Influence of fungal endophyte on plant water status, non-structural carbohydrate content and biomass partitioning in *Brachiaria* grasses grown under drought stress

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Key words
Tropical forages, endophytes, symbiosis, plant physiological responses

Abstract
Fungal endophytes are known to improve drought resistance in plants, although mechanisms for such tolerance remain unknown. A greenhouse study was performed using five *Brachiaria* grass cultivars, cvs. (*B. decumbens* cv. Basilisk, *B. humidicola* cv. Tully*, B. brizantha* cv. Marandu, *Brachiaria* hybrids cv. Cayman and cv. Mulato II) to test the influence of fungal endophyte on plant water status (measured as relative water content of leaf), total non-structural carbohydrates (TNC) content and biomass partitioning. Results on the influence of endophyte infection in only two cultivars (cv. Cayman and cv. Tully) are presented. After 24 days of drought stress, endophyte infection significantly increased relative water content in cv. Cayman and cv. Tully by 20% and 8.4%, respectively and reduced leaf dry matter content by 17% and 8.9%, respectively (p<0.05). Under well-watered condition, the endophyte significantly reduced shoot and whole plant TNC in cv. Cayman by 19.3% and 15.5%, respectively relative to uninoculated plants (p<0.05). Furthermore, stem, root and total biomass in cv. Tully were reduced due to the endophyte by 10.3% (p=0.046), 18.5% (p=0.006) and 11.9% (p=0.022), respectively under drought stress.

Introduction
Forage grasses form symbiotic associations with fungal endophytes that confer protection to plants and make them more persistent under drought (Assuero *et al*. 2006). Endophytes produce metabolites including alkaloids, carbohydrates as well as amino acids like proline and glutamic acid (Nagabhyru *et. al*. 2013). Accumulation of these compounds influences host fitness and plant physiological responses to biotic and abiotic stresses. Studies on grass-endophyte symbiosis have been extensively conducted in temperate grasses, but there remain critical knowledge gaps on the role of endophytes in the performance of tropical forages. The objective of this study was to determine the effect of the endophyte, *Acremonium implicatum*, on leaf relative water content (RWC), leaf dry matter content (LDMC), total non-structural carbohydrates (TNC) content and biomass partitioning in five *Brachiaria* grass cultivars.

Materials and Methods
The study was conducted at the International Center for Tropical Agriculture, CIAT (Palmira, Colombia) using five *Brachiaria* cultivars (*B. decumbens* cv. Basilisk, *B. humidicola* cv. Tully, *B. brizantha* cv. Marandu, *Brachiaria* hybrids cv. Cayman and cv. Mulato II). A total of 100 plants (20 plants for each cultivar) were grown in transparent plastic cylinders of 7.5 cm diameter x 120 cm length for 54 days. The cylinders were filled with 8.5 kg soil at a 2:1 ratio of soil:sand, and fertilized according to the recommended fertilizer requirement for *Brachiaria* forage grasses (Rao *et al*. 1992). Four factorial treatment combinations were applied with five replicates for each treatment combination which included: endophyte-well-watered (E+_WW), endophyte-drought stress (E+_DS) and no endophyte-well-watered (E-_WW), no endophyte-drought stress (E-_DS). Half (50) of the plants were inoculated with solutions of the fungal endophyte, *Acremonium implicatum*, while the other half (50) left uninoculated. Plant water status was estimated by leaf RWC, which was determined according to equation (i) by cutting leaf discs from five tillers and rehydrating in water-filled petri dishes for 48 hours at 4 oC; while LDMC was determined according to equation (ii).

$$\text{RWC (\%)} = \frac{(Lfw-Ldw)}{(Ltw-Ldw)} \times 100 \ldots\ldots\ldots\ldots\ldots \text{(i)} \qquad \text{LDMC (mg/g FW)} = \frac{[Ldw]}{[Lfw]} \ldots\ldots\ldots\ldots\ldots \text{(ii)}$$

Where *Lfw* = Leaf disc fresh weight; *Ltw* = Leaf disc turgid weight; *Ldw* = Leaf disc dry weight; FW = Fresh weight.

Plant tissues were oven-dried at 60 oC for 72 hours. Dry weight of the leaf discs (Ldw), as well as leaf, stem and root biomass was determined. Dry tissues were ground into powder and tissue TNC content was determined according to a modified method of Kang and Brink (1995).

Results and discussion

Although influence of endophyte was tested in five *Brachiaria* cultivars, results for only two cultivars (Cayman and Tully) with a total of 40 plants are presented here. Average results of RWC and LDMC values obtained from five sample replicates (Figs. 1 & 2) revealed that endophyte significantly increased RWC in cv. Tully and cv. Cayman under drought stress by 20% [p<0.0001] and 8.4% [p=0.013], respectively. At the same time, the endophyte reduced LDMC in cvs. Tully and Cayman by 17% (339 to 239 mg/g FW) [p<0.0001] and 8.9% (301 to 252 mg/g FW) [p=0.0001], respectively when compared with E- plants. Under well-watered condition, endophyte reduced shoot and whole plant TNC by 19.3% (119 to 80.5 mg/g of shoot biomass) [p<0.0001] and 15.5% (132.7 to 97 mg/g of total biomass) [p=0.007], respectively. Furthermore, endophyte infection reduced stem and root biomass in cv. Tully under drought stress by 10.3% (6.20 to 5.04 g) [p=0.046] and 18.5% (4.01 to 2.76 g) [p=0.006], respectively and total plant biomass subsequently reduced in E+ plants by 11.9% (12.67 to 9.98 g) than in E- plants [p=0.022].

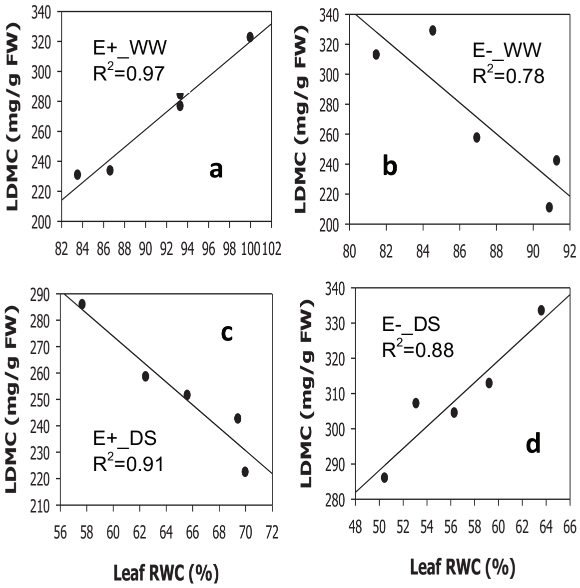

Figure 1. Relationships between RWC and LDMC in cv. Tully for E+ (a & c) and E- plants (b & d) under well-watered, WW (a & b) and drought stress, DS (c & d)

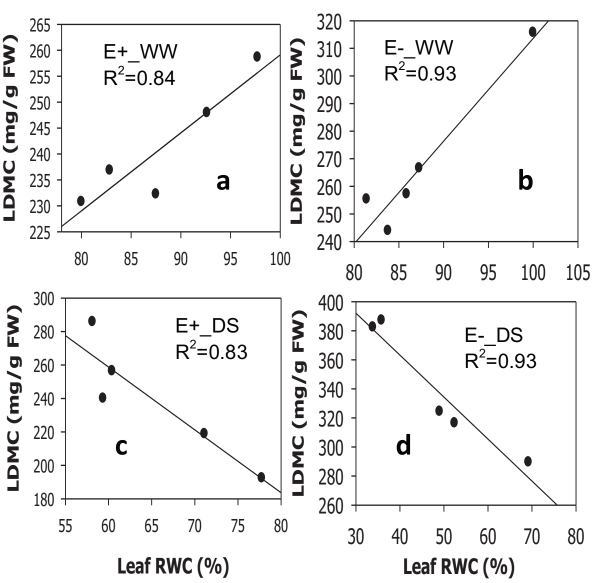

Figure 2. Relationships between RWC and LDMC in cv. Cayman for E+ (a & c) and E- plants (b & d) under well-watered, WW (a & b) and drought stress, DS (c & d)

*[Each of the five points on the graph is a sample replicate; average results are explained above]

Strong relationships were found between RWC and LDMC in both cultivars (R2=0.78 to 0.97) under well-watered and drought stress conditions (Fig. 1 & 2). Increase in RWC under well-watered condition was associated with increase in LDMC for E+ treatment contrary to E- treatment in cv. Tully. Under drought stress, LDMC decreased with increase in RWC under E+ treatment in contrast to E- treatment (Fig. 1). In Cayman (Fig. 2), increase in RWC under well-watered condition was associated with increased LDMC for both E+ and E- plants; while under drought stress, increase in RWC resulted in decrease in LDMC for both E+ and E- plants. Generally, overall trend showed that LDMC decreased as RWC increased under well-watered and drought condition (Result not shown).

Cultivars with greater resistance to drought usually maintain higher leaf RWC under drought stress (Matin *et al.* 1989) since higher RWC under water deficit may be associated with lower leaf transpiration and higher assimilation rate (Anyia and Herzog 2003). However, although cv. Tully maintained the highest leaf RWC under drought stress (61%), it showed the least resistance to drought stress since it had the lowest LDMC, leaf TNC and lowest leaf biomass.

Conclusions

Despite detection of significant endophyte effect on plant water status (RWC of leaf), LDMC, TNC and biomass in at least two cultivars (cv. Tully and cv. Cayman), no substantial positive effect of the endophyte on plant growth was found. Although this study suggests that endophytes might improve plant water status by increase in leaf RWC, it also demonstrates that endophytes might encourage high carbon losses through tissue respiration leading to reduction in TNC contents and biomass in certain cultivars. Endophytic reduction of TNC content could also reduce forage quality in some cultivars.

Acknowledgement

This project was funded by the government of Sweden through Swedish International development agency (SIDA).

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