56
4 FUNCTIONAL STRATEGIES OF RESOURCE-USE AND DROUGHT-SURVIVAL OF C4 PERENNIAL GRASS SPECIES (<i>UROCHLOA</i> SP.).....	61
Abstract.....	61

4.1 Introduction.....	61
4.2 Material and methods	63
4.2.1 Plant material	63
4.2.2 Experimental design.....	64
4.2.3 Functional leaf traits under non-limiting water conditions (Exp. 1.1)	66
4.2.4 Cultivars responses to progressive drought – Dehydration tolerance (Exp. 1.2).....	67
4.2.5 Plant survival after rehydration – Dehydration tolerance (Exp. 1.2)	67
4.2.6 Functional root traits and dehydration avoidance (Ex. 2)	68
4.2.7 Data analysis.....	70
4.3 Results	70
4.3.1 Potential of aerial biomass production and leaf traits under full irrigation..	70
4.3.2 Dehydration tolerance under progressive drought in pots	72
4.3.3 Dehydration avoidance and root traits in long tubes	75
4.3.4 Person’s correlation between traits related to resource-use and water stress response strategies	77
4.4 Discussion	79
4.4.1 Resource-use strategies varies among <i>Urochloa</i> cultivars.....	79
4.4.2 Are potential of biomass production correlated to dehydration tolerance and dehydration avoidance?	80
4.5 Conclusions.....	82
References	83
5 GENERAL CONCLUSIONS	89

RESUMO

Respostas ecofisiológicas e agronômicas de espécies de gramíneas perenes sob estresse hídrico

A partir de predições de futuros impactos climáticos, é esperado um aumento na produção anual total de forragem tropical, contudo estudos também predizem aumento da variação da produção entre e dentro dos anos. Diante desses cenários, é imprescindível buscar vantagens competitivas e o conhecimento do grau de tolerância a estresse abióticos de gramíneas tropicais. O estresse hídrico, tanto por excesso quanto por deficiência, pode reduzir a produção e a sobrevivência da planta forrageira. Assim, objetivou-se identificar as estratégias que alguns genótipos do gênero *Paspalum* e *Urochloa* utilizam para lidar com o encharcamento e/ou com a deficiência hídrica no solo. Foram conduzidos dois experimentos em casa-de-vegetação: 1 - avaliou-se características morfofisiológicas, taxa de sobrevivência e índice de recuperação em cinco genótipos de *Paspalum* sp. (BRS Guará, BGP 289, BGP 293, BGP 402 e BGP 397) e *U. brizantha* cv. Marandu sob três condições hídricas (alagado, déficit e controle), em vasos pequenos; 2 – mensurou-se em pequenos vasos a tolerância a desidratação e em longos tubos o atraso a desidratação de três cultivares de *Urochloa* ('Marandu', 'BRS Paiaguás' e 'Basilisk') e uma cultivar mediterrânea de *Dactylis glomerata* L. cv. Medly, submetidos a condições sem limitação hídrica e posteriormente sob seca prolongada. Sob déficit hídrico, os genótipos de *Paspalum* sp., especialmente o BGP 289, o BGP 402 e o BGP 397, e a cv. Marandu, apresentaram altas taxas de sobrevivência de perfilhos após reidratação. A cv. Marandu e o acesso BGP 289 não são tolerantes ao alagamento, apresentaram baixas taxas de sobrevivência e de recuperação radicular. O acesso BGP 293 não é resistente ao alagamento, porém é capaz de sobreviver a períodos de até 28 dias sob lâmina d'água. Os acessos de *Paspalum* BRS Guará, BGP 402 e BGP 397 apresentam mecanismos de resistência e sobrevivência ao alagamento, relacionados à alta concentração de clorofila na folha, de biomassa de folhas, e de recuperação radicular e sobrevivência de perfilhos após período de recuperação sob condição hídrica regular. No experimento 2, a distinção dos cultivares foi de acordo com a estratégia de uso dos recursos. Cultivares de *Urochloa* são menos tolerantes a desidratação que a cv. Medly. Mas as cultivares de *Urochloa* mais produtivas (Marandu e Basilisk) atrasaram mais a desidratação, devido ao alto investimento no sistema radicular, e conseqüentemente foram menos tolerantes a desidratação, com menores taxas de sobrevivência sob seca severa. A cv. BRS Paiaguás é menos produtiva sob irrigação regular em comparação as outras cultivares de *Urochloa*, mas sob seca aproximou-se mais da cv. Medly, mostrando-se altamente tolerante a desidratação, com altas taxas de sobrevivência sob seca severa, além de apresentar estratégia de atraso a desidratação com o alongamento de raízes ao longo do perfil do solo durante a seca. Existem diferenças entre e dentro de espécies dos gêneros *Paspalum* e *Urochloa* em relação as respostas sob duas formas de estresse hídrico. As características morfofisiológicas utilizadas neste estudo auxiliaram na compreensão dos mecanismos de resposta ao estresse hídrico e na discriminação dos genótipos.

Palavras-chave: Alagamento; Atraso a desidratação; *Brachiaria*, Estresse abiótico; Plantas forrageiras; Sistema radicular; Tolerância a desidratação

ABSTRACT

Ecophysiological and agronomic responses of perennial grass species under water stress

The total annual forage production of tropical grasslands in the Brazilian Central area is expected to increase, however, some studies also predict a higher variability of forage production between and within years. In the future, maintenance of many pastures areas will probably depend on the use of technology and increased productivity, seeking a competitive advantage concerning other activities, or even on its relocation to marginal areas where forage grasses will be more suitable to abiotic factors. The water stress, both by flooding and drought, may reduce the production and survival of grasses. The objective was to identify the strategies that some genotypes of *Paspalum* and *Urochloa* genus use to cope with the flooding and/or water deficiency in the soil. Two greenhouse experiments were conducted: 1 – morphological and physiological characteristics, survival rate and recovery index in five genotypes of *Paspalum* sp. (BRS Guar, BGP 289, BGP 293, BGP 402 and BGP 397) and *U. brizantha* cv. Marandu were evaluated under three water conditions (flooded, drought and control); 2 - dehydration tolerance and dehydration avoidance of three cultivars of *Urochloa* ('Marandu', 'BRS Paiagus' and 'Basilisk') and a cultivar Mediterranean *Dactylis glomerata* L. cv. Medly. In experiment 1, the genotypes of *Paspalum* sp., especially BGP 289, BGP 402, BGP 397 and cv. Marandu previously submitted water dficit, presented high tiller survival rates after rehydration. The cv. Marandu and BGP 289 access were not tolerant to flooding, presenting a low tiller survival rate and root recovery index. The BGP 293 access was not resistant to flooding, but it was able to survive periods up to 28 days under water depth. The accesses of *Paspalum* BRS Guar, BGP 402 and BGP 397 presented resistance mechanisms and survival to flooding, related to a high concentration of chlorophyll in the leaf, leaf biomass, and root recovery and tiller survival after a recovery period under water regular condition. In experiment 2, the distinction of cultivars was in accordance with the resource-use strategy. Cultivars of *Urochloa* were less tolerant to dehydration than cv. Medly. But the most productive *Urochloa* cultivars (Marandu and Basilisk) further delayed dehydration due to high investment in the root system, and consequently were less dehydration tolerant, with less survival rates under severe drought. The cv. BRS Paiagus was less productive under regular irrigation than other *Urochloa* cultivars, but under drought conditions it is closer to the cv. Medly, being highly dehydration tolerant, and presenting high survival rates under severe drought and dehydration avoidance strategies with roots elongation along the soil profile during the drought period. There are differences in responses to other deficit and flooding between and within the *Paspalum* and *Urochloa* genus morphophysiological traits used in this study are relevant for the understanding of the mechanisms and discrimination among genotypes.

Keywords: Abiotic stress; *Brachiaria*; Dehydration avoidance; Dehydration tolerance; Flooding; Forages grass; Root system

1 INTRODUCTION

The pastures in Brazil account for the main land use, with 48.1% of the area of agricultural establishments (INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA - IBGE, 2006), in which the native and cultivated forages represent 36.1 and 63.9%, respectively (IBGE, 2006). Plants in general are continually subject to biotic and abiotic factors in their natural environment, which impose different forms of stress that affect their physiology and production. The seasonal production of forage is one of the main factors limiting Brazilian livestock production. In almost all the national territory, the pasture areas are submitted to temporary flooding or drought periods, due to the irregularity of rainfall patterns that restrict the development of grasses, even during the rainy seasons (MATTOS et al., 2005). In addition, climate changes have affected and changed the pattern of rainfall distribution, and an increased incidence of droughts and flooded areas is expected (INTERNATIONAL PANEL ON CLIMATE CHANGE - IPCC, 2007; CARDOSO et al., 2013).

The importance of studying the responses of the *Urochloa* cultivars under water deficit is unquestionable, about 60% to 80% of the areas of cultivated pastures are occupied by species of this genus, used in establishing, formation and pasture recovery (ZIMMER; EUCLIDES, 2000). However, in the Brazilian biodiversity there are grasses of the *Paspalum* genus, from the Poaceae family, that potentially may be used for forage exploration (CHASE, 1929; ALISCIONI, 2002). Nevertheless, unawareness of these native species potential as forages and its tolerance to biotic and abiotic stress, precludes its indication in the formation of pastures. The *Paspalum* genus is an important alternative in terms of ecosystem sustainability, as the introduction of exotic species may put the flora and fauna structure at risk. The problems caused by 'stunt viruses' in *Digitaria decumbens* Pangola grass pastures (SCHANK, 1974), by spittebebug in *Urochloa* (Syn. *Brachiaria*) *decumbens* and *Urochloa* (Syn. *Brachiaria*) *humidicola* (COSENZA, 1982) and the current "death syndrome" in pastures of *Urochloa* (Syn. *Brachiaria*) *brizantha* cv. Marandu, constitute examples of the urgent need for diversification of the species currently used in the country (DIAS-FILHO, 2006).

There are two ways to increase the productivity of forage crops: the first is through the improvement of environmental conditions in which the plant develops, and the second alternative is the replacement of sensitive forage for more resistant varieties resulting from selection processes, adaptation or development of new genotypes (MILES; VALLE, 1994). For perennial cultures, high production, long-term performance and resilience are expected, and, in order to it, it is necessary to know the behavior of different genotypes of grasses under adverse water conditions and intensities. The use of survival and recovery indexes after stress can assist in the interpretation of the responses and distinguishing genotypes, since the signal the level of system compromise and persistence of the plant.

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2 LITERATURE REVIEW

2.1 *Paspalum* genus

The *Paspalum* genus includes over 400 species being able to occur across Brazil, eastern Bolivia, Paraguay, northern Argentina and Uruguay (CHASE, 1929). In Uruguay, Paraguay, Argentina and Brazil there are about three-quarters of all known species, in different ecological conditions and plant formations (MAEDA; PEREIRA, 1997). Plants may be annual or perennial. Dwarf species may be found but also cespitose plants with more than three meters high. By being native from Brazilian ecosystems, these grasses offer lower risks of causing ecological imbalance by introducing unknown diseases and pests on pastures.

Brazil is situated among the countries with the highest natural genetic diversity, much of botany and natural composition of the herbaceous communities presents a high contribution of species from the *Paspalum* genus (QUARÍN et. al., 1997). São Paulo State is the largest in the country in number of cataloged species (VALLS; OLIVEIRA, 2012). However, information about these plants is still incipient for agricultural and related research. The few *Paspalum* varieties commercially available were obtained by selection of native germplasm.

The Germplasm Bank of *Paspalum* spp., established in Embrapa Southeast Livestock of São Carlos, SP, includes more than 300 accessions of different species. Results obtained in studies from Batista and Godoy (1998, 2000) confirm the high potential of forage species of the *Paspalum* genus. The bank preserves germplasm of 13 federal units of Brazil, and the Rio Grande do Sul State is the most represented (31% of accessions) (BATISTA; GODOY, 1992). The Plicatula botanical group is the most representative of the collection, due to the forage potential of its species. Based on this biological material variability, it is possible to develop a breeding program to obtain new varieties of forage grasses (BATISTA; GODOY, 1998).

2.2 *Urochloa* genus

The grasses from the *Urochloa* genus are widely used in tropical America (MILES et al., 2004; VALLE; PAGLIARINI, 2009). From Africa, it was introduced in

South America in the eighteenth century; the genus has about 100 described species in tropical and subtropical regions (MILES et al., 1996). They are herbaceous plants with terminal inflorescences of panicle type, composed by racemes sustaining solitary uniseriate spirochetes. These species are widely used in Brazil as forages, however, about 85% consists of only two varieties, *U. decumbens* cv. Basilisk and *U. brizantha* cv. Marandu (MILES et al., 1996).

The use of *Urochloa* for the formation of pastures relates to its facility of adaptation to different acids and poor soils, drought resistance, pests and high nutritional value for cattle feed (LAPOINTE et al., 1992; KELLER-GREIN et al., 1996). In 1984, Embrapa Beef Cattle and Embrapa Cerrados released cv. 'Marandu'. Under nowadays, it is the most widely used forage resource in Brazilian pastures, and since the 80s, it dominates the national and foreign seed markets.

Marandu cultivar is a perennial plant, cespitose, robust, reaching up to 2.5 meters height. It presents climate adaptation to 3000m above sea level, and annual rainfall between 700 to 3000mm. Its main qualities are: resistance to spittlebug pastures (Homeptera: Cercopidae), palatability and nutritional quality desired for livestock (NUNES et al, 1984), persistence, regrowth capacity, tolerance to cold, drought and fire, good response to fertilization and tolerance to high aluminum and manganese content in the soil (ALCÂNTARA; BUFARAH, 1999). This cultivar is widely used and recommended for the formation of large areas of pastures intended for livestock farming (OLIVEIRA, 2007) due to its adaptation to average and low fertility soils or sandy texture soil (ALVES; SILVA FILHO, 1996), these characteristics are typical of the cerrado regions soils.

2.3 Water stress

Most cultivated plants are called mesophytes, since they require high availability of water, and those that develop in arid environments are called xerophytes, with certain specific characteristics to prevent water loss (MILBUTN, 1979). In C4 metabolism plants, such as tropical grasses, for each gram of produced organic matter, the average of 300 grams of water are absorbed by the roots and 95% is lost by the transpiration process, and the rest is used for plant metabolism and growth (TAIZ; ZEIGER, 2009).

The fast readjustments of metabolic fluxes, such as photosynthesis, respiration and transpiration, induced by changes of the incident radiation (sun, clouds), temperature or vapor pressure deficit may not be considered as stress (TAIZ; ZEIGER, 2004). Based on Levitt (1980) and Larcher (1987) definitions, the plant under stress has their balance disturbed and requires immobilization of extra resources for the normal functions of the system being maintained, resulting in increased energy expenditure in the system due to alternative metabolic pathways regulating the internal center, or even the catabolic processes that began prematurely.

The stress, due to water deficiency, can lead to dehydration, photosynthesis and growth reduction, increased tissue senescence rate and reduced leaf expansion and leaf appearance rates, that reduces leaf area in species of forage grasses (NG et al., 1975; LUDLOW; NG, 1976; BENETT; SULLIVAN, 1981; MCIVOR, 1984; VAN LOO, 1992). The deficit in the soil oxygen availability, due to water excess, is perceived in most cases, directly by the roots and indirectly by the shoot (DIAS-FILHO; LOPES, 2012) inducing to chlorosis, premature wilting and loss of photosynthetic capacity and, consequently, decrease in growth (VISSER et al., 2003).

The acclimatization constitutes the resistance mechanisms that adjust an individual organism in response to changes in environmental factors (BRAY et al., 2000). During the acclimation, an organism changes its homeostasis to accommodate the external environmental changes; it is a phenotypic response to different combinations of environmental characteristics (NILSEN; ORCUTT, 1996). The acclimatization and adaptation population level occurs through a combination of behavioral, morphological, anatomical, physiological and biochemical processes, all being dependent on molecular processes (GASPAR et al., 2002).

2.3.1 Stress by water deficit

The ability that some plants present under drought to maintain satisfactory production is usually called drought resistance (MAY; MILTHORPE, 1962). Currently, this concept has been questioned by studies with temperate and Mediterranean perennial herbaceous plants (VOLLAIRE et al., 2014). Forage plants might produce for many years and its sustainability is associated with an stable performance and long-term resiliency. Drought resistance of perennial herbaceous plants should be

analysed in a time scale and according to the magnitude of water deficit experienced. Under more intense drought, a 'trade off' is observed in some plants (POIRIER et al., 2012); they do not grow during the stress period but are able to sprout after drought mitigation. In this case, the most appropriate term seems to be drought survival.

This ability to tolerate drought is associated with specific morphological and physiological characteristics (GRIME, 2001). Plants combine characteristics and responses related to mechanisms that include specific strategies during the stress period (LEVITT 1972; LUDLOW, 1989). Avoidance and tolerance to dehydration are alternatives to prevent the fall or tolerate the loss of tissue water potential caused by cellular dehydration without occurring fatal damage to metabolic processes (LEVITT, 1980, LIDLOW, 1989; TURNER, 1997). There are two more strategies: escape, that is only detected in annual plants, and desiccation tolerance. Escape, plants take a breakout strategy presenting fast phenological development and high degree of plasticity, being able to complete their life cycle before the drought becomes severe enough to cause physiological damage (MAY; MILTHORPE, 1962). On the other extreme, the desiccation-tolerant plants are a rare group of flowering plants that can dry out under drought for long periods, but quickly revive when rehydrated, also popularly called 'resurrection plants' (GAFF, 1971; SCOTT, 2000).

The delay to dehydration corresponds to the maintenance of cell turgor and volume, these plants resist to drought keeping high water potential of tissues. For this purpose, they potentiate the water absorption by an extensive root system, and reduce the loss by transpiration through the stomatal closure or cuticle development (BLUM, 2009; WHITE; SNOW, 2012). Plants that resist to drought keeps shoot growth and tolerate dehydration of the leaf, on the other hand, plants that survive severe drought present strategies associated to delay and tolerance occurring at the level of meristematic tissue (VOLAIRE et al., 2014). These plants present a lower leaf area, accumulate carbohydrates in roots, and have high resilience due to the sharp osmotic adjustment through the accumulation of soluble sugars and amino acids in the leaves, which maintains the integrity of the meristems membranes (KRAMER, 1980; VERSLUES et al., 2006), which allows recovery after rehydration occurrence (BEWLEY, 1995, VOLAIRE; LELIÈVRE, 2001). The distinction between new and mature tissue responses is crucial for proper analysis of the strategies that the perennial grasses have under different intensities of water stress (VOLAIRE et al., 2014).

These strategies are additive and do not present a unique combination (VALLADARES; SANCHEZ, 2006; NAVAS et al., 2010). They rely on multiple and different forms of the functional characteristics, and closely relates to resource usage.

Fast-growing species show a resource-acquisition strategy, with low tissue density, low leaf dry mass content, high specific leaf area and a short organs lifespan (RYSER, 1996). A higher potential to capture light (larger photosynthetic leaf area) may confers to these species a competitive advantage in foraging for water in deep soil layers through the maintenance of higher root elongation rates during favorable seasons (PERÉZ-RAMOS et al., 2013). On the other hand, slow-growing species, exhibiting a resource-conservation strategy have lower specific leaf area associated with a reduced transpiring leaf area, and are able to maintain photosynthetic activity and carbon gain over a longer period of time under drought conditions (ESCUDEIRO et al. 2008; PERÉZ-RAMOS et al. 2013) hence able to invest for longer in root system during the drought period. Both types of resource-use strategy may exhibit dehydration tolerance associated with plant survival after rehydration (PERÉZ-RAMOS et al., 2013).

In a study with *Urochloa* genus (GUENNI et al., 2002) it was identified variability among and within species, but generally, there was a reduction of root elongation rate and concentration of the dry biomass of roots in the upper layers of soil. Santos et al. (2013) studied two cultivars of *U. brizantha* 'Marandu' and 'Piatã' in water deficit condition. It was possible to note differences in survival strategies: the Piatã grass decreased its development, indicating a conservative strategy, since the Marandu grass presented a more aggressive strategy, giving it advantages in mild stress situations, however, pledged their survival in severe stress conditions. In a study of four cultivars of the *U. brizantha* genus under water limitation (PEZZOPANE, et al., 2014) observed similar behavior among 'Marandu', 'Xaraés' and 'BRS Paiaguás' cultivars. However, 'Piatã' presented lower effect of water stress on productive and morphogenic variables that were evaluated when compared to the control treatment.

Few studies have explored alongside the characteristics that are associated with each type of drought tolerance strategy and, most of the works focus only on shoot development (PERÉZ-RAMOS et al., 2013). Root characteristics are not

prioritized as most experiments are conducted in small pots. Occasionally, when the biomass of roots is measured, leaf characteristics are not evaluated together, even recognized the connection between both for the dehydration avoidance (POOTER; MARKESTEIJN, 2008; HERNÁNDEZ et al., 2010). These parameters could aid in the detection of differences between genotypes, since the metabolic basis for handling and surviving are divergent and difficult to compare. Under mild to moderate drought, the aim is to maintain the plant productivity while persistence is the priority under severe drought (JONES et al., 1981).

Experiments with forage plants in long transparent tubes (rhizotrons) type have been done with Mediterranean species of perennial forage to eliminate differences in the pattern of growth and depth of the roots and to understand the strategies in the use of resources (VOLLAIRE; LELIÈVRE, 2001; PÉREZ-RAMOS et al., 2013). This type of trial allows the plant root system to grow into the deeper layers of the soil and increases the water absorption, which enable it to express strategies of dehydration avoidance. Drought survival strategies have been accessed under experiments in small pots, which discounts the effect of interspecies differences of root depth in water plants *status*, and allows comparisons of species-specific strategies of dehydration tolerance (VOLLAIRE; LELIÈVRE, 2001). Tolerance to dehydration is tested by measuring survival after plant rehydration, once a predetermined humidity of the soil is achieved by all genotypes (PÉREZ-RAMOS et al., 2013) or after successive rehydrations with the calculation of soil moisture associated with 50% mortality of plants (VOLLAIRE et al., 2005).

2.3.2 Stress by oxygen deficiency

Based on climate change models (GCM) for the years 2020 to 2050, an increased in areas that experience more days of flooding is expected to pastures in Colombia, Venezuela, Guyana and the region of Brazilian Cerrado (CARDOSO et al., 2013). The main way to reduce the negative impacts of excess water would be the selection or improvement of tolerant cultivars (ZHOU, 2010), and for this, the knowledge of the mechanisms that confer survival and the quantification of the stress impact on forage productivity of specific genotypes is required.

In pastures, flooding or excessive waterlogged soil can be caused naturally by intense rainy periods, poor natural soil drainage and the seasonal increase in the

level of rivers and groundwater (DIAS-FILHO, 2006). The flooding drastically reduces the diffusion of oxygen in the soil causing hypoxia, which is the major limitation of plants under this condition; it reduces the aerobic respiration of roots and absorption of water and minerals (RAO et al., 2011). CIAT is developing a multidisciplinary project along to producers of Latin American countries to identify hybrids of *Urochloa* that combine tolerance to flooding allied with high nutritional quality in order to identify desirable and necessary features in new cultivars compared to climate variability (CARDOSO et al., 2013).

Under short-term flooding events, metabolic adjustments occur in plants as an adaptive strategy so they can deal with the lack of oxygen; anaerobic root metabolism performs an important role in producing energy for survival in this situation (DREW, 1997). This process is dependent on the fermentative metabolism, an additional channel where the cell produces triphosphate adenosine (ATP) under limited or no oxygen. The alcohol dehydrogenase (ADH) and pyruvate decarboxylase (PDC) are two key enzymes that control the ethanol fermentation pathway, while the lactate dehydrogenase (LDH) is involved in the fermentation of lactic acid pathway. As a result of anaerobic catabolism, there is the production of ethanol, lactate, acetaldehyde and other organic acids that can potentially enhance the toxic effect on plant cells (DREW, 1983).

When the plant faces a relatively longer period of excess water in the soil, the most common morphoanatomic escape response to hypoxia and anoxia is aerenchyma and the formation of adventitious roots, which aim to capture and transport air oxygen to submerged tissues (ARMSTRONG et al., 1994). The porosity (aerenchyma) is also expressed in the stem base with the same function (ARMSTRONG et al., 1994), yet, many plants are able to form aerenchyma in shoot, this phenomenon facilitates the diffusion of gases throughout the plant, especially the oxygen gas and ethylene (DIAS-FILHO, 2006).

The submerged plants exhibit reactions to delay the effect of hypoxia and anaerobiosis. They usually produce long internodes, due to the growth of apical shoot parts, which rapidly reach the surface of the water and 'escape' from the hypoxic environment (SCHULZE et al., 2005). Plants under water excess exhibit this behavior, however, less prominently than the submerged. Schulze et al. (2005) describe a remarkable increase in ethylene intercellular concentration, considered as

plant hormone signal stimulating the shoot elongation. This increase in ethylene partial pressure triggers a reduction in endogenous abscisic acid level, which in turn increases the hormone responsible for shoot elongation, gibberellic acid. In addition, the persistence of auxin helps to prolong the shoot elongation; it stimulates the acidification of the cell wall, probably by activating proton pump ATP-dependent membrane in the plasma.

The ratio of aerenchyma structures in plants inhabiting wetland environments are often higher than in species that rarely or never experienced these conditions, suggesting that it is an adaptive plastic response to flooding conditions (VASELLATI, et. al., 2001; JUSTIN; ARMSTRONG, 1987). The survival of *Paspalum dilatatum* was evaluated in different topographical positions in the Argentine pampa, inherent in regime of floods and droughts, there were constitutive and plastic anatomical adaptations, as the smaller root diameter under drought and the presence of aerenchyma when flooded, which confer resilience to temporal variations of water availability and oxygen to these plants (VASELLATI et al., 2001).

Flooding may also reduce water absorption and stomatal conductance causing wilting in sensitive plants (JACKSON; DREW, 1984). The tolerance to dehydration in plants under water deficit, is commonly associated with repair and protective mechanisms that preserve the structural integrity of the meristematic tissue cells (BEWLEY, 1995). The same strategy may be adopted by plants tolerant to flooding through the air oxygen-carrying capacity to organs below ground (DIAS-FILHO, 2006), that can guarantee protection the root system and meristematic tissues.

2.4 Considerations in ecophysiological studies of forage plants under water stress

The extrapolation of data obtained under controlled conditions to explain the physiological behavior of plants under water stress in natural field conditions should be cautious, as the responses differ. The goal of the field trials is the mass selection of genotypes, while in trials in a greenhouse environment is to isolate the effects in an attempt to understand the mechanisms that plants use to cope with stress. In these environments, possible interactions are lost and water stress is greater due to restriction of pot volume, which accentuates the injuries. Although field plants can explore a greater volume of soil and delay the stress effects, everything that happens

in the pot is likely to occur in the field, but at different scales (PIMENTEL, 2008). The ideal is to evaluate variables that can be studied at different plant stages, under ideal conditions of experimentation in a greenhouse and/or field.

To understand the response of forage plants submitted to abiotic stress, it is proposed the evaluation of physiological variables that should be viewed as secondary characters in the selection of more tolerant plants (PIMENTEL, 2008). It is considered that the primary characters to be analyzed are: the morphological - leaf area, leaf position, the pattern of root system development and the biomass allocation (root: shoot ratio); - and phenological – (Maintenance of leaf area on the leaves and yield components, number of tillers and plant dry biomass). To evaluate the production stability, plants should be tested under regular irrigation conditions, shortage and excess water (BRUCE et al., 2002), since the energy costs of adjustment mechanisms is high (HSIAO, 1973, 1990) and reduces the amount of energy available for biomass accumulation in tolerant plants. The partitioning and allocation of assimilates plays an important role in these scenarios, since the model of biomass allocation is a survival strategy (SHEFFER-BASSO et al., 2002).

Regarding the strength and survival of the plant to drought, the studies must be conducted in isolation for identification of different strategies (avoidance or tolerance to dehydration). In this regard, the strategy to dehydration avoidance should be accessed under conditions which allow expression of the full root system, such as long tubes, and strategy of dehydration tolerance should be evaluated in small pots to eliminate the effect of inherent differences to the root system of each plant (VOLAIRE; LELIÈVRE, 2001; PERÉZ-RAMOS et al., 2013).

The communities of plants can show in the nature an ample combination of answers to resist water stress (LUDLOW, 1989). The understanding of characteristics associated with increased survival and long-term persistence according to water stress levels are urgent and in the future, the maintenance of many pastures areas will probably depend on the use of technology and increased productivity, seeking to competitive advantage with regards to others activities, or else, on its relocation to marginal areas where forages grasses will be more suitable to abiotic factors. From the ecological and sustainable side, native pastures have relative importance, since adaptation of ecotypes or cultivars to adverse water condition has been consistently shown to depend on the origin of genotypes

(VOLAIRE, 1995; VOLAIRE; LELIÈVRE, 1997; ANNICHIARICO et al., 2011; PECETTI et al., 2011). The development of agronomic practices, including the consortium of forage plants, is being proposed, ensuring a diversity of species that could buffer environmental fluctuations increasing the strength and resilience of pastures (VOLAIRE et al., 2014), for this reason, responses to water stress of these plants must be characterized.

2.7 Hypothesis

- ✓ There are variability in the response to water stress among and within species of *Paspalum* and *Urochloa* genus.
- ✓ Tropical genotypes a C4 species (*U. brizantha* cv. Marandu and cv. BRS Paiaguás; and *U. decumbens* cv. Basilisk) are less dehydration tolerant than a Mediterranean genotype from a C3 species (*Dactylis glomerata* cv. Medly).
- ✓ The most productive cultivars exhibit a lower dehydration tolerance.
- ✓ There is a trade-off between dehydration tolerance and dehydration avoidance under severe drought.

2.8 Objectives

- ✓ To identify the strategies that some genotypes of *Paspalum* and *Urochloa* use to deal with flooding and/or water deficit in the soil.

2.8.1 Specific objectives

- ✓ To characterize the response mechanisms of genotypes of *Paspalum* and *Urochloa* cv. Marandu under water stress, and to verify if there is variability among tropical grasses genotypes.
- ✓ To identify the intra-specific variability of dehydration tolerance and avoidance among contrasting forage grass genotypes of *Urochloa* under severe drought.

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3 MORPHOLOGICAL AND PHYSIOLOGICAL RESPONSES OF *PASPALUM* SP. ACCESSIONS UNDER WATER STRESS

Abstract

Climate change has affected rainfall distribution pattern, and is predicted to increase the incidence of droughts and flooding events. In general, tropical pastures have use limitations under conditions of water stress. Among tropical grasses stands out the genus *Paspalum* in the Americas, but the restricted knowledge on the forage potential of species from this genus hinders their indication for pasture formation, mainly in areas prone to environmental stress. The aim of this research was to characterize the response mechanisms to water stress (drought and flooding) among five accessions of *Paspalum* sp. (*P. regnellii* cv. BRS Guar and BGP 397, *P. conspersum* BGP 402 and *P. malacophyllum* BGP 289 and BGP 293), using the widely planted forage grass *Urochloa* [*Brachiaria*] *brizantha* cv. Marandu for comparisons. We measured morphological and physiological traits at the end of the stress and after the recovery period. In addition, tiller survival rate and root recovery index were calculated. All accessions of *Paspalum* sp., mainly BGP 289, BGP 402 and BGP 397, and cv. Marandu, showed survival mechanisms to water deficit. BRS Guar showed adaptive features to water excess, however, it presented low survival rate under water deficit. Under flooding, BGP 289 and cv. Marandu showed reduced growth and after the recovery period presented low plant survival and root recovery. BGP 293 spared resources during flooding, however presented high tiller survival rates. BGP 293, BGP 402 and BGP 397 showed adaptive potential strategies for flooding and drought. The observed responses suggest, genetic variability among the evaluated accessions for drought and flooding stresses.

Keywords: Abiotic stress; *Brachiaria brizantha*; Flooding; Tiller survival; Water deficit

3.1 Introduction

Future impacts on meat and milk production are expected in Latin America due to climate change (IPCC, 2007). The total annual forage production of grasslands in the Brazilian central region is expected to increase, but, a rise in the variability of forage production between and within years was also predicted for this area (ANDRADE et al., 2014, SANTOS et al., 2014). Under this scenario, the maintenance of many pastures areas will probably depend on the increased use of technology and improved productivity, seeking a competitive advantage concerning other activities, or even on the relocation of these pastures to marginal areas where forage grasses will be more exposed to abiotic stresses.

Water stress, both by flooding and drought, may reduce forage production and survival. In the north of Brazil, the death of *Urochloa* (Syn. *Brachiaria*) *brizantha* cv.

Marandu pastures have been associated to poor soil drainage and disease incidence (TEIXEIRA NETO et al., 2000, VALENTIM et al., 2000, DIAS-FILHO 2005a, 2005b, DIAS-FILHO; ANDRADE, 2005). Moderate water deficit is known to reduce green leaves production of *Urochloa* sp. plants (GUENNI et al., 2002, MELO et al., 2003, ARAÚJO et al., 2011).

Plants considered resistant to water deficit are able to maintain moderate growth under drought (MAY; MILTHORPE, 1962). Under moderate water deficits, some adaptive mechanisms may be developed (MATTOS et al., 2005a), with the objective of reducing foliar transpiration and delaying tissue dehydration. However, under more severe water deficit, drought resistance can occur in some plants combined with the ability of stopping growth in order to survive and recover once this stress is mitigated (VOLAIRE, 2013). Drought survival mechanisms include the promotion of a faster leaf osmotic adjustment, which maintains the integrity of membranes and meristematic tissues (KRAMER, 1980, VERSLUES et al., 2006), allowing the recovery after rehydration (BEWLEY, 1995, VOLAIRE; LELIÈVRE, 2001).

For dealing with flooding and its consequent commitment to the development of root system, plants use adaptive strategies to improve gas exchange and maintain energy production (ARMSTRONG et al., 1994), such as the formation of aerenchyma and adventitious roots that increases the supply of oxygen to roots (DIAS-FILHO, 2013).

There is a wide variability in adaptive responses to stress among species. The adequacy of genetic resources appears as an alternative to adapt animal production systems based on pastures to the global climate change scenarios. In the active germplasm bank of *Paspalum* at Embrapa in São Carlos, SP, Brazil, accessions collected in regions with different climatic characteristics are maintained. The characterization and classification of these accessions for water stress resistance could contribute to the development of new cultivars better adapted to current and future climate scenarios (SHEFFER-BASSO et al., 2002).

On this study, we evaluated the effects of flooding and drought on the morphological and physiological characteristics of *Paspalum* sp. accessions and *Urochloa brizantha* cv. Marandu, and their ability to recover after a period under appropriate soil water conditions. Our aim was to characterize the response

mechanisms to water stress, and verify if there is variability between tropical grasses genotypes.

3.2 Material and methods

3.2.1 Plant Material

Five accessions of *Paspalum* were used: *P. regnellii* (BRS Guar and BGP 397), *P. malacophyllum* (BGP 289 and BGP 293), *P. conspersum* (BGP 402) and *U. brizantha* cv. Marandu. Seeds of *Paspalum* accessions were obtained from the germplasm bank of *Paspalum*, located at Embrapa Southeast Livestock. These *Paspalum* genotypes were selected due to their contrasting responses to stress by flooding and drought, observed in preliminary experiments. Cultivar Marandu presents tolerance to short-term water deficit (SANTOS et al, 2013.), but does not tolerate flooding (DIAS-FILHO; CARVALHO, 2000, DIAS-FILHO, 2005), and was used for comparisons because of its better characterization and wide use in Brazilian pastures.

3.2.2 Experimental design and plant measurements

The experiment was conducted in a greenhouse, at Embrapa Southeast Livestock in So Carlos, So Paulo State, Brazil (2155'S and 4748'W). The experimental design was complete randomized blocks with factorial arrangement of 6x3x2 (six genotypes, three water conditions and two harvest dates) with three replications. The water conditions were: control (maintained at 80% of field capacity), water deficit (irrigation was stopped on day 0) and flooding (water depth of three centimeters above the soil level from day 0).

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 30 additional days, the water conditions were imposed. The pots had the capacity of eight liters and were filled with sifted soil with the following physicochemical properties: $\text{pH}_{\text{CaCl}_2}$ 6.6, OM 24 $\text{g}\cdot\text{dm}^{-3}$, $\text{P}_{\text{ressyne}}$ 67 $\text{mg}\cdot\text{dm}^{-3}$, S.SO₄ 5 $\text{mg}\cdot\text{dm}^{-3}$, K 4.5 $\text{mmolc}\cdot\text{dm}^{-3}$, Ca 50 $\text{mmolc}\cdot\text{dm}^{-3}$, Mg 23 $\text{mmolc}\cdot\text{dm}^{-3}$, H+Al 19 $\text{mmolc}\cdot\text{dm}^{-3}$, Al 0 $\text{mmolc}\cdot\text{dm}^{-3}$, CTC 97 $\text{mmolc}\cdot\text{dm}^{-3}$, V 80%, sand 604 $\text{g}\cdot\text{kg}^{-1}$, 93 silt

g.kg⁻¹ and 303 clay g.kg⁻¹. Each pot was fertilized with 2.33 g of urea (45% N), 7.78 g of simple superphosphate (18% P), 0.88 g of potassium chloride (60% K), following the recommendations of Malavolta (1986). Three times a week pots under water restriction were weighed to monitor the water content in the soil by the gravimetric method (Figure 1).

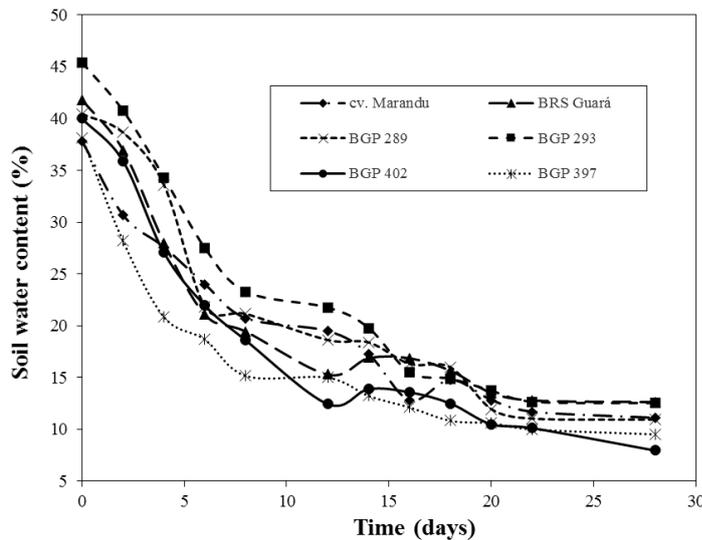


Figure 1. Soil water content (%) of pots cultivated with five genotypes of *Paspalum* sp. and *U. brizantha* cv. Marandu under drought condition. Values are the means of three replications.

At the end of the stress period, half of the pots were harvested for evaluation. Meanwhile, plants from the other half of the pots were placed close to moisture condition of 80% field capacity, and held for ten days in order to evaluate the recoverability of genotypes after stress. The plants were harvested for evaluation at the end of the recovery period. The experiment was carried out from January 21st to February 27th, 2013, totaling 28 days of water stress and 10 days of recovery period.

One tiller per pot was marked and identified with colored wires for analysis of leaf elongation rate (LER). The evaluation was performed three times a week, during the stress and the recovery periods, with a total of 15 measurements. LER was calculated as the increase on leaves 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 pot⁻¹) and the physiological variables: leaf water potential - ψ_w (MPa), leaf osmotic potential - ψ_s (MPa) and SPAD index were measured once a week (day 0, 7, 14, 21, 28 and after recovery), with a total of six evaluations. Tillers survival rate after the recovery period was estimated as the percentage of live tillers over the total number of tillers per pot.

For determining ψ_w 25mm diameter leaf discs were collected in the newest fully expanded leaf of a tiller per pot between 10 to 14 hours. The discs were immediately placed in psychrometric chambers (Wescor model C52), and the ψ_w was determined using microvoltmeter (Wescor model Psypro). The extract from the same portion of the leaves was used for the determination of ψ_w . The cellular content was collected with filter paper using a leaf press (Wescor Markhart Leaf Press model LP-27). Then, the filter paper was placed on the psychrometer for determining ψ_s (TAIZ; ZEIGER, 2004). The SPAD index (relative chlorophyll index determined based on the green color intensity of leaves) was measured by a portable absorbance-based chlorophyll meter (SPAD-502, Konica-Minolta, Osaka, Japan).

Plants were harvested at the end of water stress period or after the recovery period. The biomass was separated and dried in a forced circulation oven (at 65 °C for 72 hours), then weighed to determine the leaf, stem, roots and dead material dry biomass (g pot^{-1}). Roots were washed free of soil using a 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 ($\text{cm}^2 \text{g}^{-1}$) was calculated by dividing the area (cm^2) by the leaf dry biomass (g) obtained after drying (RADFORD, 1967). Leaf: stem relation (leaf dry biomass / stem dry biomass) and root: shoot ratio (root dry biomass / shoot dry biomass) were calculated. Furthermore, 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 analyzed following the factorial design. ANOVA were performed with PROC MIXED statistical package of SAS (SAS INSTITUTE, 2013) and the comparison of averages were done with the Tukey test, adopting a significance level of 5%.

3.3 Results

3.3.1 Morphological characteristics

Specific leaf area was affected ($P < 0.01$) by water conditions and dates of harvest. Specific leaf area was lower in water deficit ($69.11 \text{ cm}^2 \text{g}^{-1}$) than in flooded ($178.17 \text{ cm}^2 \text{g}^{-1}$) or control ($175.64 \text{ cm}^2 \text{g}^{-1}$) plants. Furthermore, the specific leaf area

was lower in plants at the end of the stress period (harvest 1: 119.63 cm² g⁻¹) than after the period of recovery (harvest 2: 163.32 cm² g⁻¹).

Leaf area showed a significant interaction ($P=0.0409$) between genotype and water condition (Figure 2). For all genotypes, leaf area was lower in plants under water restriction when compared to other water conditions. On the other hand, two patterns of response to flooding were observed: 1) Leaf area was reduced by flooding - cv. 'Marandu' ($P=0.0269$) and in *P. malacophyllum* accessions (BGP 289 ($P=0.0121$) and BGP 293 ($P=0.0387$)), 2) Leaf area did not change due to flooding condition - 'BRS Guar' ($P=0.9541$), *P. conspersum* -BGP 402 ($P=0.1520$) and *P. regnellii* - BGP 397 ($P=0.6677$). We also observed that accessions of *P. malacophyllum* (BGP 289 and BGP 393) had the same behavior. This was also observed for *P. regnellii* ('BRS Guar' and BGP 397) and *P. conspersum* (BGP 402), both species from Virgata group.

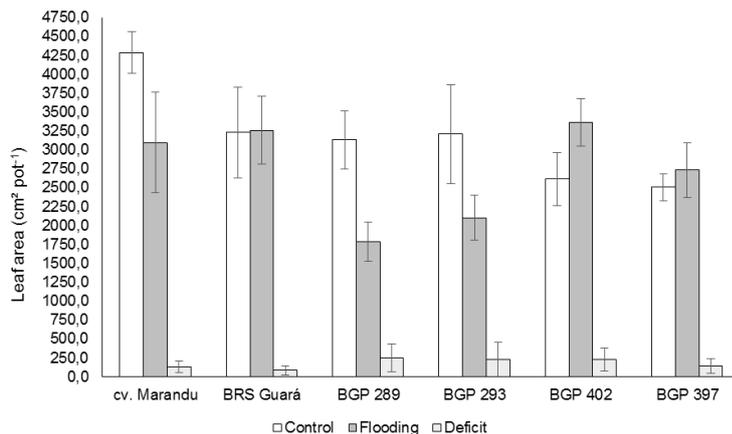


Figure 2. Leaf area (cm² pot⁻¹) of five genotypes of *Paspalum* sp. and *U. brizantha* cv. Marandu under three water conditions. Average of two harvests and three repetitions \pm means standard error.

Interactions between genotype and water condition ($P=0.0345$), and between water condition and measurement period ($P=0.055$) were significant for LER (Figure 3). The water stress declined the LER in all genotypes (Figure 3A). Under flooding, cv. Marandu ($P<0.01$), BGP 293 ($P=0.0011$) and BGP 397 ($P=0.0354$) showed reduction the LER, and BRS Guar ($P=0.0960$), BGP 289 ($P=0.6268$) and BGP 402 ($P=0.1294$) showed similar LER when compared control plants. Throughout the experiment, a decrease on LER was observed for all the conditions evaluated. LER of plants under flooding were lower than that of control plants until the fourth evaluation; after that, no significant difference was detected between control and flooded treatments until the end of the stress period, although during the recovery

period LER of control plants was slightly higher than that of flooded plants. On the other hand, unlike under progressive drought LER decreased since the beginning of the stress period and became almost null after the sixth period (Figure 3B).

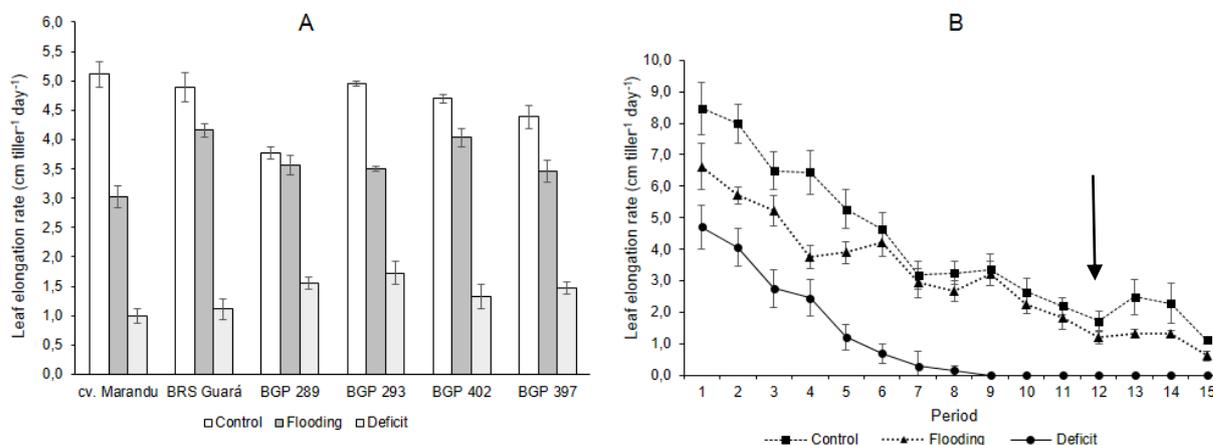


Figure 3. Leaf elongation rate (LER cm tiller⁻¹ day⁻¹) in (A) five genotypes of *Paspalum* sp. and *U. brizantha* cv. Marandu under three water conditions, and (B) plants under three water conditions during period of stress and recovery. (A) Values means of six genotypes during 12 evaluations (three times per week) and three replications \pm means standard error and (B) values are the means of six genotypes and three replications \pm means standard error. Arrow indicate the beginning of the recovery period.

A triple interaction ($P < 0.01$) was observed for the number of tillers (genotype \times water condition \times measurement period). The behavior of this variable by genotype in the three water conditions imposed along the stress and recovery periods can be observed in Figure 4.

In plants under flooding and compared the control plants, during stress the number of tillers decreased in BGP 289 ($P = 0.04$) from the third week (third period), remained stable in BGP 293 and in genotypes of Virgata Group ('BRS Guar', BGP 402 and BGP 397). And after the recovery period under draining conditions (fifth period), the number of tillers just was different in BGP 289 ($P = 0.012$), on what continued decreasing (Figure 4). In plants under drought stress, from the third week of stress (third period) were different of the control plants to cv. BGP 293, BGP 289 and BGP 402, and to others three genotypes occurred after fourth week (fourth period). However, after rehydration (fifth period), was observed the emergence of new tillers, only BRS Guar not showed signals of recovery.

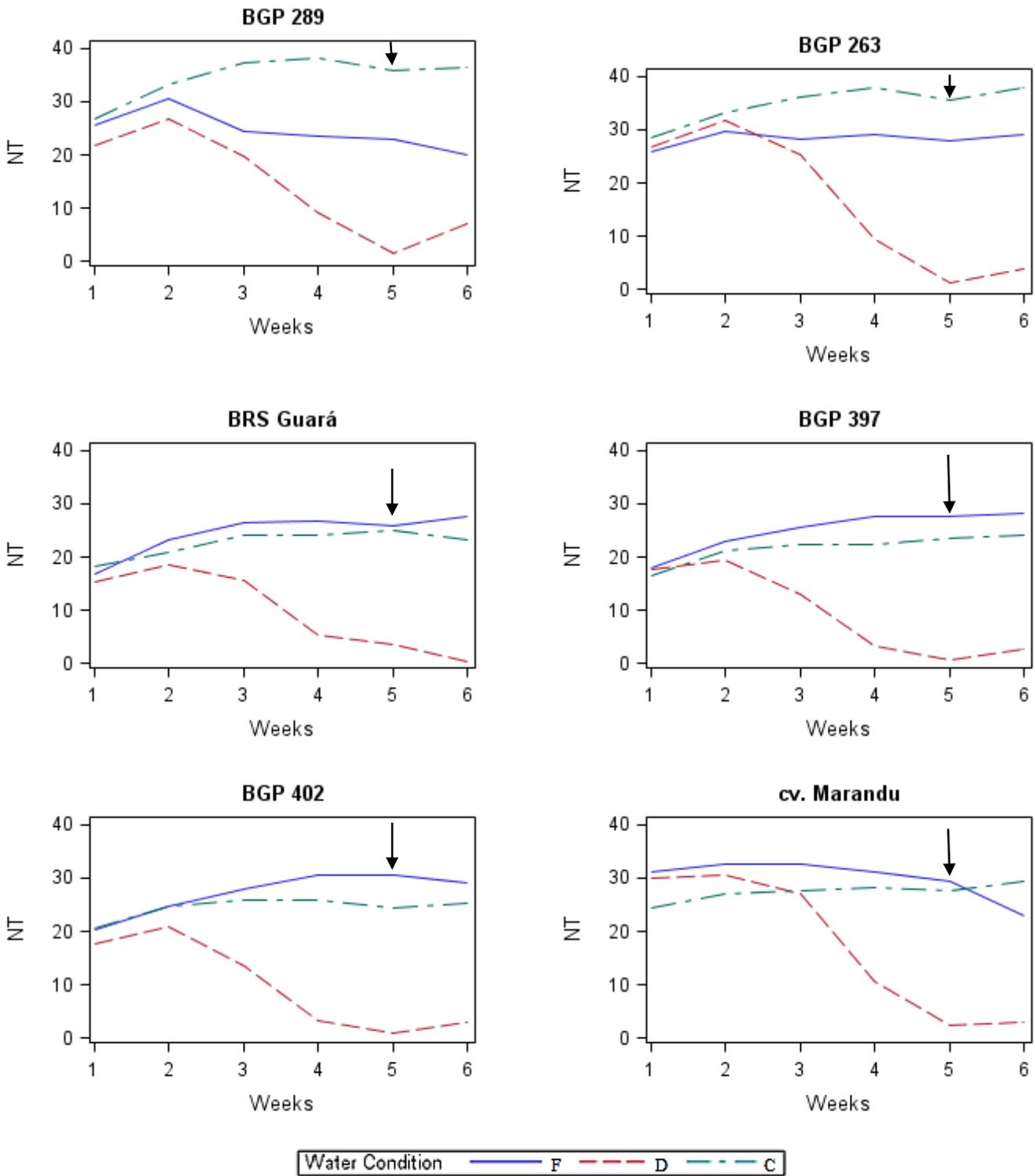


Figure 4. Number of green tillers (NT pot⁻¹) of five genotypes of *Paspalum* sp. and *U. brizantha* cv. Marandu under three water conditions (F – flooding, D – drought and C – control) during six weeks after stress imposition. *Arrows indicate the beginning of the recovery period. Values are means of three replications.

3.3.2 Physiological characteristics

SPAD index was higher ($P=0.0465$) in BGP 397 (35.21) accession than in cv. Marandu (29.51), but they did not differ from others accessions of *Paspalum* that presented intermediate value ('BRS Guar': 34.16, BGP 289: 33.51; BGP 293: 34.48; BGP 402: 34.66). A significant interaction between water condition and measurement period was observed for SPAD index ($P<0.01$). In general, the SPAD index decreased in all treatments throughout time, suggesting an overall reduction in the chlorophyll content. No difference between control and flooding treatments was observed during the experiment, but plants under drought stress presented a lower SPAD index during the fourth and fifth weeks, pointing to a decrease on chlorophyll content due to water deficit, that was recovered just after rehydration of the plants (Figure 5).

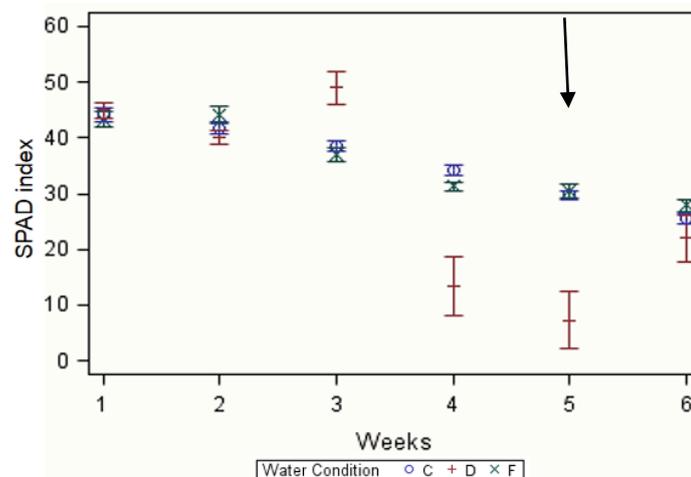


Figure 5. SPAD index of plants under three water conditions (C: control, D: drought and F: flooding) during weeks after stress imposition. Values are the means of three replications \pm means standard error. Arrow indicate the beginning of the recovery period.

Both water potential and osmotic potential showed a significant interaction between water conditions and measurement period ($P<0.01$). The pattern of response for these variables was very similar to that of the SPAD index, except that values did not decrease along the experiment for control (mean water and osmotic potentials were -2.32 ± 0.25 and -1.67 ± 0.07 MPa, respectively) and flooding (mean water and osmotic potential were -2.53 ± 0.28 and -1.68 ± 0.04 MPa, respectively) treatments. Water and osmotic potential of plants under drought conditions were lower than those under control and flooding treatments between third and fifth weeks

(mean water and osmotic potential less than -5.0 MPa and out of the range measured by the method used on this experiment); after rehydration, water and osmotic potential were recovered and no difference was observed between all three treatments.

3.3.3 Productive characteristics

Significant interactions were observed for leaf dry biomass: genotype x water condition ($P < 0.01$) and water condition x harvest date ($P = 0.0466$) (Figure 6).

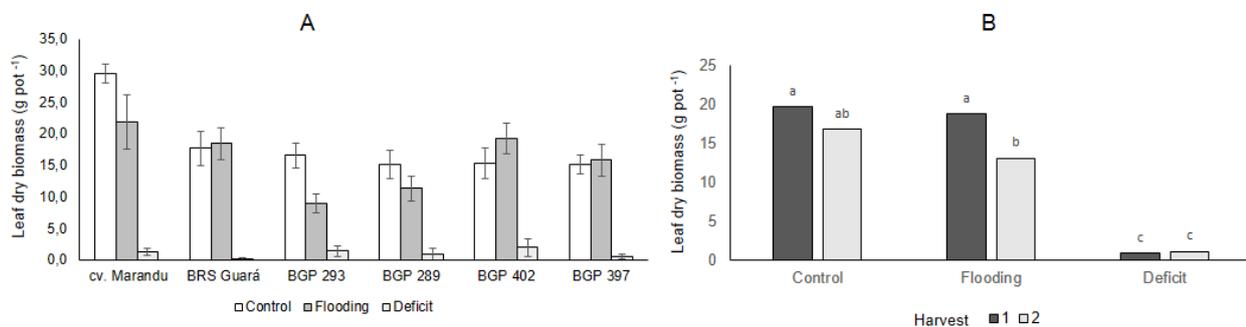


Figure 6: (A) Leaf dry biomass (g pot⁻¹) of five genotypes of *Paspalum* sp. and *U. brizantha* cv. Marandu under three water conditions, and (B) Leaf dry biomass (g pot⁻¹) under three water conditions (F – flooding, D – drought and C – control) in two harvest periods (1 - final stress, and 2 – after 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 ($P < 0.05$, Tukey test).

Drought stress reduced leaf biomass of all genotypes evaluated. While flooding reduced leaf biomass just of cv. Marandu ($P = 0.008$), BGP 293 ($P = 0.006$) and BGP 289 ($P = 0.008$) (*P. malacophyllum*) (Figure 6A). Leaf biomass was lower in the second harvest just for plants under flooding condition (Figure 6B).

Root: shoot ratio was higher ($P = 0.0185$) after the recovery period (2.99) than the end of the stress period (2.39). The others productive characteristics were affected by genotype and water condition ($P < 0.01$) (Table 1).

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

Traits	Genotypes						Condition		
	cv. Marandu	BRS Guar	BGP 397	BGP 402	BGP 289	BGP 293	Control	Flooding	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 the line differ by Tukey test at 5% probability (P <0.05).

Stem biomass and dead dry biomass were higher in cv. Marandu than in *Paspalum* accessions (Table 1). There was no difference between control and flooding treatments, however under water deficit stems biomass was lower, and dead material dry biomass was higher than under the other water conditions (Table 1).

Root dry biomass and root: shoot ratio were higher for BGP 397 and BGP 402 and lower for BGP 289; 'BRS Guar', BGP 293 and cv. Marandu showed intermediate values (Table 1). The nature of the stress affected differently root dry biomass. The highest values were found in control plants; intermediate values under flooding and the lowest root biomass values were recorded for plants under water deficit (Table 1).

After recovery period L:S ratio was lower under flooding treatment (Figure 7). Under this condition, increased values of root and decreased of leaves, suggesting that in the post-stress there was a change in the pattern of partition and allocation of photo-assimilate in these plants. The interaction between water condition and harvest (P<0.01) can be observed in Figure 7.

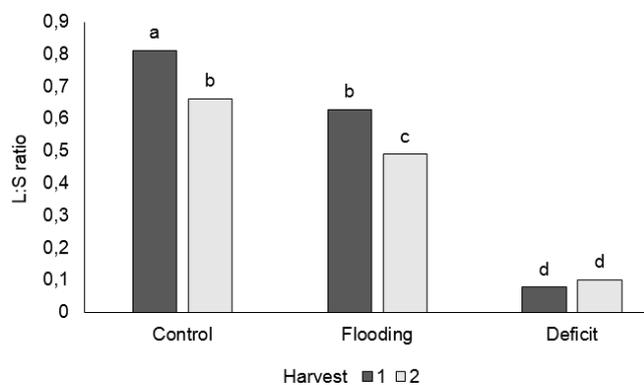


Figure 7. Leaf: stem ratio under distinct water treatments in two dates of harvest. *Different letters indicate differences among two dates (P<0.05, Tukey test).

3.3.4 Tiller survival and root recovery

Tiller survival rate under drought stress was higher in BGP 289 and lower in BRS Guar (Figure 8A). Differences between accessions from the same species It can be observed here (Figure 8; ‘BRS Guar’ and BGP 397, both *P. regnellii*, and BGP 293 and BGP 289, both *P. malacophyllum*). Under flooding, the highest tiller survival rates were observed in ‘BRS Guar’, BGP 293, BGP 402, BGP 397, and the lowest in cv. Marandu. The genotype BGP 289 did not differ from the others (Figure 8B).

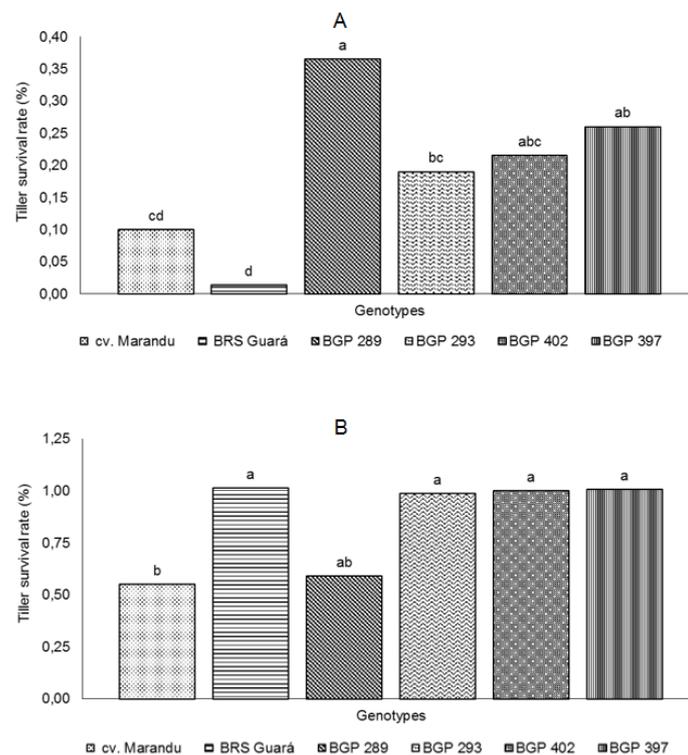


Figure 8. Tiller survival rate (%) after recovery of five genotypes of *Paspalum* sp. and *U. brizantha* cv. Marandu submitted to water deficit (A) and flooding (B) stress. *Different letters indicate differences among genotypes ($P < 0.05$, Tukey test).

The root recovery index was higher in BGP 397 and BRS Guar accessions, regardless of the stress nature (Figures 9A and B). Under deficit, Figure 9A, cv. Marandu, BRS Guar, BGP 289 and BGP 402 not showed recovery (index < 1). Since under flooding (Figure 9B) just cv. Marandu and BGP 289 not showed recovery (index < 1), suggesting that the root system of these genotypes under excess water was less damaged.

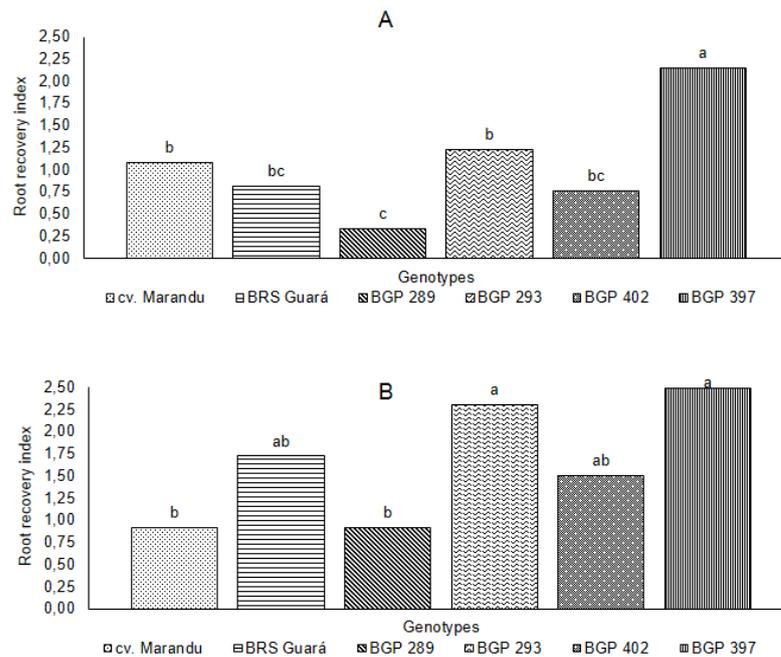


Figure 9. Root recovery index of five genotypes of *Paspalum* sp. and *U. brizantha* cv. Marandu submitted to water deficit (A) and flooding (B) stress. *Different letters indicate differences among genotypes ($P < 0.05$, Tukey test).

3.4 Discussion

3.4.1 Drought survival

Based on the observed responses, none of the evaluated genotypes can be considered resistant to drought. All genotypes under water deficit, on average, presented a reduction on shoot development (Figures 3 and 4) and SPAD index, water and osmotic potential (Figure 5) after three weeks without irrigation, and the leaf biomass production was lower than that observed in the control treatment (Figure 6). The water content in the soil during this period was less than 15%.

To maintain cell turgor and protect the photosynthetic process and other physiological activities, tolerant plants accumulate certain metabolites, which provide a decrease in osmotic potential, through a net increase in intracellular solutes (CHAVES, 1991). This mechanism helps to maintain turgor, holding cell elongation and expansion on growth regions (PREMACHANRA et al., 1992, SPOLLEN; NELSON, 1994). Despite the reduction in the osmotic potential of the plants under water deficit after the third evaluation period, this mechanism was not enough to maintain leaf growth rates.

Dehydration-tolerant species lose their leaves with increasing water deficit, as a possible mechanism for reducing transpiration from leaves and, consequently, the rate of water loss from meristems (VOLAIRE et al., 1998, VOLAIRE; LELIÈVRE, 2001, MUNNE-BOSCH; ALEGRE, 2004). This strategy was probably used by the genotypes in the present study, except for BRS Guar, since the dead biomass was higher under water deficit (Table 1) and was observed the emergence of new tillers after rehydration (Figure 4), except for BRS Guar. Drought survival, assessed by tiller survival rate in this study (Figure 8A), is a characteristic of tolerance to dehydration. This mechanism ensures maintenance of turgor and growth under moderate drought, reducing damage to meristems under severe drought and allowing recovery of plants after rehydration (WEST et al., 1990, BEWLEY, 1995, VOLAIRE; LELIÈVRE, 2001).

The root recovery rate (Figure 9A) suggests that the integrity of the meristematic tissue membranes in the root system was preserved, with the highest value recorded in BGP 397 of *P. regnellii*. Besides, allowing the synthesis of new roots, the displacement of assimilates to the shoot and the appearance of new tillers.

The accession BGP 289 of *P. malacophyllum* under drought stress showed higher tiller survival rate and lower root recovery rate, suggesting a priority for remobilizing reserves to shoot in order to quickly restore photosynthesis. Furthermore, a less shallow dense root system cannot be interpreted as a negative feature, since a deep root system requires higher energy cost for synthesis, maintenance and absorption (ADIKU et al., 2000), and plants that invest less in roots can be effective in less fertile areas and in limiting water condition, because it can be more economic on use of water (SCHENK; JACKSON, 2002). 'BRS Guar', however, besides presenting lower tiller survival rate, also had low root recovery rate, indicating that the damage caused by this stress could not be reversed.

3.4.2 Mechanisms of tolerance to excess of water

No higher plant can survive for long periods without accessing molecular oxygen. 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 tissues flooded (DIAS-FILHO, 2013). In the literature, it is described that morphological and physiological changes may occur among species or within the same species,

according to acclimation ability and because of the stress intensity (DIAS-FILHO, 2013), thereby altering the degree of tolerance to adverse conditions.

In general, it was detected the presence of adventitious roots in all genotypes, which may have promoted a greater contact between the roots and the environment, restoring partly the absorption of water, oxygen, and nutrients (DIAS-FILHO, 2006). The production of adventitious roots is a common response in plants tolerant to flooding (ARMSTRONG et al., 1994, LIAU; LIN, 2001). The lengthening of the stem and the formation of aerenchyma in the roots and the base of the stem, modifying vessel diameter, may also help on the maintenance of water, oxygen and nutrients uptake under flooding conditions (ARMSTRONG et al., 1994; VASELLATI et al., 2001, PEREIRA et al., 2008). These morphoanatomical changes considerably increase the weight and size of the stem. The length of the stem has not been evaluated in this study, however stems dry biomass did not differ between control and flooded plants (Table 1), suggesting that this phenomenon did not occurred.

The responses of *Paspalum* genotypes to flooding were similar to those observed by Cardoso et al. (2013) in *Urochloa* accessions considered tolerant. These researchers evaluated some accessions under water depth of 3cm above ground level for 21 days and reported that the genotypes with high performance showed higher chlorophyll values in the leaf, based on the SPAD index, and a higher proportion of green leaf biomass in relation to the total leaf biomass. Yet, Cardoso et al. (2013) found that accessions containing species *U. brizantha* in the hybridization were considered susceptible to flooding. Several studies have shown that such stress generally reduces the photosynthetic capacity in intolerant plants (LIAU; LIN, 2001); the main cause is the reduction in stomatal aperture (DIAS-FILHO, 2002; DIAS-FILHO; CARVALHO, 2000).

In the present study, the *Paspalum* accessions under flooding presented greater SPAD index, larger leaf area and the dry biomass of leaf was similar to the control plants, though lower values for dry biomass of dead material were observed in these plants, which may indicate low tissue renewal and resource conservation strategy.

LER is a fast tool for measuring the sensitivity to water stress, either by flooding (DIAS-FILHO, 2000) or by water deficit (LUDLOW; NG, 1976, MATTOS et al., 2005). The reduction of LER observed until the fourth evaluation and the recovery

right after, keeping the leaf elongation rate of plants under flooding very close to the control plants (Figure 3B), is another indication that plants under flooding have adaptive mechanisms to water excess. Water stress alters the expression of genes, which in tolerant plants promote metabolic, morphological and anatomical adjustment, and allows its survival and production for longer periods in unfavorable condition (ALVES et al., 2000).

The larger leaf area in the accessions of *P. regnellii* ('BRS Guar' and BGP 397) and *P. conspersum* (BGP 402) under water excess, may have guaranteed the maintenance of photosynthesis during the stress period, and thus contributed to the partitioning of photo-assimilates to the root system with the accumulation of 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 from the informal Virgata group. According Zulloaga and Morrone (2005), *P. regnellii* is a species that is found in humid areas with clay soils, in border of forests, occupying sites that were modified. The same characteristics can be found for *P. conspersum*. The increased number of tillers and tiller survival rate confirms this effect after the recovery period of the same genotypes (Figure 4 and 5).

The root: shoot ratio was also higher after recovery. The literature reports that under stress there is reallocation of biomass between the root system and the shoot (DIAS-FILHO; CARVALHO, 2000, YAMAMOTO et al., 1995), modifying the root: shoot ratio. In the post-stress, the root recovery rate of plants under flooding (Figure 9B), suggests that there was accumulation of reserves and protection of meristems, and that under the new water environment plants was possible, with variation among genotypes, to arise new roots and to remove water and nutrients to the shoot for the emergence of new tillers.

3.4.3 Genetic variability in response to *Paspalum* sp. access to water stress

There was an inversion in the performances observed between 'BRS Guar' and BGP 289 genotypes. According to the morphophysiological responses, 'BRS Guar' is a genotype with adaptive features to water excess and low survival rate after drought. In contrast, BGP 289, demonstrated a resource conservation strategy when subjected to water stress. Under deficit, it interrupted growth and senescence, as the others, however, it presented high tiller survival rate after rehydration.

Moreover, under flooding, it reduced development and after the recovery period, presented a tiller survival rate of 50% (Figure 8). This accession belongs to *P. malacophyllum* species, that according to Zulloaga and Morrone (2005), can be found in Mexico, Brazil, Paraguay, Bolivia and Argentina, in areas as hills, rocky slopes, near roads or forests.

Similarly to the BGP 289, under flooding BGP 293 accession (both from *P. malacophyllum*) spared resources during the stress period, however presented major tiller survival rates (Figure 8). In general, BGP 293, BGP 402, and especially BGP 397 accessions showed adaptive potential strategies for both types of stress that can be related to high root recovery rate (Figure 9). The high root: shoot ratio from the BGP 397 accession, may have contributed to the higher relative performance, once extensive root system can be possibly 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 a short period of time (ten days).

The results indicate that the tiller survival rate and the recovery rate of the root system (Figure 8 and 9) can contribute for the distinction of tolerant genotypes. Knowing the growth model of a species allows greater understanding of their production potential in adverse conditions (SHEFFER-BASSO et al., 2002). The monitoring of responses to water stress, from various forage materials, during development is important for screening for tolerance mechanisms that allow adequate discrimination and genotypic characterization. Finally, these results may contribute to specific improvement programs to select plants to conditions of water stress.

3.5 Conclusions

There is genetic variability between accessions evaluated in terms of response to deficit stress and excess water. 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 flooding. The BGP 293 accession is not resistant to flooding, however, it is able to survive periods of up to 28 days under water depth. The genotypes of *Paspalum regnellii* ('BRS Guará' and BGP 397) and *P. conspersum*

(BGP 402) both species from Virgata group, present resistance mechanisms and survival to flooding.

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4 FUNCTIONAL STRATEGIES OF RESOURCE-USE AND DROUGHT-SURVIVAL OF C4 PERENNIAL GRASS SPECIES (*UROCHLOA* SP.)

Abstract

Drought events interfere directly on productivity and functionality of the plants. Two general strategies have been described: dehydration avoidance, that allows the plant to maintain higher water status for a longer period of time through increased water uptake; and dehydration tolerance, through specific mechanisms that ensure turgor and growth maintenance under moderate drought and reduce damage in basal meristematic tissues under severe drought, allowing the plant to recover when rehydration occurs. The aim this study was to identify the intra-specific variability of dehydration tolerance, in small pots, and avoidance, in long tubes, among contrasting forage grass genotypes of *Urochloa* (*U. brizantha* cv. Marandu, *U. brizantha* cv. BRS Paiaguás and *U. decumbens* cv. Basilisk) under severe drought. It was characterized the predominant resource-use strategies of three cultivars of *Urochloa* and compared to that of a highly dehydration tolerant *Dactylis glomerata* L. cv. 'Medly' successively submitted to non-limiting water supply and then prolonged drought under standardized experimental conditions. In general, *Urochloa* cultivars were less dehydration tolerant to drought than Mediterranean cultivar cv. Medly. The variations in response to drought among and within *Urochloa* cultivars were in terms of resource use strategy. The most productive cultivars, cv. Marandu and cv. Basilisk, were more 'dehydration avoiders' and did not survive under severe drought. Conversely, cv. BRS Paiaguás was less productive and more dehydration tolerant, resulting in higher survival under severe drought. The results show that there is not a single strategy to effectively cope with drought and reveal the diversity of adaptive mechanisms among *Urochloa* cultivars.

Keywords: Biomass production; *Brachiaria*; Dehydration avoidance; Dehydration tolerance; Root system; Severe drought; Tropical grasses

4.1 Introduction

Among the abiotic conditions that periodically impose challenges to tropical species, drought events interfere directly on productivity and functionality of the plants. To cope with drought, plant strategies are complex at all levels of organization in space and time, and they are considered multigenic traits (BLUM, 1997). The ability of a plant species to deal with drought has been associated with specific morphological and physiological traits (GRIME, 2001; REICH, 2014), which root traits are crucial since they are associated with water uptake efficiency (COMAS et al., 2013; PADILLA et al., 2013).

Two general strategies have been described for delaying or withstanding plant dehydration in perennial plant species (LEVITT, 1980; LUDLOW, 1989; TURNER, 1997): (i) dehydration avoidance, that allows the plant to maintain higher water status for a longer period of time through increased water uptake (GARWOOD; SINCLAIR, 1979) or reduced water loss (VOLAIRE et al., 1998; MARTINEZ-FERRI et al., 2000; FERRIO et al., 2003); and (ii) dehydration tolerance, through specific mechanisms that ensure turgor and growth maintenance under moderate drought and reduce damage in basal meristematic tissues under severe drought (WEST et al., 1990), allowing the plant to recover when rehydration occurs (BEWLEY, 1995; VOLAIRE; LELIÈVRE, 2001). However, plants in nature may exhibit a wide range of combined responses to withstand drought (LUDLOW, 1989).

The major factors propelling plant functioning are resource availability and disturbances (GRIME, 1979; WESTOBY, 1998; LAVORELAND; GARNIER, 2002), and traits associated with these factors define the range of strategies observed for dealing with stressful conditions (ACKERLY, 2004). To characterize plants strategies it is necessary to analyze both leaf and root traits. Fast-growing species present a resource-acquisition strategy, with low tissue density, low leaf dry mass content, high specific leaf area and short organs lifespan (RYSER, 1996). A higher potential to capture light (larger photosynthetic leaf area) may confers to these species a competitive advantage in foraging for water in deep soil layers through the maintenance of higher root elongation rates during favorable seasons (PEREZ-RAMOS et al., 2013). Conversely, slow-growing species exhibiting a resource-conservation strategy have lower specific leaf area associated with a reduced transpiring leaf area, and are able to maintain photosynthetic activity and carbon gain over a longer period of time under drought conditions (ESCUDEIRO et al., 2008; PEREZ-RAMOS et al., 2013) therefore being able to invest for longer in root system during the drought period. Both types of resource use strategies may exhibit dehydration tolerance associated with plant survival after rehydration (PEREZ-RAMOS et al., 2013).

High temperatures and availability of moderate-to-high-light levels are the principal determinants of C4 species biogeographical distribution (STILL et al., 2003; PAU et al., 2013). Water availability is not clearly reported as a key-factor for the distribution of C4 species, although most native C4 species are located in tropical and subtropical areas with high rainfalls and only few are reported in Mediterranean

and arid areas (STILL et al., 2013). While perennial C3 species in Mediterranean areas have developed strategies to survive chronic intense summer droughts (VOLAIRE, 2009), the plant response of C4 species to cope and survive severe droughts are little explored whereas it could impact their future adaptation to extreme drought events under climate change (IPCC, 2014). In Brazil, *Urochloa* (Syn. *Brachiaria*) species are the most cultivated forage grasses (MACEDO, 2009), *Urochloa brizantha* cv. Marandu (Syn. *Brachiaria brizantha*) and *Urochloa decumbens* (Syn. *Brachiaria decumbens*), cover almost 72 million hectares of pastures (ZIMMER et al., 2011b). A wide genetic variability has been shown among and within species of this genus (GUENNI et al., 2002).

The objective of this study was to identify the intra-specific variability of dehydration tolerance and avoidance among contrasting forage grass genotypes of *Urochloa* under severe drought. It was characterized the predominant resource-use strategies of three cultivars of *Urochloa* (*U. brizantha* cv. Marandu, *U. brizantha* cv. BRS Paiaguás and *U. decumbens* cv. Basilisk). The plant strategies of these C4 cultivars was compared to that of a highly dehydration tolerant cultivar (cv. 'Medly' from Mediterranean origin) of a C3 species (*Dactylis glomerata* L.) successively submitted to non-limiting water supply and then prolonged drought under standardized experimental conditions. The hypotheses were that (1) tropical genotypes of a C4 species are less dehydration tolerant than a Mediterranean genotype from a C3 species; (2) the most productive cultivars exhibit a lower dehydration tolerance and (3) there is a trade-off between dehydration tolerance and dehydration avoidance under severe drought.

4.2 Material and methods

4.2.1 Plant material

Three perennial *Urochloa* cultivars used as pastures in Brazil (*U. brizantha* cv. Marandu, *U. brizantha* cv. BRS Paiaguás and *U. decumbens* cv. Basilisk) and the Mediterranean cultivar *D. glomerata* cv. Medly were compared under full irrigation and drought conditions. *U. brizantha* cv. Marandu and *U. decumbens* cv. Basilisk have a high potential of forage production, but are not adapted to long droughts

(GUENNI et al., 2002; SANTOS et al., 2013). *U. brizantha* cv. BRS Paiaguás was recommended as a new cultivar based on its forage production quality, especially during the dry season, which results on improved animal production when compared to other *U. brizantha* cultivars (VALE et al., 2013). *D. glomerata* is a C3 species and the Mediterranean cv. Medly was used as control since it showed a high dehydration tolerance in former experiments (POIRIER et al., 2012).

4.2.2 Experimental design

Two experiments were performed in controlled conditions in a glasshouse at the CNRS-CEFE campus in Montpellier (France, 43°38'N, 3°52'E).

1. Small pots experiment: (1.1) initially, plants were compared under full irrigation to measure aerial biomass production and leaf traits in optimum conditions for two cycles of leaf growth. (1.2) Then, the dehydration tolerance was measured after cessation of irrigation in the same small pots used to eliminate the differences effect of root depth on water availability (VOLLAIRE; LELIÈVRE, 2001; PÉREZ-RAMOS et al., 2013). Plant survival was measured after successive rehydration performed at decreasing soil water contents (SWC), and to calculate the soil moisture associated with 50% plant mortality (VOLLAIRE et al., 2005, 2014).
2. Long tubes experiment: successively under full irrigation (2.1), and then during cessation of irrigation (2.2), the analysis of root trait and dehydration avoidance were carried out in conditions allowing the full expression of root length and density (1.3 m long tubes) to measure root traits and water uptake (VOLLAIRE, 2014).

In early April 2014, seeds of all four cultivars were sown in trays in a glasshouse. Tillers were then randomly separated and transplanted as monocultures into pots of 7L (09/09/2014) and long tubes (09/19/2014) which were filled with a similar amount of substrate composed of 69.5% sand, 13.2% clay and 17.3% loam, which were fertilized before starting the experiment (50 kg ha⁻¹ N, P and K). This substrate was chosen to facilitated drainage and root sampling.

(i) Thirteen small pots per cultivar (0.25 m height × 0.15 m diameter; 2 plants per pot; thirteen repetitions per cultivar), were used for the measurements of leaf traits and above ground biomass production under non-limiting water supply (Exp.

1.1) and subsequently for monitoring plant water status after progressive drought imposition in ten small pots per cultivar (Exp. 1.2), for estimating specific dehydration tolerance of the species for discounting the effect of interspecific differences in rooting depth on the state of water in the plant.

(ii) Six transparent long tubes per cultivars (1.30 m height × 0.12 m diameter; 2 plants per pot) covered with opaque film to protect roots from sun radiation and inclined at ~15° from the vertical were used to measure root traits (Exp. 2).

The experiments started on October 6 2014 in a glasshouse. The mean (minimum–maximum) temperatures were maintained at 27.7 °C (27.4–28.1°C) during the day and at 23.8 °C (23–24.2°C) during the night. The position of pots and tubes was rotated to avoid the effect of possible small differences in environmental conditions.

The pots and tubes were equally watered two and three times per week and maintained at field capacity during eight weeks. On November 26 2014, irrigation was stopped in ex. 1.2 and 2.2 in order to analyze responses to drought. At that date, all pots were standardized at 40% of soil water content (SWC) at field capacity before cessation of irrigation. During the drought period, the pots (ex. 1.2) were weighed to determine soil water content (g H₂O/g dry soil, %) three times a week by gravimetric method. Soil water was monitored to enable the right interpretation of performance differences among cultivars without the confounding effects associated with differences in soil water availability (JONES, 2007). The kinetics of soil water content (SWC) is presented in Figure 1.

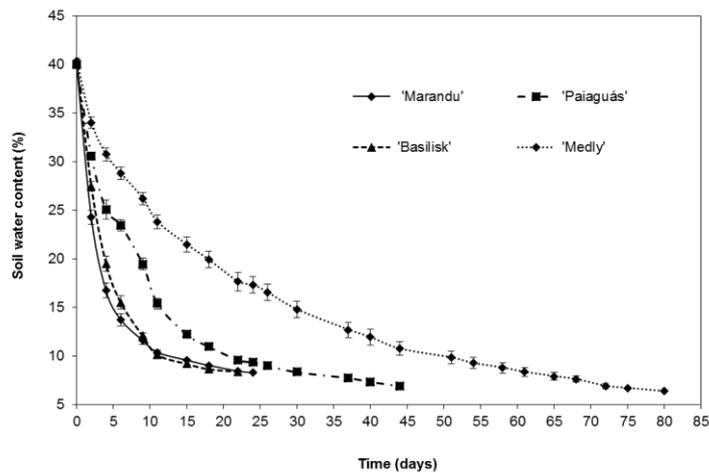


Figure 1 - Kinetics of soil water content for the four studied species after drought imposition in small pots (Exp. 1.2). 'Marandu': *U. brizantha* cv. Marandu; 'Paiguás': *U. brizantha* cv. BRS Paiguás; 'Basilisk': *U. decumbens* cv. Basilisk; 'Medly': *D. glomerata* cv. Medly.

4.2.3 Functional leaf traits under non-limiting water conditions (Exp. 1.1)

During the initial period of irrigation (from October 6 to November 24), the following quantitative traits were measured: plants maximum vegetative height (MH) per pot (2 plants per pot x 13 pots per cultivars) was determined with a ruler (precision of 0.1cm) three times per week; the maximum leaf elongation rate (MLER) was evaluated three times per week on marked tillers per cultivar. The number of tillers (NT) per plant was registered weekly. In addition, photosynthesis ($\text{mmol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) and stomatal conductance ($\text{mol m}^{-2} \text{ s}^{-1}$) were measured by an Infrared Gas Analyser-IRGA (LI 6400 XT; LiCor) on the youngest fully expanded leaf of each plant after vegetative height stabilized (06/11/2014) and on a sunny day. The temperature inside the IRGA leaf chamber was adjusted to 18.91°C, the vapor pressure deficit was maintained at 3.91 kPa and light, provided by LEDs emitting in the blue-red spectrum connected to the sampling chamber, was maintained at 1200 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$.

During the second growth cycle (11/19/2014) it was determined in 10 fully expanded leaves per cultivars, (10 pots per cultivars): the water-saturated specific leaf area (SLA; leaf area per unit of dry leaf mass; $\text{cm}^2 \text{ g}^{-1}$), the leaf dry matter content (LDMC; dry mass per unit of fresh mass; mg g^{-1}) and the leaf thickness (LT; μm), following the protocol described by Garnier et al. (2001) and leaf thickness was estimated as $1/(\text{SLA} \times \text{LDMC})$ (VILE et al., 2005). Leaf projected area was determined with an area meter (Delta-T Devices, model MK2, Cambridge, UK). All

the leaf samples were weighed, oven-dried at 60°C for 48 hours and then reweighed. On the same leaf samples, the leaf nitrogen concentration (N; g hg⁻¹) of *Urochloa* cultivars was also determined by the Kjeldahl method (SILVA, 1981). Plants were cut in 11/24/2014 (just before stopping irrigation) at either 25 cm (*Urochloa* sp.) or 8 cm (*D. glomerata*) plant height according to their different growth habit so that aerial and biomass production was determined (g DM pot⁻¹).

4.2.4 Cultivars responses to progressive drought – Dehydration tolerance (Exp. 1.2)

Three pots per cultivars were kept fully irrigated as control for three weeks and the aerial biomass of each pot was measured at the end of this period. The other ten pots per cultivars were used to assess the progressive drought impact on plants.

Predawn leaf water potential (LWP) and leaf relative water content (LRWC) were measured at five dates as drought progressed. LWP was measured on three green leaves per cultivars (same pots) with a Scholander-type (PMS Instruments Company, model 1000, Corvallis, Oregon, USA) pressure chamber. LRWC was measured as follows: $LRWC = (FW - DW)/(HW - DW)$, where FW (fresh weight) was obtained by weighing leaves immediately after harvesting; DW (dry weight) was obtained just after oven-drying the sampling leaves for 48 hours at 60°C; and HW (weight at full hydration) was measured after full rehydration, after cutting the end of the leaves in test tubes filled with deionized water during 24 hours in the dark (GARNIER et al., 2001). LRWC was also measured early after drought imposition and just before the pots were rehydrated. During the drought, the plants maximum vegetative height (MH) per pot (2 plants per pot x 10 pots per cultivars) was measured three times per week, as well as the maximum leaf elongation rate (MLER).

4.2.5 Plant survival after rehydration – Dehydration tolerance (Exp. 1.2)

Plant rehydration was performed successively when SWC reached a range from 13% until 6% associated with increasing plant senescence. Each pot was rehydrated and maintained under full irrigation during three weeks. After three weeks

of recovery, plant survival was assessed as the percentage of green tillers recovering (ratio between number of green tillers and total tillers) for each plant per pot after full rehydration. The ratio of aerial recovery biomass after rehydration and the biomass produced during the same period by the control of fully irrigated plants (BAR/BC) allowed the detection of possible compensatory growth after drought.

4.2.6 Functional root traits and dehydration avoidance (Ex. 2)

Root elongation was periodically monitored (biweekly) on long tubes (same protocol as in pots). Root elongation rate (RER; $\text{cm cm}^{-2} \text{ day}^{-1}$) and maximum root depth (MDR; cm) were measured during the irrigation period (seven weeks) and the drought period (eight weeks).

Half of the tubes (12 tubes) were harvested in 11/24/2014 (just before stopping irrigation); either at 25 cm (*Urochloa* sp.) or 8 cm (*D. glomerata*) plant height, and aerial biomass production was determined (g DM tube^{-1}). In 01/22/2014, after first signs of leaf wilting in some cultivars LWP and LRWC were measured. Both at the end of the irrigation period and at the end of the drought period, plants were harvested for measuring live and dead aerial biomass. In order to characterize root biomass distribution, the entire soil column was extracted from each tube and then divided into five sections of variable length (0–25, 25–50, 50–75, 75–100 and 100–130 cm). In each soil, section samples were collected to determine the final soil water content, after drying at 80°C for 72 hours (Figure 2).

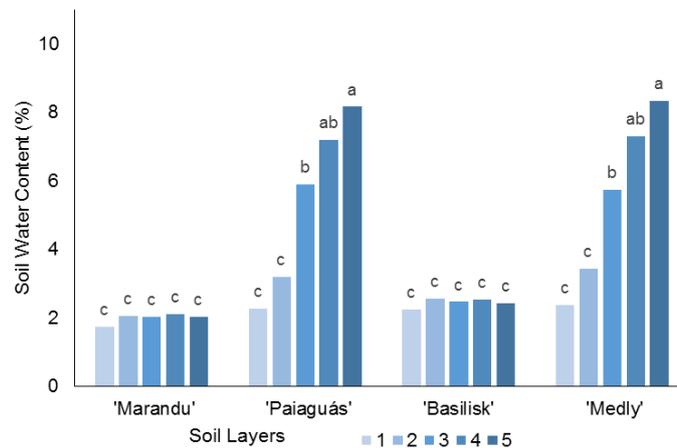


Figure 2 - Soil water content for the four studied species at the end experiment in each soil layers (Exp. 2). Soil layer: 1: 0-25 cm; 2: 25-50 cm; 3: 50-75 cm; 4: 75-100 cm; and 5: 100-130 cm. 'Marandu': *U. brizantha* cv. Marandu; 'Paiaguás': *U. brizantha* cv. BRS Paiaguás; 'Basilisk': *U. decumbens* cv. Basilisk; 'Medly': *D. glomerata* cv. Medly

For each soil section, roots were carefully washed free of soil in water and a representative sub-sample of fresh roots of the first soil layer was frozen. The root material harvested was weighed, oven-dried at 60°C for 48 hours and then reweighed. The subsample of roots from the first layer was then scanned at 400 dpi (see HUMMEL et al., 2007 for methodological details). The image analysis software Winrhizo (ver. 2003b, Regent Instruments Inc., Quebec, Canada) was used to determine length, mean diameter, area, volume of roots (as the sum of the areas and volumes in the different diameter classes), and root thickness. A few root functional traits were calculated from these measurements: specific root length (SRL; root length per unit of dry root mass; m g^{-1}), specific root area (SRA; root area per unit of dry root mass; $\text{cm}^2 \text{g}^{-1}$), mean root diameter (mm), percentage of very fine roots (<0,02 mm), fine roots (<1 mm), thick roots (1-2 mm), and big roots (2-3 mm), tissue mass density (TMDr; the ratio of root dry mass to fresh volume; g cm^{-3}) and root dry matter content (RDMC; root dry mass per unit of root fresh mass; mg g^{-1}).

Finally, the root mass fraction was calculated (RMF, root dry mass per unit of total plant dry mass (g g^{-1}), which indicates the proportional biomass investment in the underground compartment, and root: shoot ratio (R:S; g g^{-1}). Root biomass distribution along the whole soil profile was used to calculate the 95% rooting depth (D95; cm).

4.2.7 Data analysis

Statistical analyzes were accomplished with statistical package SAS (Statistical Analysis System, version 6.0). Comparisons between cultivars were tested by using analysis of variance (one-way ANOVA) and post hoc Tukey test, adopting a 5% significance level.

For the comparison of cultivars after rehydration following drought (dehydration tolerance), the plant survival was estimated using a sigmoid function to identify the SWC associated with 50% tiller survival after rehydration (SWC50). In addition, three categories of final SWC values were considered: $SWC \leq 8$, 5% , 8 , $5\% < SWC < 10\%$ and $SWC \geq 10\%$, as a factorial arrangement with three final SWC and four genotypes. Differences in recovery biomass indexed on biomass control (BAR/BR) for each of these three ranges of SWC were tested by analysis of variance (one-way ANOVA) and post hoc Tukey tests, adopting a 5% significance level.

Pearson's coefficients of correlation among plant traits aimed to identify which leaf traits were best associated with aerial biomass production potential, with dehydration tolerance (SWC50), with dehydration avoidance (D95 under irrigated and drought periods) and finally to identify if aerial biomass production under full irrigation, dehydration tolerance and avoidance were correlated (only traits which were significant in previous correlations).

As major morphological differences were observed between 'Medly' and *Urochloa* cultivars, all statistical analysis were performed first considering all four cultivars together and secondly comparing the three *Urochloa* cultivars. Results presented are generally based on the comparison of all four cultivars.

4.3 Results

4.3.1 Potential of aerial biomass production and leaf traits under full irrigation

Urochloa cultivars differed from 'Medly' for all morphological traits (Table 1). For most of the traits, no differences were observed among *Urochloa* sp. cultivars (maximum vegetative height, maximum leaf elongation rate and stomatal conductance, leaf dry matter content, specific leaf area, leaf thickness and specific

leaf biomass; Table 1). However, the number of tiller and aerial biomass production were higher for 'Basilisk' than for 'Marandu' (Table 1).

Photosynthesis efficiency was similar between the *U. brizantha* cultivars ('Marandu' and 'BRS Paiaguás') and higher than that of 'Basilisk' (Table 1), suggesting that photosynthesis may be a species inherent response. Leaf nitrogen concentration was the highest for 'BRS Paiaguás', intermediate for 'Marandu' and the lowest for 'Basilisk' (Table 1).

Table 1. Plant traits and Pearson's correlation coefficients between aerial biomass production under full irrigation.

Traits	'Marandu'	'Paiaguás'	'Basilisk'	'Medly'	Correlation ABP
MH	91.50 ^a	89.13 ^a	90.87 ^a	37.48 ^b	0,84
MLER	3.96 ^a	3.63 ^a	4.31 ^a	1.68 ^b	0,7
NT	40.65 ^b	45.58 ^a	45.33 ^a	31.86 ^c	0,37
PS	31.76 ^a	34.06 ^a	24.64 ^b	12.68 ^c	0,58
SC	0.17 ^b	0.17 ^b	0.16 ^b	0.22 ^a	-0,61
LDMC	0.19 ^b	0.18 ^b	0.19 ^b	0.22 ^a	-0,37
SLA	32.76 ^b	34.34 ^b	33.62 ^b	42.36 ^a	-0,65
LT	0.16 ^a	0.16 ^a	0.16 ^a	0.11 ^b	0,81
LMA	0.03 ^a	0.03 ^a	0.03 ^a	0.02 ^b	0,58
N	28.48 ^{ab}	30.64 ^a	26.84 ^b	—	-0,53
ABP	14.00 ^b	9.36 ^c	19.07 ^a	1.07 ^d	

Letters show significant differences among the four genotypes, according to one-way ANOVA and Tukey tests ($P < 0.05$); Significant correlations are shown in bold ($P < 0.05$); Analysis of N were tested only *Urochloa* cultivars; MH: maximum vegetative height (cm); MLER: maximum leaf elongation rate (cm d⁻¹); NT: number of the tillers (pot⁻¹); PS: Photosynthesis (mmol m⁻² s⁻¹); SC: stomatal conductance (mol m⁻² s⁻¹); LDMC: leaf dry matter content (g g⁻¹); SLA: specific leaf area (m² kg⁻¹); LT: leaf thickness (μm); LMA: specific leaf biomass per unit area (kg m⁻²); N: leaf nitrogen concentration (g kg⁻¹); ABP: Aerial biomass production: dry biomass above cutting height in second cycle (g pot⁻¹); 'Marandu': *U. brizantha* cv. Marandu; 'Paiaguás': *U. brizantha* cv. BRS Paiaguás; 'Basilisk': *U. decumbens* cv. Basilisk; 'Medly': *D. glomerata* cv. Medly.

Aerial biomass production was positively correlated to maximum vegetative height, maximum leaf elongation rate, photosynthetic efficiency, leaf dry matter content, specific leaf area, leaf thickness and leaf area, while it was negatively correlated to stomatal conductance and specific leaf biomass (Table 1). The aerial biomass production of 'BRS Paiaguás' is 40% to 100% lower than of 'Basilisk' and 'Marandu', respectively. In these environmental conditions, 'Medly' produces very little aerial biomass.

4.3.2 Dehydration tolerance under progressive drought in pots

Maximum vegetative height and maximum leaf elongation rate decreased during drought, but were similar among *Urochloa* cultivars (Table 2). The first wilting signs were observed at around 10 days after irrigation cessation in 'Marandu' (~12% SWC) and 'Basilisk' (~13% SWC). The last pot of each of these cultivars was rehydrated after 24 and 22 days, respectively. The first signs of wilting in 'BRS Paiaguás' occurred after 20 days of drought when SWC was ~11%, and the last pot of this cultivar was irrigated on day 44 of drought. 'Medly' started wilting at ~11% SWC, after 43 days of drought. Some 'Medly' pots were rehydrated when reached a SWC between at around 13-12% to allow comparisons between *Urochloa* and *D. glomerata* cultivars at similar soil water contents.

Table 2. Plant traits measured under drought and after rehydration in pots and Pearson's correlation coefficients between dehydration tolerance (soil water content corresponding to 50% tiller survival after rehydration - SWC50) and other variables.

Traits	'Marandu'	'Paiaguás'	'Basilisk'	'Medly'	Correlation	
					Traits	SWC50
MH	54.41 ^a	55.94 ^a	56.42 ^a	25.29 ^b	MLER	0,42
MLER	2.15 ^a	1.66 ^a	1.73 ^a	0.54 ^b	LWP	-0,75
SWC50	8.55 ^a	7.53 ^c	8.26 ^{ab}	7.69 ^{bc}	LRWC	-0,91
					BAR/BC	0,64

Letters show significant differences among the four cultivars, according to one-way ANOVA and Tukey tests ($P < 0.05$); MH: maximum vegetative height (cm); MLER: maximum leaf elongation rate (cm d^{-1}); SWC50: soil water content corresponding to 50% tiller survival after rehydration (%); BAR/BC: biomass after rehydration on aerial biomass production of control irrigated plants; LWP: leaf water potential (MPa); LRWC: leaf relative water content (%); 'Marandu': *U. brizantha* cv. Marandu; 'Paiaguás': *U. brizantha* cv. BRS Paiaguás; 'Basilisk': *U. decumbens* cv. Basilisk; 'Medly': *D. glomerata* cv. Medly.

Leaf relative water content and leaf water potential decreased after irrigation cessation for all cultivars (Figure 3). As a function of declining SWC, the evolution of water status seems similar for all cultivars. 'Medly' and 'BRS Paiaguás' cultivars used less water during the early part of the drought (Figure 3), than 'Marandu' and 'Basilisk'. However, after ~20 days of stress or ~12% SWC, leaf relative water content of 'BRS Paiaguás' significantly decreased.

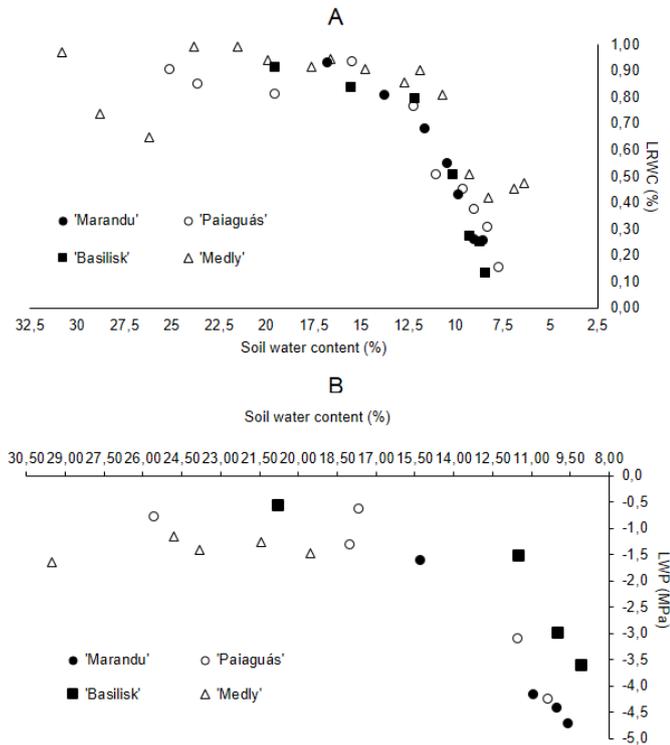


Figure 3. A - Leaf relative water content (LRWC) and B - Leaf water potential (LWP) for the four genotypes after drought imposition in pots. 'Marandu': *Urochloa brizantha* cv. Marandu; 'Paiaguás': *U. brizantha* cv. BRS Paiaguás; 'Basilisk': *U. decumbens* cv. Basilisk; 'Medly'; *D. glomerata* cv. Medly.

Forage senescence began to increase significantly at around 10% SWC. All cultivars were able to recover following rehydration after a period of severe drought (Fig. 5), but plant survival rates, measured through the tiller survival rate, differed significantly among cultivars, as may be seen with the calculation of SWC associated with 50% tiller survival (Table 2, Figure 4). Marandu ($8.55\% \pm 0.20$) and 'Basilisk' cultivar ($8.26\% \pm 0.26$) have a similar dehydration tolerance. 'BRS Paiaguás' cultivar ($7.53\% \pm 0.12$) was significantly more dehydration tolerant than 'Marandu' and 'Basilisk', but not than 'Medly' ($7.69\% \pm 0.46$). The SWC₅₀ of 'Medly', although with a high standard error, was lower than that of 'Marandu' but not significantly different from that of 'Basilisk' and 'BRS Paiaguás'.

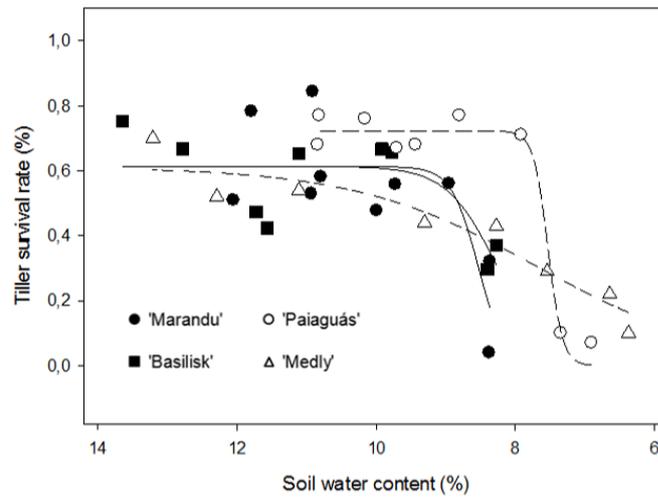


Figure 4. Tiller survival rate (%) after rehydration as a function of soil water content before rehydration. For four cultivars 'Marandu': *U. brizantha* cv. Marandu; 'Paiguás': *U. brizantha* cv. BRS Paiguás; 'Basilisk': *U. decumbens* cv. Basilisk; 'Medly': *D. glomerata* cv. Medly.

At each level of final SWC, the biomass indexed recovery DBAR/DBC was greater for 'BRS Paiguás' than for 'Marandu' and 'Basilisk' (Figure 5). 'Medly' ranked second for biomass recovery.

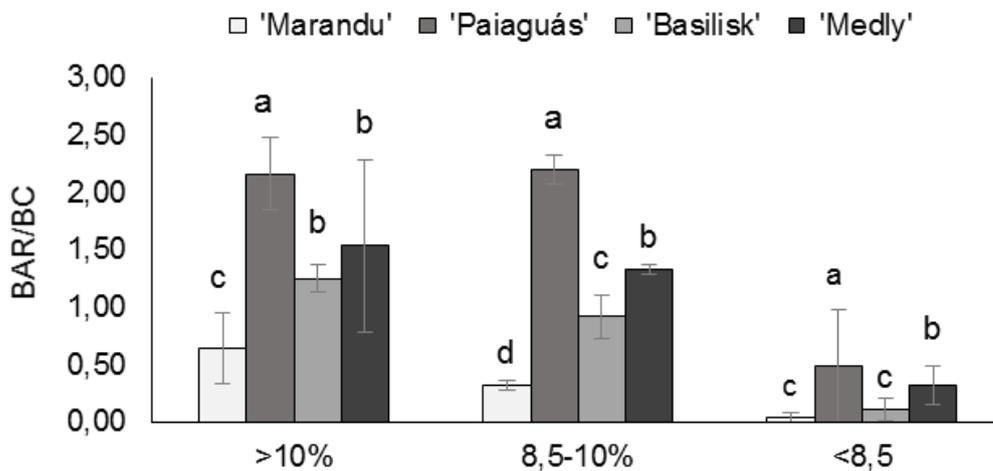


Figure 5. Ratios between aerial biomass after rehydration and aerial biomass of control irrigated plants (DBAR/DBC) for four cultivars at three levels of soil water content (SWC; above 10%, from 8.5 to 10%, and below 8.5%). 'Marandu': *U. brizantha* cv. Marandu; 'Paiguás': *U. brizantha* cv. BRS Paiguás; 'Basilisk': *U. decumbens* cv. Basilisk; 'Medly': *D. glomerata* cv. Medly.

The measurement of SWC₅₀, as the main variable for dehydration tolerance, was negatively correlated with DBAR/DBC, LWP and LRWC (Table 2).

4.3.3 Dehydration avoidance and root traits in long tubes

Under non-limiting water supply, the root elongation rate (RER) was the highest for 'Marandu' and 'Basilisk', intermediate for 'BRS Paiaguás' and the lowest for 'Medly' (Table 3). As a result maximum root depth (MDR) and 95% rooting depth (D95) were 1.5 to 3 fold higher for 'Marandu' and 'Basilisk' than for 'BRS Paiaguás' and 'Medly' (Table 3).

Under drought, the RER of 'Marandu', 'Basilisk' and 'Medly' decreased more than of 'BRS Paiaguás' (Table 3). This cultivar exhibited higher values of RER during the drought period compared to all the other cultivars and reached the deepest soil layer (127.60 cm) that was similar to the rooting depth of other *Urochloa* cultivars, while 'Medly' roots reached a maximum of 88 cm (Table 1). However, the 95% root systems depth (D95) was similar between 'BRS Paiaguás' and 'Medly' at the end of the drought period (Table 3).

The root: shoot ratio and root mass fraction varied between treatments (Table 3). Under full irrigation, they were higher for 'Marandu' while after drought imposition they were higher for 'Medly'. At similar dates, leaf hydration (LRWC) and leaf potential (LWP) were significantly higher in 'BRS Paiaguás' and 'Medly', since they both maintained greater SWC for longer time (Table 3, Figure 2).

Regarding root traits of the 0-20 cm soil layer and for irrigated plants, 'Medly' exhibited higher specific root area and length, root tissue density and percentage of very fine roots than *Urochloa* cultivars (Table 3). Mean root volume, percentage of fine root and mean root diameter were higher in *Urochloa* cultivars (Table 3). During drought, root dry mass content was higher for 'Medly', while the other traits followed the same pattern observed before drought. Total water uptake during drought was around 15% higher for 'Marandu' and 'Basilisk' than for the other two cultivars.

Table 3. Aerial biomass and root biomass – root depth at whole plant level. Root traits were measured on 0-25 cm depth roots in a long tubes experiment under successively full irrigation and drought. And leaf relative water content and leaf water potential measured in final drought period.

Traits	Full irrigation				Drought			
	Marandu'	'Paiaguás'	'Basilisk'	'Medly'	'Marandu'	'Paiaguás'	'Basilisk'	'Medly'
RER	2.66 ^a	1.83 ^b	2.42 ^a	1.34 ^c	0.34 ^b	0.88 ^a	0.38 ^b	0.38 ^b
MRD	125.17 ^a	85.85 ^b	113.93 ^a	63.00 ^c	127.70 ^a	127.60 ^a	127.80 ^a	84.00 ^b
DLB	1.80 ^a	0.48 ^b	0.90 ^{ab}	0.37 ^b	2.08 ^{ab}	1.50 ^{b^c}	2.85 ^a	0.19 ^c
DDB	–	–	–	–	0.10 ^a	0.07 ^a	0.13 ^a	0.03 ^b
R:S	0.86 ^a	0.54 ^b	0.49 ^b	0.54 ^b	0.71 ^{ab}	0.48 ^b	0.42 ^b	0.91 ^a
RMF	0.46 ^a	0.35 ^b	0.33 ^b	0.35 ^b	0.41 ^{ab}	0.32 ^{bc}	0.30 ^c	0.47 ^a
D95	90.28 ^a	38.16 ^b	82.93 ^a	35.39 ^b	107.50 ^a	55.33 ^b	99.85 ^a	40.62 ^b
RDMC	187.48 ^a	179.16 ^a	193.02 ^a	205.95 ^a	260.39 ^b	208.40 ^b	228.77 ^b	347.79 ^a
SRL	60.23 ^b	78.19 ^b	86.65 ^b	292.01 ^a	71.00 ^b	62.50 ^b	96.78 ^b	230.73 ^a
SRA	49.94 ^c	59.75 ^{bc}	72.25 ^b	111.94 ^a	50.49 ^b	51.91 ^b	59.36 ^b	83.23 ^a
Volume	0.51 ^a	0.23 ^b	0.29 ^b	0.10 ^c	0.32 ^a	0.26 ^{ab}	0.23 ^b	0.10 ^c
RTD	0.15 ^b	0.20 ^{ab}	0.16 ^b	0.26 ^a	0.28 ^b	0.25 ^b	0.27 ^b	0.42 ^a
Vfine	14.74 ^b	12.68 ^b	11.62 ^b	69.31 ^a	14.70 ^c	9.92 ^c	28.15 ^b	72.92 ^a
Fine	82.27 ^a	86.37 ^a	87.57 ^a	30.68 ^b	84.89 ^a	89.66 ^a	71.43 ^b	27.08 ^c
Thick	2.38 ^a	0.94 ^{ab}	0.81 ^{ab}	0.002 ^b	0.41 ^a	0.42 ^a	0.42 ^a	0.00 ^b
Big	0.61 ^a	0.01 ^b	0.00 ^b	0.00 ^b	–	–	–	–
Diam	0.26 ^a	0.24 ^a	0.27 ^a	0.12 ^b	0.21 ^b	0.25 ^a	0.20 ^b	0.11 ^c
LRWC					0.53 ^b	0.99 ^a	0.62 ^b	0.88 ^a
LWP					4.87 ^b	0.52 ^a	3.09 ^{ab}	1.48 ^{ab}
ETP					4156.61 ^a	3471.22 ^b	4069.40 ^a	3453.51 ^b
WUEa					2.0x10 ^{-3a}	1.1x10 ^{-3b}	1.8x10 ^{-3a}	3.4x10 ^{-4c}
WUEt					3.6x10 ^{-3a}	1.6x10 ^{-3b}	2.6x10 ^{-3b}	6.6x10 ^{-4c}

Letters show significant differences among the four genotypes, according to one-way ANOVA and Tukey tests ($P < 0.05$). DLB: dry life biomass (g tube^{-1}); DDB: dry dead biomass (g tube^{-1}); RER: root elongation rate (cm d^{-1}); MDR: maximum depth root (cm); R:S: root:shoot ratio; RMF: root dry mass per unit of total plant dry mass (g g^{-1}); D95: 95% rooting depth (cm); RDMC: root dry matter content – root dry mass per unit of root fresh mass (mg g^{-1}); SRL: Specific root length – root length per unit of dry root mass (m g^{-1}); SRA: Specific root area - root urea per of dry root mass ($\text{cm}^2 \text{g}^{-1}$); Volume: mean root volume (cm^3); RTD: Root tissue density – the ratio of root dry mass to fresh volume (g cm^3); Vfine: percentage of very fine root < 0.02 mm (%); Fine: percentage of fine root < 1 mm (%); Thick: percentage of thick root 1-2 mm (%); Big: percentage of big root 2-3 mm (%); Diam: mean root diameter (mm); LRWC: leaf relative water content (%); LWP: leaf water potential (MPa); ETP: evapotranspiration (g); WUEa: water use efficiency on the total aerial biomass ($\text{g DM g H}_2\text{O}^{-1}$); WUEt: water use efficiency on the total mass of the plants – root + shoot ($\text{g DM g H}_2\text{O}^{-1}$); 'Marandu': *U. brizantha* cv. Marandu; 'Paiaguás': *U. brizantha* cv. BRS Paiaguás; 'Basilisk': *U. decumbens* cv. Basilisk; 'Medly': *D. glomerata* cv. Medly.

There were some differences between Pearson's correlation analysis performed with all four cultivar and only with *Urochloa* cultivars (Table 4). The main differences were observed on the correlations between D95 and variables measured during irrigated and drought periods (Table 4).

Table 4. Pearson's correlation coefficients to traits related with dehydration avoidance for four cultivars and for *Urochloa* cultivars.

	D95-IR -4 cvs.	D95-IR- <i>Urochloa</i>	D95-DR -4 cvs.	D95-DR- <i>Urochloa</i>
LRWC	-0.89	-0.59	-0.81	-0.84
LWP	-0.63	-0.59	-0.63	-0.63
D95 _{IR}	1	1	0.38	0.15
D95 _{DR}	0.38	0.15	1	1
RER _{IR}	0.62	0.59	0.9	0.85
RER _{DR}	-0.43	-0.57	-0.23	-0.93
MDR _{IR}	0.62	0.59	0.9	0.85
MDR _{DR}	0.26	0.008	0.87	0.28
R:S _{IR}	0.74	0.74	0.46	0.32
R:S _{DR}	0.17	0.94	-0.46	0.07
DLB _{IR}	0.9	0.9	0.43	0.21
DLB _{DR}	0.16	-0.2	0.9	0.77
DDB _{DR}	0.03	-0.11	0.47	0.31
RMF _{IR}	0.77	0.77	0.46	0.31
RMF _{DR}	0.26	0.93	-0.46	0.03
RDMC _{IR}	-0.43	-0.35	0.06	0.57
RDMC _{DR}	0.01	0.62	-0.56	0.31
SLR _{IR}	0.24	-0.69	-0.14	0.51
SLR _{DR}	-0.34	-0.21	0.66	0.51
SRA _{IR}	-0.77	-0.73	-0.16	0.45
SRA _{DR}	0.37	-0.23	-0.63	0.1
Volume _{IR}	0.96	0.96	0.37	-0.005
Volume _{DR}	0.57	0.83	0.65	-0.1
RTD _{IR}	-0.81	-0.83	-0.3	0.17
RTD _{DR}	-0.21	0.36	-0.63	0.48
Vfine _{IR}	-0.67	-0.69	-0.13	0.57
Vfine _{DR}	0.36	-0.28	0.59	0.76
Fine _{IR}	0.65	0.57	0.12	-0.59
Fine _{DR}	0.37	0.29	0.59	-0.77
Thick _{IR}	0.96	0.94	0.28	-0.12
Thick _{DR}	-0.02	-0.26	0.67	0.41
Big _{IR}	0.72	0.68	0.41	0.29
Diam _{IR}	0.76	0.71	0.19	-0.45
Diam _{DR}	0.18	-0.21	0.42	-0.94
SWC	-0.54	-0.45	-0.94	-0.96

Significant correlations are shown in bold ($P < 0.05$); variables 'IR' mean during irrigated period and 'DR' during drought period; LRWC: leaf relative water content (%); LWP: leaf water potential (MPa); D95: 95% rooting depth (cm); RER: root elongation rate (cm d^{-1}); MDR: maximum depth root (cm); R:S: root:shoot ratio; DLB: Dry life biomass (g tube^{-1}); DDB: dry dead biomass (g tube^{-1}); RMF: root dry mass per unit of total plant dry mass (g g^{-1}); RDMC: root dry matter content – root dry mass per unit of root fresh mass (mg g^{-1}); SRL: Specific root length – root length per unit of dry root mass (m g^{-1}); SRA: Specific root area - root area per of dry root mass ($\text{cm}^2 \text{g}^{-1}$); Volume: mean root volume (cm^3); RTD: Root tissue density – the ratio of root dry mass to fresh volume (g cm^3); Vfine: percentage of very fine root < 0.02 mm (%); Fine: percentage of fine root < 1 mm (%); Thick: percentage of thick root 1-2 mm (%); Big: percentage of big root 2-3 mm (%); Diam: mean root diameter (mm); SWC: Soil water content (%).

4.3.4 Person's correlation between traits related to resource-use and water stress response strategies

Person's correlation showed that aerial biomass production under irrigation, dehydration tolerance and dehydration avoidance were correlated. Biomass production was positively correlated with SWC50, showing that the most productive cultivars under irrigation were the least tolerant to severe dehydration. Moreover, a

high dehydration avoidance was negatively correlated to a high dehydration tolerance since D95, under irrigated and drought conditions, was positively correlated to biomass production and SWC50 (Table 5). And belowground in long tubes, root traits (D95_{DR}) was highly correlated with WUE_t. Genotypes with more aerial production also were the that showed greater more root systems allowing a greater WUE_t.

Table 5. Pearson's correlation coefficients to traits related of biomass production under full irrigation, dehydration tolerance and dehydration avoidance for four cultivars.

	ABP	SWC50	D95-IR	D95-DR	EPT	WUE _a	WUE _t
ABP	1,00	0.56	0.82	0.89	0.76	0.82	0.72
SWC50	0.56	1,00	0.82	0.69	0.82	0.63	0.68
D95-IR	0.82	0.82	1,00	0.88	0.87	0.82	0.82
D95-DR	0.88	0.69	0.88	1,00	0.94	0.91	0.87
MH	0.84	0.35	0.60	0.75	0.56	0.81	0.74
EPT	0.77	0.82	0.87	0.94	1,00	0.87	0.88
WUE _a	0.82	0.63	0.82	0.91	0.87	1,00	0.97
WUE _t	0.72	0.68	0.82	0.87	0.88	0.97	1,00
MLER	0.7	0.19	0.44	0.65	0.41	0.69	0.63
PS	0.58	0.20	0.39	0.43	0.29	0.59	0.58
SC	-0.61	-0.37	-0.40	-0.54	-0.53	-0.67	-0.58
LMA	0.58	0.56	0.55	0.55	0.52	0.64	0.64
SLA	-0.65	-0.49	-0.59	-0.54	-0.47	-0.64	-0.62
LT	0.81	0.36	0.61	0.77	0.59	0.86	0.78
BAR/BC	-0.48	-0.92	-0.75	-0.68	-0.83	-0.68	-0.74
LRWC	-0.63	-0.91	-0.89	-0.81	-0.89	-0.68	-0.72
LWP	-0.38	-0.75	-0.67	-0.63	-0.75	-0.70	-0.76
RER-IR	0.85	0.82	0.92	0.90	0.89	0.92	0.92
RER-DR	-0.18	-0.68	-0.63	-0.23	-0.42	-0.11	-0.20
MDR-IR	0.85	0.82	0.92	0.90	0.89	0.92	0.92
R:S-IR	0.18	0.42	0.50	0.46	0.57	0.60	0.75
DBF-IR	0.26	0.50	0.58	0.43	0.56	0.61	0.76
RMF-IR	0.20	0.42	0.52	0.46	0.57	0.59	0.74
SRL-DR	-0.73	-0.22	0.47	-0.66	-0.44	-0.73	-0.67
Diam-DR	0.55	-0.03	0.23	0.42	0.16	0.54	0.47

Significant correlations are shown in bold ($P < 0.05$); In variables 'IR' mean during irrigated period and 'DR' during drought period; ABP : Aerial biomass production: dry biomass above cutting height in second cycle (g pot^{-1}); SWC50: soil water content corresponding to 50% tiller survival after rehydration (%); D95: 95% rooting depth (cm); MH: maximum vegetative height (cm); MLER: maximum leaf elongation rate (cm d^{-1}); PS: Photosynthesis ($\text{mmol m}^{-2} \text{s}^{-1}$); SC: stomatal conductance ($\text{mol m}^{-2} \text{s}^{-1}$); LMA: specific leaf biomass per unit area (kg m^{-2}); SLA: specific leaf area ($\text{m}^2 \text{kg}^{-1}$); LT: leaf thickness (μm); BAR/BC: aerial recovery biomass after rehydration aerial biomass of irrigated control plants; LRWC: leaf relative water content (%); LWP: leaf water potential (MPa); RER: root elongation rate (cm d^{-1}); MDR: maximum depth root (cm); R:S: root:shoot ratio; DLB: Dry life biomass (g tube^{-1}); RMF: root dry mass per unit of total plant dry mass (g g^{-1}); SRL: Specific root length – root length per unit of dry root mass (m g^{-1}); Diam: mean root diameter (mm); ETP: evapotranspiration (g); WUE_a: water use efficiency on the total aerial biomass ($\text{g DM g H}_2\text{O}^{-1}$); WUE_t: water use efficiency on the total mass of the plants – root + shoot ($\text{g DM g H}_2\text{O}^{-1}$); MDRdif: difference maximum depth root between drought and irrigated conditions (cm); RERdif: difference root elongation rate between drought and irrigated conditions (cm d^{-1}).

4.4 Discussion

4.4.1 Resource-use strategies varies among *Urochloa* cultivars

Urochloa cultivars explored more the available resources, adopting a resource acquisition strategy when compared to 'Medly'. Plants maximum height, leaf maximum elongation rate and number of tillers were higher for *Urochloa* cultivars (Table 1), suggesting a higher photosynthetic leaf area and capture of light, and a lower transpiring leaf area of these cultivars when compared to the Mediterranean 'Medly' cultivar. Furthermore, root elongation rate of *Urochloa* cultivars was higher than 'Medly'.

Traits of fast-growing species, that maximize light capture through an investment in leaves production (WRIGHT et al., 2004; PÉREZ-RAMOS et al., 2013) represent a competitive advantage in foraging for water and nutrients since greater C acquisition could allow the maintenance of higher root elongation rates in full irrigation and a deepest exploitation of soil profile (PÉREZ-RAMOS et al., 2013). Despite the benefits, however, a larger photosynthetic surface implies higher transpiring leaf area (LAMONT et al., 2002; ESCUDERO et al., 2008) consequently, in wasteful use of water.

Although a higher SLA and lower thickness are related most closely with a resource acquisition strategy (PEREZ-RAMOS et al., 2013), differences observed in the presented experiment does not seem to be related to the genotypes resource use strategy. Ripley et al. (2008), observed a higher specific leaf area of C3 *Alleoteropsis semilata* subspecies compared to the C4 subspecies. The higher specific leaf area and lower leaf thickness of the C3 'Medly' compared to C4 *Urochloa* cultivars may be related to a photosynthetic group more than to a resource use strategy.

Resource use strategies also seems to vary between *Urochloa* cultivars. 'BRS Paiaguás' traits observed aboveground and underground during long tube experiments were more similar to 'Medly' than to others *Urochloa* cultivars, suggesting that this plant uses a conservation of resource use strategy. Besides, a higher leaf nitrogen concentration of 'BRS Paiaguás' suggest that it accumulates and preserves resources in the same way as nutrient saver grasses (Table 1).

The discrimination of two groups in this study suggest that different strategies (acquisitive versus conservative) in full irrigation, evaluated in small pots and long tubes, could be useful to understand plant performances under subsequent drought imposition.

4.4.2 Are potential of biomass production correlated to dehydration tolerance and dehydration avoidance?

Dehydration avoidance strategies may be related to mechanisms that improve water acquisition or water conservation. Experiments in small pots avoids the expression of water acquisition mechanisms, while it allows the evaluation of the water conservation components of dehydration avoidance strategy (SUGIYAMA; NIKARA, 2004). 'BRS Paiaguás' and 'Medly' cultivars maintained long-term water uptake and water tissues status, high LRWC and LPW values, thus ensuring survival for longer periods than 'Marandu' and 'Basilisk' (Figure 3, 4 and 5). The main mechanism of drought survival used by 'Medly' and 'BRS Paiaguás' seems to be related to reduction of water consumption by limiting biomass production (Table 1), which is positively correlated with transpiration (RITCHIE, 1983). These mechanisms, in addition to a greater senescence at higher SWC, reduce water loss and are considered a component of dehydration avoidance. Differently, 'Marandu' and 'Basilisk' extracted more water from soil during the initial phase of water restriction (Figure 1), and showed an aggressive response, with high vegetative growth, leaf elongation rate and maximum vegetative height (Table 1) during low and moderate drought, but reduced survival under severe water deficit (Figure 4 and 5), (SINCLAIR; LUDLOW, 1986). The increase on senescence observed on 'Marandu' and 'Basilisk' plants under severe drought conditions, seems to be a prelude of plant death in susceptible cultivars instead of a strategy to avoid it (NORTON et al., 2014) by a reduction on water consumption.

Results from the long tubes experiment suggest that, the drought sensibility of the 'Marandu' and 'Basilisk', is due to its roots being able to access water in the soil profile. The ability to extract more water from deeper zones of the soil profile is one strategy of dehydration avoidance to maintain high water status (CHAVES et al., 2003), and it could also be employed by perennial species to keep a consistent water supply to attend the demand during water deficit (VOLLAIRE et al., 2009), it reinforces

the result that Marandu and Basilisk exhibit a more efficient dehydration avoidance than 'BRS Paiaguás' and 'Medly'. Under a long and severe stress, they use up all water to keep growing with a high WUE. On the other hand they have a lower dehydration tolerance. There is a trade-off between both strategies. This mechanism may be activated just by plants cultivated on deep soils. Otherwise, this mechanisms of dehydration avoidance may be more harmful than beneficial, as this plants may be considered less efficient on delaying leaf dehydration than the species exhibiting a resource conservation strategy (PEREZ-RAMOS et al., 2013).

Besides a lower production, 95% of 'BRS Paiaguás' and 'Medly' roots were placed in the most superficial layers of the soil, what ensured a high water saving strategy (Table 1; Figure 2). Shallow roots could be more efficient in water limited and less productive sites (SCHENK; JACSON, 2002), once the energy costs for construction, maintenance and resource uptake may be non-viable or wasteful in this type of habitat (ADIKU et al., 2000).

In this study, the greater values related with water status (LRWC and LWP) were negatively correlated with D95 during irrigated period, and positively correlated with SWC50 after rehydration period (Table 5); LRWC was also negatively correlated with aerial biomass production (Table 5). These suggest the existence of a trade-off between the traits favoring fast-growing species and plant survival. Both 'BRS Paiaguás' and 'Medly' cultivars saved energy and water during the irrigated period, allowing root development and better exploitation of soil and water during the drought period, which confirms that maximizing water uptake during drought is an important mechanism for maintenance of the leaf lamina hydration (PASSIOURA, 1981; VOLAIRE et al., 2009; LELIERE et al., 2011). The *Urochloa* cultivars showed greater levels of water stress in leaf (Figure 3), that may be related to higher cost of total root system maintenance, and it consequently conducted to a rapid exhaustion of the plant after water restriction.

Compensatory growth was observed in all cultivars after rehydration (Figure 5), but as drought level was intensified, compensatory growth could only be observed on more tolerant cultivars. This tolerance was estimated as the soil moisture associated with 50% of plant survival evaluated after rehydration. Those cultivars that showed lower SWC50 are dehydration tolerant due to preservation of meristems hydration status. Basal tissues exhibit the greatest osmotic adjustment relative to

other tissues during drought events (MUNNS et al., 1979; MATSUDA; RIAZI, 1981; WEST et al., 1990), and can often still regenerate when the adult laminae are dead (VAN PEER et al., 2004). Besides, this mechanism has been shown to be potentially efficient in adapted genotypes, since high concentrations of fructans and dehydrins contribute to osmoregulation and membrane stabilization of these tissues (HINCHA et al., 2000, 2002), and high carbohydrate reserves are associated with superior plant resilience and recovery after severe drought (VOLLAIRE et al., 2014). This plant response illustrates the trade-off between growth and mortality (Sibly, 1989); it shows the best plants that survive a stress are those that allocate the least resource to growth (REICH, 2014).

Cultivar 'BRS Paiaguás' showed the lower SWC50, followed by 'Medly'. Imposed water deficit was not severe enough to cause total senescence of 'Medly' plants. Besides, greater SWC50 than 'BRS Paiaguás', 'Medly' plants showed dehydration tolerance through less pronounced decrease in the survival rate of tillers when compared with 'BRS Paiaguás' (Figure 4). Based on the knowledge of these critical levels of water stress for each cultivar, management strategies could also be employed by perennial species to keep a consistent water supply to attend the demand during water deficit (VOLLAIRE et al., 2009).

The results of this study confirm that there are wide genetic variability among and within species of *Urochloa* genus in response to water stress (GUENNI et al., 2002). Dehydration avoidance and tolerance are clearly not achieved by a single combination of traits (VALLADARES; SANCHES-GOMEZ, 2006; NAVAS et al., 2010). However, it may be considered that 'Marandu' and 'Basilisk' are more dehydration avoiders, while 'BRS Paiaguás' and 'Medly' are more tolerant to dehydration, determined by how much it regulates its water potential as water soil deficit increased (KRAMER, 1980; LUDLOW, 1989). And also by contrast on the depth of the root system, 'Marandu' and 'Basilisk' are efficient in water soil extraction, if established in deep soils.

4.5 Conclusions

In general, *Urochloa* cultivars (C4) are less dehydration tolerant to drought than Mediterranean cultivar cv. Medly (C3). However, there are variations among and within *Urochloa* cultivars in terms of resource use strategy and response to drought.

The most productive cultivars, *Urochloa brizantha* cv. Marandu and *Urochloa decumbens* cv. Basilisk, are more 'dehydration avoiders' and have a low dehydration tolerance associated to a lesser survival under severe drought. Conversely, *Urochloa brizantha* cv. BRS Paiaguás is less productive and more dehydration tolerant hence able to survive at lower dehydration under severe drought.

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5 GENERAL CONCLUSIONS

In future, the maintenance of many pastures areas will probably depend on the of technology and increased productivity, seeking a competitive advantage with regards to others activites, or else, on its relocation to marginal areas where forages grasses will be more suitable to abiotic factors. The identification of tropical forage genotypes more tolerant to cope with abiotic stress is essential for the buffering of environmental fluctuations and to guarantee resilience of grasslands.

Results from the study with *Paspalum* accessions suggest that there are intra- and interspecific variability among genotypes. In this experiment, cv. Marandu showed sensitivity to both kinds of water stress studied (drought and flooding). BRS Guar showed to be tolerant to flooding and BRA 23469 showed high drought survival rates. Yet, BGP 397, BGP 402 and BGP 293 acessions have adaptive responses to both flooding and to drought. The results indicate that tiller survival rate and recovery index of the root system may contribute to the distinction among genotypes in terms of response to water stress and that species *Paspalum* genus may be potentially used to improve genetic diversification of Brazilian grasslands and its resilience.

Traditionally, tolerance to water deficit has been evaluated as the capacity to maintain plants growth and production under stressful conditions. Under extreme drought conditions, predicted by future climate change scenarios, the ability to survive severe drought seems to be more important to guarantee grassland resilience and long-term production than the capacity to keep elevated growth rates. Drought survival of *Urochloa* cultivars and of *D. glomerata* was evaluated by a method that allows the characterization of diferent strategies to cope with severe drought conditions. Using small pots to analyse dehydration tolerance traits and long tubes to analyse dehydration avoidance traits, it was possible to determine mechanisms used by cv. BRS Paiagus to be more drought tolerant and to survive under severe drought conditions.

The soil water content associated with 50% tiller survival after rehydration (SWC50) was the main trait used to evaluated dehydration tolerance, and seems to be suitable to discriminate among cultivars. The cv. BRS Paiagus showed the lower SWC50, due to the preservation of meristems hydration status. Two groups of

genotypes could be discriminated during the study: cv. Marandu and cv. Basilisk that were considered more dehydration avoider'; and: cv. BRS Paiaguás and cv. Medly that were classified as 'more tolerant to dehydration'.

A trade-off between growth and mortality was also observed during this experiment. The most productive cultivars, that allocated most resources to growth, had the lower capacity to survive under severe drought conditions.

It was concluded that there are variability among and within species from *Paspalum* and *Urochloa* in terms of water stress responses. This genetic diversity may contribute to specific improvement programs to select plants more suitable to extreme climate conditions.