Endophytic Fungi and Ergot Alkaloids in Native Turkish Grasses

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Mature inflorescences of 76 samples of ten genera of native grasses were collected in the Ankara province of Turkey. Microscopic examination of individual seeds revealed the presence of endophytic fungi in four seed samples, The endophytes were isolated in pure culture and identified. Seeds were also ground and subjected to ELISA and HPLC analyses to confirm the levels of ergot alkaloids typically produced by endophytic fungi of grasses.
KEY WORDS: Endophytes; *Neotyphodium* spp.; ergot alkaloids; ergopeptine; ELISA; HPLC.

Clavicipitaceous grass endophytes of the genus *Neotyphodium* have a worldwide distribution and infect many grass host genera (11). Of the economically important forage genera serving as hosts, *Lolium* and *Festuca* have been of particular interest (1,5,10), mainly due to the toxicosis that infected plants can cause in grazing animals. Endophytes have mutualistic-symbiotic relationships with their grass host, producing deterrents to some insects and plant diseases (3). Endophytes of the genus *Neotyphodium* produce numerous alkaloids, among them ergopeptine and related alkaloids which have been the subjects of intensive research. A high performance liquid chromatography (HPLC) method was used (12) to detect ergopeptine alkaloids from tall fescue seed (*Festuca arundinacea*) and identified ergovaline in the infected grass and seeds. Various grass species and cultivars infected by *Neotyphodium* spp. were examined and 60% of the associations were found to produce ergovaline (9). HPLC combined with electrospray ionization mass spectrometry (ESI-MS) was used to identify ergot alkaloids in tall fescue by Shelby *et al.* (8). Immunoassays have also been developed for a number of ergot alkaloids (2,4,6). A monoclonal antibody was developed by Shelby and Kelley (7), which is cross reactive with many of the ergot alkaloids produced by clavicipitaceous endophytes.

This study constituted a brief survey of native Turkish grasses for the presence of endophytic fungi and ergot alkaloids using microscopy, culture, ELISA and HPLC techniques. Plant material was collected at random in June and July 1997, from different locations in Ankara province. Ten plant genera and 11 plant species were collected from 21 sites. Of the 76 cereal and grass accessions collected, 36 belonged to the genus *Aegilops*, two belonged to genera of *Hordeum*, and 38 were temperate grasses (*Amblyopyrum, Bromus, Eremopoa, Festuca, Poa, Stipa, Taeniatherum*).

Mature seeds along with the inflorescence were placed in envelopes and assigned accession numbers. Data on collection date, geographic location and altitude were recorded. Grasses were identified by Musa Dogan from the Botanical Department of Gazi University and Aysegul Yildirim from the Plant Protection Research Institute of Ankara. The seeds were stored at 5°C until tested for infection.

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Seeds were soaked in 5% NaOH overnight (8 h minimum) at room temperature and then washed thoroughly in running tap water. They were then placed on a microscope slide in a drop of aqueous aniline blue stain (1 g aniline blue + 100 ml water + 200 ml lactic acid), squashed under a cover slip, and examined at ×200 magnification for the presence of fungal hyphae.

Endophytic fungi were isolated from the remaining seeds of samples with visible endophyte infection. Approximately 70–80 seeds were surface-sterilized by soaking them in 50% Clorox (commercial bleach, NaOCl) for 20 min, then transferred into 70% ethanol for 5 min, following which they were placed on tissue paper for ~5 min in a laminar flow cabinet. Seeds were plated on MS (Murashige & Skoog Basal Salt Mixture, Sigma M5519 + Agar 12 g/l) in petri dishes and incubated at 25°C. Inoculated plates were examined daily for contaminating fungi. Endophytic fungi could be observed from some seeds after 14 days. These were transferred, examined by light microscopy, and identified. Endophytes were also isolated from plants grown from seeds. Stems and leaf sheaths were collected after ~30 days of growth, sterilized with 10% Clorox solution for 5 min, plated on MS, incubated and isolated as described previously.

For the ELISA test, seeds were ground, and 1 g of each sample was placed in a disposable plastic cup. The enzyme linked immunosorbent assay (ELISA) procedure as described by Shelby and Kelley (7), was followed. Each test sample was placed in one well of four ELISA plates (four replications), and compared with positive controls of tall fescue infected with *Neotyphodium coenophialum*. Seeds scoring positive by the ELISA test were further analyzed by HPLC (8) to determine specific alkaloid content. Of the 76 samples tested, only four were found to contain endophytic fungi (Table 1). When examined by ELISA for total ergot alkaloid content, only one accession (no. 44) was found to contain ergot alkaloids. The sensitivity of the ELISA test also precluded the possibility of infected seed not being detected by microscopy. When the ELISA-positive seed were also tested by HPLC for the presence of ergovaline, none was found (data not shown), suggesting that this endophyte–host combination produces a unique spectrum of alkaloids.

TABLE 1. Endophytes and ergot alkaloids from Turkish grasses

Accession no.	Grass species	Endophyte species	ELISA value[z]
37	*Festuca callieri*	*Neotyphodium typhinum, Acremonium ochraceum*	0.852 a
44	*Amblyopyrum muticum*	*Acremonium bactrocephalum, Paecilomyces inflatus*	0.387 b
54	*Aegilops triuncialis*	*Acremonium ochraceum*	0.679 a
76	*Aegilops cylindrica*	*Acremonium bactrocephalum*	not tested
+Control	*Festuca arundinacea*	*Neotyphodium coenophialum*	0.456 b
−Control	*Festuca arundinacea*	none	0.817 a

[z] Mean of four replications of the competitive indirect ELISA for total ergot alkaloids. Absorbance at 405 nm. Means followed by the same letter do not differ significantly (P= 0.05).

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