SEMI-HYDROPONIC CULTIVATION AS A SELECTION TOOL FOR ALUMINUM TOLERANCE IN FORAGE GRASSES

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ABSTRACT

The constant improvement of selection methods is necessary in order to optimize efficiency of breeding programs for aluminum tolerance. Thus, the aim of this study was to establish a vase volume to the ideal semi-hydroponic cultivation of forage grasses subjected to stress by aluminum in nutrient solution. The experimental design completely randomized, with four replicates, each replicate consisting of one plant per vase. The treatments were arranged in a factorial design 4x5, being the first factor consists of four genotypes of forage grasses *Brachiaria Humidicola*; *Brachiaria brizantha* cv. Piatã; *Panicum maximum* cv. Massai and *Panicum maximum* cv. Mombaça) and the second consisting of five separate volumes plastics vases where seedlings were grown (0.2; 0.25; 0.3; 0.35 and 0.4 dm^3). The grasses grown in a semi-hydroponics system irrigated with nutrient solution rich in aluminum (3 mg L^{-1}). Was measured plant height, dry weight of aereal part and root length. The semi-hydroponic cultivation in aluminum increased nutrient solution is effective in differentiation of forage grasses genotypes in relation to aluminum tolerance. Pots volumes near 0.3 dm^3 promote greater development for root and aerial part attributes in forage grasses grown in nutrient solution with aluminum toxicity.

Keywords: toxic aluminum, abiotic stress, nutrient solution, forage production

RESUMO

CULTIVO SEMI-HIDROPÔNICO COMO FERRAMENTA DE SELEÇÃO PARA TOLERÂNCIA AO ALUMÍNIO EM GRAMÍNEAS FORRAGEIRAS

O aprimoramento constante das ferramentas de seleção se faz necessário, no sentido de otimizar a eficiência de um programa de melhoramento para tolerância ao alumínio. Desta forma, o objetivo deste trabalho foi estabelecer um volume de vaso ideal ao cultivo semi-hidropônico de gramíneas forrageiras submetidas ao estresse por alumínio em solução nutritiva. O delineamento experimental utilizado foi o inteiramente casualizado, com quatro repetições, sendo cada repetição constituída por uma planta por vaso. Os tratamentos foram dispostos em esquema fatorial 4x5, sendo o primeiro fator constituído por quatro genótipos de gramíneas forrageiras (*Brachiaria Humidicola*; *Brachiaria brizantha* cv. Piatã; *Panicum maximum* cv. Massai e *Panicum maximum* cv. Mombaça) e o segundo constituído de cinco volumes distintos de vasos plásticos onde as plântulas foram cultivadas (0,2; 0,25; 0,3; 0,35 e 0,4 dm^3). As plântulas foram cultivadas em sistema semi-hidropônico com solução nutritiva rica em alumínio (3 mg L^{-1}). Foram mensuradas a altura de plantas, massa seca da parte aérea e comprimento de raiz. O cultivo semi-hidropônico foi eficaz na diferenciação de genótipos de gramíneas forrageiras em relação à tolerância ao alumínio. Volumes de vasos próximos a 0,3 dm^3 favoreceram o desenvolvimento dos atributos do sistema radicular e da parte aérea em gramíneas forrageiras cultivadas em solução nutritiva com a toxidez de alumínio.

Palavras-chave: Alumínio tóxico, estresse abiótico, solução nutritiva, produção de forragem

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INTRODUCTION

In Brazil, the beef cattle production is characterized by farming systems that use grasslands, providing 99% of the diet for cattle herds. The pastures managed in extensive system, with low use of fertilizers or, in most cases, without the addition of these inputs, so that the productivity levels of livestock systems are low, and over time production, declines are evident (DIAS-FILHO, 2011).

New areas opening for cattle pasture is getting more restrict, and gradually limited to marginal areas with low fertile soil and high acidity, rich in aluminum and other toxic elements (MACEDO, 2009). Aluminum is toxic to most species of cultivated plants by promoting the inhibition of root growth and, consequently, hindering the development of plants (CANÇADO *et al*., 2001). Thus, the operation of these pasture land need, increasingly, the selection of forage that is adapted to those conditions (EUCLIDES, 2000).

Character aluminum tolerance in forage species has received attention from breeding programs (OLIVEIRA *et al*., 2013; BITENCOURT *et al*., 2011; MATINS *et al*., 2011). According to Ryan *et al*. (1993), the Al^{3+} is present predominantly in the root area of the plant, and the apexes of the roots its critical site of toxicity. Thus, methods that measure restricting root growth receive more attention as a parameter in the evaluation of Al^{3+} toxicity (CRESTANI *et al*., 2011; CRESTANI *et al*., 2009; VOSS *et al*., 2006; MAZZOCATO *et al*., 2002).

Reviews in field conditions have traditionally been used in the plant tolerance study about Al^{3+} toxic, by depicting more faithfully the natural growing conditions (MISTRO et al., 2001; CAMARGO *et al*., 1995; FERREIRA *et al*., 1986). However, most interference from external factors of hard control in this type of study priority the cultivation in nutrient solution to facilitate the work by environment control and ease visualization of Al^{3+} effect on interested attribute (LANA *et al*., 2013; MACEDO *et al*., 2011; REIS *et al*., 2009). Crestani *et al*. (2009) signalize that growing in nutrient solution also allows evaluating large number of genotypes in short period of time in the early stages of plant development, providing significant improvements to the efficiency of selection.

Different methods based on cultivation in nutrient solutions have been developed in evaluation of tolerance and sensitivity to Al^{3+} in annual and perennial species. Cardoso *et al*. (2004) proposed semi-hydroponics method in nutrient solution in which the species of interest is transplanted, still in seedling stage for a polyethylene vase containing an inert substrate, suspended over a container containing nutrient solution. In this way, contact with the substrate allows plant root system realize gas exchange, while the rest of the roots are immersed in the nutritive solution prepared for each study. For toxicity studies Al^{3+}, Furlani and Hanna (1984) showed nutrient solution composed of a series of chemical elements essential for plant associated with the use of aluminum - potassium decahydrate $(AlK(SO_4)_2.12H_2O)$ as element source.

However, in all hydroponics system, priority should be given a homogeneous distribution of root system on the nutrient solution. Whereas the availability of nutrients to plants is also affected by morphological and physiological attributes of each plant species (SCHENK and BARBER, 1979), attributes such as length, volume and surface roots must be considered in experimental design. Moreover, plant roots have a high demand for oxygen by the high respiration rate (MARSCHNER, 1995), provide the necessary aeration system becomes essential to plant development in full growth medium.

The constant improvement of methodologies is necessary in order to optimize the evaluation process efficiency and selection of genotypes tolerant to aluminum. Thereby, the aim of this study was to establish the ideal vase volume semi-hydroponic cultivation of forage grasses subjected to stress by aluminum in nutrient solution.

MATERIALS AND METHODS

The experiment was conducted in greenhouse at the Experimental Station of the Federal University of Tocantins, Campus of Gurupi, at coordinates 11°43’45” latitude and 49°04’07” longitude. The experimental design completely randomized, with four replicates, each replicate consisting of one plant per vase.

The treatments were arranged in a factorial 4 x 5, the first factor consists of four genotypes of forage grasses (*Brachiaria humidicola*; *Brachiaria brizantha* cv. Piatã; *Panicum maximum* cv. Massai and *Panicum maximum* cv. Mombaça) and the second consisting of five different volumes of plastic pots where seedlings were grown (0.2; 0.25; 0.3; 0.35 and 0.4 dm^3).

The seeds of the genotypes germinated on filter paper, spaced from each other in one centimeter. The paper was rolled up and moistened with distilled water. These rolls were placed in germination chamber at 25°C, as described by Brasil (1992). After 60 hours, the rollers were removed and the germinating plants were selected for uniformity. Subsequently, these plantlets were placed individually in plastic vases.

In cultivation of seedlings of grasses used the double vase methodology adapted from Cardoso *et al*. (2004), where cylindrical plastic pots of 75 dm diameter containing washed sand, were superimposed with the aid of a support, a polyethylene tray measuring 45 cm long, 30 cm wide and 12.0 cm deep, containing nutrient solution (Figure 1). In shared double vases (20 plots per tray) of the roots grown in liquid medium and solid medium part as a way to avoid the necessity of artificial aeration of the solution.

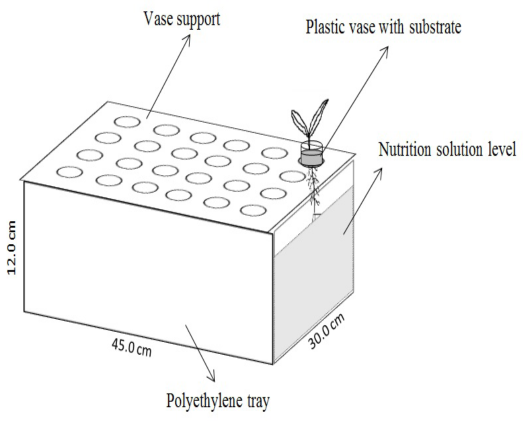

Figure 1. Illustration of double vase shared, adapted from Cardoso *et al*. (2004).

Seedlings received daily irrigation with nutrient solution similar to that defined by Furlani and Hanna (1984), however, with a 50% reduction in the concentration of calcium. The aluminum stress was simulated by adding to the nutritive solution at a single concentration of 3 mg L^{-1}, in the form of aluminum - potassium decahydrate $(AlK(SO_4)_2.12H_2O)$.

After 25 days elapsed germination, the plants were picked, separate the shoot roots, and evaluated the plant height (PH), measured in centimeters from the ground base to the apex of the larger sheet; and the root length (RL), measured in centimeters from the insertion base end to the main root.

Subsequently they were identified accommodated in paper bags and placed in a forced ventilation oven at a temperature of 60°C for 72 hours. After this period, the material was weighed to determine the dry weight of the aerial part (DWAP).

The data were submitted to regression analysis, assessing the significance of the betas and the coefficients of determination using the statistical program SigmaPlot 11.0 software (SIGMAPLOT, 2008).

RESULTS AND DISCUSSION

All genotypes showed quadratic response depending to the increase in the volume of growing vases. The determination coefficients were significant ($p < 0.05$) in all genotypes. The same occurred in both regression coefficients (β^{-1} and β^{-2}). The increase in the vases caused a significant effect on the root length (RL) of cultivated grasses (Figure 2).

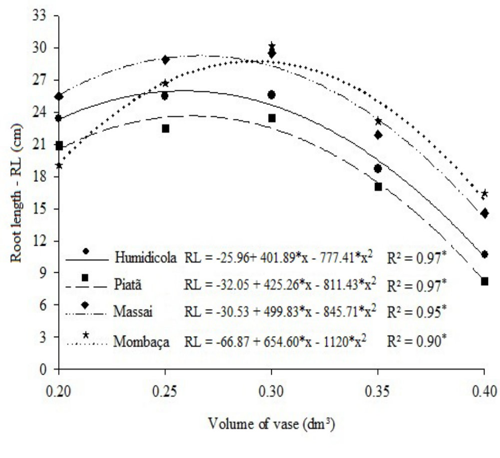

Figure 2. Root length of forage grasses genotypes depending on the volume of vases for semi-hydroponic cultivation in nutrient solution, 25 days after germination, Gurupi-TO 2015.

By observing the maximum response point in RL of the four genotypes, it appears that there are differences in vase volume ideal for the full root development. Grasses *B. humidicola*, Piatã and Massai had similar maximum points (0.258; 0.262 and 0.266 dm^3, respectively), while Mombaça grass was more demanding in volume vase, reaching maximum response point RL with vases of 0.292 dm^3.

The four grass species decreased in the RL when grown in vases 0.2 dm^3. This behavior can have occurred by the fact that this volume vase has not accommodated a sufficient root portion to find the respiration rate of the root system. Low availability of oxygen to roots can be because small volume of substrate available for the roots, it undertook the process of gas exchange by root metabolism. With lack of oxygen, oxidative phosphorylation is blocked and metabolism starts work anaerobically, severely affecting plant growth (SAIRAM *et al.*, 2008).

Similar reduction in RL occurred in the four genotypes grown in pots of 0.35 dm^3 and 0.4 dm^3. However, when subjected to higher volumes vase, the root system shows growth and space exploration laterally, less inducement to deepen roots, resulting in delay of contact with the nutrient solution. Since most of the absorbent root hairs do not exploit the nutrient solution, with an expected frame nutritional failure and consequent decrease in plant growth. According to Barber (1995), the plants tend to uncontrolled emission of roots, as a mechanism to increase efficiency in the interception of nutrients. Prado *et al.* (2011), evaluating seedlings *Panicum maximum* cv. Tanzânia in nutrient solution, observed reductions of up to 87% in root length, due to weak absorption of macronutrients.

As for plant height (PH), all genotypes showed quadratic response due to the increase in the volume of growing vases. The determination coefficients were significant ($p<0.05$) in all genotypes. The same observed in both regression coefficients (Figure 3).

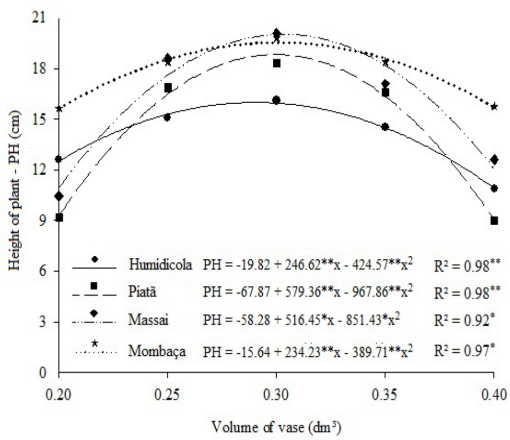

Figure 3. Height of plant genotypes of forage grasses depending on the volume of vases to semi-hydroponic cultivation in nutrient solution, 25 days after germination, Gurupi-TO 2015.

It showed shorter variation in the volume of vases that provided maximum increase in PH (4.48% difference between the four genotypes) compared to the volumes observed for RL. The genotypes *B. humidicola*, Piatã, Massai and Mombaça have peak in PH with potted 0.290; 0.299; 0.303 and 0.301 dm^3, respectively.

The results observed in PH are reflections of root development of genotypes. In the 0.2 dm^3 vases, the shoot elongation was compromised by the low metabolic rate, as a result of hypoxia that the root system was subjected. Studies show that lack of oxygen in the soil is able to significantly reduce the photosynthetic capacity in non-tolerant grasses (CAETANO and DIAS-FILHO, 2008; SOUSA and SODEK, 2002). Ramos *et al.* (2011), by evaluating physiological and metabolic changes *B. Brizantha* plants subjected to conditions of root hypoxia, observed a significant reduction in photo synthetic rate, due to stomatal imbalances when compared to plants under normal conditions.

About the dry weight of the aerial part (DWAP), all genotypes showed quadratic response due to the increase in the volume of growing vases. The determination coefficients were significant ($p<0.05$) in all genotypes. The same observed in both regression coefficients (Figure 4).

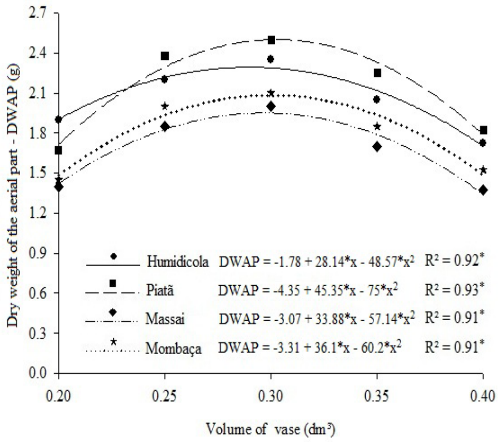

Figure 4. Dry weight of the aerial part of forage grasses genotypes depending on the volume of vases to semi-hydroponic cultivation in nutrient solution, 25 days after germination, Gurupi-TO 2015.

The grasses *B. humidicola*, Piatã, Massai and Mombaça show maximum increment of points similar DWAP (0.289; 0.302; 0.296 and 0.299 dm^3, respectively), with change of 4.5% between them.

Overall, the genotypes grown in pots of 0.2 dm^3 compromise the results presented at the three characteristics studied. This presents insufficient dimension to accommodate a substrate portion that allows the plant to perform the process of breathing and gas exchange medium by full root tissue, not supplying the breathing demand of the plant. Deficit in the biochemical phase of photosynthesis, providing energy for cell metabolism is precarious, and results in lower PH and DWAP, directly correlated to energy supply (TAIZ and ZEIGER, 2009).

When grown in vases 0.35 dm^3 and 0.4 dm^3, genotypes found aggravating for its vegetative growth, a decrease in all attributes, indicating that these volumes are too big for semi-hydroponic cultivation. In that condition, the initial root portion is located exclusively in the substrate layer with reduced exposure of roots to nutrient solution, damaging essential nutrients absorption for metabolism. According to Tavares *et al*. (2012), in an experiment with genre grasses *Cynodon* and *Digitaria*, the layer of 0-10 cm responsible for about 70% of the mass and number of roots, responsible for 49% of absorption and accumulation of nutrients in shoot.

Despite the four species have presented different responses depending on the vase volume used in semi-hydroponic cultivation, it can say that volumes close to 0.3 dm^3 promoted greater development for the studied attributes. However, it is important to alert that for each genotype, there is an ideal volume for better measurement of stressful effect on the plant, and it is up to the researcher to carry out prior determination of the ideal vase volume according to the genotype studied. In addition to satisfying reliability and experiment significance, the use of vases with smaller volumes can have real gains, with economy vases substrate and physical space, and temporal gains, with the agility to obtaining results.

For situations of stress for aluminum, where all treatments are equally exposed to stressful situation, the use of vases with ideal volume gives real answers of the effect of toxic aluminum in the metabolism of genotypes, excluding external factors that can distort the observations and macular selection tolerant individuals.

CONCLUSIONS

- The semi-hydroponic cultivation in aluminum increased nutrient solution is effective in differentiation of forage grasses genotypes in relation to aluminum tolerance.
- Vase volumes near 0.3 dm^3 promote greater development for root and aerial part attributes in forage grasses grown in nutrient solution with aluminum toxicity.

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