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Phytochemistry 66 (2005) 195–202
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Distribution of the fungal endophyte *Neotyphodium lolii* is not a major determinant of the distribution of fungal alkaloids in *Lolium perenne* plants

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Received 4 September 2004; received in revised form 25 November 2004

Abstract

The relationships of the distributions of the insect and mammalian mycotoxins, lolitrem B and ergovaline, and the insect-feeding deterrent, peramine, with the distribution of fungal mycelium were investigated in three genotypes of perennial ryegrass (*Lolium perenne* L.) infected with the endophyte *Neotyphodium lolii*. In planta levels and distribution of the endophyte and of the three alkaloids were assessed in parallel, and different spatial or temporal concentration gradients were observed for each. Variation in the tissue distribution of the endophyte accounted only for 20%, 6%, and 31% of the variation in ergovaline, lolitrem B, and peramine, respectively. Alkaloid–endophyte ratios, determined in individual grass tissues, showed distinct in planta distribution patterns for each alkaloid and differed in magnitude among genotypes. The ergovaline–endophyte ratio was higher in the very basal plant tissues than in the apical tissues, while the lolitrem B and peramine ratios tended to be higher in apical tissues. The lolitrem B–endophyte ratio increased with leaf age, while no consistent temporal trends were detected for the other alkaloids. The results indicate that endophyte colonisation is a minor determinant of alkaloid levels, and that accumulation of the alkaloids relative to the endophyte mycelium is affected by plant genotype and tissue in a manner specific to each alkaloid. Possible factors in the regulation of alkaloid levels in the grass plant are discussed.

Keywords: *Lolium perenne*; *Neotyphodium lolii*; Poaceae; Alkaloid distribution; Ergot alkaloids; Indole-diterpenoids; Ergovaline; Lolitrem B; Peramine; GUS-reporter gene

1. Introduction

Neotyphodium lolii (Latch, Christensen, & Samuels) Glenn, Bacon & Hanlin is a fungal endophyte symbiotic with perennial ryegrass (*Lolium perenne* L.) (Christensen et al., 1993). In this symbiosis, the biologically active alkaloids, ergovaline, peramine and lolitrem B, are produced (Fig. 1). The ergopeptine, ergovaline, and the indole-diterpenoid, lolitrem B, have been associated with toxic effects on mammals (Bush et al., 1997; Rowan, 1993; Tor-Agbidye et al., 2001) and insects (Ball et al., 1997b; Prestidge and Gallagher, 1988). The pyrrolopyrazine alkaloid, peramine, deters feeding of the Argentine stem weevil (*Listronotus bonariensis*), a major insect pest on perennial ryegrass (Rowan, 1993). Despite the mammalian toxicities associated with ergovaline and lolitrem B, endophyte infection is considered to provide a net benefit in agricultural settings (Bacon, 1993; Bush

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doi:10.1016/j.phytochem.2004.11.021

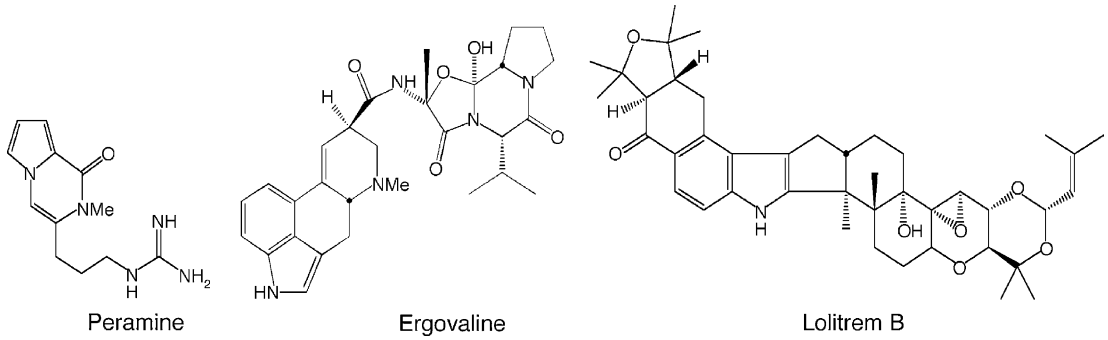

Fig. 1. Structures of the alkaloids produced in *N. lolii*-infected perennial ryegrass.

et al., 1997; Easton, 1999). Therefore, current strategies for forage grass improvement focus on maintaining the benefits of endophyte infection, while minimising negative effects caused by the accumulation of ergovaline and lolitrem B. One approach has been the identification of endophyte strains that do not lead to the accumulation of these toxins in the host plant and inoculation of these strains into endophyte-free grass plants (Bouton et al., 2002; Tapper and Latch, 1999). Perennial ryegrass cultivars infected with endophytes that do not produce the mammalian toxins have been shown to be free from animal toxicity and to maintain significant levels of resistance to insect herbivory (Fletcher, 1999; Popay et al., 1999).

An alternative approach is to manipulate the levels and distribution of alkaloids in the plant, hence, the balance between different alkaloid pathways in the symbiosis, by selection of suitable plant–endophyte associations. Genetic and in vitro studies have indicated that the endophyte is the producer of all three alkaloids (Gurney et al., 1994; Rowan, 1993; Scott, 2001). Both plant genotype and tissue type influence endophyte and alkaloid levels (Easton et al., 2002; Keogh et al., 1996; Roylance et al., 1994), and some correlations between alkaloid levels and endophyte levels were suggested by earlier studies (Ball et al., 1995; Easton et al., 2002; Keogh et al., 1996). It is still unclear, however, whether alkaloid levels in the plant are simply a function of the level of endophyte colonisation, or if the plant genotype/tissue inhabited by the endophyte has an influence on alkaloid levels, for example, by modulating alkaloid biosynthesis in the fungal mycelium or alkaloid degradation and translocation. Until recently, a lack of sensitive high-resolution methods for mapping of endophyte and alkaloid distributions has prevented researchers from addressing this key question. However, such methods have now become available. Tan et al. (2001) have shown that the in planta distribution of endophytes transformed with a constitutively expressed β-glucuronidase (GUS) gene (Jefferson, 1987) can be precisely mapped with quantitative GUS activity assays. We have also recently developed sensitive micro-scale extraction methods for extraction and quantification of ergovaline and peramine (Spiering et al., 2002) and lolitrem B (Tapper and Latch, 1999). Here, we have combined these methods to investigate the relationship between alkaloid and endophyte levels. This study is the first assessing the distributions of all of the major alkaloids produced in *N. lolii*-infected perennial ryegrass together with the distribution of the endophyte. Our findings suggest that levels and distribution of the endophyte is only a minor factor in determining alkaloid levels in a given plant genotype or tissue.

2. Results and discussion

2.1. GUS activity is a quantitative indicator for endophyte levels in different plant genotypes and tissues

Alkaloid levels in endophyte–grass associations are affected by the plant genotype (Adcock et al., 1997; Easton et al., 2002). To represent different spectra of the alkaloids in different endophyte–plant associations, we performed a pre-screening. Tissue concentrations of GUS activity, ergovaline, peramine, and lolitrem B were determined in 15 genotypes infected by the GUS-transformant KS1. Three genotypes, Nui D, Nui UIII, and Nui UIV, showing distinct differences in alkaloid and GUS activity concentrations (data not shown) were selected for detailed dissection. To minimise environmental effects in the experiments, plants of the selected genotypes were introduced into a controlled-environment cabinet.

As shown by Tan et al. (2001), GUS activity closely follows the in planta distribution of KS1 hyphae in the ryegrass genotype Nui D, suggesting that GUS could be used as a quantitative marker for endophyte levels. We confirmed this for all genotypes (Nui D, Nui UIII, and Nui UIV) used in this study. We determined GUS activity and endophyte levels (as hyphal counts in cross-sections) in 29 tissues (sheath and blade from first and third mature leaf) from all three genotypes. Linear regression analysis indicated a first-order relationship