Neotyphodium spp.–grass association in natural grassland in Argentina

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Abstract

The agronomic importance of the grass-endophyte association became apparent after it was linked to livestock diseases. This paper reports the incidence of endophytes in natural grasslands in Argentina from data collected through grass seed collections, tillers and culms from natural grasslands. A total of 159 samples were analysed: 144 grass collections from 30 species were obtained from accessions (germplasm bank of EEA Balcarce-INTA), and 15 samples were collected in the field in the province of Buenos Aires (Balcarce and Ayacucho). The samples were analysed according to Saha *et al.* (1988) or Belanger (1996). Endophytes were found in only nine of the 30 species of accessions analysed; accessions of *Lolium multiflorum* showed the highest incidence of endophyte infection, ranging from 57 to 100%. Of the 15 field populations collected: *Festuca arundinacea* and *L. multiflorum* samples were infected with infection ranging from 89 to 100%; populations of *Bothriochloa*, *Deyeuxia*, *Piptochaetium*, *Sporobolus*, *Stipa megapotaia*, *S. papposa*, were endophyte free. The economic losses to the livestock industry and the potential benefits of endophytes as biological agents to improve plant fitness and pest resistance of forage grasses increases the importance of determining the incidence of fungal endophytes in grass germplasm collections and natural grasslands.

Keywords: native grasses, endophytes, naturalised grasses, Argentina

Introduction

Native and naturalised grasses are relevant for soil conservation, revegetation, and also important reserves of genetic diversity of forage grasses. In the pampas alone, the Poaceae family includes approximately 190 native species and 40 introduced species, where the best represented genera are *Stipa* (25), *Poa* (8), *Piptochaetium* (8), *Aristida* (6) and *Melica* (6). Other characteristic grass genera are *Bothriochloa*, *Briza*, *Eragrostis*, *Panicum*, *Paspalum* and *Sporobolus*.

Grass-endophyte associations are widely distributed (White 1988). The endophytes that are seed transmitted are classified in the genus *Neotyphodium* Glenn, Bacon & Hanlin (= *Acremonium* Link Seccion *Albo-lanosa* Morgan-Jones & W. Gams) (Schardl 1996). The relationship between the plant and the fungi is symbiotic and mutualistic; where the plants colonised with endophytes receive the benefits of increasing growth, biomass, drought tolerance (Arachevala *et al.* 1989; Malinowski & Belesky 2000) and resistance to nematodes (West *et al.* 1987), insects and a wide range of animal herbivores (Cheplick & Clay 1988) and pathogens (Clarke *et al.* 2006). There have also been registered positive effects in intra and interspecific competitive ability in plants colonised with *Neotyphodium* (Marks *et al.* 1991). On other hand, the endophytes benefit from the association with nutrition, dispersal and survival (Siegel *et al.* 1987).

In Argentina, as in other areas of the world, forage grasses have received more attention than native grasses. Tall fescue represents 30% of the total cultivated pasture area mainly in the pampas, and the economic losses to the livestock industry due to tall fescue infection have been estimated at US\$ 54 million /year (De

Battista *et al.* 1995). Consequently some seed regulations were established and only seed lots with less than 5% infection were recommended for use in seeding pastures. However, in a survey, 31% of the seed lots of tall fescue offered in the Argentinean market showed infection percentages outside the tolerance range (De Battista *et al.* 1997).

The economic losses to the livestock industry and the potential benefits of endophytes as biological agents to improve plant fitness and pest resistance of forage grasses increases the importance of determining the incidence of fungal endophytes in germplasm collections of grasses. The largest collection of grass germplasm in Argentina is maintained at the Instituto Nacional de Tecnología Agropecuaria (INTA), localized in EEA-Balcarce for the pampa region.

Materials and Methods

A total of 159 samples were analysed. Seed samples of 144 grass collections from 30 species were directly obtained from accessions of the Germplasm Bank at Estación Experimental Agropecuaria de Balcarce (BAL), INTA and 15 samples were collected in the field in the province of Buenos Aires (Balcarce and Ayacucho). The accessions of BAL Germplasm bank were collected in native and naturalised grasslands from eight provinces ranging from 22° 17' S and 41° 19' S and 57° 18' W and 71° 33' W and from sea level to 3724 meters (Fig. 1). Table 1 lists the species sampled and collection identification number.

Seed samples were analysed according to Saha *et al.* (1988), 20-60 seeds were soaked overnight in 5% sodium hydroxide at 22°C, rinsed with tap water and stained for several minutes with aniline blue or rose bengal. Seeds were then rinsed, squashed and mounted on slides and examined microscopically at 100-400 x magnification. In field collections, 25 plants (seeds or culms and tillers)/population were sampled and analysed according to Saha *et al.* (1988) or Belanger (1996).

Results

From a total of 144 germplasm accessions (Table 1), 80% were from to the province of Buenos Aires, 8% Rio Negro, 3% La Pampa and Córdoba, 2% Mendoza and Jujuy, and 1% San Luis and Chubut. Endophytes were found in only nine of the 30 species of accessions analysed, *Bromus catharticus* (15% infection), *Lolium multiflorum* Lam. (57-100% infection) and *Thinopyrum ponticum* (= *Agropyron elongatum*) (8% infection) and all infected samples were from Buenos Aires province. Overall, the infection levels were low for *Thinopyrum ponticum* (8%) and *Bromus catharticus* (15%). Accessions of *L. multiflorum* showed the highest incidence of endophyte infection with infection rates that ranged from 57 to 100%.

The 15 field populations collected were from Buenos Aires province. *Festuca arundinacea* and *Lolium multiflorum* samples were infected and the level of infection ranged from 89 to 100%. Populations of *Bothriochloa laguroides*, *Deyeuxia viridiflavescens*, *Piptochaetium stipoide*, *Sporobolus indicus*, *Stipa megapotaia*, *Stipa papposa*, were endophyte free.

Table 1 Native grass populations analysed for endophyte presence, from Buenos Aires, Argentina (†) and from Germplasm bank Balcarce (BAL) INTA, Argentina (‡).

Native grass species	N	Location	Grass species	N	Location
<i>Alopecurus magellanicus</i> (‡) Alo 2343.	1	Rio Negro (3)	<i>Festuca</i> sp. (‡) Alo s/n	1	Rio Negro (3)
<i>Bothriochloa lagunoides</i> (†) Population A and B.	2	Buenos Aires (1)	<i>Festuca pampeana</i> (‡) CIA 1056.	1	Buenos Aires (1)
<i>Bromus auleticus</i> (‡) CIA: 1041, 1059	2	Buenos Aires (1)	<i>Festuca ventricola</i> (‡) CIA 1058.	1	Buenos Aires (1)
<i>Bromus brevis</i> (‡) CIA: 996, 1012, 1014, 1016, 1020, 1022, 1028, 1029, 1031, 1033, 1034, 1063.	12	Buenos Aires (1) Rio Negro (3) La Pampa (2)	<i>Hordeum chilense</i> (‡) Alo 2340	1	Rio Negro (3)
<i>Bromus catharticus</i> (‡) CIA: 1036, 1002, 1086, 1011, 1095, CI 1013, 1040, 1035-36, 995, CIB: 27, 36, CCS: 1246, 1265, CI 1623, CICO: 1699, 1700.	17	Buenos Aires (1) Jujuy (7)	<i>Paspalum dilatatum</i> ssp. <i>dilatatum</i> (‡) CIA: 1641, 2483, CI 1722.	3	Buenos Aires (1)
<i>Bromus lithobius</i> (‡) Alo 2359.	1	Rio Negro (3)	<i>Paspalum dilatatum</i> ssp. <i>flavescens</i> (‡) CIA: 1626, 1634, 1687, 1689, 1691, 2484, CI1721.	7	Buenos Aires (1)
<i>Briza rufa</i> (‡) Alo 1554 B	1	Buenos Aires (1)	<i>Paspalum notatum</i> (‡) CIA: 1627, 1636, 1656.	3	Buenos Aires (1)
<i>Briza subaristata</i> (‡) Alo 1553 B, CIA: 983, 1062, Oka: 7953, 7961	5	Buenos Aires (1) Córdoba (6)	<i>Paspalum urvillei</i> (‡) CIA: 1635, 1653.	2	Buenos Aires (1)
<i>Danthonia cirrata</i> (‡) Alo 2462.	1	Buenos Aires (1)	<i>Piptochaetium stipoides</i> (†) Population A and B.	2	Buenos Aires (1)
<i>Deschampsia caespitosa</i> (‡) var. <i>caespitosa</i> Alo 2341.	1	Rio Negro (3)	<i>Poa iridifolia</i> (‡) CIA: 1090, 1042.	2	Buenos Aires (1)
<i>Deauxia viridiflavescens</i> (†) Population B.	1	Buenos Aires (1)	<i>Sorghastrum pellitum</i> (‡) Alo 2460.	1	Buenos Aires (1)
<i>Elymus gavanus</i> (‡) Alo 2352.	1	Rio Negro (3)	<i>Sporobolus indicus</i> (†) Population A and B	2	Buenos Aires (1)
<i>Elytrigia scaberrima</i> (‡) CIA: 398, 404; Alo: 1355, 1377, 1557.	5	Buenos Aires (1)	<i>Stipa megapontica</i> (†) Population A and B	2	Buenos Aires (1)
<i>Elytrigia scaberrima</i> (‡) CI: 368, 451, 464, 472, 477; C/C 896.	6	Buenos Aires (1)	<i>Stipa papposa</i> (†) Population A and B.	2	Buenos Aires (1)
<i>Avena barbata</i> (‡) Alo: 1828, 1833, 1831, 1852, 1853, 1919, 1918, 1917; AIG 1788; AIC: 1981, 2022, 2025, 2111; CIA: 980, 982.	1	Buenos Aires (1) La Pampa (2) Rio Negro (3) Mendoza (4)	<i>Lolium multiflorum</i> (‡) AICN 1313; CIA 263; Nu: 185, 304; CIAE 216; Alo 1285, 2480, CI 1559, Population A and B	8	Buenos Aires (1)
<i>Bromus hordaceus</i> (‡) CIA 1049	1	Buenos Aires (1)	<i>Phalaris aquatica</i> (‡) Nu: 210, 220; CIB: 54, 89; Bal 10897; CIA 347.	6	Buenos Aires (1)
<i>Bromus racemosus</i> (‡) Alo 2438	1	Chubut (8)	<i>Poa pratensis</i> (‡) Alo 2360.	1	Rio Negro (3)
<i>Dactylis glomerata</i> (‡) CIA: 338, 330, 319; Nu 219.	4	Buenos Aires (1)	<i>Thinopyrum ponticum</i> (‡) Evans: 60, 61, 67, 70, 75, 76, 103, 104, 105; CIB: 9, 42, 44, 55, 57, 64, 68, 74 c, 87, 90, 99; Alo: 1361, 1426; CIA 420; Nu: 338, 345 a, b y c; CIA: 1640, 1661, 1669, 1672, 1674, 1682.	33	Buenos Aires (1) La Pampa (2)
<i>Festuca arundinacea</i> (†) Population A and B	2	Buenos Aires (1)			

Table 1 continued... (£) Collected by and number of collection (Alo: Alonso; AIG: Alonso, Ispizúa, Guma; AIC: Alonso, Clausen; CIA: Clausen, Alonso; CI: Clausen; CIB: Celina Borrajo; CCS: Clausen, Carranza, Stábile; Cla: Clausen; Oka: Okada; ClCo: Clausen, Colabelli; CIC: Clausen, Castaño; AICN: Alonso, Clausen, Nuciari; Nu: Nuciari; CIAE: Clausen, Alonso, Echarte; Bal: Banco Balcarce; Evans: Evans); (¥) Samples from natural grassland in province of Buenos Aires, no for Germplasm Bank (sampling Colabelli and Fernández. Population A: Ayacucho; Population B: Balcarce); (♣) Provinces in the map, N population sampled

Discussion

Endophyte status in native species

Considering native and naturalised grasses, to date, endophytes have been reported in at least 38 species of native grasses in Argentina mainly in the genera *Festuca*, *Poa*, *Bromus*, *Melica*, *Phleum*, *Briza* and *Hordeum* (Bertoni *et al.* 1993; Lugo *et al.* 1998; Cabral *et al.* 2000; Colabelli *et al.* 2001; Vila Aiub *et al.* 2001; Torres *et al.* 2004; Iannone *et al.* 2005; Iannone & Cabral 2006). Our populations of *B. auleticus* (native) and *B. hordaceus* (naturalised) from the humid pampa were free of infection. However, there are several reports of the presence of *Neotyphodium* in the genera *Bromus* (White 1987; Nelson *et al.* 1993). Species reported infected are *B. auleticus* and *B. setifolius* (Cabral *et al.* 1999, 2000), *B. catharticus* and *B. hordaceus* (Piontelli & Toro 1992) and *B. brachyanthera* (Iannone *et al.* 2005).

The genus *Festuca* comprises 25 species in Argentina. Native species, *F. pampeana* and *F. ventanica*, were free of infection as previously reported by Bertoni *et al.* (1993) in a study of South American species of *Festuca* while other species, such as *F. dissitiflora*, *F. argentina* and *F. hieronymii*, are known to show high levels of infection and to be associated with animal toxicosis (Cabral *et al.* 1999).

Two species of *Briza* were not infected. There are no previous data about *B. rufa* while *B. subaristata* has been reported as uninfected (Lugo *et al.* 1998) and infected (Iannone *et al.* 2005). *Poa iridifolia* and *P. pratensis* (naturalised) were uninfected. Several *Poa* species have been reported infected; e.g. *Poa pratensis* (Nan 2000), *P. bergii*, *P. bonariensis*, *P. lanigera*, *P. lanuginosa*, *P. af. ligularis* and *P. resinulosa* (Iannone *et al.* 2005) or not infected (Sun & Breen 1993), in agreement with the present survey. Populations of *Agropyron* were reported infected (Nelson *et al.* 1993; Nan 2000).

Endophyte status in naturalised species

Naturalised populations of *Lolium multiflorum* Lam. have previously been reported to be infected (Piontelli & Toro 1992), with infection levels that range between 4–90%. *Neotyphodium occultans* in *L. multiflorum* seems to maintain viability longer than perennial ryegrass and tall fescue endophyte, in seed storage at room temperature (Medvescigh *et al.* 2004). This information, together with its wide distribution in naturalised populations can be an indication of some adaptive advantage of being infected (Maidana *et al.* 1999).

Perennial ryegrass represents about 6% of the total cultivated area. It is used in pure stands or in mixed swards, and infection by the endophyte *Neotyphodium lolii* is widespread. Commercial seed lots have shown relatively high levels of infection, ranging from 1–67% (Torres *et al.* 2004). In Argentina, there are reports of toxicity but it seems that the adaptive advantage of *N. lolii*-perennial ryegrass is not as remarkable as in New Zealand grasslands in improving host fitness or persistence (De Battista *et al.* 1997).

Seed storage

Previous studies on seed storage and germplasm collections have shown that storage temperature and seed moisture content are

relevant for conserving endophyte viability. Endophyte viability in tall fescue and perennial ryegrass decrease with time being close to 0 after 14–16 months of storage at ambient temperature (Williams *et al.* 1984). Do Valle Ribeiro (1993) reported that the endophyte viability remains the same in perennial ryegrass seed storage for 6–7 years at 3–5°C and a relative humidity of 10%. In general, endophyte viability is more susceptible than seed viability to the effects of higher temperatures and higher seed moisture content, with important implications for the conservation as seed of indigenous or naturalised grasses that harbour endophytes (Rolston *et al.* 2002). Our data indicate that storage conditions at the Germplasm Bank in Balcarce where samples are stored at 5°C in aluminium envelopes favoured endophyte viability loss.

This survey adds to similar observations of native grasses in other geographic areas which suggest that, if endophytes are present, they occur at low frequencies or the infections levels show a great variation between populations in native and naturalised grasses. We intend to characterise the accessions of the main germplasm bank in Argentina for endophyte presence. One of the future objectives will be to implement a PCR-based method to detect symbiotic *Neotyphodium* endophytes in grass seed accessions.

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FIG.1. Map of República Argentina showing collection places

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