Rev Argent Microbiol. 2016;**48(2)**:154–160

asociación argentina de microbiología

REVISTA ARGENTINA DE MICROBIOLOGÍA

www.elsevier.es/ram

ORIGINAL ARTICLE

Endophytic fungi from selected varieties of soybean (*Glycine max* L. Merr.) and corn (*Zea mays* L.) grown in an agricultural area of Argentina

María L. Russo[a,*], Sebastián A. Pelizza[a,b], Marta N. Cabello[a,d], Sebastián A. Stenglein[c], María F. Vianna[a], Ana C. Scorsetti[a]

[a] *Instituto de Botánica Carlos Spegazzini (Facultad de Ciencias Naturales y Museo-Universidad Nacional de La Plata), La Plata, Argentina*
[b] *Centro de Estudios Parasitológicos y de Vectores (CEPAVE), CCT La Plata CONICET-UNLP, La Plata, Argentina*
[c] *Laboratorio de Biología Funcional y Biotecnología (BIOLAB)-CICBA-INBIOTEC, Facultad de Agronomía de Azul, Cátedra de Microbiología, UNCPBA, República de Italia 780, 7300 Azul, Argentina*
[d] *Comisión de Investigaciones Científicas (CIC) de la Provincia de Buenos Aires, Argentina*

Received 12 September 2015; accepted 11 November 2015
Available online 1 April 2016

KEYWORDS
Endophytic fungi;
Soybean;
Corn;
Diversity

Abstract Endophytic fungi are ubiquitous and live within host plants without causing any noticeable symptoms of disease. Little is known about the diversity and function of fungal endophytes in plants, particularly in economically important species. The aim of this study was to determine the identity and diversity of endophytic fungi in leaves, stems and roots of soybean and corn plants and to determine their infection frequencies. Plants were collected in six areas of the provinces of Buenos Aires and Entre Ríos (Argentina) two areas were selected for sampling corn and four for soybean. Leaf, stem and root samples were surface-sterilized, cut into 1 cm^2 pieces using a sterile scalpel and aseptically transferred to plates containing potato dextrose agar plus antibiotics. The species were identified using both morphological and molecular data. Fungal endophyte colonization in soybean plants was influenced by tissue type and varieties whereas in corn plants only by tissue type. A greater number of endophytes were isolated from stem tissues than from leaves and root tissues in both species of plants. The most frequently isolated species in all soybean cultivars was *Fusarium graminearum* and the least isolated one was *Scopulariopsis brevicaulis*. Furthermore, the most frequently isolated species in corn plants was *Aspergillus terreus* whereas the least isolated one was *Aspergillus flavus*. These results could be relevant in the search for endophytic fungi isolates that could be of interest in the control of agricultural pests.

* Corresponding author.
E-mail address: leticiarusso@conicet.gov.ar (M.L. Russo).

http://dx.doi.org/10.1016/j.ram.2015.11.006

PALABRAS CLAVE
Hongos endófitos;
Soja;
Maíz;
Diversidad

Hongos endófitos aislados de cultivares de soja (*Glycine max* L. Merr) y maíz (*Zea mays* L.) presentes en áreas agrícolas argentinas

Resumen Los hongos endófitos son ubicuos y se encuentran en el interior de los tejidos de las plantas de manera asintomática. Se sabe muy poco acerca de la diversidad y la función de estos hongos, particularmente en especies de importancia económica. El objetivo de este trabajo fue determinar la diversidad y la frecuencia de colonización de hongos endófitos en raíces, tallos y hojas de 2 variedades de maíz y de 4 variedades de soja; las muestras se tomaron de 6 áreas diferentes ubicadas en las provincias de Buenos Aires y Entre Ríos (Argentina). Con un bisturí estéril se obtuvieron porciones de 1 cm^2 de raíz, tallo y hoja, que fueron colocados en placas con agar papa dextrosa más antibiótico.

Las especies de hongos fueron identificadas a partir de características morfológicas y moleculares. La colonización de hongos endófitos en soja estuvo influenciada por la variedad y por el tipo de tejido, en tanto que en el maíz solo hubo influencia del tipo de tejido. El mayor número de endófitos se encontró en los tallos de ambas especies. El aislamiento más frecuente en todas las variedades de soja fue *Fusarium graminearum* y el menos frecuente *Scopulariopsis brevicaulis*. En ambas variedades de maíz la especie con mayor frecuencia de aislamiento fue *Aspergillus terreus* y la de menor fue *Aspergillus flavus*. Estos resultados son relevantes para la búsqueda de especies de hongos endófitos que podrían ser de interés en el control de plagas agrícolas.

Introduction

By the year 2020, the supply of food especially of cereals, will have to increase about 70% in developing countries to secure food for the projected population of 6500 million people. It is expected that most of this increase in food supply will come from developing countries[33]. Soybean and corn are the extensive major crops in Argentina, providing a high percentage of the basic food needs of the population. The most important crops in Argentina are soybean and corn with 18 and 3.4 million sown hectares, respectively[19].

Symptomless internal colonization of healthy plant tissues by fungi is a widespread and well-documented phenomenon. Increasing interest in the ecological roles of these fungi has stimulated research in recent years since they might have plant growth – promoting activity[13]. Endophyte is an all-encompassing topographical term that includes all those organisms that during a variable period of their life symptomlessly colonize the living internal tissues of their hosts[24]. It is hypothesized that fungal endophytes, in contrast to known pathogens, generally have far greater phenotypic plasticity and thus more options to interact with their host than pathogens[27]. Since the 1970s several reports have shown that these fungal endophytes play important roles in protecting their host against predators and pathogens[25]. Endophytic fungi that infect plants are ubiquitous in all environments studied[7,24,28]. Although the diversity and function of fungal endophytes that infect grasses are well documented, little is known about the diversity and function of fungal endophytes in plants, particularly in economically important species[13,26]. Some fungal endophytes can reportedly reduce plant diseases and enhance plant growth and may be the basis for emerging methods to improve plant growth and production[12,17,18,20]. For example treatment of soybean [*Glycine max* L. (Merr)] with culture filtrate from the endophyte *Cladosporium sphaerospermun* increased plant height[3,11,20,21]. Although soybean and corn are major world crops, there is very limited knowledge of their fungal endophyte community.

The goal of this study was to isolate fungal endophytes from leaves, stems and roots of four soybean and two corn varieties grown in agricultural sites of the provinces of Buenos Aires and Entre Rios, Argentina, and to determine their colonization frequencies.

Materials and methods

Sample collection

The plants were collected during January and February 2013 in six locations in the soybean and corn cropping area of the provinces of Buenos Aires and Entre Rios (Argentina)[5] (Table 1). The region's climate is temperate with an average temperature of 17 °C and an average annual rainfall of 1000 mm. Two areas were selected for sampling corn and four for soybean sampling, since soybean varieties are more predominant in Argentina. Plants of both species were grown in monoculture fields with a history of annual corn and soybean rotation. Ten plants without symptoms of disease were randomly selected from each plot in soybean cultivars DM 3810, DM 4210, DM 4670 (Don Mario Co., Buenos Aires province, Argentina), NA 5009 (Nidera Semillas Co., Buenos Aires province, Argentina) and ten plants were selected from corn cultivars NK 900 (Sygenta Semillas Co., Argentina) and DK 747 (Dekald®, Argentina). All samples were collected at 60–70 days after germination, cut at the soil line,

Table 1 Locations where different soybean and corn plants were sampled

Variety	Locality	GPS coordinate
Soybean		
DM3810 (Don Mario, Argentina)	Alberti, Buenos Aires	35° 1′ 53″ S 60° 16′ 49″ O
DM4210 (Don Mario, Argentina)	Bragado, Buenos Aires	35° 6′ 59″ S 60° 28′ 45″ O
DM4670 (Don Mario, Argentina)	Salliqueló, Buenos Aires	36° 45′5″ S 62° 57′ 32″ O
NA5009 (Nidera, Argentina)	Victoria, Entre Ríos	32° 37′ 0″ S 60° 10′ 0″ O
Corn		
DK747 (Monsanto)	Olascoaga, Buenos Aires	35°14′ 14″ S 60° 36′ 36″ O
NK900 (Syngenta)	Las Cuevas, Entre Ríos	32° 21′ 00″ S 60° 28′ 60″ O

immediately placed on ice and stored up for 72 h at 4 °C until processed according to Impulliti and Malvick[13].

Isolation and identification of endophytic fungi

Endophytic fungi were isolated according to the protocols described by Pimentel et al.[25]. All leaf, stem and root samples were washed twice in distilled water, then surface-sterilized by immersion for 1 min in 70% (v/v) ethanol, 4 min in sodium hypochlorite (3%, v/v available chlorine) and then washed three times in sterilized distilled water for each time. After surface sterilization, samples were cut into 1 cm^2 pieces with a sterile scalpel and aseptically transferred to plates containing potato dextrose agar (PDA, Britania S.A., Buenos Aires, Argentina) to which a 0.1% stock consisting of 0.02 g of each of two antibiotics (chloramphenicol and streptomycin) dissolved in 10 ml sterile distilled water was added, followed by filter sterilization through a 0.2-μm filter (Syringe filter sterile, E-Chrom Tech, Taiwan); 1 ml of this was added to each litre of medium, to suppress bacterial growth[31]. Aliquots from the third wash were plated onto PDA to check that surface sterilization had been effective. A total of 1080 fragments were plated (18 from each of the 60 plants investigated). To facilitate isolation of endophytic fungi, the plates were incubated in the dark at 25 °C. The plates were checked everyday for up to ten days after incubation and any fungi present was isolated, purified and then maintained at 4 °C on PDA slopes for further identification. Percentage colonization was defined for each variety as the total number of fragments colonized by fungi in relation to the total number of fragments × 100[25].

The species were identified using both morphological and molecular data.

Morphological identification of the isolates was done by growing them on PDA plates or in microculture[14] and examining the colonies for asexual or sexual reproductive structures using optical microscopy and taxonomic keys. Species were identified according to Leslie and Summerell[15] and Domsch et al.[8].

Genomic DNA of monosporic cultures was obtained according to Stenglein and Balatti[29]. To confirm morphological identifications, a PCR was carried out in an XP thermal cycler (Bioer Technology Co, Hangzhou, China) to amplify the ITS rDNA region using primer pairs ITS5 (5′-GGAAGTAAAAGTCGTAACAAG G-3′) and ITS4 (5′-TCCTCC GCT TATTGATATGC-3′)[32]. For *Fusarium* species confirmation the translation elongation factor (EF-1α) region was amplified using primers EF1 (5′-ATGGGTAAGGA(A/G)GACAAGAC-3′) and EF2 (5′-GGA(A/G)GTACCAGT(G/C)ATCATGTT-3′)[22]. The PCR reactions, the fragment purifications and sequencing were performed according to Canel et al.[6] and Stenglein et al.[30].

The similarities of the fragment with previously published sequence data were examined with BLASTn[1] on the NCBI web page.

Diversity was assessed using the Shannon Index[16] (for the cultivars and tissue types).

The differences between fungi isolates and frequency of colonization for the different varieties were tested using two-way analysis of variance (ANOVA) and their means were compared by the LSD test ($p < 0.05$) using the Infostat software.

Results

From the soybean plants sampled, 11 fungal species were isolated and identified using both morphological and molecular data. In all soybean cultivars, *Fusarium graminearum* was the most frequently isolated species sampled while *Scopulariopsis brevicaulis* was the least frequently isolated one (Table 2). Furthermore, in the corn plants sampled, 7 fungal species were isolated (six species belonging to Ascomycota and one to Zygomycota (Table 3)), being *Aspergillus terreus* the most frequently isolated species and *Aspergillus flavus* the least frequently isolated one (Table 3). All endophytic fungal species were deposited in the strain culture collection of the Spegazzini Institute, La Plata, Argentina (LPSC) (Tables 2 and 3).

Table 2 Colonization percentage of different fungal species isolated from roots, stems and leaves from four different soybean cultivars sampled

Fungal species	Colonization percentage (%)											
	DM3810			DM4210			DM4670			NA5009		
	Root	Stem	Leave	Root	Stem	Leave	Root	Stem	Leave	Root	Stem	Leave
LPSC1187 *Alternaria alternata* (Fr.) Keissl	–	–	–	–	25	26.6	–	–	–	–	–	–
LPSC1186 *Arthrinium phaeospermun* (Corda) Ellis	–	–	–	–	20	33.3	–	–	–	–	–	–
LPSC1181 *Aspergillus niger* Tiegh	–	10	38.3	–	13.3	41.6	–	–	–	–	–	–
LPSC1182 *Clonostachys rosea* (Link) Schroers	16	–	–	–	–	–	–	–	–	–	15	30
LPSC1178 *Curvularia lunata* (Wakker) Boedijn	–	6.6	46.6	–	38.3	–	–	–	–	–	38.3	–
LPSC1188 *Fusarium graminearum* Schwabe	20	45	–	3.3	10	30	20	20	10	11.6	25	31.6
LPSC1184 *Fusarium equiseti* (Corda) Sacc	–	–	58.3	–	–	–	–	–	–	–	–	–
LPSC1191 *Fusarium oxysporum* Schlecht	–	10	21.6	–	–	–	–	33.3	8.3	–	–	–
LPSC1185 *Macrophomina phaseolina* (Tassi) Goid	13.3	28.3	–	–	–	–	10	13.3	28.3	–	–	–
LPSC1189 *Scopulariopsis brevicaulis*(Sacc) Bainier	–	–	–	–	–	–	–	41.6	–	–	–	–
LPSC1179 *Trichoderma saturnisporum* Hammill	–	–	–	–	–	–	–	–	–	10	16.6	38.3

With regard to fungal diversity in soybean plants, cultivar DM3810 showed the highest diversity while in cultivar NA5009 we observed the lowest diversity, with a Shannon's index of 3.09 and 1.93, respectively. Moreover, corn cultivar DK747 showed the highest fungal diversity whereas the lowest diversity was observed in cultivar NK900, with a Shannon's index of 1.84 and 1.67, respectively. Furthermore, based on the Shannon's index, the greatest fungal diversity in all the soybean cultivars sampled was observed in the stems and then in the leaves whereas the lowest diversity occurred in the roots, except for cultivar DM4670, where we observed increased diversity in the roots rather than in the leaves with a Shannon's diversity index of 1.35 and 1.18, respectively. With respect to the fungal diversity in the roots, stems and leaves of corn plants, most fungal diversity was observed in the stems of both DK747 and NK900 cultivars

Table 3 Colonization percentage of different fungal species isolated from roots, stems and leaves from two different corn cultivars sample

Fungal species	Colonization percentage (%)					
	DK747			NK900		
	Root	Stem	Leaf	Root	Stem	Leaf
LPSC1183 *Aspergillus flavus* Link	8.3	35	–	–	–	–
LPSC1180 *Aspergillus terreus* Thom	8.3	30	28.8	10	43.3	–
LPSC1193 *Bipolaris tetramera* (McKinney) Shoemaker	–	31.6	–	21.6	23.3	–
LPSC1223 *Clonostachys rosea* (Link) Schroers	–	–	–	–	18.3	31.6
LPSC1224 *Fusarium graminearum* Schwabe	–	–	–	–	28.3	18.3
LPSC1190 *Fusarium proliferatum* (Matsush) Nirenberg	26.6	43.3	28.3	–	25	26.6
LPSC1192 *Mucor circinelloides* Tiegh	–	25	–	10	33.3	–

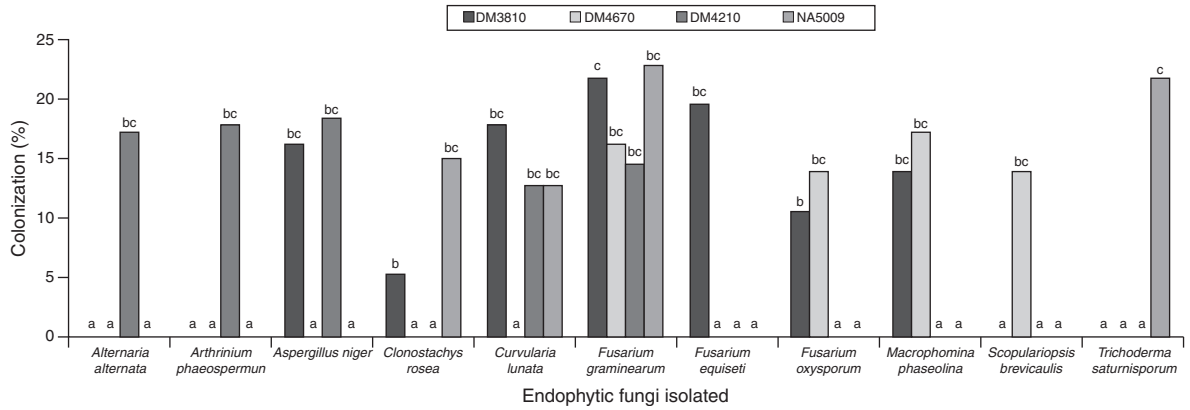

Figure 1 Colonization percentage of endophytes isolated from soybean plants of four cultivars DM3810, DM4670, DM4210, NA5009. Different letters indicate statistically significant differences between the groups (LSD test, $p < 0.05$).

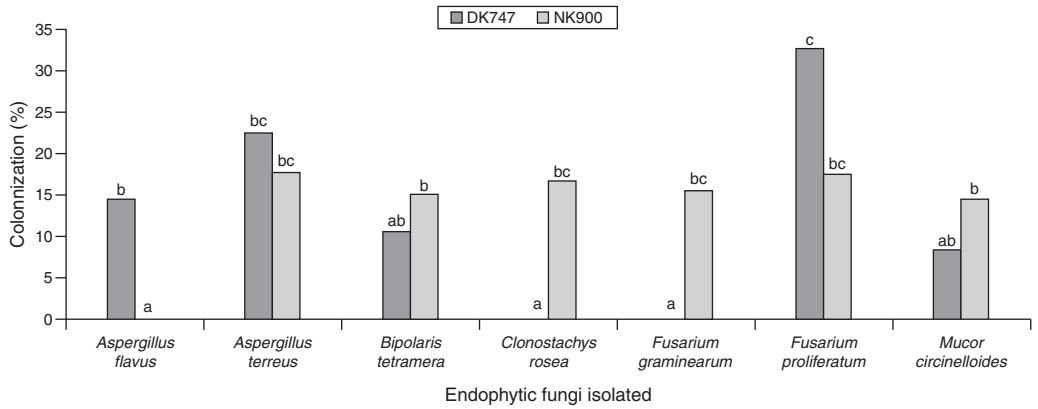

Figure 2 Colonization percentage of endophytes isolated from corn plants of two cultivars DK747, NK900. Different letters indicate statistically significant differences between the groups (LSD test, $p < 0.05$).

with a Shannon's diversity index of 2.27 and 2.49, respectively, then in leaves and the lowest diversity occurred in roots.

Fungal isolates identification was confirmed at molecular level and submitted to GenBank (Accession numbers: KF753941–KF753956). Fungal endophyte colonization in soybean plants was influenced by the cultivars, showing significant differences between varieties ($F = 4.17$, df = 3, $p = 0.0063$), fungi isolates ($F = 6.93$, df = 10, $p < 0.0001$) and in the interaction among them ($F = 7.12$, df = 30, $p < 0.0001$) (Fig. 1). Corn plants showed no significant differences between cultivars ($F = 1.34$, df = 1, $p = 0.2500$), however, they did instead among fungi isolates ($F = 4.07$, df = 6, $p = 0.0009$) and in the interaction among cultivars and fungi isolates ($F = 4.12$, df = 6, $p = 0.0008$) (Fig. 2).

Discussion

Studies of fungal endophytes in many environments are an active area for research; however, the endophytes in soybean and corn have never been systematically characterized. This work expands our understanding of endophytic fungi in soybean and corn plants. We have focused on roots, stems and leaves because many soybean and corn pathogens commonly colonize these organs. The fungal endophytes identified in this study are not known to be soybean and corn pathogens, and the functional associations between these fungi and soybean and corn plants are unknown. Furthermore, it is important to mention that none of the plants used in this study had symptoms of disease. The most prevalent endophytic fungal species isolated in the organs (root, stem and leaf) in all soybean cultivars was *F. graminearum* and in the two corn cultivars was *A. terreus*. Pimentel et al.[25]; Impullitti and Malvick[13] found that *Cladosporium* was the endophytic fungal genus most frequently identified from leaves and stems of soybeans grown in Brazil and Minnesota, USA whereas Pan et al.[23] found this genus in leaves and stems from corn in Minnesota, USA. In this study only one species of Zygomycota was isolated in maize plants; species from this Phylum were isolated as endophytes in *Dactylis glomerata* L. and other plants[18].

The endophytic fungal species detected in plants may be influenced by many factors, including the type of tissue sampled, the time when plants were assayed, perhaps the climate and location in which they were grown[13], whether the plant is grown in a monoculture or polyculture, the plant age or cropping history of the field[2,4,9,27]. Soybean and maize used in this study were grown in a monoculture and in fields that had a history of corn, wheat and soybean rotations.

Fisher et al.[10] observed that parts of corn-stems nearer to the soil showed a lower incidence of fungal infection and explained that this probably was due to these parts of the stem having an increased frequency of bacteria that inhibited fungal colonization. This could explain why we obtained the greatest number and diversity of isolates from stems in different soybean and corn cultivars, than in leaves and roots.

A greater number of fungi such as endophytes in stems were also observed in soybean in Brazil whereas endophytic bacteria in maize were found in the USA[10,25]. These studies also suggested that endophytes may exclusively colonize certain tissues, for example, *Colletotrichum* was only isolated from soybean leaves and not from the stems cultivated in Brazil[25]. In our study, *Fusarium equiseti* was only isolated from leaves of soybean plants and *S. brevicaulis* only from stems.

Endophytes may be important organisms to improve a sustainable production of crops, although their identities and functions in a range of plants are just beginning to be revealed. This is the first time that we study the natural endophytes placed in roots, stems and leaves of the main soybean and maize cultivars in Argentina. Species could be determined by classical taxonomy and the use of molecular techniques for each of the isolates obtained. In addition, we determined the colonization percentage, the fungal diversity in the different organs of every plant studied, the differences between fungi isolates and the frequency of colonization for different varieties using the ANOVA analysis. Future research should be conducted to determine which of these fungal natural endophytes could be used for both biological control and plant growth promotion.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this investigation.

Confidentiality of data. The authors declare that no patient data appears in this article.

Right to privacy and informed consent. The authors declare that no patient data appears in this article.

Conflict of interest

The authors declare that they have no conflicts of interest.

Acknowledgements

This study was partially supported by Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET PIP0009), Agencia de Promoción Científica y Tecnológica (PICT 2013-0543), Comisión de Investigaciones Científicas de la Provincia de Buenos Aires (CICPBA), Universidad Nacional de La Plata (UNLP, 11/N 773) and Rizobacter Argentina S.A.

References

1. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol. 1990;215:403–10.
2. Arnold AE, Herre EA. Canopy cover and leaf age affect colonization by tropical fungal endophytes: ecological pattern and process in *Theobroma cacao* (Malvaceae). Mycologia. 2003;95:388–98.
3. Arnold AE, Mejia LC, Kyllo D, Rojas EI, Maynard Z, Robbins N, Herre EA. Fungal endophytes limit pathogen damage in a tropical tree. Proc Natl Acad Sci USA. 2003;100:15649–54.
4. Arnold AE, Maynard Z, Gilbert GS, Coley PD, Kursar TA. Are tropical fungal endophytes hyperdiverse? Ecol Lett. 2000;3: 267–74.
5. Cabrera AL, Willink A. Biogeografía de América Latina. Monografía 13, Serie de Biología. Washington, DC: OEA; 1973. p. 120.
6. Canel RS, Wagner JR, Stenglein SA, Ludemann V. Indigenous filamentous fungi on the surface of Argentinean dry fermented sausages produced in Colonia Caroya (Córdoba, Argentina). Int J Food Microbiol. 2013;164:81–6.
7. Carroll GC. Fungal endophytes in stems and leaves: from latent pathogen to mutualistic symbiont. Ecology. 1988;69:2–9.
8. Domsch KH, Gams W, Anderson TH. Compendium of soil fungi. Eching, Germany: IHW-Verlag; 2007. p. 859.
9. Elamo P, Helander ML, Saloniein I, Neuvonen S. Birch family and environmental conditions affect endophytic fungi in leaves. Oecologia. 1999;118:151–6.
10. Fisher PJ, Petrini O, Scott HML. The distribution of some fungal and bacterial endophytes in maize (*Zea mays L.*). New Phytol. 1992;122:299–305.
11. Hamayun M, Afzal Khan S, Ahmad N, Tang DS, Kang SM, Na CI, Sohn EY, Hwang YH, Shin DH, Lee BH, Kim JG, Lee IJ. *Cladosporium sphaerospermum* as a new plant growth-promoting endophyte from the roots of *Glycine max* (L.) Merr. World J Microbiol Biotechnol. 2009;25:627–32.
12. Hardoim PR, Van Overbeek LS, Elsas JDV. Properties of bacterial endophytes and their proposed role in plant growth. Trends Microbiol. 2008;16:463–71.
13. Impullitti AE, Malvick DK. Fungal endophyte diversity in soybean. J Appl Microbiol. 2013;114:1500–6.
14. Larran S, Perelló A, Simón MR, Moreno V. Isolation and analysis of endophytic microorganisms in wheat (*Triticum aestivum* L.) leaves. World J Microbiol Biotechnol. 2002;18:683–6.
15. Leslie JF, Summerell BA. The *Fusarium* laboratory manual. Ames IA, USA: Blackwell Publishing; 2006. p. 388.
16. Magurran AE. Measuring biological diversity. Malden MA/Oxford, UK: Blackwell Publishing; 2004. p. 256.
17. Marquez LM, Redman RS, Rodriguez RJ, Roossinck MJ. A virus in a fungus in a plant: three-way symbiosis required for thermal tolerance. Science. 2007;315:513–5.
18. Marquez SS, Bills GF, Zabalgogeazcoa I. The endophytic mycobiota of the grass *Dactylis glomerata*. Fungal Divers. 2007;27:171–95.
19. Ministerio de Agricultura Ganadería y Pesca (MAGyP). 2012. www.minagri.gob.ar.
20. Mejía LC, Rojas EI, Maynard Z, Bael SV, Arnold AE, Hebbar P, Samuels GJ, Robbins N, Herre EA. Endophytic fungi as biocontrol agents of *Theobroma cacao* pathogens. Biol Control. 2008;46:4–14.
21. Narisawa K, Kawamata H, Currah RS, Hashiba T. Suppression of *Verticillium* wilt in eggplant by some fungal root endophytes. Eur J Plant Pathol. 2002;108:103–9.
22. O'Donnell K, Kistler HC, Cigelnik E, Ploetz RC. Multiple evolutionary origins of the fungus causing Panama disease of banana: concordant evidence from nuclear and mitochondrial gene genealogies. Proc Natl Acad Sci USA. 1998;95:2044–9.

23. Pan JJ, Baumgarten AM, May G. Effects of host plant environment and *Ustilago maydis* infection on the fungal endophyte community of maize (*Zea mays*). New Phytol. 2008;178:147–56.
24. Petrini O. Fungal endophytes of tree leaves. In: Andrews J, Hirano S, editors. Microbial ecology of leaves. New York: Springer; 1991. p. 179–97.
25. Pimentel IC, Glienke-Blanco C, Gabardo J, Stuart MR, Azevedo JL. Identification and colonization of endophytic fungi from soybean (*Glycine max* (L.) Merril) under different environmental conditions. Braz Arch Biol Technol. 2006;49:705–11.
26. Rodriguez RJ, White JF, Arnold AE, Redman RS. Fungal endophytes: diversity and functional roles. New Phytol. 2009;182:314–30.
27. Roy KW, Baird RE, Abney TS. A review of soybean (*Glycine max*) seed, pod, and flower mycofloras in North America, with methods and a key for identification of selected fungi. Mycopathologia. 2001;150:15–27.
28. Schultz B, Boyle C. The endophytic continuum. Mycol Res. 2005;109:661–86.
29. Stenglein S, Balatti P. Genetic diversity of *Phaeoisariopsis griseola* in Argentina as revealed by pathogenic and molecular markers. Physiol Mol Plant Pathol. 2006;68:158–67.
30. Stenglein SA, Rodriguero MS, Chandler E, Jennings P, Salerno GL, Nicholson P. Phylogenetic relationships of *Fusarium poae* based on EF-1α and *mtSSU* sequences. Fungal Biol. 2010;114:96–106.
31. Vega FE, Posada F, Aime MC, Pava-Ripoll M, Infante F, Rehner SA. Entomopathogenic fungal endophytes. Biol Control. 2008;46:72–82.
32. White TJ, Bruns T, Lee S, Taylor J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, editors. PCR protocols: a guide to methods and applications. New York, USA: Academic Press; 1990. p. 315–22.
33. Yudelman M, Ratta A, Nygaard D. Pest management and food production: looking to the future. Food, Agriculture, and the Environment Discussion Paper No 25. Washington, DC: International Food Policy Research Institute; 1998. p. 1–49.