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Fungal Endophyte Enhances Biomass Production and Essential Oil Yield of East Indian Lemongrass

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Abstract

East Indian lemongrass (*Cymbopogon flexuosus* (Stued.) Wats.) is one of the most important aromatic grasses cultivated for the production of lemongrass oil in tropical and subtropical regions of the world. A fungal endophyte, *Balansia sclerotica* (Pat.) Hohn. establishes a perennial association with the commercially grown east Indian lemongrass cv. Kerala local (syn.=OD-19). Endophyte-infected plants produced 195% more shoot biomass and 185% more essential oil than the endophyte-free control plants when grown experimentally under glasshouse conditions. The essential oil extracted from the endophyte-infected plants is qualitatively identical with that of endophyte-free plants. Thin layer chromatography analyses also confirmed that the essential oil from endophyte-infected lemongrass is free of toxic ergot alkaloids. Endophyte infection induced vivipary in east Indian lemongrass. Endophyte-infected plants can be propagated vegetatively. The results of the present investigation indicate that *B. sclerotica*-infected east Indian lemongrass has potential for agricultural exploitation.

Keywords: Fungal endophyte, *Balansia sclerotica*, *Cymbopogon flexuosus*, east Indian lemongrass, biomass production, essential oil content, vivipary induction

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1. Introduction

Fungal endophytes are a group of fungi that form mutualistic associations with grasses and sedges. They are clavicipitaceous fungi that exist perennially in the aerial tissues of the host plants. Five genera, *Atkinsonella* Diehl, *Balansia* Speg., *Balansiopsis* Hohnel, *Epichloe* (Fr.) Tul. and *Myriogenospora* Atk., with approximately 30 species are included in this group. Current interest in fungal endophytes derives from the recent discovery of many benefits they confer on their host plants and the agricultural potential of plants infected by them (Siegel et al., 1987; West et al., 1988; Arechavaleta et al., 1989; Kimmons et al., 1990; Hill et al., 1991). Diehl (1950) described 17 species of *Balansia* of which 13 are endemic to the USA and other regions of the western hemisphere, the remaining 4 species occur in India (Singh and Pavgi, 1972). Their association may be either complete, i.e. endophytic or localized, i.e. epibiotic.

The genus *Cymbopogon*, belonging to family Andropogoneae, contains six important aromatic grasses which produce a number of essential oils of significant commercial values. *Cymbopogon flexuosus* (Steud.) Wats., commonly known as east Indian lemongrass is cultivated commercially in India and several other South-east Asian countries. The essential oil obtained from the east Indian lemongrass serves as a chief source of citral (75–85%), which is a basic raw material for the synthesis of β-ionones, a precursor for the synthesis of a number of aromatic compounds and vitamin A. It is also used extensively in the perfume, soap and cosmetic industries. East Indian lemongrass cv. Kerala local (syn.=OD-19) is known to be infected by *Balansia sclerotica* (Pat.) Hohn., which causes grassy-shoot malformation (Janardhanan et al., 1991). The association is perennial, endophytic and leads to the production of sterile flowers, abnormal inflorescences and shortened internodes. In this communication, we report for the first time the ability of a fungal endophyte to enhance shoot biomass production and essential oil yield of commercially grown east Indian lemongrass cv. Kerala local.

2. Material and Methods

Planting, growth and biomass production

Pathogenicity of *B. sclerotica* was established on the susceptible genotype, Kerala local (syn.=OD-19) by artificial inoculation of the healthy inflorescences with an aqueous spores suspension of the fungus (Absar, 1991; Janardhanan et al., 1991). Since the infection by *B. sclerotica* on the host was extremely low (3–4%), two kinds of plant progenies, one was *Balansia*-infected

and other was *Balansia*-free, were derived from the seeds of inoculated inflorescences of the same mother plant. These two categories of plant clones of east Indian lemongrass cv. Kerala local were used for the present investigations. Strong tillers (slips) from the infected clones were separated and washed thoroughly to remove soil particles. One slip was planted in ten-replicated 25 cm earthen pots filled with garden soil after sterilization by steam at 121°C for 2 hrs. Similarly, an equal number of slips from a healthy, endophyte-free clone was planted in another set of ten earthen pots, which served as control. The endophyte-free nature of the control plant was confirmed by histopathological and microbiological methods as described by Janardhanan et al. (1991). The plants were grown in the glasshouse and irrigated when necessary with tap water. After one year, growth was determined by measuring height and number of tillers/plant, while biomass production was estimated by harvesting herbs 15 cm above the soil.

Yield of essential oil and its analysis for citral content

Freshly harvested endophyte-infected and endophyte-free plants were separately chopped into small pieces and placed into round bottom flasks for the extraction of essential oil by hydrodistillation using Clevenger's apparatus. Essential oil yield was calculated on fresh weight basis. Citral content of the essential oil was determined by gas liquid chromatography (GLC) using a Perkin-Elmer Model 3920-B equipped with thermal conductivity detector (TCD) and a 2 m × 3 m stainless steel column packed with 10% carbowax 20 M on chromosorb W (80–100 mesh).

Determination of the presence of toxic ergot alkaloids in the endophyte-infected plants

To establish the presence of toxic ergot alkaloids in the endophyte-infected plants, samples of infected as well as healthy leaves and culms of the same age were lyophilized and powdered separately. An amount of 20 g sample was extracted with 100 ml of ethanol three times after adjusting to pH 8–9 with aqueous ammonia. Ethanolic extracts were pooled together and filtered through Whatman filter paper No.1. Filtrate was evaporated to dryness under vacuum at 40°C. The concentrated extract was then dissolved in ethyl acetate (50 ml) and shaken with 20 ml of 2% tartaric acid. The aqueous tartaric acid fraction after adjusting to pH 8–8.5 was extracted with 50 ml of chloroform three times. The moisture in chloroform extract was removed by adding anhydrous sodium sulphate and the solvent was removed at 40°C under vacuum.

The dried extracts from the endophyte-infected and endophyte-free plants were dissolved in a small amount of chloroform: ethanol (90:10) and spotted on TLC plate coated with silica gel G. LAD and ILAD were used as standards. The chromatogram was developed in chloroform: methanol (80:20) and sprayed with Ehrlich's reagent for detecting the presence of toxic ergot alkaloids.

Determination of the presence of toxic ergot alkaloids in the essential oil

The essential oil extracted from *B. sclerotica*-infected lemongrass was tested for the presence of toxic ergot alkaloids. One ml essential oil was taken into a test tube, diluted to 10 ml with a solution of methanol-acetic acid-water (40:10:50 v/v), and shaken well. One ml of this mixture was removed to another test tube and 2 ml van Urk reagent was added, mixed thoroughly and observed after 30 min for typical colour reaction of ergot alkaloids. The essential oil extracted from endophyte free healthy plants and addition of toxic ergot alkaloids into essential oils from healthy plants were used for comparison. Samples of essential oil from the endophyte-infected plant was further analyzed by thin layer chromatography (TLC) for detecting the presence of ergot alkaloids. An amount of 1 ml essential oil was thoroughly mixed with 10 ml of ethanol and pH of the solution was adjusted to 9–10. Then the solution was extracted with solvent such as chloroform for three times. The extract was combined, concentrated and extracted with 2% aqueous tartaric acid. This solution was then made alkaline (pH 9–10) and extracted again with chloroform three times and evaporated to dryness. The residue was dissolved in small amount of chloroform:ethanol (10:10) and 10 µl was spotted on TLC plates coated with silica gel G. Similar treatment given to the oil samples from the endophyte-free plants with and without addition of ergotamine and pure ergometrine (Sigma) served as control. Chromatogram was developed in chloroform:methanol (80:20) and TLC plates were air dried and sprayed with Ehrlich's reagent for the detection of ergot alkaloids.

Induction of vivipary in east Indian lemongrass

We observed a few induced viviparous plantlets on the aborted inflorescences of infected plants. To establish whether *B. sclerotica* infection induced vivipary in east Indian lemongrass cv. Kerala local, a number of branchlets from the inflorescences of an endophyte-infected plant were removed and the basal portions of the branchlets were placed in sterile distilled water. An equal number of cuttings from an endophyte-free plant treated similarly served as control.

3. Results

Effect of B. sclerotica infection on biomass production of east Indian lemongrass

Lemongrass plants generated from the "slips" of *B. sclerotica*-infected mother plants showed typical symptoms of endophytic infection. The inflorescences that developed on these plants were malformed and the flowers were either aborted or sterile. The endophyte-infected plants were found to grow more vigorously, produced larger numbers of tillers and a more bushy growth with significantly higher biomass than endophyte-free plants (Fig. 1a). These plants were indistinguishable from endophyte-free plants in the initial stages of growth until flowering. However, plants developed from the slips of endophyte-free mother plants were normal and healthy (Fig. 1b). Histopathological observations revealed the presence of typical hyphae of the fungal endophyte in the tissues of infected host plants (Fig. 1c). The fungal endophyte infection induced 400% more tillering and 195% more biomass production (Table 1). Since lemongrass can be propagated vegetatively, the infection is carried through generation after generation. This suggests that the fungal endophyte establishes an intimate physiological relationship with the host plants. The endophyte-infected plants can be propagated routinely.

Effect of B. sclerotica infection on the yield and quality of essential oil

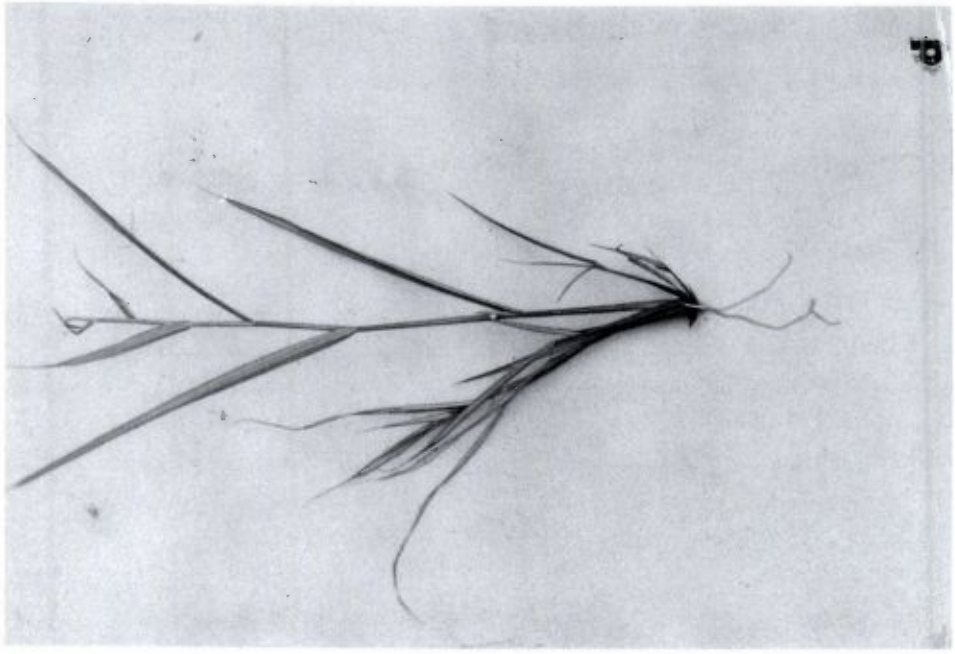
The endophyte-infected plants were found to produce 185% more essential oil than endophyte-free plants. GLC analyses showed that the endophyte did not

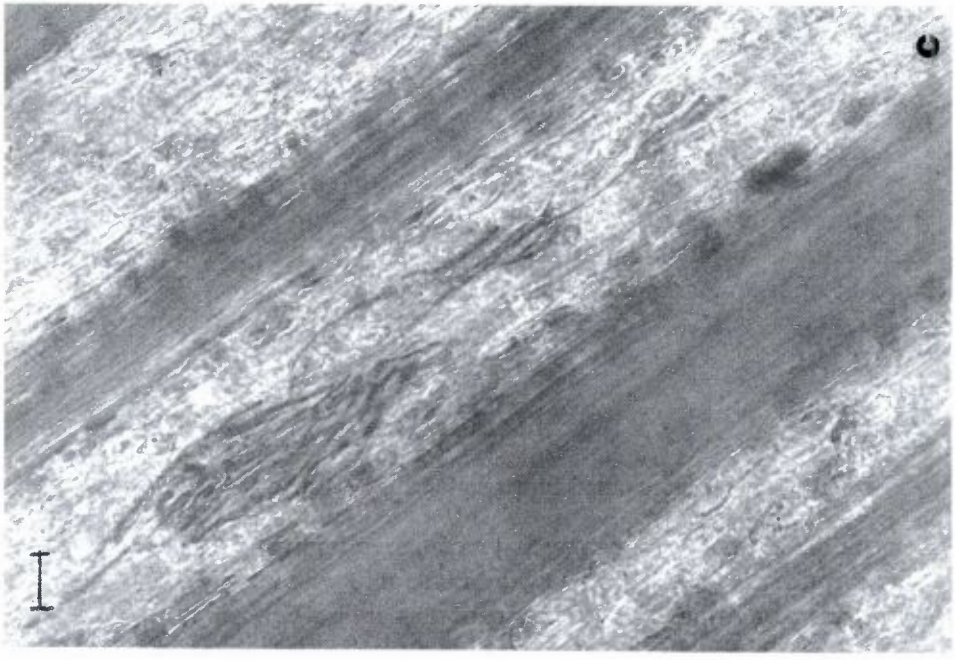
Table 1. Effect of endophytic infection by *Balansia sclerotica* on the biomass and essential oil of east Indian lemongrass cv. Kerala local

Parameter of responses	Endophyte-infected plants (mean±SE)	Endophyte-free plants (control) (mean±SE)
No. of tillers	41.00±4.00	10.00±1.00
Height (cm)	44.70±0.77	59.10±1.91
Fresh weight (g)	90.00±2.4	46.30±5.9
Dry weight (g)	38.76±1.86	15.76±0.67
Essential oil content (%)	0.65±0.02	0.35±0.02
Citral content (%)	84.78±0.098	88.43±0.32

SE = standard error.

Figure 1. a) East Indian lemongrass cv. Kerala local infected by *B. sclerotica* showing abnormal and deformed inflorescence, grassy-shoot symptoms and profuse tillering, b) endophyte-free control plant, c) endophytic hyphae of *B. sclerotica* in the leaf sheath of infected lemongrass plant (bar = 50 μm), and d) induced vivipary due to the endophyte-infection showing emergence of roots from the basal portion of branchlets.





cause any qualitative deterioration of the essential oil constituents. The citral content of the essential oil extracted from endophyte-infected lemongrass plants was a little higher than that of endophyte-free plants (Table 1).

Determination of the presence of toxic ergot alkaloids in the B. sclerotica infected lemongrass

Ethanolic extracts of *B. sclerotica*-infected lemongrass and control plants yielded a greenish substance when the solvent was completely evaporated. The major part in the residue appeared to be chlorophyll. Ergot alkaloids were recovered in aqueous tartaric acid from the residue by dissolving crude ethanolic extract into ethyl acetate and extraction with tartaric acid. This process removed the coloring materials. Extraction of the aqueous tartaric phase with chloroform at pH 8–8.5 brought the ergot alkaloids into the solvent system. A light brown residue containing ergot alkaloids was obtained from the endophyte-infected samples, while there was no detection of the ergot alkaloids in the samples of endophyte-free plants, as confirmed by TLC (Fig. 2).

Detection of ergot alkaloids in the essential oil of endophyte-infected plants

Although several species of *Balansia* have been shown to produce ergot alkaloids *in vitro* and *in vivo* (Clay et al., 1985; Bacon et al., 1986), the essential oil extracted from *B. sclerotica*-infected lemongrass was found to be free of ergot alkaloids as confirmed by TLC. Thus, the quality of the essential oil of east Indian lemongrass was not affected by *Balansia* infection.

Induction of vivipary in lemongrass

Emergence of roots was observed from all the endophyte-infected branchlets (Fig. 1d) 3–4 days after they were placed in water, indicating the potential viviparous nature of branchlets of aborted inflorescences. Branchlets of inflorescences from endophyte-free plants treated in the same manner did not show the emergence of roots.

4. Discussion

Fungal endophyte infection is of particular importance in the east Indian lemongrass cv. Kerala local because it enhances the economic value of the variety. Infection enhances total biomass by increasing the number of tillers/plant. Infection-induced sterility might result in more vigorous

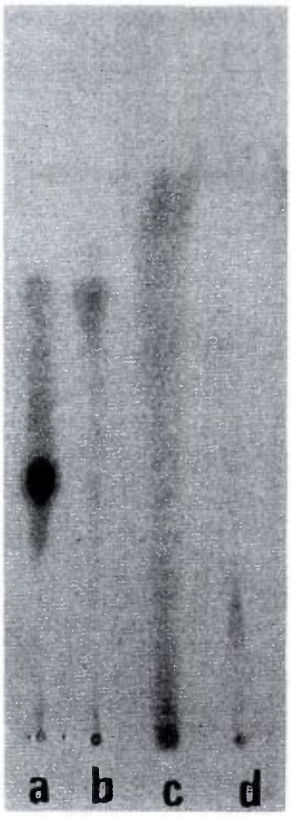


Figure 2. TLC plate showing presence of ergot alkaloids in the *Balansia*-infected east Indian lemongrass. Standard lysergic acid amide (a), iso-lysergic acid amide (b), crude leaf extracts of *Balansia*-infected (c) and of healthy (d) lemongrass (control).

vegetative growth of infected plants due to reallocation of resources from reproduction to vegetative growth. Enhanced essential oil production by the endophyte-infected lemongrass plants shows the influence of the fungus on the host metabolism. The altered morphology of infected plants might be a contributing factor for higher essential oil production as the infection induces profuse production of tiny leaves. The intimate physiological relationship between the *B. sclerotica* and lemongrass seems to draw a parallel with the plant microbe complexes involving mycorrhizal fungi and nitrogen fixing bacteria. The usual animal toxicoses observed with *Balansia*-infected grasses are not associated with the lemongrass, because animals do not graze on this

plant. The endophyte infection on east Indian lemongrass producing typical grassy-shoot symptoms is still a disease.

The induction of vivipary in the *B. sclerotica* infected east Indian lemongrass seems to be due to increase in the IAA content. Induced vivipary has been reported in *Cyperus virens* infected with *B. cyperi* (Clay, 1986). Vivipary has been artificially induced in grasses by altering the normal hormonal balance of the plant (Junttila, 1985). This suggests that altered hormonal balance in *B. sclerotica* infected lemongrass plants is responsible for grassy shoot symptoms. Based on the results of the present investigation it has been concluded that *Balansia* infection can impart beneficial effects on biomass production and essential oil yield of east Indian lemongrass cv. Kerala local.

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