Available online at www.sciencedirect.com
ScienceDirect
Environmental and Experimental Botany 63 (2008) 183–199
Environmental and Experimental Botany
www.elsevier.com/locate/envexpbot

Neotyphodium fungal endophytes confer physiological protection to perennial ryegrass (*Lolium perenne* L.) subjected to a water deficit

Heike Hahn[a,b], Michael T. McManus[a], Kristina Warnstorff[c], Brendon J. Monahan[a,1], Carolyn A. Young[a,2], Elizabeth Davies[d], Brian A. Tapper[d], Barry Scott[a,*]

[a] *Institute of Molecular BioSciences, Massey University, Private Bag 11 222, Palmerston North, New Zealand*
[b] *Martin-Luther-University Halle-Wittenberg, Faculty of Natural Sciences III, Institute of Agricultural and Nutritional Sciences, Crop Science, Ludwig-Wucherer-Strasse 2 D-06108 Halle, Germany*
[c] *Martin-Luther-University Halle-Wittenberg, Faculty of Natural Sciences III, Institute of Agricultural and Nutritional Sciences, Biometrics and Informatics in Agriculture Group, Ludwig-Wucherer-Strasse 2 D-06108 Halle, Germany*
[d] *AgResearch, Grasslands Research Centre, Private Bag 11 008, Palmerston North, New Zealand*

Received 15 February 2007; received in revised form 1 October 2007; accepted 8 October 2007

Abstract

While it is generally accepted that *Neotyphodium lolii* and related epichloë endophytes are mutualists that provide important benefits to their perennial ryegrass (*Lolium perenne*) host plants under conditions of biotic stress, relatively little is known about the effect of endophyte on the host under conditions of abiotic stress. Using genetically identical endophyte infected (E+) and uninfected (E−) clones of perennial ryegrass from a natural and a synthetic association grown under conditions of water stress in a controlled environment, we show that *N. lolii* had minor effects on morphological responses (leaf elongation rate and ground biomass production) but had more pronounced effects on physiological responses (water use efficiency, relative water content and osmotic potential) by the host. The effects were most marked in the natural association. While levels of proline increased in response to water stress, the presence of endophyte had no effect on those levels. The effect of water stress on endophyte bioprotective metabolites was also examined. Ergovaline levels in pseudostem tissue increased in response to increasing water stress for both *N. lolii*/*L. perenne* associations but lolitrem B levels only increased in the natural association. No differences in steady state levels of transcripts from genes known to be required for the synthesis of these alkaloids were observed in response to water stress. This study demonstrated that *N. lolii* can confer protection to perennial ryegrass from water stress and that levels of the bioprotective metabolites, lolitrem B and ergovaline were altered in response to this abiotic stress in a manner that was specific for each symbiotic association.

Keywords: *Neotyphodium lolii*; Water stress; Perennial ryegrass; Endophyte; Lolitrems; Ergovaline; Proline

1. Introduction

Perennial ryegrass (*Lolium perenne* L.) is one of the most important plant species in forage ecosystems. It has become naturalised in many different parts of the world, and is particularly well adapted to temperate regions. It is an outcrossing species with considerable genetic variability both within and between cultivars (Casler, 1995; Kubik et al., 2001). As a species it can tolerate a wide range of environmental and grazing conditions, attributes that have undoubtedly contributed to its ecological success. Many of the benefits of this grass species in forage ecosystems, especially those related to field persistence and stress tolerance, have been attributed to the presence within the host grass of the symbiotic fungus, *Neotyphodium lolii* (Easton, 1999).

N. lolii is an obligate seed-borne biotrophic fungus that colonises the intercellular spaces of the basal meristem, and the leaves, culms and inflorescences that arise from this meristem (Christensen et al., 2002; Philipson and Christey, 1986). The hyphae are distributed within the vegetative tillers in a pronounced basal to apical concentration gradient, and tend to be more abundant in mature leaf tissue (Herd et al., 1997; Keogh

* Corresponding author. Tel.: +64 6 3505168; fax: +64 6 3505668.
E-mail address: d.b.scott@massey.ac.nz (B. Scott).
[1] Present address: Department of Genetics, Harvard Medical School, New Research Building, Room 239, 77 Avenue Louis Pasteur, Boston, MA 02115, USA.
[2] Present address: Forage Improvement Division, The Samuel Roberts Noble Foundation, Inc. 2510 Sam Noble Pky, Ardmore, OK 73401, USA.

0098-8472/$ – see front matter
doi:10.1016/j.envexpbot.2007.10.021

et al., 1996; Tan et al., 2001). Numerous studies have established that *N. lolii* and related epichloë endophytes are mutualists that provide a wide range of important benefits to their host plants (Clay, 1990; Easton, 1999), particularly under conditions of biotic or abiotic stress. The most well documented benefit of *N. lolii* to the perennial ryegrass host is protection from insect herbivory (Latch, 1993; Prestidge and Gallagher, 1988), a biological effect ascribed to endophyte synthesis of various alkaloids *in planta*.

Three main classes of biologically active metabolites have been identified in endophyte infected perennial ryegrass, including peramine, the only known pyrrolopyrazine, ergot alkaloids, principally ergovaline, and indole-diterpenes, principally lolitrem B (Lane et al., 2000; Rowan, 1993). Peramine has been shown to be a potent feeding deterrent of adult Argentine stem weevil (ASW; *Listronotus bonariensis*) (Rowan et al., 1990), an economically important exotic pest of perennial ryegrass in New Zealand. The presence of *N. lolii* within the grass protects the host from ASW and several other insect herbivores (Latch, 1993; Prestidge and Ball, 1993). Lolitrem B also has biological activity against ASW larvae (Prestidge and Gallagher, 1988) but is better known as the causative agent of the neuromuscular disorder known as ryegrass staggers, associated with sheep grazing the base of ryegrass dominant pastures after long periods of water stress or following fresh re-growth after periods of water stress (Fletcher and Harvey, 1981; Fletcher and Easton, 1997). The importance of ergot alkaloids toward ecological fitness of the host grass has not been well defined but this group of compounds are known to be primarily responsible for tall fescue toxicosis, a livestock disorder associated with the presence of *N. coenophialum* endophyte in tall fescue (*Festuca arundinaceae*) (Bacon et al., 1977). The presence of ergopeptines in perennial ryegrass potentially exacerbates ryegrass staggers, as they induce heat stress in grazing livestock (Fletcher and Easton, 1997).

Alkaloid levels in endophyte infected perennial ryegrass are affected by season (Ball et al., 1995; di Menna and Waller, 1986), environment (Barker et al., 1993; Lane et al., 1997), and plant genotype (Easton et al., 2002; Spiering et al., 2005). The concentrations of ergovaline were significantly increased in response to addition of N fertilizer and under conditions of water stress (Barker et al., 1993; Lane et al., 1997). The level of alkaloids found in endophyte-infected perennial ryegrass varies with tissue type and leaf age, and tends to correlate with endophyte levels (Ball et al., 1995; Easton et al., 2002; Keogh et al., 1996). However, recent work by Spiering et al. (2005) showed that there were distinct *in planta* distribution patterns for each alkaloid that differed in magnitude among genotypes. The ergovaline/endophyte ratio was higher in basal tissue, whereas lolitrem B and peramine to endophyte ratios tended to be higher in apical tissues. The lolitrem B/endophyte ratio increased with leaf age, a result consistent with the demonstration that high endophyte metabolic rates are maintained after hyphal extension and leaf growth ceases (Tan et al., 2001).

Genes for the biosynthesis of ergot alkaloids (Panaccione et al., 2001; Wang et al., 2004), indole-diterpenes (Young et al., 2005; Young et al., 2006) and peramine (Tanaka et al., 2005) have now been cloned. Expression analysis has shown that the genes for all three pathways are preferentially and highly expressed *in planta* (Tanaka et al., 2005; Young et al., 2006), suggesting that plant-specific signalling is required for expression of these pathways. However, the biochemical and physiological processes that control the biosynthesis of these metabolites are largely unexplored.

In addition to bioprotective benefits, several lines of evidence suggest that epichloë endophytes may alter physiological or morphological properties of host plants, especially under conditions of environmental stress. These endophyte mediated abiotic benefits are most thoroughly documented for the *N. coenophialum*-tall fescue association (Malinowski and Belesky, 2000). Tolerance of tall fescue to water stress is probably the most thoroughly documented example, where it has been shown that endophyte infection can improve host tolerance to, and recovery from, drought (Arachevaleta et al., 1989; Malinowski and Belesky, 2000; West, 1994). In contrast, in associations between *N. lolii* and *L. perenne* the effects of endophyte on the host under conditions of water stress are less clear. Although there is evidence that *N. lolii* can improve host tolerance to drought (Amalric et al., 1999; Ravel et al., 1997), other studies have shown either no (Barker et al., 1997; Hume et al., 1993), or even negative effects of the endophyte (Cheplick, 2004; Eerens et al., 1998). With exception of one (Amalric et al., 1999), all these studies were carried out under either field or greenhouse conditions that were subject to variable environmental conditions and even where clonal E+ and E− material was used, very strong genotype effects were observed (Cheplick, 2004; Cheplick et al., 2000).

The objectives of this study were to determine whether (i) *N lolii* was able to confer morphological or physiological protection to the perennial ryegrass host from water stress using E− and E+ ramets derived from natural and synthetic symbiotic associations grown under controlled environmental conditions, and (ii) whether the levels of bioprotective metabolites and the expression of the corresponding genes changed in response to water stress in natural and synthetic symbiotic associations grown under controlled environmental conditions.

2. Materials and methods

2.1. Plant material

Two perennial ryegrass (*Lolium perenne* L. cv Grassland Nui) genotypes, G1146 and G1056, were used. G1146 is a naturally occurring symbiotum (symbiotic association between *Neotyphodium lolii* and *L. perenne*), infected with a *N. lolii* endophyte (Latch et al., 1984) of the loA taxonomic group (Christensen et al., 1993) that was isolated and designated as strain PN2309. G1056 is a synthetic symbiotum generated by inoculating an endophyte-free (E−) perennial ryegrass seedling (Latch and Christensen, 1985), with an *N. lolii* strain PN2191 (Lp19) of the loB taxonomic group. Genetically identical E− clones of both genotypes, designated G1146E− and G1056E−, were generated by Benlate treatment (Latch and Christensen, 1982).