NOTAS CIENTÍFICAS

FUNGAL ENDOPHYTES IN THE TROPICAL GRASSES *BRACHIARIA BRIZANTHA* CV. MARANDU AND *B. HUMIDICOLA*[1]

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ABSTRACT - A preliminary survey of fungal endophytes was conducted with species of *Brachiaria* cultivated at two active pastures in the State of Pará in the Brazilian Amazonia. Endophytic species, mostly fungi imperfecti, were isolated from leaves of *Brachiaria brizantha* cv. Marandu and *B. humidicola*. However, *Acremonium*-like (clavicipitaceous endophytic anamorph) was isolated only from the leaf sheaths of *B. brizantha* cv. Marandu.

FUNGOS ENDOFÍTICOS NAS GRAMÍNEAS TROPICAIS *BRACHIARIA BRIZANTHA* CV. MARANDU E *B. HUMIDICOLA*

RESUMO - Foi conduzido um levantamento preliminar dos fungos endofíticos presentes em duas espécies de *Brachiaria* cultivadas em pastagens em utilização no estado do Pará, na Amazônia brasileira. Várias espécies endofíticas foram isoladas das folhas de *B. brizantha* cv. Marandu e *B. humidicola*. No entanto, apenas em *B. brizantha* cv. Marandu foi isolada uma espécie com características morfológicas típicas de *Acremonium*.

Endophytic fungi are known to colonize the interior of apparently healthy plant tissues. These asymptomatic infections have been reported to be widespread in nature, and symptoms are expressed only under certain ecological or physiological conditions (Petrini, 1991).

The interest in the association between forage grasses and endophytic fungi is mainly due to the production of toxic alkaloids within the above ground tissues of certain grasses by the fungi. The fungal presence may increase plant resistance to insect herbivores (Siegel et al., 1990), enhance tolerance to environmental abiotic stresses (Bacon, 1993) and reduce grazing animal production due to the toxicity (Siegel & Schardl, 1991; Osborn et al., 1992). These systemic infections are caused by members of the Clavicipitaceae, tribe Balansiae. These are typical grass endophytes developing a systemic infection within the plant tissues that is carried to a new plant through the seeds. The asexual state of most of these fungi comprise species that belong to the form-genus *Acremonium* section *Albo-lanosa* Morgan-Jones & Gams (Morgan-Jones & Gams, 1982), and they are called clavicipitaceous endophytes.

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A great number of investigations on the distribution of clavicipitaceous endophytes derives from studies conducted in temperate regions with the forage grasses *Festuca arundinacea* (Schreb.) and *Lolium perenne* L. (Clay, 1987, 1988, 1989, 1990a; Fribourg et al., 1991; Fritz & Collins, 1991; White et al., 1992). Conversely, reports on endophyte-infected grasses from tropical regions are scarce. Only recently the presence of clavicipitaceous endophytes had been reported from a warm-season perennial grass (White Junior et al., 1990). On the other hand, tropical endophytes of woody plants have been the subject of several studies. For example: Palmae (Rodrigues & Samuels, 1990; Rodrigues, 1994), Araceae, Bromeliaceae and Orchidaceae (Petrini & Dreyfuss, 1981), Piperaceae and Crassulaceae (Dreyfuss & Petrini, 1984), and *Stylosanthes* (Pereira et al., 1993).

The knowledge on endophytes from tropical forage grasses could be of a great potential to agronomists and plant breeders for the manipulation and/or a better understanding of resistance mechanisms to insect attacks (e. g., spittlebug attacks in *Brachiaria* spp.) or toxicity symptoms in grazing animals.

Forage grasses of the genus *Brachiaria* are of growing importance in tropical America and particularly in Brazil (Miles & Lapoint, 1992). *Brachiaria brizantha* (Hochst. ex A. Rich) Stapf cv. Marandu and *B. humidicola* (Rendle) Schweickerdt have been widely adopted in Brazilian Amazonia (Dias-Filho, 1983, 1986). Both species are known to be relatively resistant to spittlebug (*Deois incompleta*) attacks although, in some situations, *B. humidicola* pastures can be severely damaged by this pest (Dias-Filho, 1983, 1986).

The mechanisms that may be involved in the resistance of forage grasses to spittlebug attacks, although extensively studied (Lapoint & Miles, 1992) are still poorly understood. It is possible that some plant compounds that confer antibiotic resistance to spittlebugs attacks may be related to endophyte infestation in resistant grasses.

In this study, the results of a preliminary survey on the fungal endophytes in *B. brizantha* cv. Marandu and *B. humidicola*, sampled from pasture areas in Brazilian Amazonia are reported. The objective was to determine if these plant species are infected by endophytes. This information could be of great importance, since the presence of certain endophytic species could be related to the potential of antibiotic resistance properties to herbivore insects or to the potential of poisoning of mammalian grazers in these forage grasses.

Leaves (blade, sheath, node and internode) from *B. humidicola* were collected from ten random locations in a 25-year-old pasture located in Belém, state of Pará, Brazil. Leaves (blade, sheath, node and internode) from *B. brizantha* were sampled from a five-year-old pasture located in Redenção, state of Pará, Brazil. Both sampling were made during the dry season.

Leaves were divided into five segments (blade, transition, sheath/blade, sheath, node and internode) and processed within 24 hours of sampling according to the procedures described in Leuchtmann & Clay (1988). The

standard medium used for the identification of the fungal isolates was cornmeal dextrose agar. Cultures were grown in 9 cm plastic petri dishes, and incubated at room temperature.

As far as we are aware this is the first report on the occurrence of endophytic fungi infecting *B. brizantha* cv. marandu and *B. humidicola*. The majority of the endophytic fungi isolated from both grass species belong to the Deuteromycetes (either anamorphic states of the Ascomycetes or anamorphs with unknown sexual state) and to a much lesser extent to the Ascomycetes (Table 1). A closely similar morphological and cultural characteristics of a typical *Acremonium* section *Albo-lanosa*, as discussed by White Junior et al. (1987), was revealed by an endophyte present only within the leaf sheath segments of *B. brizantha* cv. marandu.

Although the presence of *Acremonium* (clavicipitaceous endophytic anamorph) was detected only in *B. brizantha* cv. marandu and with a low frequency of infection during this investigation, its potential ecological role should not be underestimated. It could be possible that the relatively small sample size, and the fact that *Acremonium*-type endophyte is extremely slow growing in culture and as a result likely to be obscured by some fast-growing fungi, may have contributed to its low frequency during this preliminary survey. This low frequency could also be explained by differences in habitat, in which a woodland would favor a high frequency of endophytic infection in contrast to grassland habitat of pooid grasses as discussed by Clay & Leuchtmann (1989).

TABLE 1. Fungal endophytes isolated from five different leaf segments of *Brachiaria brizantha* and *B. humidicola*.

Taxa	Leaf segments				
	Blade	Transition sheath/blade	Sheath	Node	Internode
B. brizantha					
Acremonium-like			x		
Colletotrichum gloeosporioides (Penz.) Sacc.			x		
Fusarium sp.				x	x
Phoma sp. 2		x	x		
Stagonospora sp.		x	x		
Trichoderma viride Pers.:Fr.		x			
B. humidicola					
Curvularia cf. *pallescens* Boedijn	x	x	x		
Fusarium sp.				x	x
Leptosphaeria sp.				x	
Phoma sp. 1				x	
Phoma sp. 2		x	x	x	
Phomopsis sp.		x	x		
Physalospora sp.	x				

Although the occurrence in grass species of endophytic fungi that belong to the Balansiae and their anamorphs has been associated with increased insect resistance, other fungal species unrelated to the clavicipitaceous endophytes could have similar effect as discussed by Clay (1990b). The absence of *Acremonium*-type endophyte from *B. humidicola* and its low frequency found in *B. brizantha* make it not significant for toxin production. However, the other endophytic fungi isolated during this survey could be able to produce toxins active against herbivores, being beneficial to the host grasses. For example, *Fusarium* spp. have been reported to produce trichothecene mycotoxins which could act as insect deterrant (Clay, 1990b), and we have isolated a species of *Fusarium*, from both grasses, that could be tested for toxin production.

The beneficial or harmful effect caused by the fungal endophytes found in the forage grasses investigated in this study remains unknown. Additional studies need to be conducted in order to establish the agro-ecological significance of these findings in pasture environments.

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