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ENDOPHYTE OCCURRENCE IN BREEDING STRAINS OF MEADOW FESCUE (*FESTUCA PRATENSIS*) CV. 'PASJA'[1]

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Abstract

Breeding strains of cv. 'Pasja' (meadow fescue) were examined for *Neotyphodium uncinatum* occurrence in plants and seeds using immunoblots assays. Differences in endophyte infection of strains were detected. The majority of strains (70%) contained endophytes both in plants and seeds. In some of them (16.7%) endophyte was detected only in plants. Some of tested strains (12.5%) were free from *Neotyphodium*. Mean level of endophyte infection for basic material of 'Pasja' was 46% (ranging from 0 to 90%). The distribution of strains infection may support hypothesis that different share of seeds from breeding strains in basic material of cultivar is the main reason for variable endophyte occurrence in seed lots from different harvest years and different sites.

Key words: endophytes, fungi, grass, infection, *Neotyphodium uncinatum*

Introduction

Many grass species, including meadow fescue (*Festuca pratensis*) often harbour asymptomatic, asexual endophytic forms of fungi from the genus *Neotyphodium* (formerly *Acremonium*) (Zabalgogeazcoa et al. 2003, Prończuk 2005). Endophytes are defined as fungi that live their entire life cycle within the aerial portion of the host grass, forming nonpathogenic, systemic, and usually intercellular associations (Malinowski and Belesky 2000).

The benefits conferred to grasses by their fungal symbiont are well documented and may include insect deterrence and drought resistance (Bush et al. 1997, Morse

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et al. 2002). Fungal endophytes are well known also for increasing plant performance, competitive ability, especially under stress, and increasing resistance to pathogens, which is very important particularly in turfgrasses (Saha et al. 1994, Faeth and Sullivan 2003). However, there may be disadvantages when endophyte-infected grasses are used for fodder, as the occurrence of endophytes in tall fescue (*Festuca arundinacea*) and perennial ryegrass (*Lolium perenne*) may cause serious problems to livestock, such as fescue toxicosis or ryegrass staggers syndrome due to the production of toxic alkaloids (Burke et al. 2002). *Neotyphodium* endophytes show considerable variation in alkaloid production and are used for improving turf and fodder grass quality (Leuchtmann et al. 2000). The mutualistic specialization of endophyte fungi was also reported. Tall fescue may be infected with *N. coenophialum*, meadow fescue with *N. uncinatum* and perennial ryegrass with *N. lolii* (Prończuk 2005).

Our recent results suggested different endophyte infection in seed lots of a single cultivar from different harvest years, sites and seed companies (Wiewióra et al. 2006). Variable level of endophyte occurrence in perennial ryegrass lines was reported by Wheatley (2005). Seed infection rate in fodder cultivars as 'Pasja' is important due to livestock problems. According to Kemp et al. (2007) seed lines infected with endophyte in 70% or more are considered to be highly infected, in less than 25% – low infected and in 5% or less – nil infected.

In many cases, cultivar consists of few to several breeding strains. It is therefore possible that due to different seed yield from particular breeding strain and with different endophyte occurrence, results in average endophyte infection of cultivar varying for seed harvest years or/and sites.

The aim of our study was to determine endophyte infection of all breeding strains of cv. 'Pasja' (meadow fescue), both in plant and in seed.

Materials and methods

Objects of the study were seeds and plants of the meadow fescue (*F. pratensis*) cv. 'Pasja', previously known as endophyte host (Pańka et al. 2004, Wiewióra et al. 2006). Materials consisted of the 24 strains of breeding material of the cultivar, kindly provided by breeder. Cultivar 'Pasja' during initial phases of reproduction consisted of breeding strains and each strain is in a form of spaced plants (ca. 30 clones per strain).

To determine endophyte infection in plant and seed of particular strains, immunoblots assays were used (Hill et al. 2002, International rules... 2007). Commercial phytoscreen *Neotyphodium* immunoblot test kits for seed and for plant tillers were used for quantitative determination of *Neotyphodium* in plant samples and seeds harvested from these plants.

The principle of the immunoblot assay is extraction and attachment of proteins from the *Neotyphodium* endophyte in infected seed and tillers of plants to a solid membrane support. Monoclonal antibodies specific to different epitopes of the

Neotyphodium protein are added and bind to the proteins. Analysis was performed in four replications of 25 seeds per strain and 25 tillers per plant from three plants per strain (75 tillers per strain). Analytical procedures followed recommendations given by immunoblot producer (cat. #ENDO7971 for seed; cat. #ENDO7973 for tillers, Agrinostics Ltd. Co., Watkinsville, GA 30677, USA) (according to International rules... 2007). Spermann correlation coefficient (r) was calculated with SAS® statistical package (SAS 9.1... 2004, SAS/STAT 9.1... 2004).

Results

Average endophyte infection rate of tested breeding strains of cv. 'Pasja' by *N. uncinatum* was similar both in seeds and plants (46.0 and 47.5%, respectively) and both values were highly positively correlated ($r = 0.96$, $p > 0.001$).

Distribution of endophyte in seed and plant from particular strains was also similar (Table 1). Relatively high number of strains (13) was infected with high (range 60–100%) intensity as well as with low intensity or absence of fungus (nine strains with range 0–19%), both in seeds and in plants. Medium infection intensities (20–59%) were observed only in two out of all (24) tested strains (Table 1, Fig. 1).

Table 1

Frequency of endophytes in breeding strains of cv. 'Pasja'

Range of infection	Seed infection		Plant infection	
	number of strains	%	number of strains	%
0	7	29.2	3	12.5
1–19	2	8.3	5	20.8
20–39	1	4.2	2	8.3
40–59	1	4.2	1	4.2
60–79	9	37.5	10	41.7
80–100	4	16.6	3	12.5
Total	24	100.0	24	100.0

In less than one-third (29.2%) of tested strains endophyte infection occurred only in plants or did not occur at all (Table 2). If infection intensity in plants was higher than 8% it could be also detected in seeds. Relatively low plant infection (from 16 to 24%) resulted also in low seed infection (from 7 to 30%).

Discussion

Average endophyte infection rate as for 'Pasja' was also reported by Cagas (2005) for Czech ecotypes of perennial ryegrass.

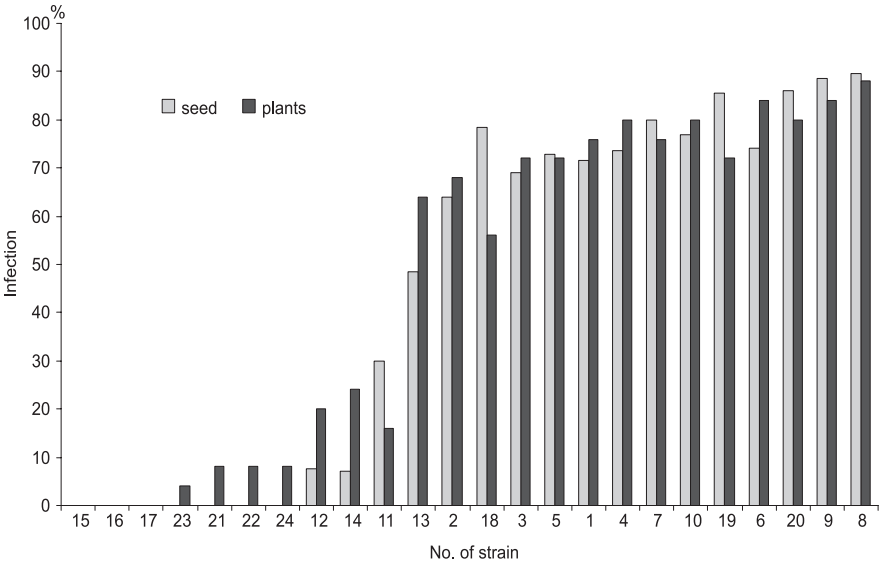

Fig. 1. Endophyte infection of breeding strains of meadow fescue (*Festuca pratensis*) cv. 'Pasja'

Similar endophyte distribution in seeds and plants of particular strains (with the majority of strains infected with high intensity) support the hypothesis that different levels of endophyte infection in seed lots from different breeding strains may result in different proportion of seed from particular strains in total seed yield. It could be due to local factors (different weather, soil, fertilization etc.) affecting seed yield. Endophyte viability in seeds is also strongly reduced during storage, and decreases rapidly after 18 to 24 months of storage in ambient conditions (Wheatley 2005). In our investigation seeds were examined about four months after harvest. During this time seeds were stored in refrigerator in ca 5°C. Bouter and van Klooster (1996) suggested that endophyte viability decreased after one year of storage and was greatest at higher temperatures. Therefore, additional differences in endophyte infection of various seed lots may result from seed storage duration and conditions. Otherwise, our hypothesis would not be supported if endophyte infection would be rather stable in all strains of cultivar.

None of the strains in question displayed seed infection alone, what is obvious in terms of vertical transmission of symbiotic fungi in host plant from the base of

Table 2

Distribution of endophyte infection in breeding strains of cv. 'Pasja'

Endophyte presence	Number of strains	%
Not present in seed and plant	3	12.5
Only in seed	0	0.0
Only in plant	4	16.7
In seed and plant	17	70.8
Total	24	100.0

steam up to inflorescence and seed (Faeth and Sullivan 2003). Endophyte infected parent plants usually produce strongly infected seeds (Paul et al. 2000, Wheatley 2005).

Endophyte occurrence in plant tissues or seed is more or less stable during further seed regeneration (Cagas 2005). An increase could be expected mostly where the parental generation has the lowest occurrence of endophytes.

An appropriate description of average endophyte infection of cultivar consisting of differently infected breeding strains is possible only in case of the same harvest year and one seed field. Moreover, such description is valid only for a short time, due to rapid endophyte deterioration during seed storage. Neither at the early stages of breeding process nor during cultivar regeneration, breeders of cv. 'Pasja' monitored endophyte infection (Ostrowska, personal communication 2004). It is therefore clear that such procedure should become routine, if endophytes are not required on pastures or in hay, providing that in endophyte-infected meadow fescue the fungal component of the association seems to play a key role in synthesis of loline alkaloids (Wilkinson et al. 1997). However, some isolates of *N. uncinatum* may produce ergovaline and chanoclavine (Fliger et al. 2000). According to Strickland et al. (1996) loline alkaloids have negligible effects on livestock compared with ergot or lolitrem alkaloids. Further research is also necessary to examine stability of endophyte infection in course of time and generations.

Streszczenie

WYSTĘPOWANIE ENDOFITÓW W RODACH HODOWLANYCH KOSTRZEWY ŁĄKOWEJ (*FESTUCA PRATENSIS*) ODMIANY 'PASJA'

Badano występowanie *Neotyphodium uncinatum* w roślinach i nasionach rodów hodowlanych kostrzewy łąkowej odmiany 'Pasja', stosując metodę immunologiczną. Stwierdzono różnice w występowaniu endofitu w rodach. U większości z nich (70%) grzyb zasiedlał zarówno rośliny, jak i nasiona. U kilku rodów (16,7%) stwierdzono jego obecność tylko w roślinach. Jedynie trzy rody spośród 24 badanych (12,5%) były całkowicie wolne od endofitu. Średni poziom infekcji materiału bazowego w 2007 roku wyniósł 46% (u poszczególnych rodów od 0 do 90%). Obserwowane różnice w stopniu infekcji rodów i różny udział rodów w materiale bazowym odmiany mogą być przyczyną różnic w zawartości *N. uncinatum* w materiale siewnym kostrzewy łąkowej 'Pasja' pochodzącym z różnych plantacji i rozmaitych lat zbioru.

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