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Adaptive responses of *Brachiaria* grasses to hypoxia stress

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Introduction

It is likely that oxygen shortage in waterlogged soils is the most limiting factor for plant growth, restricting root aerobic respiration and adenosine triphosphate (ATP) production (Vartapetian and Jackson 1997). When oxygen becomes limiting for oxidative phosphorylation, plant cells depend on alternative metabolic pathways to produce ATP (Rocha et al. 2010). The induction of fermentative metabolism is considered of adaptive value to maintain ATP production under oxygen-limited conditions. Ethanol is the main end product of fermentative metabolism in plants. Alcohol dehydrogenase (ADH) is a key enzyme in ethanolic fermentation. Roots can sustain aerobic respiration under oxygen deficiency if aerenchyma is present. Aerenchyma commonly refers to tissue containing air-filled spaces that provide oxygen under oxygen-limited conditions (Colmer and Voesenek 2009). The main objective of the present study was to determine morpho-physiological adaptive responses of 7 *Brachiaria* genotypes to hypoxia stress.

Materials and Methods

The material used in this study included 7 *Brachiaria* genotypes with different levels of known waterlogging tolerance: tolerant *B. humidicola* cvv. Tully and Llanero (accessions CIAT 679 and CIAT 6133, respectively); moderately tolerant *B. decumbens* cv. Basilisk (CIAT 606) and *B. brizantha* cv. Toledo (CIAT 26110); and sensitive *B. brizantha* cv. Marandu (CIAT 6294), *B. ruziziensis* (Br 44-02) and *Brachiaria* hybrid cv. Mulato II (CIAT 36087). Vegetative propagules were used for experiments. After 8 days of rooting in low ionic strength nutrient solution (Wenzl et al. 2003), 12 propagules of each genotype were selected for homogeneity and placed in 12 L plastic containers (6 plants of each genotype per container) with renewed nutrient solution and grown under aerated or hypoxic conditions. Hypoxia was achieved by previously flushing N_2 through the solution for 4 hours. Agar (1% w/v) was added to the hypoxic nutrient solution to simulate lack of gas convection in waterlogged soils (Wiengweera et al. 1997). Containers were arranged in a completely randomized design. Two harvests were made at 3 and 10 days of growth and roots were separated from shoots. To examine root ADH, root samples were collected from the first 4 cm from the root tip. The remaining root segments were placed in 50% ethanol solution for later use. ADH activity was measured according to Bergmeyer (1974). Roots conserved in ethanol solution were used for quantification of aerenchyma development. Three roots were randomly selected and cross-sections were taken at 1 cm from the root base. Cross-sections were viewed under a light microscope equipped with a digital camera (Nikon, Coolpix 4500, Osaka, Japan). The percentage of aerenchyma (expressed per unit cross-sectional area) in each digital picture was determined using ImageJ software (version 1.41, National Institutes of Health, Bethesda, USA).

Data were analyzed to generate mean values, standard deviation and analysis of variance (ANOVA) using R (v. 2.15.2). Log transformation was carried out to ensure normality of data. Differences between genotypes were analyzed with the least significant difference (LSD) at α=0.05 and α=0.01.

Results and Discussion

Plants grown under hypoxic conditions for 3 and 10 days had higher values of root ADH activity on average than plants grown under aerated conditions, but there were no significant differences among treatments or genotypes (Figure 1). Root ADH tended to decrease with time under hypoxic conditions. Growth under hypoxic conditions resulted in higher root aerenchyma formation than in aerated plants after 3 days ($P<0.05$) with further increases ($P<0.05$) by 10 days, and the extent was greater in tolerant genotypes (Figure 1). Lower values of root ADH after 10 days compared with 3 days of growth under hypoxic conditions suggest that O_2 diffusion in roots was presumably improved by increased formation of root aerenchyma.

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Conclusions

This study suggests that roots of *Brachiaria* grasses grown under hypoxic conditions experience an increase in ethanolic fermentation irrespective of known tolerance level to waterlogging. However, root ADH was not a good indicator of waterlogging tolerance in *Brachiaria* grasses and may not serve as a useful screening procedure for evaluating tolerance of hypoxia/waterlogging. Increased aerenchyma improves internal root aeration to sustain root aerobic respiration under oxygen-deficient conditions. The most waterlogging-tolerant genotypes (*B. humidicola* CIAT 679 and CIAT 6133) developed more aerenchyma in roots, but differences among less-tolerant genotypes could not be explained by this mechanism alone. Even with aerenchyma present, it is likely that root tips will experience some degree of O_2 deprivation (Colmer and Voesenek 2009). This suggests that the presence of both adaptive responses, namely increased root ADH and aerenchyma formation, may contribute to the fitness of *Brachiaria* grasses under oxygen-deficient conditions. Increased aerenchyma formation may contribute to longer-term tolerance of hypoxic conditions.

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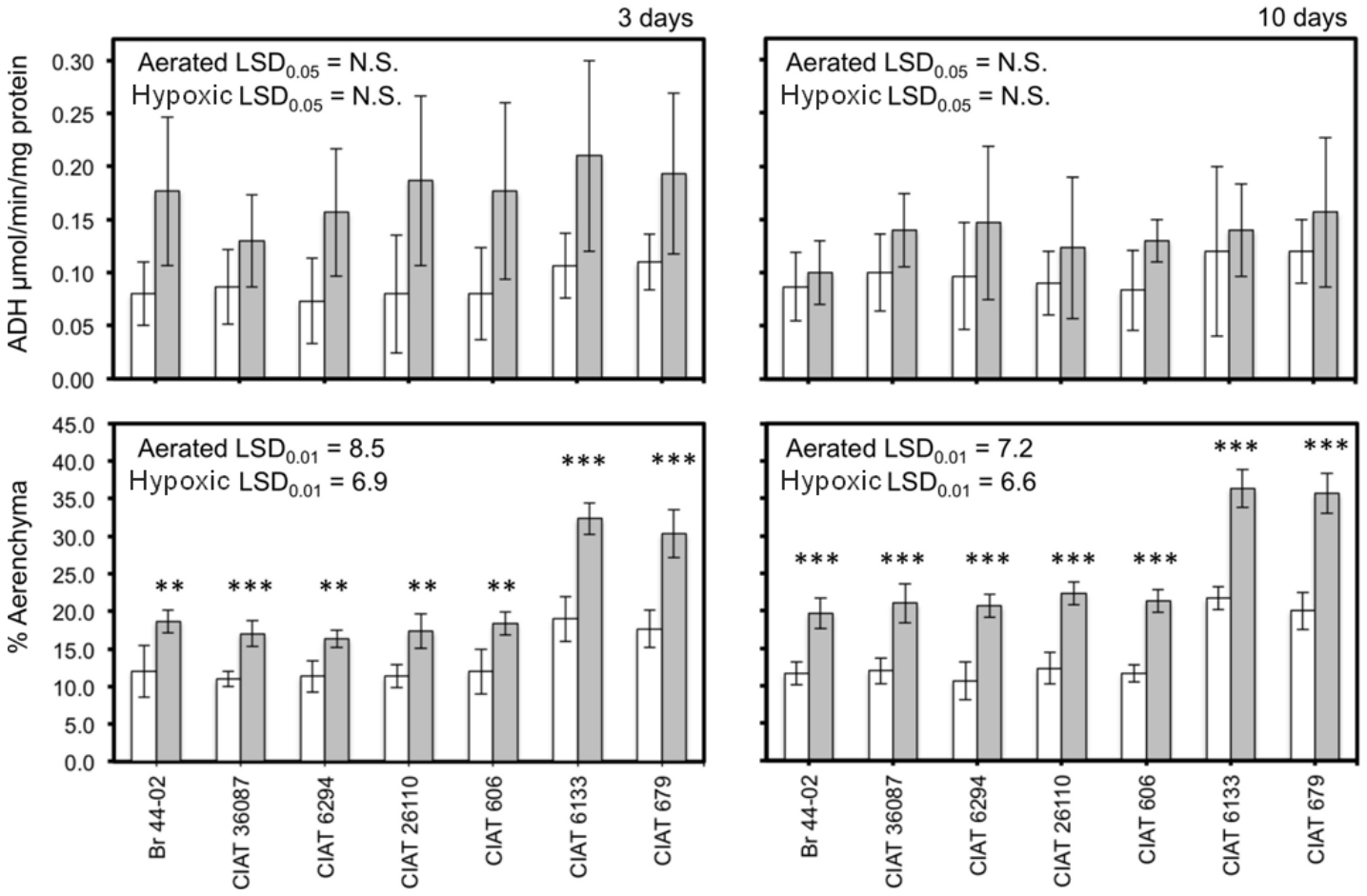

Figure 1. Differences in root ADH activity and aerenchyma formation among 7 *Brachiaria* genotypes grown under aerated (□) or hypoxic (■) conditions after 3 and 10 days of growth. Columns represent means, and error bars, their standard deviation. **, *** represent significant differences between treatments at $\alpha<0.05$ and 0.01, respectively; N.S., not significant.

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