**Abstract**

The diversification of forage grasses is a strategic solution to obtain higher productivity in diverse environments. In this regard, the objective of the present study was to evaluate in a glasshouse study the flooding tolerance of 9 cultivars of forage grasses. The study was conducted using a complete randomized design with a 9 x 3 factorial arrangement: 9 cultivars (Brachiaria brizantha cvs. Marandu, Piatã e Xaraés; hybrid Brachiaria cv. Mulato II; B. humidicola cvv. Llanero and Tupi; B. ruziziensis cv. Common; Panicum maximum cvv. Massai and Tanzânia) and 3 soil water levels: a) minimal water for development (50% of field capacity); b) field capacity; and c) flooded soil (2 cm above soil level), with 3 replicates. Forage accumulation, plant height and root accumulation were evaluated. All cultivars grew well in soil at 50% field capacity highlighting their adaptation to mildly dry conditions. Under flooded conditions, B. humidicola cvv. Llanero and Tupi showed no reduction in forage dry matter production, while shoot growth of cvv. Marandu, Piatã, Tanzânia and Xaraés was significantly reduced (P<0.001) by 71.3, 94.0, 81.2 and 77.2%, respectively. Root mass was reduced about 30% in flooded plants relative to those grown at 50% field capacity. While all cultivars could be used where soil moisture is marginal for production, cvv. Llanero, Tupi and Massai would be most suitable where flooding could occur during the growing season. Field studies are needed to verify these glasshouse findings.

**Resumen**

La diversificación de las gramíneas forrajeras es una solución estratégica para obtener una mayor productividad en ambientes diferentes. En condiciones de invernadero en la Universidad del Estado de Mato Grosso, Alta Floresta, Mato Grosso, Brasil, se realizó un estudio con el objetivo de evaluar la tolerancia de 9 cultivares de gramíneas forrajeras tropicales a condiciones de inundación controlada. Los tratamientos se dispusieron en un diseño completamente al azar con arreglo factorial 9 x 3: nueve cultivares (Brachiaria brizantha cvs. Marandu, Piatã e Xaraés; Brachiaria híbrido cv. Mulato II; B. humidicola cvv. Llanero y Tupi; B. ruziziensis cv. Común; y Panicum maximum cvs. Massai y Tanzania) y 3 niveles de agua en el suelo: a) cantidad de agua considerada como mínima para el crecimiento de las plantas (50% de capacidad de campo); b) suelo a capacidad de campo; y c) suelo inundado con una lámina de 2 cm sobre el nivel del suelo, con 3 repeticiones. Como parámetros se midieron la producción de forraje, la altura de planta y la acumulación de raíces. Todos los cultivos presentaron buen desarrollo cuando el suelo se encontraba a 50% de capacidad de campo, lo cual muestra su adaptación a condiciones ligeramente secas. Bajo condiciones de inundación, B. humidicola cvs. Llanero y Tupi no mostraron reducción en la producción de materia seca de forraje, mientras que el crecimiento de los cvs. Marandu, Piatã, Tanzânia y Xaraés se redujo significativamente (P<0.001) en 71.3, 94.0, 81.2 y 77.2%, respectivamente. En plantas bajo condiciones de inundación, la producción de masa de raíces se redujo aproximadamente 30% en comparación con los resultados obtenidos a 50% de capacidad de campo. Aunque todos los cultivares evaluados se podrían utilizar bajo condiciones de humedad marginal en el suelo, la siembra de los cvs. Llanero, Tupi y Massai sería la más indicada en zonas sujetas a inundaciones frecuentes. Se necesitan estudios a nivel de campo para verificar estos resultados de invernadero.

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Introduction

Animal husbandry is one of the most important activities in Brazil, and pastures are the main feed source for the Brazilian cattle herd. This results in lower costs of production than in intensive or semi-intensive systems, where animals are confined and fed grain. In a large part of Brazil such as in Mato Grosso State, livestock production is currently almost exclusively on Brachiaria brizantha cv. Marandu pastures.

The establishment of pastures in the Amazon region often occurs in areas with poorly-drained and/or waterlogged soils, and with high precipitation rates (over 500 mm/month during the rainy season). Thus, pastures are often flooded for extended periods, resulting in slow pasture growth, low forage quality and high risks of degradation. Temporary flooding and waterlogging of the soil can result in serious damage to livestock production, e.g. the Marandu Death Syndrome (MDS) (Pedreira et al. 2014).

According to Dias-Filho (2006), the relationship between MDS and the low adaptation of B. brizantha cv. Marandu to soil flooding is that the excess water in the soil acts as a predisposing factor to the onset of the problem, i.e. as a ‘trigger’. Under such conditions (excess moisture or rainfall), fungi present in the soil become pathogenic owing to the condition of hypoxia or anoxia in some forage plants lowering their resistance. In view of this problem, the only practical solution is to use pasture species with greater tolerance to poorly-drained and/or temporarily waterlogged soils.

Therefore, this study aimed to evaluate the flooding tolerance of 9 commercial cultivars of forage grasses.

Material and Methods

The experiment was carried out under a shelter at the State University of Mato Grosso (UNEMAT), Alta Floresta, MT, Brazil, from August to December 2012. According to the Köppen classification, the region has a tropical rainy climate (Aw type), with a well-defined dry and rainy period, and an average annual precipitation of 2,200 mm. The temperature varies from 18 to 40 ºC, averaging 26 ºC (INMET 2014).

The soil utilized was a clayey dystrophic hapludox collected from a gully. It was sieved to remove undesirable particles such as gravel and wood chunks, autoclaved for 20 min at 120 ºC at 0.5 kgf/m² for soil biological sterilization and then stored in a clean, sterilized plastic container.

The experimental design was a 9 x 3 factorial arrangement: 9 cultivars of tropical grasses [Brachiaria brizantha (now: Urochloa brizantha) cvv. Marandu, Piatã and Xaraés; hybrid Brachiaria cv. Mulato II; B. humidicola (now: U. humidicola) cvv. Llanero and Tupi; B. ruziziensis (now: U. ruziziensis) cv. Common (subsequently referred to as Ruziziensis); and Panicum maximum (now: Megathyrsus maximus) cvv. Massai and Tanzânia] and 3 soil water levels. The water levels were classified as: a) minimal water for development, in which irrigation was estimated to achieve 50% of field capacity; b) soil at field capacity, with irrigation estimated at 0.5 kg water/kg dry soil (equivalent to -0.01 MPa); and c) flooded soil, with irrigation applied to achieve 2 cm of water above the soil surface. Three replicates were used, totaling 81 experimental units.

Each experimental unit was characterized by a rectangular pot with a volume of 3 L filled with 2 L of sterilized soil, into which the grass seeds were sown. The undrained/undrilled pots were irrigated until they reached the desired moisture level. Thirty seeds were sown in each pot at 1 cm depth, with subsequent thinning to leave 15 plants/pot. At planting, each pot received 75 g of fertilizer formulated with N:P:K 04:14:08.

The seeds were sown on 25 May 2012, and all pots were irrigated for 30 days to ensure establishment. After a standardization harvest on 24 September 2012, treatments were imposed and 3 harvests were made subsequently: on 5 and 23 November and on 13 December 2012, which allowed 3 regrowth periods and a total of 80 days of exposure to treatments.

At each harvest, the forage was cut with scissors at 10 cm from ground level and dried in a forced-circulation oven at 55 ºC until constant weight to determine dry matter (DM) accumulation. Plant height was determined in 10 plants from each pot by measuring from ground level to the tip of the longest leaf using a measuring tape.

After the third harvest, the roots of all plants from each pot were collected, cleaned and dried in a forced-circulation oven at 55 ºC to constant weight to determine DM root mass.

The data were analyzed using a mixed-models method with special parametric structure in the covariance matrix, through the MIXED procedure of SAS software (Littell et al. 2006). Akaike’s information criterion was used to choose the covariance matrix (Wolfinger and O’Connell 1993). Treatment means were estimated using “LSMEANS”, and “PDIFF” was used to compare them. Significance was detected at the 0.05 level of probability.
Results

The results reported here refer to the measurements performed at the end of the third evaluation cycle, when the plants had been subjected to a total of 80 days of water stress (treatments).

There were significant cultivar x water level interactions in terms of forage DM accumulation (P<0.001; Table 1). At 50% of field capacity all cultivars grew satisfactorily with highest yields from Tupi, Llanero and Tanzânia (13.5–15.8 g DM/pot) and least from Massai, Mulato II and Ruziziensis (5.7–8.3 g DM/pot; P<0.05). As water availability increased, growth of Piatã, Xaraés, Marandu and Tanzânia declined significantly (P<0.05) so that in the flooded situation, DM yields were very low (0.6–3.0 g DM/pot). At the other end of the scale, yields of Llanero, Tupi and Massai were not affected by flooding (P>0.05).

There were no significant cultivar x water level interactions for root mass, which, however, differed among cultivars (P<0.001) and soil water levels (P=0.029) (Table 2). There was a gradation in root mass from highest in Llanero to lowest in Tupi, with root mass in the latter being only 23% of that of Llanero. This fact can be associated with differential increases of adventitious roots under flooded conditions.

At 50% field capacity, Tanzânia produced the tallest plants (P<0.05; Table 3), followed by Llanero, Tupi and Piatã and then the remaining cultivars (P<0.05). Under flooded conditions, height of Tupi, Massai and Mulato II actually increased (P<0.05), while height of most other cultivars declined, the most marked reduction (about 50%) being in Piatã.

Table 1. Forage dry matter accumulation (g DM/pot) in grass cultivars in pots at the end of the third regrowth cycle (80 days) under varying levels of water availability.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Water levels</th>
<th>50% Field capacity</th>
<th>Field capacity</th>
<th>Flooded$^1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Massai</td>
<td>5.7 Da$^2$</td>
<td>6.8 CDa</td>
<td>8.1 BCa</td>
<td></td>
</tr>
<tr>
<td>Tanzânia</td>
<td>15.8 Aa</td>
<td>2.2 Db</td>
<td>3.0 Db</td>
<td></td>
</tr>
<tr>
<td>Piatã</td>
<td>10.9 ABCa</td>
<td>9.4 ABCa</td>
<td>0.6 Db</td>
<td></td>
</tr>
<tr>
<td>Xaraés</td>
<td>9.8 BCDa</td>
<td>5.9 CDab</td>
<td>2.2 Db</td>
<td></td>
</tr>
<tr>
<td>Marandu</td>
<td>10.1 BCDa</td>
<td>4.5 CDab</td>
<td>2.9 Db</td>
<td></td>
</tr>
<tr>
<td>Mulato II</td>
<td>7.3 CDa</td>
<td>7.8 BCa</td>
<td>4.1 CDa</td>
<td></td>
</tr>
<tr>
<td>Ruziziensis</td>
<td>8.3 CDa</td>
<td>5.8 CDa</td>
<td>4.7 CDa</td>
<td></td>
</tr>
<tr>
<td>Piatã</td>
<td>40.42</td>
<td>31.57</td>
<td>824</td>
<td></td>
</tr>
<tr>
<td>Marandu</td>
<td>29.94</td>
<td>50.38</td>
<td>27.95</td>
<td></td>
</tr>
<tr>
<td>Xaraés</td>
<td>44.71</td>
<td>19.72</td>
<td>20.06</td>
<td></td>
</tr>
<tr>
<td>Mulato II</td>
<td>27.54</td>
<td>19.96</td>
<td>12.74</td>
<td></td>
</tr>
<tr>
<td>Ruziziensis</td>
<td>46.26</td>
<td>34.97</td>
<td>24.50</td>
<td></td>
</tr>
<tr>
<td>Tupi</td>
<td>9.01</td>
<td>10.89</td>
<td>8.21</td>
<td></td>
</tr>
<tr>
<td>Llanero</td>
<td>52.78</td>
<td>30.12</td>
<td>42.38</td>
<td></td>
</tr>
</tbody>
</table>

1Water to 2 cm above soil level.
2Means followed by the same upper-case letter within columns (cultivars) and lower-case letter within rows (water levels) do not differ statistically according to the F test at a significance level of 5%.

Table 2. Root dry matter mass (g DM/pot) of grass cultivars in pots at the end of the third regrowth cycle (80 days) under varying levels of water availability.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Water level</th>
<th>50% Field capacity</th>
<th>Field capacity</th>
<th>Flooded$^1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Massai</td>
<td>35.19</td>
<td>45.84</td>
<td>39.04</td>
<td></td>
</tr>
<tr>
<td>Tanzânia</td>
<td>31.87</td>
<td>11.99</td>
<td>35.56</td>
<td></td>
</tr>
<tr>
<td>Piatã</td>
<td>40.42</td>
<td>31.57</td>
<td>824</td>
<td></td>
</tr>
<tr>
<td>Marandu</td>
<td>29.94</td>
<td>50.38</td>
<td>27.95</td>
<td></td>
</tr>
<tr>
<td>Xaraés</td>
<td>44.71</td>
<td>19.72</td>
<td>20.06</td>
<td></td>
</tr>
<tr>
<td>Mulato II</td>
<td>27.54</td>
<td>19.96</td>
<td>12.74</td>
<td></td>
</tr>
<tr>
<td>Ruziziensis</td>
<td>46.26</td>
<td>34.97</td>
<td>24.50</td>
<td></td>
</tr>
<tr>
<td>Tupi</td>
<td>9.01</td>
<td>10.89</td>
<td>8.21</td>
<td></td>
</tr>
<tr>
<td>Llanero</td>
<td>52.78</td>
<td>30.12</td>
<td>42.38</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>35.30 a</td>
<td>28.38 ab</td>
<td>24.29 b</td>
<td></td>
</tr>
</tbody>
</table>

1Water to 2 cm above soil level.
2Means followed by the same upper-case letter within columns (cultivars) and lower-case letter within rows (water levels) do not differ statistically according to the F test at a significance level of 5%.
Table 3. Plant height (cm) of grass cultivars in pots at the end of the third regrowth cycle under varying levels of water availability.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>50% Field capacity</th>
<th>Field capacity</th>
<th>Flooded¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Massai</td>
<td>39.5 Db²</td>
<td>47.4 BCDa</td>
<td>48.7 BCa</td>
</tr>
<tr>
<td>Tanzânia</td>
<td>65.8 Aa</td>
<td>44.9 Db</td>
<td>44.1 Db</td>
</tr>
<tr>
<td>Piatã</td>
<td>50.8 Ba</td>
<td>44.9 Db</td>
<td>25.7 Gc</td>
</tr>
<tr>
<td>Marandu</td>
<td>44.5 Ca</td>
<td>39.4 Eb</td>
<td>32.6 Fc</td>
</tr>
<tr>
<td>Xaraés</td>
<td>44.9 Ca</td>
<td>46.7 CDa</td>
<td>37.0 Eb</td>
</tr>
<tr>
<td>Mulato II</td>
<td>45.6 Cb</td>
<td>51.2 ABa</td>
<td>44.6 CDb</td>
</tr>
<tr>
<td>Ruziziensis</td>
<td>51.7 Ba</td>
<td>47.8 ABCDab</td>
<td>45.5 CDb</td>
</tr>
<tr>
<td>Tupi</td>
<td>48.6 BCb</td>
<td>51.7 Aab</td>
<td>52.6 Ba</td>
</tr>
<tr>
<td>Llanero</td>
<td>50.3 Bb</td>
<td>49.5 ABCb</td>
<td>56.7 Aa</td>
</tr>
</tbody>
</table>

¹Water to 2 cm above soil level.
²Means followed by the same upper-case letter within columns (cultivars) and lower-case letter within rows (water levels) do not differ statistically according to the F test at a significance level of 5%.

Discussion

Under flooding, plants can produce numerous metabolic signs in response to the reduction in the endogenous levels of soil oxygen. During this period of stress, architecture, anatomy, metabolism and growth patterns change as a survival strategy (Bailey-Serres and Voosenk 2008).

Excess water in the soil and filling of the macro- and micropores lead to a decrease in the diffusion of the oxygen necessary for root respiration, causing hypoxia (low concentration of O2) or anoxia (absence of O2) in the soil (Thomson and Greenway 1991; Armstrong et al. 1994). Replacement of the oxygen consumed by microorganisms from the soil and by the roots of plants occurs slowly due to the low diffusion of oxygen in the water (Dias-Filho 2012). Thus, stomatal conductance is decreased, which reduces the rate of photosynthesis and consequently growth rates (Baruch 1994; Huang et al. 1994; Dias-Filho 2002; Mattos et al. 2005), culminating in a decrease in accumulation of shoots and roots (Dias-Filho 2002). While these symptoms of flooding were observed in our study, they did not occur in all cultivars.

Cultivars Llanero, Tupi and Massai remained productive even under flooded conditions (Table 1), which is very important for the production systems based on grasslands in this region of Brazil. This fact is attributed to important morpho-physiological adaptations such as physical acclimatization and coping with a flooded environment by increasing the amount of adventitious roots, as was evidenced for Llanero (Dias-Filho 2002; Jackson and Colmer 2005; Mattos et al. 2005). Plants with this ability are more tolerant of anoxia (Gibbs and Greenway 2003) and have the capacity to prevent or repair oxidative damage during re-aeration (Blokhina et al. 2001; 2003).

This oxidation (production of toxic compounds) results from the anaerobic fermentation performed by soil microorganisms, to produce Mn2+, Fe2+, S2−, H2S and carboxylic acids as products of their metabolism (Jackson and Colmer 2005). When accumulated in the soil, these elements cause severe damage to the plants.

It is of interest that the 2 Panicum maximum cultivars used in our study reacted differently to flooding. While Tanzânia suffered severely, Massai was not affected. Silva et al. (2009) evaluated 7 P. maximum cultivars and accessions (including Massai, Mombaça and Tanzânia) and observed that flooding reduced total forage production significantly. In contrast, the poor tolerance of Brachiaria brizantha cultivars in our study under flooding agrees with the findings of Dias-Filho (2002), who evaluated the morpho-physiological responses of 5 accessions of B. brizantha planted in pots under flooded and well-drained soil conditions and observed that flooding reduced net photosynthesis and chlorophyll contents significantly.

Flooding of soils negatively affects leaf elongation rate (LER) and leaf blade senescence rate (Mattos et al. 2005). Thus, Dias-Filho and Carvalho (2000) suggest that LER should be employed as a mechanism for early detection of tolerance of flooding in Brachiaria species. Unfortunately, limited resources did not allow us to measure this parameter.

Silva et al. (2009) observed a reduction in accumulation of roots of Tanzânia under flooded conditions. Similarly, Silva et al. (2007) reported an abrupt drop in production of root biomass by cv. Tupi after the second week of waterlogging, as compared with B. humidicola cv. Common, which showed an initial reduction and then stabilization. In our study, Tupi and Llanero presented the highest root production, independent of water level, an
Grass cultivars under flooding

An important issue to consider when establishing a pasture in the Brazilian Amazon region.

Silva et al. (2007) found that B. brizantha cv. Marandu suffered a dramatic reduction in root biomass production from the fourth week of flooding, and Caetano and Dias-Filho (2008) reported that this cultivar was quite susceptible to reduction of root production in waterlogged and/or flooded soils. Likewise, cultivar Xaraés showed a linear decrease in root biomass under flooding, to 14.2% of biomass of dry roots in unflooded soil by 8 weeks. Our findings support these outcomes, clearly demonstrating that Marandu and Xaraés should be avoided in grassland systems experiencing high levels of rainfall and waterlogging and/or flooding.

In plants that go through relatively long periods of flooding, one of the most common morpho-anatomical responses to hypoxia and/or anoxia is the formation of aerenchyma (Evans 2003; Colmer and Voesenek 2009; Takahashi et al. 2014). Those aerenchyma are mainly of the lysigenous type: they are formed by dead cortical cells (Yamauchi et al. 2013). This occurs due to the increase in the synthesis of ethylene caused by increased activity of ACC synthase, resulting in increased cellulase activity, which in turn contributes to degradation of the cell wall and formation of aerenchyma (Takahashi et al. 2014). There is a second type, the schizogenous aerenchyma, originating from the connection of cells, which form continuous columns for the storage and transport of gases (Joly 1991; Colmer et al. 2004). Furthermore, adventitious roots to capture and transport oxygen to the submerged tissues can be developed (Dias-Filho 2012).

The accumulated ethylene may reduce the roots’ extension, whereas the soil carbon dioxide may severely damage roots, because high concentrations of CO₂ can reduce pH (Colmer and Voesenek 2009). In the presence of Ca²⁺, ethylene forms calcium carbonate, increasing the pH and making some nutrients unavailable (Jackson 2004) and reducing root growth. These factors can be potentiated by an increase in temperature, which increases the enzymatic activity of plants (Taiz and Zeiger 2004), and the presence of organic matter, a substrate for aerobic microorganisms (Jackson 2004).

The degradation of nitrogen compounds (proteins and peptides) of the soil organic matter by microorganisms reduces them to nitrite and nitric oxide (NO), which interact indirectly through the reaction with superoxide favoring nitrogen-reactive species, which are capable of reacting with DNA, proteins and lipids. Likewise, high concentrations of NO result in production of peroxynitrite, which has a catalytic effect on lipids, DNA and proteins, causing cell death (Wink and Mitchell 1998).

Another explanation for the low root biomass production is the presence of hydrogen peroxide in the root apoplast. Evaluating 2 tolerant (Iris pseudacorus and Oryza sativa) and 2 intolerant (Iris germanica and Triticum aestivum) species under anoxia by soil flooding, Blokhina et al. (2001) observed that, in all tested plants, H₂O₂ was increased in plasma membranes and in the root apoplast. According to Visser and Voesenek (2004), this system works as a rheostat system that regulates the endogenous levels of H₂O₂, a crucial factor for plant tissue, because high increases in H₂O₂ concentrations in plant tissue can trigger the death of cells.

Cultivars less tolerant of soil waterlogging can exude ethanol from the roots, which is attractive to zoospores of pathogenic fungi and which supplies substrate for the colonization of mycelia in the plant tissue (Dias-Filho 2006). The morpho-physiological alterations brought about by soil flooding associated with the attack of phytopathogenic fungi in intolerant forages can lead to their death, e.g. the Marandu Death Syndrome (Pedreira et al. 2014).

An interesting finding was the good performance at 50% field capacity relative to that at field capacity. Overall most cultivars performed better at 50% field capacity than at field capacity. This suggests that soils do not have to be flooded to produce some reduction in plant growth. The correct choice of species and cultivars is the most important technical aspect when a pasture-based livestock production system is to be established in an environment prone to flooding. Forage grasses like Tupi, Llanero and Massai showed promising results and should be tested under field conditions in some of these environments.

Conclusions

This study has revealed wide variation in production of important pasture grasses, under flooded conditions. An interesting outcome was the production levels obtained in all cultivars in soils at 50% of field capacity. When soil water was limited, most cultivars had acceptable growth rates. Increasing the level of soil water to field capacity produced little change in forage growth under the experimental glasshouse conditions and often reduced growth to some extent. This highlights the adaptation of all cultivars tested to situations where only moderate levels of soil water are available in the growing season. However, under flooded conditions only a few cultivars produced satisfactory levels of DM. Brachiaria humidicola cvv. Llanero and Tupi continued to produce well after 80 days of flooding. Panicum maximum cv. Massai also appeared to be quite tolerant of flooding for extended periods, while the
remaining cultivars performed poorly under flooding and cannot be recommended for sowing in areas with deficient soil drainage or flooding problems. How these controlled situation results can be extrapolated to field situations warrants evaluation.

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