

Fungal endophytes from the aerial tissues of important tropical forage grasses *Brachiaria* spp. in Kenya

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Introduction

Most, if not all plants in natural ecosystems are symbiotic with mycorrhizal fungi and/or fungal endophytes. This association between plant and fungi is believed to be over 400 million years old when plants first colonized the land (Redecker *et al.*, 2000). These fungal symbionts play important roles on plant ecology, fitness, and evolution; shaping plant communities; and the community structure and diversity of associated organisms. The importance of fungal endophytes in cool season grasses production and effects of endophyte on the livestock health (Examples fescue toxicosis and ryegrass staggers) have been extensively investigated (Smith *et al.*, 2009; di Menna *et al.*, 2012). However, current understanding of fungal endophyte community of warm season grasses (including *Brachiaria* spp.) and the impact of these endophytes on livestock production is very limited. Therefore, this study aims to analyse fungal endophytes community inhabiting in the aerial tissues of important tropical grasses *Brachiaria* species from Kenya, an East African country representing place of origin for most of the *Brachiaria* species.

Materials and Methods

Brachiaria plant samples for this study were: apparently healthy looking aerial portion of thirty naturally grown plants five plants from experimental station (Kenya Agriculture and Livestock Research Organization – Katumani) and fifteen germplasms of African origin from CIAT genebank. Samples processing, fungal isolation, DNA extraction, PCR amplification, sequencing, sequence analysis, and identification were performed as described previously (Ghimire *et al.*, 2011) except that PrepMan Ultra Sample Preparation Reagent (Applied Biosystems, Foster City, CA) and CLC Main Workbench 6.8.4 (<http://www.clcbio.com>) were used in DNA extraction and sequence analysis, respectively. Shannon–Wiener Index (H') was used to estimate species diversity. The seeds were surface sterilized in 1.9% sodium-hypochlorite solution for 30 min and rinsed 3 times in sterile water, blot dried and plated on PDA plate amended with antibiotics. Plates were incubated at room temperature up to 45 days and emerged fungal colonies were harvested for identification and subsequent analysis.

Results and Discussion

The 18S rRNA gene (partial), ITS1, 5.8S rRNA gene, ITS2, and 28S rRNA gene (partial) sequences of 94 fungal isolates from *Brachiaria* species revealed them into 46 distinct taxa (Fig. 1). These taxa belonged to two phyla, six classes, 14 orders, 22 families and 32 genera. Ascomycota and Basidiomycota constituted 96.8% and 3.2% of the population, respectively. Similarly, Dothideomycetes, Eurotiomycetes, Microbotryomycetes, Sordariomycetes, Tremellomycetes and Ustilaginomycetes were six classes detected in the study representing 51.1%, 5.3%, 1.1%, 40.4%, 1.1% and 1.1% of the population, respectively. Despite substantial differences in number of taxa belonging to Ascomycota and Basidiomycota each phylum was represented by three classes. Pleosporales (33%), Hypocreales (23.4%) and Capnodiales (13.8%) were the most frequent orders among 14 orders detected. Similarly, frequently detected families included Davidiellaceae (13.8%) and Pleosporaceae (13.8%), Incertae sedis I (12.8%) and Montagnulaceae (10.6%). *Cladosporium*, *Microsporaopsis*, *Acremonium*, *Fusarium* and *Alternaria* were the most frequently detected genera in this study. The phylogenetic relationships among the fungal isolates have been illustrated using 46 representative fungal taxa (Fig. 2). The Shannon Diversity Index (H') for this microbial community was 3.54.

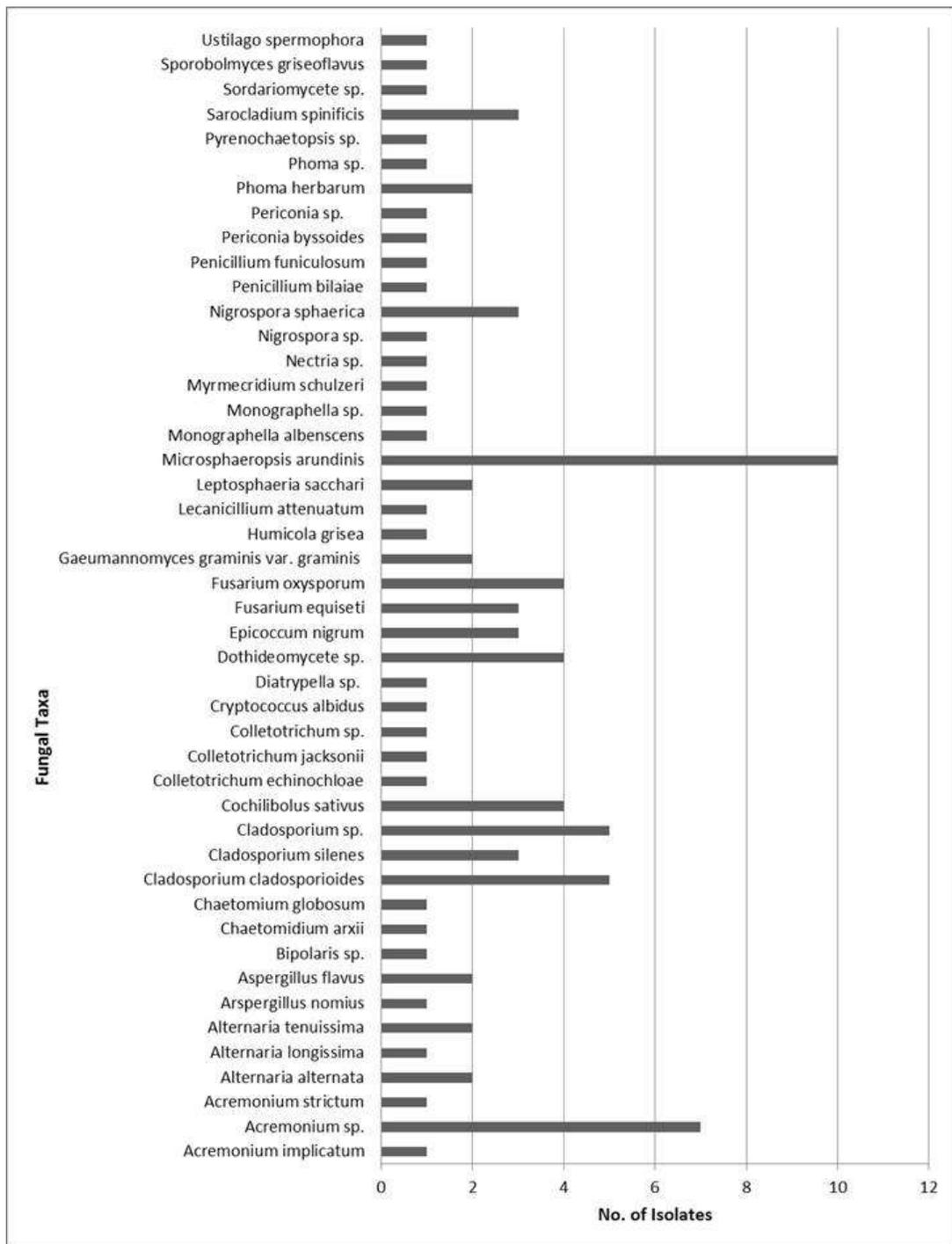


FIG1 Frequency of different taxa isolated from the aerial tissues of *Brachiaria* spp. in Kenya

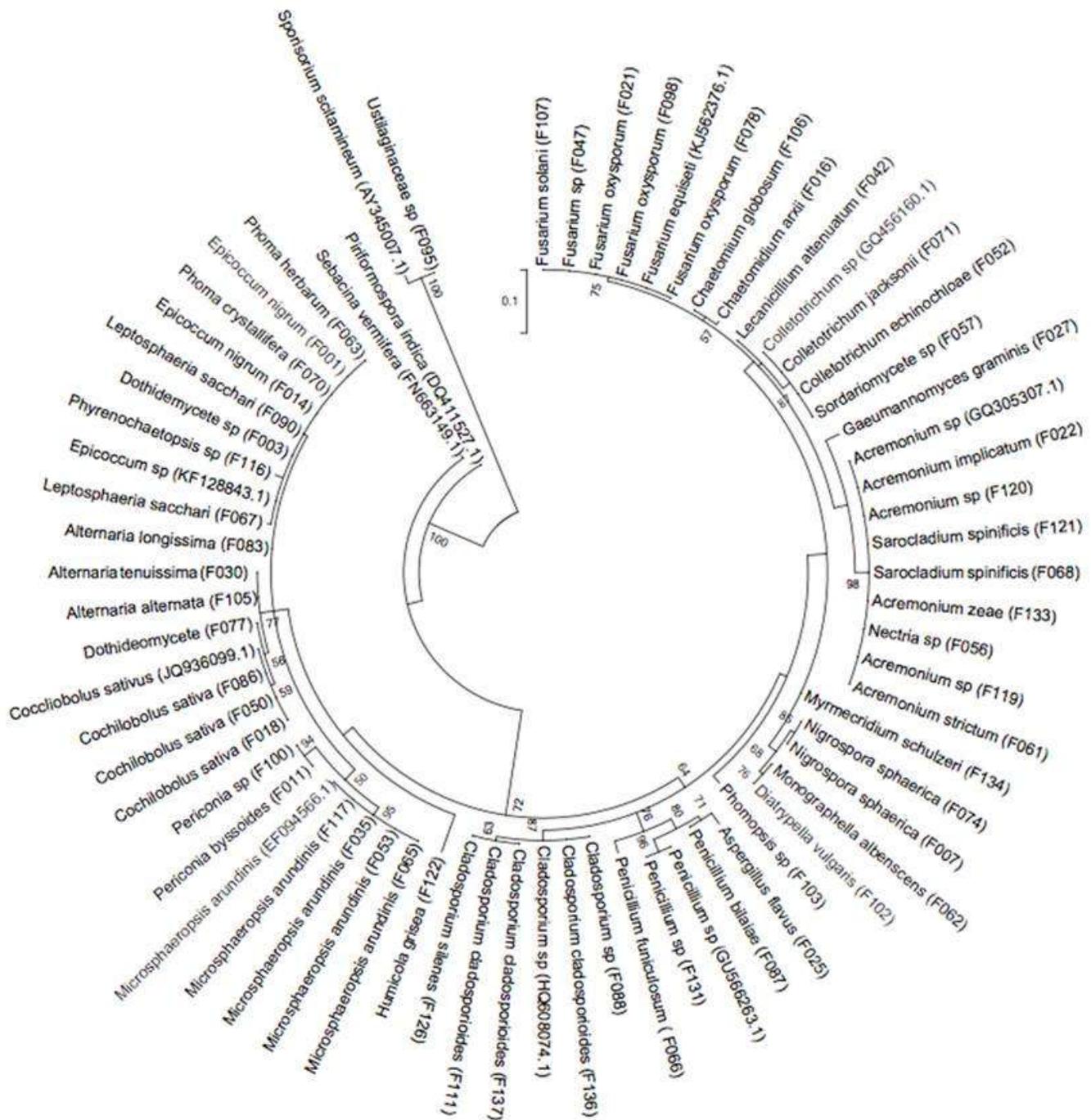


FIG2 Phylogenetic trees of the fungal isolates from above ground part of *Brachiaria* grasses. The evolutionary analysis was inferred using the maximum likelihood method, based on the Kimura 2-parameter substitution model at 1000 bootstraps.

This is the first study that documents the endophytic fungal community from the aerial tissues of *Brachiaria* grasses from Africa, the native home of most *Brachiaria* species. Endophytes isolated from *Brachiaria* grasses belonged to Ascomycota or Basidiomycota, and Ascomycota constituted about 97% of the fungal population. The taxonomic affiliations of these endophytes clearly showed them all as Class 3 nonclavicipitaceous endophytes. These Class 3 endophytes occur primarily or exclusively in the above-ground tissues, forms localized infection on host plants, and transmit horizontally. They have extremely high *in planta* biodiversity and their ability to confer benefits or costs on hosts are not necessarily habitat-specific. The Class 3 endophytes reproduce by hyphal fragmentation and/or by the production of sexual or asexual spores on dead or senescent tissue. The current understanding on ecological roles of these hyper diverse, Class 3 endophytes is very limited.

Brachiaria grasses harbor diverse group of endophytic fungi in above ground aerial tissues. The commonly isolated endophytes in the order of high to low occurrence were from the genus *Cladosporium*, *Microspora*, *Acremonium*, *Fusarium*, *Alternaria*, *Cochliobolus*, *Nigrospora*, *Aspergillus*, *Colletotrichum*, *Incertae sedis*, *Epicoccum*, *Phoma* and *Sarocladium*. Other eighteen genera were represented once or twice in the population. *Cladosporium* is cosmopolitan fungi in environment reported as causal agent of plant diseases and agent for respiratory health risk in susceptible human. Members of *Cladosporium* are known to be endophytes in many plant species. Endophytic status of *C. cladosporioides*, *C. silenes* and *Cladosporium* sp. has been reported from medicinal plant, *Panax ginseng*. *Microspora arundinis*, the second most frequently isolated endophyte from Brachiaria grasses form endophytic relationships with mangrove plant, *Laguncularia racemosa*. However, *M. arundinis* is associated with soft tissue infections in immunosuppressed patients and subcutis infection in cats.

Members of *Acremonium* formed the third largest group of endophytes in Brachiaria grasses. Some *Acremonium* species establish endophytic association with plant and provide fitness advantages to the host. For example, *Acremonium implicatum* is endophyte of Brachiaria grasses and transmits vertically through seeds, and confers protection against fungal pathogen *Drechslera* spp. the causal agent of leaf spot disease (Kelemu *et al.*, 2001). Similarly, *Acremonium zeae* has been considered as a protective endophyte of maize and displays antifungal activity against kernel rotting and mycotoxin producing fungi *Aspergillus flavus* and *Fusarium verticillioides*, and interferes with *A. flavus* infection and aflatoxin contamination of pre-harvest maize kernels. Our study demonstrates a substantial presence of *Acremonium* spp. in the native Brachiaria populations. It was very surprising that *A. implicatum*, a common endophyte of Brachiaria species in South America was not detected at all in African Brachiaria ecotypes, and the only one *A. implicatum* isolate detected in this study was isolated from the foliage tissue of the first year crop of hybrid Mulato grown from the seeds imported from South America. *Sarocladium spinificis* that shows a close relationship to *Acremonium* species detected in this study was recently reported as endophyte of coastal grass *Spinifex littoreus* in Taiwan. Moreover, *Sarocladium* accommodate seven species that were formerly placed under *Acremonium*. Other endophytic fungi isolated from Brachiaria grasses are believed to be non-systemic, generalist grass endophytes as reported for several temperate and tropical grasses.

Phylogenetic analysis of the 94 isolates representing 46 putative species showed them into six major clades each representing the unique class. Dothideomycetes, Eurotiomycetes and Sordariomycetes were divided into sub clades showing relationships among taxa at lower taxonomic levels. A study on fungal endophytes of eleven understory forest grasses isolated 94 putative species among 402 fungal isolates belonging 14 orders. Similarly, the shoot endophyte community of switchgrass was composed of 51 putative species from 9 orders (Ghimire *et al.*, 2011).

Conclusion

Brachiaria harbor complex and diverse fungal community possibly with varied roles to host plant ranging from plant pathogen to beneficial organism. This study demonstrated *Acremonium* as the third most frequently occurring genus after *Cladosporium* and *Microspora*, and *Acremonium implicatum* was not recovered from local ecotypes. The association of other *Acremonium* species with local ecotypes necessitates further research to understand whether they provide similar benefit to host as reported for *A. implicatum*. Like in other tropical grasses endophytic taxa recovered in this study were generalist and no *Epichloë* species was detected. This study presents the first hand inventory of the aerial endophytes community of Brachiaria grasses and opens opportunities for further research on the utilization of these microbes for agricultural and industrial applications.

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