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Characterization of dark septate endophyte fungi associated with cultivated soybean at two growth stages

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ABSTRACT

Dark Septate Endophytes (DSE) is a diverse group of Ascomycetes that colonize the roots of a wide range of plants species. They can be found in all ecosystems, from deserts to the tropics, but also in agroecosystems associated to the crops. Despite the ubiquitous of these fungi, only a few major crops were assessed for this association in field conditions. In general terms, a complex consortium of DSE fungi were observed to colonize the roots of some crops. In this work we study the DSE community associated to soybean in the most productive area of this crop in Argentina. We hypothesized that DSE root colonization level, and the identity/frequency of the isolates switch according to the growth stage of the crop. A total of 34 dark fungal colonies were isolated from two growth stages, being only six isolates asyntomatic in the resynthesis assay. Some taxa as *Boeremia* sp., *Cadophora* sp., *Coniothyrium* sp., *Corynespora cassicola, Peyronellaea* sp. and *Phaeosphaeria* sp. were exclusively isolated from seedlings, supporting the hypothesis of DSE fungal consortium switching. The soybean pathogens *Corynespora cassicola* and *Macrophomina phaseolina* were isolated from young and mature plants respectively. Additional studies should focus on a fine analysis of the dynamics of these fungi, considering also the driving factors that could determine these changes. To understand these mechanisms may be fundamental for a better sustainable management of the crop.

1. Introduction

The dark septate endophytes (DSEs) are a polyphyletic group of fungi including species from phylogenetically distant phyla. They are characterized by their darkly pigmented hyphae due to the presence of melanin, and colonize inter and intracellular parenchymatic root tissue, often forming microsclerotia (Jumpponen and Trappe, 1998). DSE fungi are widely distributed and registered in more than 600 phylogenetically diverse host species (Addy et al., 2005; Mandyam and Jumpponen, 2005). Several studies related to the mycorrhizal status of plant species on diverse biomes have included these endophytic fungi within their surveys, evidencing their abundance in plant communities worldwide (Fracchia et al., 2009; Rodriguez et al., 2009). Particularly, DSEs were predominantly observed in roots of plants growing in stressful and nutrient-limited environments (Lugo et al., 2009; Newsham et al., 2009; Silvani et al., 2013). This suggests a possible mitigating and relieving effect of DSEs in such extreme conditions, functionally behaving as a mycorrhizal symbiosis in some plants species (Porras-Alfaro and Bayman, 2011). Moreover, the broad geographic distribution of DSE fungi would be indicating low host specificity. For instance, the most studied DSE taxon, the *Phialocephala fortinii–Acephala applanata* complex, has been found in diverse hosts and biomes of the northern hemisphere (Grünig and Sieber, 2005).

When considering crop plants, the information related to their association with DSEs is scarce, and limited to descriptive analyses of the colonization of DSE on root samples from the field (Fernandes et al., 2015; Muthukumar and Tamilselvi, 2010), or to genomic analyses where sequences related to DSE taxa were detected (Bokati et al., 2016; Detheridge et al., 2016; Likar et al., 2008). Several studies have also focused on the interaction effects of isolated DSE strains from diverse origins on crop plants mainly as biotic/abiotic stress relievers (Narisawa et al., 2004; Su et al., 2013) as well as promoters of plant growth under greenhouse conditions (Della Monica et al., 2015; Yuan et al., 2010a). Although some isolates of DSE have demonstrated potential as bioinoculants in commercial crops, there are still many questions to be solved (Lugtenberg et al., 2016). One of them is related to the consortium concept of this fungal group, since commonly a number of species are associated simultaneously in the same root

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system, therefore the associated DSEs need to be addressed deeply for each crop and throughout the plant growth. On the other hand, as DSE fungi are established in root tissues, the nature of the association (i.e. biotrophic, necrotrophic) throughout the plant life cycle needs also to be taken into account (Rodriguez et al., 2009).

Soybean has emerged as one of the most important agricultural crop worldwide, being South America the main production area (Confalone et al., 2010). In Argentina, since 1970 the planted area destined for this crop have increased uninterruptedly, reaching more than 20 million hectares in 2016. This have made soybean in Argentina the first crop per cultivated area and tons of production (Ministerio de Agroindustria, 2017), with the genetically modified (GM) soybean (glyphosate herbicide tolerant) varieties representing over 90% of the planted area (Leguizamón, 2014). Currently, the standard culture system for soybean crops include commercial *Bradyrhizobium* spp. inoculation and no-tillage farming, thus increasing crop efficiency and grain yields (Díaz Zorita and Fernández Canigia, 1999). Despite the enormous economic importance of soybean, the knowledge regarding its associated fungal root endophyte community is almost limited to their symbiosis with arbuscular mycorrhizal (AM) fungi. Since the early works of Ross and Harper (1972), Safir et al. (1972) and Bethlenfalvay et al. (1983), the published studies have been focused mainly on the nutritional aspects of the AM symbiosis and the associated tolerance to stress conditions (Porcel and Ruiz-Lozano, 2004; Wang et al., 2011). When considering other root associated fungi, Russo et al. (2016) reported recently the isolation and identification of endophytic fungi in leaves, stems and roots of different soybean cultivars. Within them, the soybean pathogen *Macrophomina phaseolina* was identified as the only dark fungi associated with root tissues. However, it could be expected that a greater diversity of DSEs colonize soybean roots, interacting with their host throughout the plant life cycle.

The main objective of this work is to study the dynamics and composition of the DSE communities associated with soybean crop in the most productive area of this legume in Argentina. We set up the hypothesis that the DSE root colonization level as well as the identity/frequency of the isolates change with the growth stage of the plant. To test this hypothesis, we select a study area within the core region for soybean planting in Argentina, and assessed root colonization level and DSE community composition from plants sampled at two different growth stages (seedlings and mature plants). Additionally, we undertake a resynthesis assay under laboratory conditions with each of the fungal isolates and soybean plants, in order to attempt an approach of the nature (symptomatic/asymptomatic) of the dual association.

2. Materials and methods

2.1. Experimental site and sampling

The experimental site lies within an intensive soybean crop area located in the north of the Buenos Aires province (34° 08′ S–59° 16′ W). This area corresponds to the humid Pampa, the most productive region for soybean planting in Argentina. The soil is a Typical Argiudol pH 6.7 and 4–5% organic matter content; (see Etcheverry and Génova (2015) for general edaphic characteristics). The climate is temperate, with temperatures varying between 5 °C and 32 °C and mean annual precipitation of 980 mm. The field is cropped under no-tillage management, since more than 15 yr, and the entire crop rotation is soybean followed by a double crop of wheat/soybean. The soybean planted is the GM cultivar RR (Roundup Ready)® resistant to glyphosate, and the sowing is carried out inoculating the grain (140 kg ha^{-1}) with a commercial formulation of *Bradihizobium* spp. Glyphosate is applied before sowing and along crop development.

To sample soybean roots for the DSE colonization assessment and fungal isolation, two growth stages were selected: 4 leaves seedlings (stage 1–45 days old) and mature plants with pods (stage 2–135 days old). The plants were collected in November 2010 for stage 1 and in February 2011 for stage 2. A total of 25 whole plants were collected for each sampling date, following a 500 m transect, digging up 5 individuals every 100 m. Only healthy plants without disease symptoms were chosen. The plant material was stored up for 24 h at 4 °C until processed in the laboratory.

Table 1
Fungal endophytes taxa isolated from soybean roots: phylum, order, close relative taxa and GenBank accession numbers.

Phylum	Order	Close relative	GenBank accession numbers
Ascomycota	Pleosporales	*Alternaria alternata*	KX784250–KX784254, KX784256, KX784257–KX784261
Ascomycota	Pleosporales	*Alternaria arborecens*	KX784249, KX784255
Ascomycota	Pleosporales	*Boeremia* sp.	KX784233, KX784237
Ascomycota	Pleosporales	*Coniothyrium* sp.	KX784247
Ascomycota	Pleosporales	*Corynespora cassicola*	KX784231, KX784232
Ascomycota	Pleosporales	*Curvularia trifolii*	KX784248
Ascomycota	Pleosporales	*Paraphoma radicina*	KX784239–KX784246
Ascomycota	Pleosporales	*Peyronellae* sp.	KX784234–KX784236
Ascomycota	Pleosporales	*Phaeosphaeria* sp.	KX784238
Ascomycota	Capnodiales	*Cladosporium* sp.	KX784262
Ascomycota	Botryosphaeriales	*Macrophomina phaseolina*	KX784263
Ascomycota	Helotiales	*Cadophora* sp.	KX784264

2.2. Root colonization

Root samples were separated carefully from the rest of the plant, and cleaned with running tap water to remove adhering soil particles. A root subsample of each individual was separated to dye and quantify the DSE colonization level. These roots were cleared and stained as described by Phillips and Hayman (1970). Briefly, samples were cleared in 10% KOH at 90 °C for 30 min, followed by staining with trypan blue, distained with distilled H_2O and stored in lactic acid. In order to quantify the proportion of colonized root relative to the total root lenght, ten stained fragments from each plant were observed under binocular microscope at 200× magnifications, according to the method of McGonigle et al. (1990).

2.3. Fungal isolation

For DSE isolation we used the remaining root subsamples from the samples described above. Roots were surface sterilized by washing with 70% ethanol for 2 min, sodium hypochlorite solution 10% for 15 min and in an antibiotic solution (0.05% w/v Penicillin, 0.05% w/v Ampicillin, 0.05% w/v Streptomycin, 0.05% w/v Tetracycline) during 15 min, and then rinsed in abundant sterile distilled water. Under laminar flow, the sterilized fragments (2–3 cm) were cut in 2 mm pieces, discarding the ends, and inoculated in droplets of 0.35% w/v Gel-Gro® and 0.02% $MgSO_4$, in Petri dishes (Silvani et al., 2008). Twenty-five root fragments of each plant were incubated in the dark at 28 °C and checked every day, using a binocular microscope. The emerging fungi from the ends of the fragments were transferred individually to Petri plates with malt extract agar (MEA) medium at pH 6.5, and incubated in the dark at 25 °C. The dark pigmented colonies were finally transferred to glass tubes with the same medium and stored at 4 °C.

2.4. Molecular identification

To perform the molecular identification, DNA was extracted from all isolates, amplified, and sequenced. The fungal isolates were cultured in

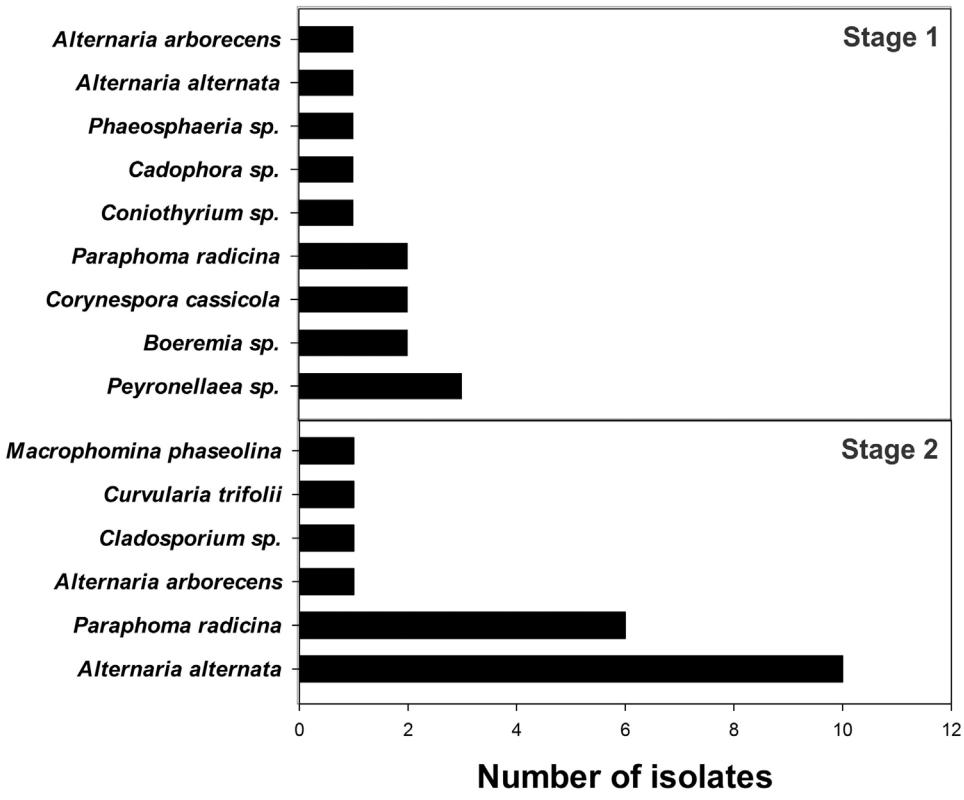

Fig. 1. Frequency of root endophytes isolated from two growth stages of soybean plants growing in an agricultural landscape.

extract broth 20% (w/v) under static conditions at 25 °C for one week. Then, the genomic DNA was extracted using UltraCleanTM Microbial Isolation Kit (MO BIO). The rDNA ITS region was amplified by polymerase chain reaction (PCR) with primers ITS4 and ITS5 (White et al., 1990). PCR amplification was performed in 50 µl of a mixture containing 1 µl of 10 mM dNTP, 10 µl of 10× Iproof buffer, 0.5 µl of 100 µM primers, 1 unit of iProofTM High Fidelity DNA Polymerase (BIO RAD), and 2 µl of genomic DNA. The thermal cycler parameters were programmed for 1 cycle of denaturation at 98 °C for 3 min, 35 cycles of denaturation at 98 °C for 10 s, annealing at 54 °C for 30 s, extension at 72 °C for 20 s, and a final extension at 72 °C for 10 min. The PCR-amplifications were purified with UltraCleanTM PCR Clean-up DNA Purification Kit (MO BIO) and sequenced on genotyping service of FCEN (UBA). The obtained sequences were compared with sequences present in the GenBank. Subsequently finding sequences were performed for molecular phylogenetic analyzes (Aveskamp et al., 2010; de Gruyter et al., 2010; Hyde et al., 2013). Sequences were aligned with MAFFT program version 6 (Katoh and Toh, 2008) available online (http://mafft.cbrc.jp/alignment/server/), assigning a cost to the opening 15 and 6 to the extent of the gaps. The alignments were subjected to a neighbor-joining analysis using the heuristic search option and phylogenetic trees were inferred in MEGA v. 5 (Tamura et al., 2013). The support nodes obtained were calculated by the Bootstrap analysis with 1000 replicates (Felsenstein, 1985). When phylogenetic analysis not resolved at species level, it was supplemented with identification based on morphological characters (Woudenberg et al., 2013). To compare the degree of similarity and diversity of communities, Jaccard similarity index (J) and Simpson's diversity index (1-D) were calculated (Whittaker, 1972). Jaccard similarity index reports similarity based on presence/absence data only and Simpson's diversity index is a measure of diversity which takes into account the number of species present, as well as the relative abundance of each species.

2.5. Resynthesis in vitro

This assay was performed to evaluate the resynthesis characterization of each fungal isolate, focusing on root colonization patterns and particular symptoms observed on inoculated soybean plants. Soybean seeds (transgenic RR) were surface sterilized with a solution of 10% NaOCl for 15 min, washed with abundant sterile distilled water and incubated at 25 °C in Petri dishes containing water agar. Once their cotyledons were completely green, the seedlings were inoculated depositing the emerging roots directly on an actively growing fungal colony (MEA). These plates were placed in a growth chamber with 16 h light and 8 h dark at 25 °C for 48 h. As control we used seedlings deposited directly on MEA and without fungi. Finally, after the inoculation time, the seedlings were transferred axenically to 250 ml flasks, with 100 ml of agarized Murashige and Skoog medium at pH 5.5. The flasks were sealed with parafilm and incubated for 40 day in a culture chamber with 16 h light and 8 h dark at 25 °C. For each isolate, 5 replicates were performed. At the time of harvesting, the general condition of the plant and the presence of disease symptoms in shoot and root were noted.

3. Results

3.1. Root colonization

The stained roots from both plant stages showed high levels of DSE colonization. In stage 1, the level reached 62 ± 12% and in stage 2, a value of 89 ± 10% of total root colonization (mean ± SD, N = 25). Microsclerotia were absent in stage 1 root samples, meanwhile in mature plants these structures colonized sporadically the parenchyma tissues. Not all microsclerotia belonged to the same morphotype. Melanized and hyaline septate hyphae were observed under bionocular microscope in all root samples, often as a continuum between both morphotypes, being this feature more frequent in seedlings than in mature soybean plants. When the hyphal connection was evident, it was

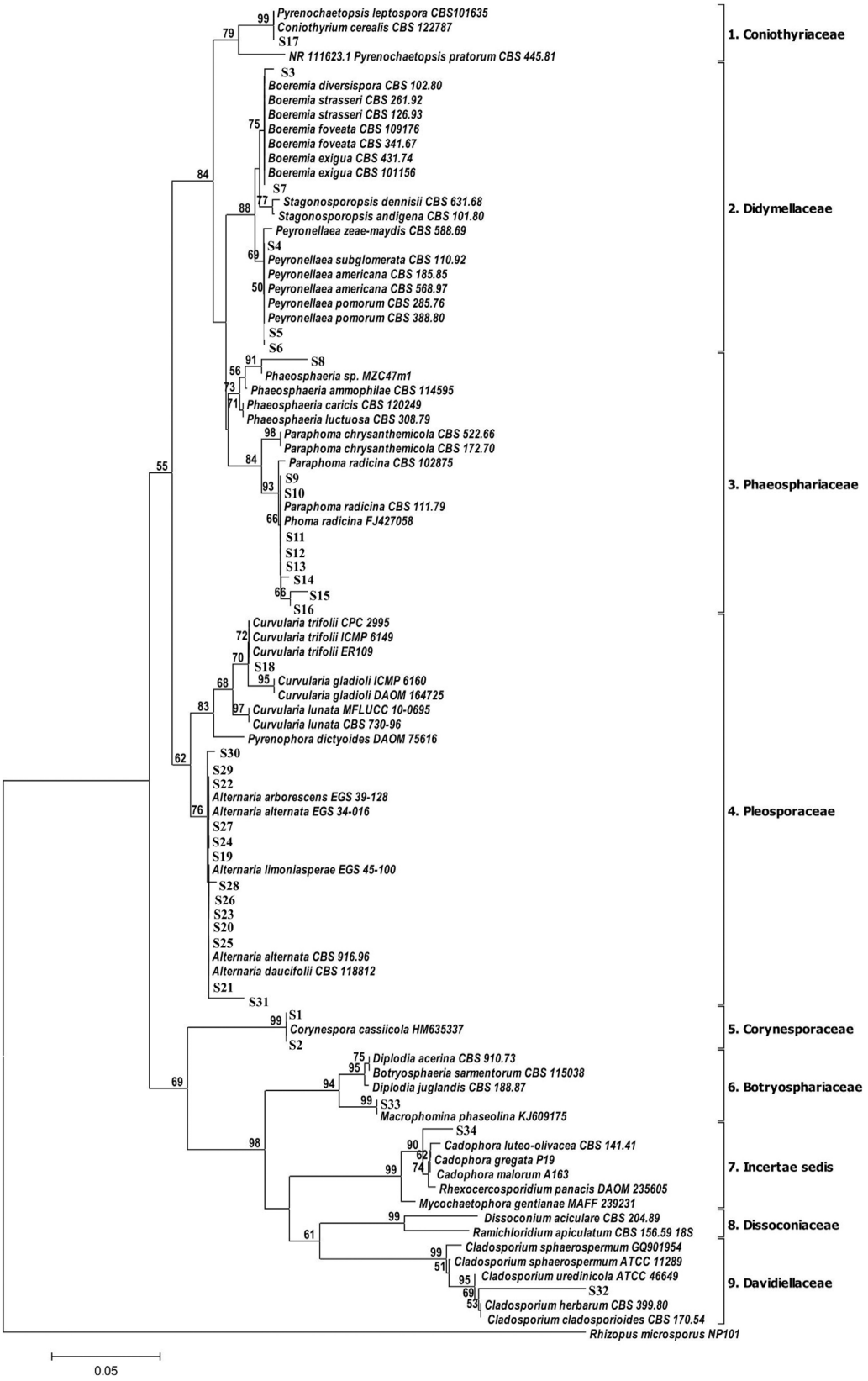

Fig. 2. Neighbour-joining tree based on the fungal rDNA internal transcribed spacer (ITS) with name of strains. Numbers above branches refer to bootstrap values.

taken into account for DSE colonization measurement in the same root fragments.

3.2. Diversity of DSE fungal communities

A total of 34 dark septate isolates were obtained from the root samples. All fungal isolates were cultivated and preserved in MEA, and subsequently deposited at the fungal collection of the Centro Regional

Table 2
In *vitro* resynthesis in solid MS (Morashige-Skoog) medium of each fungal isolate; record of five replicates (symptomatic/asymptomatic) and main characterization of disease symptoms on soybean plants.

Fungalspecies	Strain code/GenBank accession numbers	Stage 1			Stage 2		
		Asymptomatic	Symptomatic	Observations[a]	Asymptomatic	Symptomatic	Observations[a]
Alternaria alternata	**S20** KX784250	–	–	–	5/5	–	–
	S21 KX784251	–	–	–	–	4/5	chlorosis (S)
	S22 KX784252	–	–	–	–	5/5	inhibition of development (S,R), defoliation (S)
	S23 KX784253	–	–	–	–	5/5	inhibition of development (S,R), defoliation (S)
	S24 KX784254	–	3/5	chlorosis (S)	–	–	–
	S26 KX784256	–	–	–	–	3/5	chlorosis (S)
	S27 KX784257	–	–	–	–	3/5	tissue disorganization (R)
	S28 KX784258	–	–	–	–	5/5	inhibition of development (R)
	S29 KX784259	–	–	–	–	4/5	tissue disorganization (R)
	S30 KX784260	–	–	–	–	5/5	inhibition of development (R), defoliation (S)
	S31 KX784261	–	–	–	–	5/5	necrosis (S,R)
Alternaria arborecens	**S19** KX784249	–	–	–	–	3/5	chlorosis (S), inhibition of development (S,R)
	S25 KX784255	5/5	–	–	–	–	–
Boeremia sp.	**S3** KX784233	–	3/5	chlorosis (S), papillae (R)	–	–	–
	S7 KX784237	–	5/5	chlorosis (S), tissue disorganization (R)	–	–	–
Cadophora sp.	**S34** KX784264	–	4/5	papillae (R)	–	–	–
Cladosporium sp.	**S32** KX784262	–	–	–	5/5	–	–
Coniothyrium sp.	**S17** KX784247	5/5	–	–	–	–	–
Corynespora cassicola	**S1** KX784231	–	4/5	leaf spot (S), chlorosis (S), tissue disorganization (R)	–	–	–
	S2 KX784232	–	5/5	leaf spot (S), chlorosis (S), tissue disorganization (R)	–	–	–
Curvularia trifolii	**S18** KX784248	–	–	–	–	5/5	necrosis (S,R)
Macrophomina phaseolina	**S33** KX784263	–	–	–	–	5/5	necrosis (S,R)
Paraphoma radicina	**S9** KX784239	–	–	–	–	3/5	inhibition of development (S), papillae (R)
	S10 KX784240	–	–	–	–	4/5	inhibition of development (R), papillae (R)
	S11 KX784241	–	5/5	papillae (R)	–	–	–
	S12 KX784242	–	–	–	–	5/5	papillae (R)
	S13 KX784243	–	–	–	–	5/5	necrosis (S,R)
	S14 KX784244	–	–	–	5/5	–	–
	S15 KX784245	–	–	–	–	5/5	inhibition of development (S), papillae (R)
	S16 KX784246	–	3/5	necrosis (S,R)	–	–	–
Peyronellaea sp.	**S4** KX784234	–	3/5	chlorosis (S)	–	–	–
	S5 KX784235	–	4/5	necrosis (S,R)	–	–	–
	S6 KX784236	3/5	–	–	–	–	–
Phaeosphaeria sp.	**S8** KX784238	–	5/5	tissue disorganization (R)	–	–	–
Total (%)[b]		3 (21)	11 (79)		3 (15)	17 (85)	

[a] (S) shoot, (R) root.
[b] Number of isolates with asymptomatic or symptomatic responses.

de Investigaciones Científicas y Transferencia Tecnológica (CRILAR) in La Rioja, Argentina. The molecular analyses showed that all the isolates belonged to four orders of the Ascomycota, with 31 (91.17%) strains belonging to the Pleosporales order, and the remainders three to the orders Capnodiales, Botryosphaeriales and Helotiales (Table 1, Fig. 2). A total of 11 genera and 12 species of dark colonies were isolated and identified. Within the Alternaria complex, two species (*A. alternata* and *A. arborescens*) were identified by morphological characters, complementing the phylogenetic analysis. When considering the growth stages of the surveyed plants, 14 fungi were isolated from stage 1, and 20 from stage 2. The diversity of both communities assessed by the Simpson index was 0.88 for stage 1 and 0.61 for stage 2 (Fig. 1). In stage 1 all species were relatively equally represented, being *Peyronellaea* sp. the most abundant strain with 3 isolates. In stage 2, the frequency was characterized by two dominant species; *Paraphoma radicina* (6 isolates, 30%) and *A. alternata* (10 isolates, 50%). In addition, according to the Jaccard index, the similarity of the communities was 0.5. The species *P. radicina*, *A. alternata* and *A. arborescens* were isolated from both sampling stages, meanwhile the strains of the genera *Boeremia* sp., *Cadophora* sp., *Coniothyrium* sp., *Peyronellaea* sp., *Corynespora cassicola* and *Phaeosphaeria* sp. were found exclusively in plants from stage 1, and *Curvularia trifolii*, *Cladosporium* sp. and *Macrophomina phaseolina* exclusively in stage 2.

3.3. Resynthesis assay

In the resynthesis assay, only six isolates (17.6%), three from each plant growth stage (21% stage 1; 15% stage 2), were asymptomatic when inoculated on *in vitro* soybean plants (Table 2). The remaining 28 (82.4%) isolates produced some kind of symptomatology. From 11 isolates of *A. alternata*, only the strain S20, isolated from mature soybean plants, was asymptomatic, as well as one *P. radicina* strain (S14) of eight isolates of this genus. The other species that did not produced symptoms were *A. arborecens* (S25), *Coniothyrium* sp. (S17), *Peyronellaea* sp. (S6) and *Cladosporium* sp. (S32). The main features of root colonization by some of the fungal isolates are shown in Fig. 3.

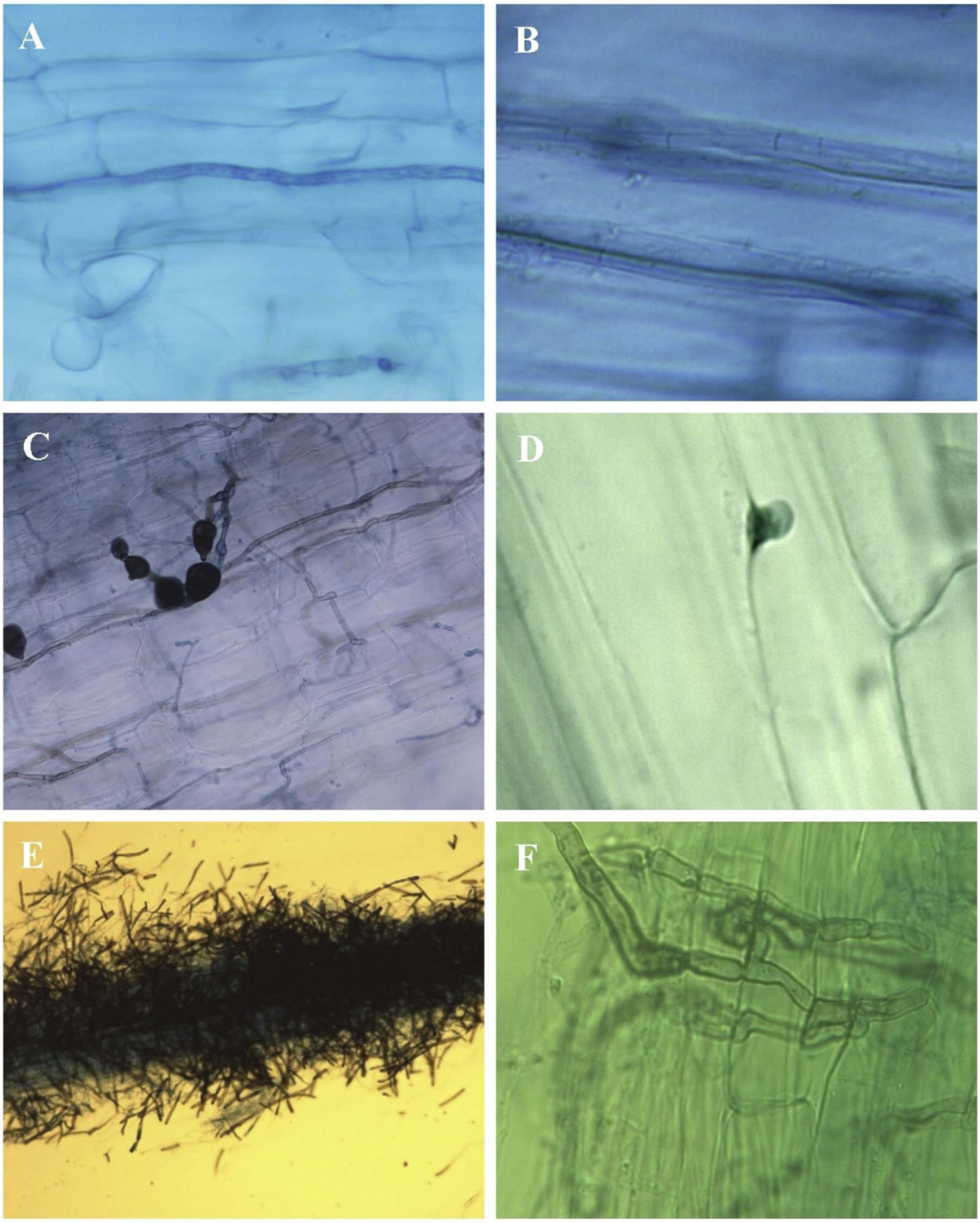

Fig. 3. Root colonization of the resynthesis in vitro assay with soybean plants: a *Cladosporium* sp. (S32); b *Coniothyrium* sp. (S17); c Conidia in root surface and intraradical colonization by *A. arborescens* (S28); d Papillae induced by *P. radicina*; e,f *C. cassicola* (S2) colonizing profusely root surface (e) and intraradical tissues (f).

Asymptomatic *Cladosporium* sp. (S32) and *Coniothyrium* sp. (S17) fungi colonized the root parenchymal tissue with melanized and septate hyphae, running longitudinally mainly in the intercellular space (Fig. 3A and B). These two isolates colonized parenchymal cells without any necrotic evidence. The isolate S25 of *A. arborecens* colonized the roots forming conidia on the root surface (Fig. 3C). When considering the symptoms, the most frequent was chlorosis followed by defoliation and root tissue disorganization. However, not all isolates were equally effective in producing the symptoms on soybean plants; in some cases, the five replicated individuals showed symptoms (i.e. *A. alternata* S22, *P. radicina* S15), meanwhile in others only 3 of 5 plants were infected (i.e. *P. radicina* S9, *Peyronellaeae* sp. S4). The formation of papillae was observed in soybean roots inoculated with several fungal species (i.e. *Cadophora* sp. S34, *P. radicina* S11 and S15; Fig. 3D). Finally, isolates belonging to the genera *Boeremia, Curvularia, Corynespora, Paraphoma, Phaeosphaeria* and *Alternaria* produced necrosis and tissue disorganization on roots, thus clearly behaving as plant pathogens. Fig. 3E and F show a soybean root totally colonized on the surface (Fig. 3E) and intraradically (Fig. 3F) by *C. cassicola* (S2) before tissue disorganization was evident.

4. Discussion

In this study, using classical methodologies of root fungal staining and isolation, we found that soybean roots from the rich soils of the Pampas region of Argentina are profusely colonized by dark septate hyphae and associated with a complex and rich DSE fungal consortium. In spite of the increasing interest on the DSE fungi, ours and only a handful of other studies were undertaken directly under crop conditions (Das and Chaudhuri, 2009; Das and Kayang, 2010; Yuan et al., 2010b; Zhang et al., 2014). When considering soybean as the subject of study, the species was assessed only for endophytic fungi from aerial parts (Chen et al., 1996), and only recently the work of Russo et al. (2016) considered DSE fungi associated to the roots.

Our results show that the growth stage of the plant should be considered as an important factor determining the level of root fungal endophyte colonization and the diversity of the DSE community associated to the roots. The host species/genotypes as well as the edaphic and climatic conditions are all variables that clearly drive the fungal biota associated with the roots of the plant species. The study of Russo et al. (2016) found differences between fungal communities associated to the stems, leaves and roots from three different soybean cultivars. As other driving factor, crop management has also been shown as determinant for the community of mycorrhizal fungi associated with the plant. No-tillage practices, agrochemical applications or the irrigation regime has been demonstrated to modify the root endophytic fungal communities associated with the roots (Druille et al., 2013, 2016; Galván et al., 2009; Jansa et al., 2006; Sadowsky et al., 2012). Beside these factors, the growth stage of the plants has been poorly studied in order to evaluate the dynamics of root endophytic fungal groups, in particular for the DSE community. As was hypothesized, some fungal taxa as *Boeremia* sp., *C. cassicola, Peyronellaea* sp. and *Phaeosphaeria* sp. were isolated only from soybean seedlings (stage 1), meanwhile other taxa as *Cladosporium* sp., *C. trifolli* and *M. phaseolina* were exclusively found in mature plants. These results might be suggesting a switch of at least a part of the fungal community associated with the roots of soybean throughout the plant development. Although there are no references on DSE fungal succession on other crops, it has been proved in other root fungal associations such as in orchid mycorrhiza (McCormick et al., 2006) and ectomycorrhizal fungi of tree plantations (de Mendonça Bellei et al., 1992; Peay et al., 2011). An important feature to be considered is the variability in the distribution of endophytic fungi on soil. Khidir et al. (2010) found that even adjacent conspecific plants in a natural habitat showed a great variation in their root associated fungal community composition, including DSE fungi. However, we selected carefully a soybean crop field with a long history of plowing and cultivation (> 50 years), and no-tillage management for at least 15 years. In this context, we expected *a priori* a more homogeneous distribution of the soil microbiota.

In contrast to other surveys (Detheridge et al., 2016; Yuan et al., 2010b), no basidiomycotan fungi were isolated from the root samples. Although the study focused on DSE culture, leaving aside hyaline fungi, care was taken to detect strains of the *Thanatephorus* complex, which may be melanized late in agar plate culture. This fungal group comprises pathogenic strains, but also melanized asymptomatic fungi that colonize the roots and were considered as DSE by some authors (Brundrett, 2006). Some of the isolated taxa as *A. alternata*, *M. phaseolina* and *C. cassicola* are well known pathogens of soybean crops (Almeida et al., 2001; Smith and Carvil, 1997). The existence of avirulent strains of fitopathogenic fungi has been demonstrated in several studies (Bao and Lazarovits, 2001), being considered as potential bioprotectors in crop species. Nevertheless, as the status of the interaction between fungal endophytes and host may be transient, pathogenic fungi could remain latent within the plant tissues and express their virulence in late stages of the crop, or under certain environmental conditions (Schulz and Boyle, 2005). In the resynthesis assay, *M. phaseolina*, and *C. cassicola* behaved as plant pathogens, producing chlorosis and tissue disorganization. However, the inoculation of an *A. alternata* strain (S20) and the *A. arborecens* isolate (S25) did not produce any symptomatology, meanwhile all other *Alternaria* strains did it. This genus comprises a wide complex group, with known aggressive pathogen strains, and is also a ubiquitous taxon recorded in root endophytic surveys (Khidir et al., 2010; Yuan et al., 2010b). *Cladosporium* is a genus of endophityc strains, that colonizes even aerial organs as leaves and stems of soybean (Impullitti and Malvick, 2013). The inoculation of the isolate S32 did not evidence any symptomatolgy on soybean plants, behaving as a true DSE fungus, as their hyphae were septate and darkly pigmented. When considering all the isolates obtained in the resynthesis assay from each growth stage, we found only slight differences between them regarding the observation of symptoms. In stage 1, 79% of the isolates produced symptoms, and 85% produced them in stage 2.

5. Conclusion

We can assert that soybean roots from rich soils of the Pampas crop production area are colonized by several septate and melanized fungal taxa; comprising pathogens but also asymptomatic strains. An imbalance of this consortium in a defined growth stage of the plant, could be a determining factor for the crop health. Future studies focusing on the fine dynamics of these fungal communities throughout the plant development could help to understand environmental or intrinsic host plant factors that may have an influence on them.

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