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 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 fermentation 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 seven *Brachiaria* genotypes to hypoxia stress.

Materials and methods

The material used in this study included seven *Brachiaria* genotypes with different levels of known waterlogging tolerance. Genotypes included tolerant *B. humidicola* (CIAT 679 and CIAT 6133); moderately tolerant *B. decumbens* (CIAT 606) and *B. brizantha* (CIAT 26110); and sensitive *B. brizantha* (CIAT 6294), *B. ruziziensis* (Br 44-02) and *Brachiaria* hybrid (CIAT 36087). Vegetative propagules were used for experiments and allowed to root in low ionic strength nutrient solution (Wenzl *et al*. 2003). After 8 days of rooting in nutrient solution, 12 propagules of each genotype were selected for homogeneity. Selected propagules were placed in 12L plastic containers (6 plants of each genotype per container) with renewed nutrient solution and grown under aerated or hypoxic (1% agar deoxygenated nutrient solution) conditions. Hypoxia was achieved by previously flushing N_2 through the solution for 4 hours. Containers were arranged in a completely randomized design. Two harvests were made at 3 and at 10 days of growth under aerated or hypoxic conditions. 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 ethanol solution of 50% 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 that was 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).

Statistical analysis

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 $\alpha = 0.05$ and $\alpha = 0.01$.

Results

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

Conclusions

The present study suggests that roots of *Brachiaria* grasses grown under hypoxic conditions involve 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 therefore may not serve as a useful screening procedure for evaluating tolerance to 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 presence, it is likely that root tips will experience some degree of O_2 deprivation (Colmer and Voesenek 2009).

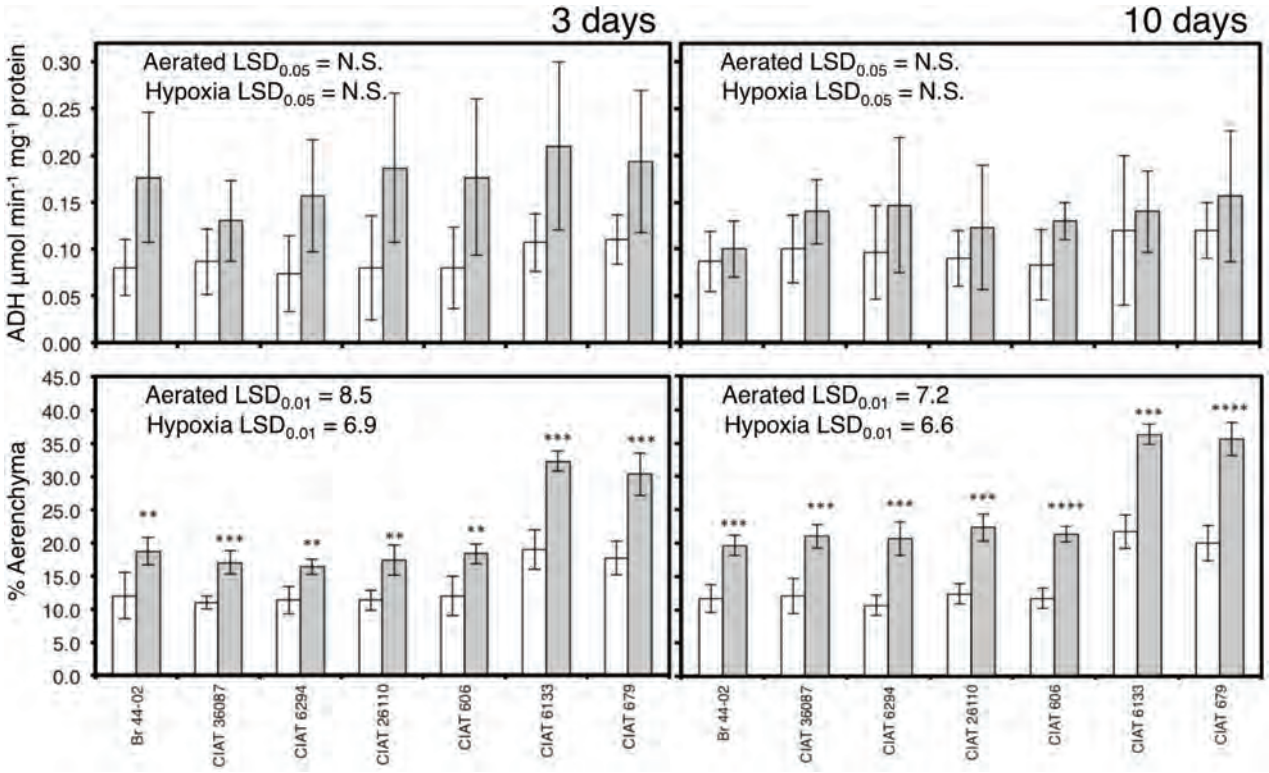

Figure 1. Differences in root ADH activity and aerenchyma formation among seven *Brachiaria* genotypes grown under aerated (□) or hypoxic (■) conditions. Columns represent means and error bars, their standard deviation. **, * represent significant differences between treatments at P < 0.05 and 0.01, respectively.**

This suggests that the presence of both adaptive responses, increased root ADH and aerenchyma formation, may contribute to the fitness of *Brachiaria* grasses under oxygen deficient conditions. However, increased aerenchyma formation may contribute to longer-term tolerance to hypoxic conditions.

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