Plant Physiology and Biochemistry 144 (2019) 100–109

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

The fungal endophyte *Fusarium solani* provokes differential effects on the fitness of two *Lotus* species

Amira Susana Nieva[a], Juan Manuel Vilas[a], Andrés Gárriz[a], Santiago Javier Maiale[a], Ana Bernardina Menéndez[b], Alexander Erban[c], Joachim Kopka[c], Oscar Adolfo Ruiz[a,d,*]

[a] Instituto de Investigaciones Biotecnológicas-Instituto Tecnológico de Chascomús (IIB-INTECH), Av. Intendente Marino km 8.2, Chascomús, 7130, Argentina
[b] Departamento de Biodiversidad y Biología Facultad de Ciencias Exactas y Naturales Universidad de Buenos Aires, PROPLAME-PRHIDEB (CONICET), Argentina
[c] Max Planck Institute of Molecular Plant Physiology, Am Mühlenberg 1, 14476, Potsdam, Germany
[d] Instituto de Fisiología y Recursos Genéticos Vegetales-Instituto Nacional de Tecnología Agropecuaria (IFRGV-INTA), Camino 60 cuadras km 5.5, Córdoba, 5119, Argentina

ARTICLE INFO

Keywords:
Lotus tenuis
Lotus japonicus
Fusarium solani
Endophytes
Plant *fitness*

ABSTRACT

The interactions established between plants and endophytic fungi span a *continuum* from beneficial to pathogenic associations. The aim of this work was to isolate potentially beneficial fungal endophytes in the legume *Lotus tenuis* and explore the mechanisms underlying their effects. One of the nine fungal strains isolated was identified as *Fusarium solani* and shows the highest phosphate-solubilisation activity, and also grows endophytically in roots of *L. japonicus* and *L. tenuis*. Interestingly, fungal invasion enhances plant growth in *L. japonicus* but provokes a contrasting effect in *L. tenuis*. These differences were also evidenced when the rate of photosynthesis as well as sugars and K contents were assessed. Our results indicate that the differential responses observed are due to distinct mechanisms deployed during the establishment of the interactions that involve the regulation of photosynthesis, potassium homeostasis, and carbohydrate metabolism. These responses are employed by these plant species to maintain *fitness* during the endophytic interaction.

1. Introduction

The interactions established between soil-borne microorganisms and plant roots have been evaluated intensively. Many of these interactions play important roles for plant growth and development, as a huge repertoire of fungal and bacterial species provide the host with several benefits (Rodriguez et al., 2009). For instance, the mutualistic interaction established with mycorrhizal fungi conduces to important improvements in plant nutrition and tolerance to stress (Evelin et al., 2009). On the other hand, it is also known that the biological interplay between plant roots and microbes may result in pathogenesis, that is, a type of interaction where microbes proliferate and cause detrimental effects in the plant host (Saikkonen et al., 1998; Douglas, 2010; Kiers et al., 2010). Interestingly, some microbial strains may establish mutualistic interactions with a group of plant species but cause diseases in very closely related species. Much of what is known on the mechanisms underlying this *continuum* mutualism-pathogenesis remains still unknown.

Legume plants constitute a major group of plants mostly used for grain and pasture production, with the ability to establish symbiotic interactions with nitrogen-fixing soil bacteria known as rhizobia. The molecular events occurring during the establishment of mutualism between legume and rhizobia have been widely explored in model plants such as *L. japonicus* (Handberg and Stougaard, 1992), demonstrating that a complex and dynamic molecular dialogue determines the outcome of the interaction. Other members of the genus *Lotus* such as *L.*

Abbreviations: ITS, Internal Transcribed Spacer gene; TEF, alpha Transcription Elongation Factor gene; Pn, Net photosynthesis; GS, stomatal conductance; C_i, CO^2 in the sub-stomatal cavity; PSII, Photosystem II; *OJIP*, O, Initial fluorescence, at 50 μs or less, J-I, intermediate levels at 2 ms and 30 ms; P, maximal fluorescence; Fv/Fm ($\Phi_{;Po}$), Maximum quantum yield of primary photochemistry; PIabs, Performance Index on the Absorption Basis; ABS, Absorption flux; Sm, normalized total complementary area above the *OJIP* transient; TRo, Trapped energy flux; ETo, Electron transport flux; DIo, Dissipation flux; RC, Reaction centre; CSo, Cross section; $\Psi_{;Eo}$, Electron transport from *quinone a* to plastoquinone efficiency; $\Phi_{;Eo}$, maximum yield of electron transport from *Qa* to plastoquinone primary photochemistry; GC-EI/TOF-MS, gas chromatography coupled to electron impact ionization/time-of-flight mass spectrometry; N, Total Nitrogen; P, Total Phosphorus; K, Total Potassium

* Corresponding author. Instituto de Investigaciones Biotecnológicas-Instituto Tecnológico de Chascomús (IIB-INTECH), Av. Intendente Marino km 8.2, Chascomús, 7130, Argentina.
E-mail address: ruiz@intech.gov.ar (O.A. Ruiz).

https://doi.org/10.1016/j.plaphy.2019.09.022
Received 16 July 2019; Received in revised form 31 August 2019; Accepted 16 September 2019
Available online 17 September 2019
0981-9428/

tenuis and *L. corniculatus* (Waldst. and Kit., syn. *Lotus glaber*; Kirkbride, 2006) are economically important legume species used in pasture systems in a diverse range of landscapes due to their abilities to grow successfully in adverse environmental conditions (Escaray et al., 2012). In this trend, *L. tenuis* is a naturalized legume in lowland grasslands in the Argentinean Flooding Pampa (García et al., 2008; Antonelli et al., 2016, 2019), the most important area devoted to cattle production in this country. This area is characterized by flooding and drought periods, conducing to restrictive soil conditions such as high salinity and alkalinity as well as the presence of low levels of phosphorous (P). Even though P deficiency is usually overcome with the use of fertilizers, a large proportion of the soluble inorganic phosphates is immobilized in soils and result still unavailable for plants. Therefore, agricultural microbiologists have tried to identify P-solubilising microbes to increase its availability, thus avoiding the excessive use of P-based fertilizers. Besides these practises, pasture promotion is a widely-used agricultural approach in the Flooding Pampa to improve the implantation of *L. tenuis*, consisting in the use of herbicides to remove weeds and promote *L. tenuis* growth. As a consequence, the intensive cultivation of *L. tenuis* causes modifications in the diversity of soil fungal communities, with a reduction in biodiversity and an increase in the relative abundance of species belonging to the genus *Fusarium* (Nieva et al., 2018).

The fungal genus *Fusarium* is distributed in a wide range of geographical and climatic conditions, and its members interact with a large group of plant hosts (Backhouse et al., 2001). In this trend, several species of this genus are the main pathogens of important crops, such as *F. graminearum* in cereals (McMullen et al., 1997) and *F. oxysporum*, which causes vascular wilt diseases in a wide variety of plant species (Di Pietro et al., 2001). This genus has been proposed as a model system for soil-borne pathogens (Roncero et al., 2003), and the mechanisms involved in their virulence were deeply studied (Berrocal-Lobo and Molina, 2008). In addition, despite of the economical loses that these fungi cause in important plant species, their capacity to grow as endophytes producing symptomless infections has also been reported (Kuldau and Yates, 2000). These kinds of interactions are poorly known, but usually improve tolerance to soil contaminants, increase abiotic as well as biotic stress resistance, and even may enhance plant growth (Clay, 1988; Malinowski and Belesky, 1999; Saikkonen et al., 1998; Clay and Schardl, 2002). Interestingly, some of the molecular processes triggered in plants during the interaction with these non-pathogenic endophytic strains are quite analogous to those elicited against pathogens. For instance, both type of microbes leads to the regulation of the oxidative balance and activation of the salicylic and jasmonic acid signalling pathways (Saikkonen et al., 2013). Nevertheless, studies on the molecular events occurring in the interactions between plants and non-pathogenic *Fusarium* are still scarce.

In this work, we isolated endophytic fungal strains from the root system of *L. tenuis* plants growing in the Flooding Pampa soils in order to characterize the nature of the species predominating in this agroecosystem and also assess the beneficial/detrimental effects they exert on *Lotus* species. These fungal strains were evaluated first on the bases of their ability to solubilise phosphate and to convey plant tolerance to alkalinity and salinity. Interestingly, we found that a P-solubilising strain was also able to invade inner tissues of *L. japonicus,* but provoked differential responses in *Lotus* species. These differences seemed to be due to the deployment of distinct nutrient metabolizing mechanisms. Our results shed more light on the current knowledge of the molecular events explaining the mutualism-pathogenesis *continuum* in plant-microbe interactions.

2. Materials and methods

2.1. Fungal isolation

In order to isolate fungal endophytic strains, *L. tenuis* plants growing at the INTECH Experimental field (−35.622076 W, −57.993875 S, Flooding Pampa, Buenos Aires, Argentina) were collected, and the root system was separated from the shoots and washed under tap water. The roots were cut in several pieces of 1 cm length and placed in ethanol (70%) and NaClO solution (10%) during 1 and 3 min, respectively, and then rinsed several times with sterile distilled water. No fungal growth was evidenced after plating aliquots of this final wash in Potato-Dextrose Agar (PDA, Britania Lab, Argentina) medium, used to control the efficiency of the surface sterilizing procedure. Each root fraction was placed in PDA containing 1 mg/L gentamicin, and incubated in a growth chamber at 28 °C until the apparition of fungal mycelia. The isolated strains were stored in PDA at 4 °C.

2.2. Identification and characterization of fungal strains

For the evaluation of the phosphate solubilisation capability, one plug of 1 cm^{-2} of mycelia growing in PDA medium of each fungal strain was inoculated in liquid NBRIP medium (Nautiyal, 1999) supplemented with 10 g of glucose per litter and shacked during 7 days at 30 °C. A plug of fresh PDA medium was used as control. After the incubation period, an aliquot of the supernatant was collected to evaluate the amount of H_3PO_4 according to the procedures described by Murphy and Riley (1962). The mycelia were filtered and dried in order to determine the amount of fungal biomass generated and further used as reference to normalize the ppm P solubilised by gr of fungal biomass.

Two strains, according to the highest phosphate solubilisation capability, were selected for further analysis and identified through amplification and sequencing of the Internal Transcribed Spacer sequences ITS1-ITS2 of the ribosomal 18S gene (White et al., 1990) and the alpha Transcription Elongation Factor (α-TEF, Geiser et al., 2004) gene. These regions were amplified in a reaction mix (25 µl of final volume) consisting in: 1 µl of template, 1.5 µl MgCl2 (25 mM), 2.5 µl buffer reaction (10x), 0.2 µl Taq polymerase, 0.2 µl dNTP (10 mM), 0.5 µl of the primers ITS5 (5′ GGAAGTAAAAGTCGTAACAAGG′3) and ITS4 (5′TCCTC CGCTTATTGATATGC ′3) (White et al., 1990), or EF1 (5′ATGGGTAA GGA(A/G)GACAAGAC′3) and EF22 (5′AGGAACCCTTACCGAGCTC′3) (Geiser et al., 2004), in order to amplify the ITS and α-TEF genes, respectively. The following program was used to amplify the ITS gene: 95 °C during 5 min, 38 cycles of: 94 °C 30s, 53 °C 30s, 72 °C 45 s and a final extension of 72 °C 10 min. In turn, the thermal cycles of amplification for α-TEF were 94 °C for 5 min, 38 cycles of 94 °C 45 s, 56 °C 30 s, 72 °C 2 min, and a final extension of 72 °C 2 min. In order to assign taxonomic identities to fungal strains, the databases *UNITE* (version 2013, Nilsson et al., 2018) and *FUSARIUM ID* (Geiser et al., 2004) were used.

The growth tolerance of the selected strains to different concentrations of NaCl was evaluated in PDA medium supplemented with 0, 100, 150 and 200 mM NaCl. The ability to growth in a wide range of pHs was also determined using malt extract agar medium supplemented with buffer solutions according to the procedure described by Nagai et al. (1995). The strains were cultivated in each medium at 30 °C during 7 days. At the end of this period, fungal growth was assessed through the calculation of the mycelial area using the *ImageJ* software v 1.47.

2.3. Plant growth and inoculation

Seeds of *L. tenuis* (cv. *Nahuel*) and *L. japonicus* (ecotype *Gifu B-129*) were scarified with concentrated H_2SO_4 for 5 min and washed ten times with distilled water. Then, seed surface was sterilized with NaClO (5%) and rinsed several times with sterile distilled water. Sterilized seeds were incubated in sterile water overnight and placed in Petri dishes containing water-agar medium (0.5%). Plates were incubated during 15 days in a grow chamber with a 16/8 h photoperiod at 24 °C/19 °C (day/night), light intensity of 240 mol $m^{-2}s^{-1}$ and 60% humidity, until the radicle and trifoliate leaf developed. Then, seedlings were transferred to pots containing sterile sand-perlite substrate (2:1) and irrigated with

nutritive *Evans* solution (Evans et al., 1970). Fungal inoculation was carried out immediately after transplanting. In order to prepare fungal inoculum, the selected strain was cultivated on PDA medium at 30 °C for 14 days. Then, plugs of medium (0.5 × 0.5 cm) were cut out from the borders of the colony and used to inoculate plants. Plugs of fresh PDA medium were used in control plants. After 37 days, roots were washed under tap water and separated from the shoots. An aliquot of the roots was surfaced sterilized and treated as described above in order to re-isolate fungi growing in inner tissues to check its endophytic nature. Fractions of roots were stained with Trypan blue according to the procedures explained by Phillips and Hayman (1970) for microscopic analysis. Each plant fraction (roots and shoots) was placed at 70 °C during 48 h for dry weight assessment. Foliar area was measured in the second oldest leaf of main shoots.

2.4. Assessment of net photosynthesis rate, stomatal conductance and Chlorophyll a transient fluorescence

Carbon exchange, net photosynthesis (Pn), CO_2 in the sub-stomatal cavity (Ci) and stomatal conductance (GS) were determined using an infrared gas analyser (Portable Photosynthesis System, MA, USA) supported with LED lights with an intensity of 240 mol $m^{-2}s^{-1}$, according to the procedures described by Calzadilla et al. (2016).

Chlorophyll a fluorescence transients were determined using a Portable Plant Efficient Analyzer (PEA, Hansatech Instruments Ltd., UK). Leaves were adapted to dark before measurement during 20 min and exposed to a light intensity of 3500 µmol $m^{-2}s^{-1}$ for 3 s. Parameters derived from the *OJIP* test (O: Initial fluorescence, at 50 µs or less, J-I: intermediate levels at 2 ms and 30 ms, P: maximal fluorescence) were analysed in order to characterize the status of the photosystem II (PSII) in plants as described by Vilas et al. (2018): Fv/Fm (Φ_{Po}), Maximum quantum yield of the primary photochemistry; PIabs, Performance Index on the Absorption Basis; ABS, contribution of the absorption of light energy; Sm, normalized total complementary area above the *OJIP* transient; TRo, trapping of excitation energy, ETo, conversion of excitation energy to electron transport; DIo, Dissipation flux; RC, reaction centre, CSo, Cross section; Ψ_{Eo}, Electron transport from *quinone a* to plastoquinone efficiency; Φ_{Eo}, maximum yield of electron transport from *Qa* to plastoquinone primary photochemistry.

2.5. Quantification of plant photoassimilates

The amount of fructose, glucose, sucrose and maltose was determined through gas chromatography coupled to electron impact ionization/time-of-flight mass spectrometry (GC-EI/TOF-MS, Agilent 6890N24, Agilent Technologies, Böblingen, Germany). Samples of leaves and roots were collected from fungal-inoculated or mock plants and immediately deep frozen in liquid nitrogen. Polar metabolites were extracted in chloroform: methanol as previously reported (Lisec et al., 2006) with the modifications described by Dethloff et al. (2014). GC-EI/TOF-MS chromatograms were processed through a standardized data analysis procedure using ChromaTOF (v. 4.32; LECO, St. Joseph, USA) and TagFinder software (Luedemann et al., 2008). Compounds were identified by mass spectral and retention time index matching to the reference collection of the Golm metabolome database (GMD, http://gmd.mpimpgolm.mpg.de/; e.g. Hummel et al., 2010) and to the mass spectra of the NIST08 database (http://nistmassspeclibrary.com/).

2.6. Macronutrient quantification

Total nitrogen (N) was quantified in 50 mg of dry leaves samples. With this purpose, samples were digested in concentrated H_2SO_4 according to the Kjeldahl method adjusted for plant determinations (Bremner and Mulvaney, 1982). For Total phosphorus (P) and potassium (K), samples were digested with HNO_3 (Merck, Germany) and diluted in 50 ml of distilled water. These macronutrients were quantified directly with a Microwave Plasma–Atomic Emission Spectrometry analyzer (MP-AES Agilent Technologies, Germany).

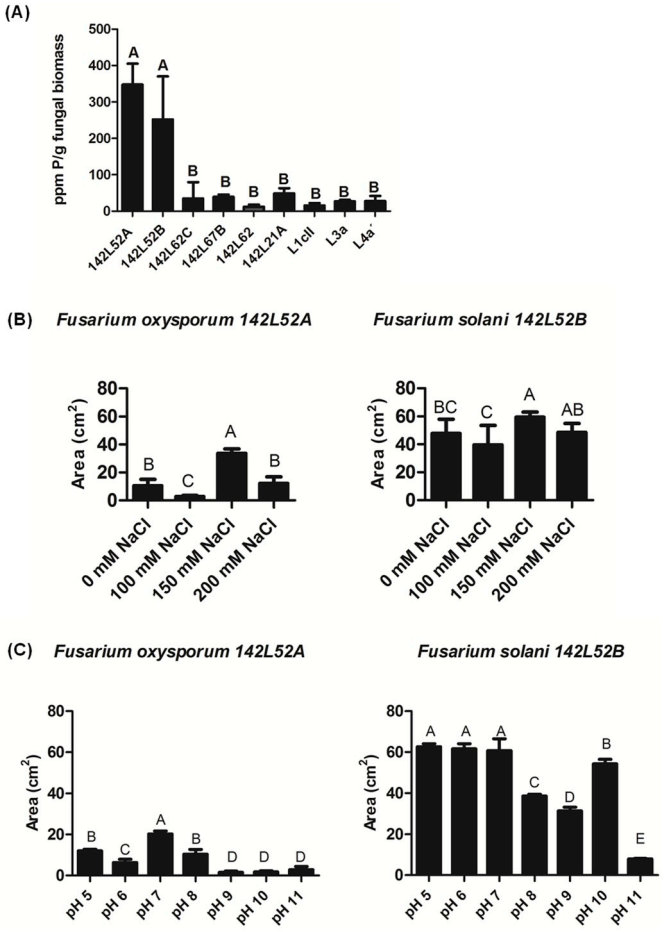

Fig. 1. Performance of the isolated strains according to: (A) P solubilising activity: P was determined on nine fungal strains isolated from healthy roots of *L. tenuis* plants growing in alkaline al saline soils, after 7 days of fungal growth on NBRIP medium by blue molybdenum technique. Five biological replicates were measured (mean ± standard deviation). Different letters indicate grows that are different according to the Tukey Test ($p < 0.05$); (B) Capacity of growth in different NaCl concentrations: PDA medium supplemented with either no additional NaCl (0 mM) or, 100 mM NaCl, 150 mM NaCl and 200 mM NaCl; (C) Capacity of growth in a wide range of pH: Malt medium (MA) supplemented with buffer solutions, according to Nagai et al. (1997) in order to obtain media ranging from pH 5 to 11. Fungal growth areas were measured in five biological replicates (mean ± standard deviation), after 7 days of incubation. Different letters indicate conditions that are significantly different according to the Tukey Test ($p < 0.05$).

3. Results

3.1. Isolation and characterization of fungal strains from the roots of L. tenuis

We were able to isolate 9 different fungal strains from healthy roots of *L. tenuis* plants growing in soils from the Flooding Pampa, Argentina. As these soils are characterized by a reduced availability of P, we were prompted to investigate whether these fungal strains were able to increase P availability though mineral phosphate solubilisation. As shown in Fig. 1A, all these strains showed phosphate solubilisation activity *in vitro*. Among them, the strains *142L52A* and *142L52B* showed a remarkable activity for phosphate solubilisation. These fungal strains were identified as *Fusarium oxysporum* and *F. solani*, respectively, by

sequencing the ITS region and TEF gene and comparing these data to the UNITE and Fusarium ID databases (Supplementary material). Moreover, as *L. tenuis* usually thrives in areas from the Flooding Pampa dominated by high salinity and alkalinity conditions, we also evaluated the capacity of *142L52A* and *142L52B* to growth in restrictive ranges of pH and NaCl concentrations. Our experiments showed that *142L52A* has a low growth rate compared to *142L52B*, with and optimum pH of 7 (Fig. 1B and C). Interestingly, even though 100 mM NaCl causes a 50% reduction in growth, this parameter is normalized or even improved at higher salt concentrations. In turn, *142L52B* shows similar growth rates between pH 5–7, and seems to tolerate quite well up to pH 10. On the other hand, it grows at normal rate in all the salt concentrations tested in our experiment, indicating that it has a high potential to tolerate the saline/alkaline conditions prevailing in soils of the Flooding Pampa. On these bases, we selected this strain for further experiments.

3.2. Effects of F. solani 142L52B inoculation on plant growth

In order to reveal the effects of *F. solani 142L52B* on plant physiology, we inoculated *L. tenuis* seedlings with mycelial plugs and incubated the plants for 37 days in a growth chamber. We also inoculated *L. japonicus* seedlings as the analysis of this interaction would be facilitated by the use of different genetic approaches available in this model plant. As shown in Fig. 2A, fungal inoculation caused contrasting effects on the growth of the two *Lotus* species. In this trend, *F. solani 142L52B* enhanced growth in *L. japonicus*, whereas it provokes growth impairment in *L. tenuis*. This effect was consistent with the changes observed in the biomass of roots and shoots between infected and control plants (Fig. 3A and B). Interestingly, even though the total number of leaves and lateral shoots did not change in both species in response to fungal inoculation (Fig. S1), a reduction in the foliar area of *L. tenuis* was documented (Fig. 3C).

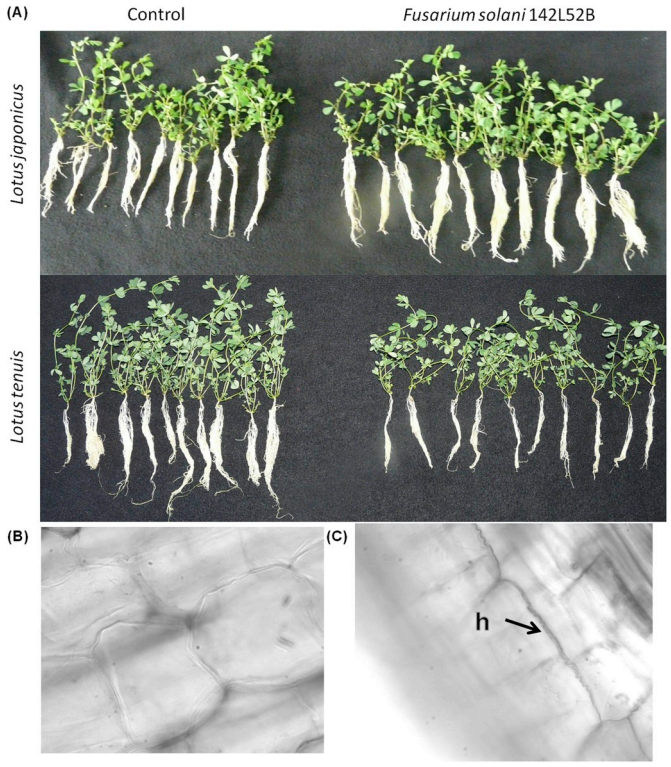

Fig. 2. Mock and *F. solani*-infected Lotus japonicus plants after 37 days of incubation. Seedlings were transferred to pots containing sterile sand-perlite (2:1). Roots were inoculated with plugs of non-inoculated PDA medium (Control) and plugs obtained from a *F. solani 142L52B*-inoculated PDA plate. (A) Whole plants after the growth period. (B). Cortex cells of roots of control plants (400x). (C) Root cortex cells after *F. solani 142L52B* infection. h: hyphae infecting the intercellular space of root cortex cells.

In addition, with the purpose to confirm the endophytic nature of the fungal strain, we attempted to re-isolated it from infected roots after the growth period of 37 days. This analysis confirmed that *F. solani 142L52B* colonize inner root tissues of *L. tenuis*, but also demonstrated that it is equally able to colonize *L. japonicus* roots. Microscopy analysis showed that the colonization is mostly intracellular and located only in the cortex cells of roots (Fig. 2B and C). Besides, the fungus is unable to reach shoot tissues and is limited to the root system (Data not shown).

3.3. Effect of F. solani on photosynthesis and stomatal conductance of Lotus plants

Several reports demonstrated that photosynthesis might be affected by endophytic microbes (Bacon, 1993; Marks and Clay, 1996; Pinto et al., 2000), which could be a consequence of the presence of the fungus as it may constitute an additional carbon sink. Besides, the microorganism could also affect stomatal opening, increasing the flux of CO_2 and water (Richardson et al., 1993). Our results showed that the net photosynthesis rate (Pn), measured by the rate of CO_2 exchange is significantly improved in *L. japonicus* infected by *F. solani 142l52B* compared to mock-inoculated controls (Fig. 4A). In turn, fungal colonization showed no effect on Pn in *L. tenuis* plants. The increment in Pn in *L. japonicus* agrees with the decrease in the amount of CO_2 in the substomatal cavity (C_i) (Fig. 4C), which reflects the movement of CO_2 to the leaf mesophyll and is associated to higher C fixation rates. In turn, inoculation with F. *solani 142L52B* did not provoke any alteration in the stomatal conductance (GS) in both plant species (Fig. 4B).

In addition, we analysed the Chlorophyll *a* fluorescence *OJIP* transient to determine the operational status of the photosynthetic apparatus. This non-invasive method provides valuable structural and functional parameters associated to the plant photosynthetic machinery (Berger et al., 2007). The *OJIP* test indicated that the maximum quantum yield of primary photochemistry (Fv/Fm) of both plant species is not affected by the fungal inoculation (Fig. 5), indicating that the fungus do not cause considerable effects on photosynthesis. Accordingly, neither of the parameters evaluated were modified in *L. japonicus*. However, absorption and conversion of energy (PI abs, ABS/RC, DIo/RC, TRo/RC and ETo/RC) were significantly altered in *L. tenuis*, suggesting that this strain may cause a perturbation in the PSII performance.

3.4. Changes of the content of photoassimilates and ions in response to fungal inoculation

Different plant metabolites such as sugars are good reporters to estimate the potential cost of plant-microbe interactions, as their concentration correlates with plant's energetic status. As shown in Fig. 6, the amount of fructose, glucose, sucrose and maltose in leaves were significantly higher in *L. japonicus* plants infected with *F. solani 142L52B* than in mock-inoculated control plants. In contrast, fungal colonization led to a reduction in the concentration of these compounds in *L. tenuis*. In agreement with the increment in glucose and fructose, the amount of sugar precursors, such as fructose-6-phosphate and glucose-6-phosphate varied in both species. The concentrations of sugars detected in roots were also modified in both species. Thus, there was a significant increment in the concentration of glucose and fructose in *L. japonicus*, while the concentration of sucrose was diminished in *L. tenuis* (Fig. 6).

In turn, considering the ability of *F. solani 142L52B* to solubilise phosphate, we assessed the amount of P in leaves of *L. japonicus* and *L. tenuis* in order to evidence a positive effect of the endophyte in the incorporation of this element. In addition, the amount of other essential macronutrients, such as N and K were included in our analysis as it has been reported that these elements may be modified during plant biotic

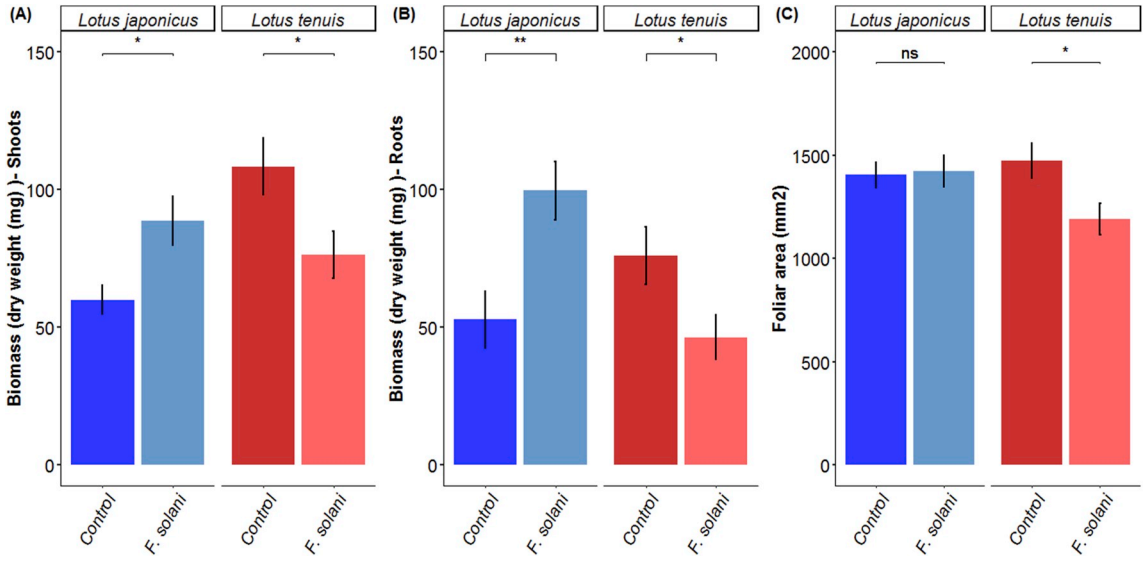

Fig. 3. *F. solani 142L52B* provokes contrasting changes in biomass production in *L. tenuis* and *L. japonicus*. Biomass of shoots (A) and roots (C) of *L. japonicus* (blue) and *L. tenuis* (red) plants under mock and fungal-inoculated conditions. (C) Foliar area of the second oldest leaf belonging to the main shoot of *L. japonicus* and *L. tenuis* plants expressed as cm^2. Statistical differences between controls and *F. solani* treatments were assessed using Student's test (mean ± standard deviation, n = 10). * ($p < 0.05$), ** ($p < 0.01$). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article).

interactions, such as those established between *Acremonium* and tall fescue (Lyons et al., 1990), and *Peniciullium* and *Aegiceras corniculatum* (Xu et al., 2007). Our analysis showed that the effects of the fungal inoculation on N and P contents were not statistically different (Fig. 7A and B). These results indicate that there is no apparent benefit provided by the endophyte on the P and N nutrition. However, fungal inoculation leaded to a reduction in the amount of K in leaves of *L. japonicus*, while an increment in the concentration of this element was detected in *L. tenuis* (Fig. 7C).

4. Discussion

4.1. *Fusarium solani: a plant partner in the restrictive soil environments*

In this study, we isolated and identified different fungal strains from healthy roots of *L. tenuis* growing in saline-alkaline lowland soils of the Flooding Pampa, Argentina. These strains were able to solubilise phosphate and, one of them identified as *F. solani*, showed high tolerance to high alkalinity and salinity conditions *in-vitro*. *F. solani* is an important pathogen affecting crops belonging to the *Solanaceae* family, such as *Solanum tuberosum*, *S. melongena, Lycopersicon esculentum* and *Capsicum anuum* (Romberg and Davis, 2007). This fungal species is also able to infect other plant families, including legumes, such as *Pisum sativum* and *Glycine max,* causing the so-called sudden death syndrome (Matuo and Snyder, 1973; Burke and Hall, 1991; Ruan et al., 1995). Despite of this, it has been also reported that strains of *F. solani* survive without causing negative effects as endophytes in different plant species such as *Camptotheca acuminata* (Kusari et al., 2009), *L. esculentum* (Kavroulakis et al., 2007; Imazaki and Kadota, 2015), *Taxus baccata* (Tayung et al., 2011) and *Apodytes dimitata* (Shweta et al., 2010). In the same line of evidences, it has been proved the ability of *F. solani* strains to cause symptomless colonizations in model legumes such as *L.*

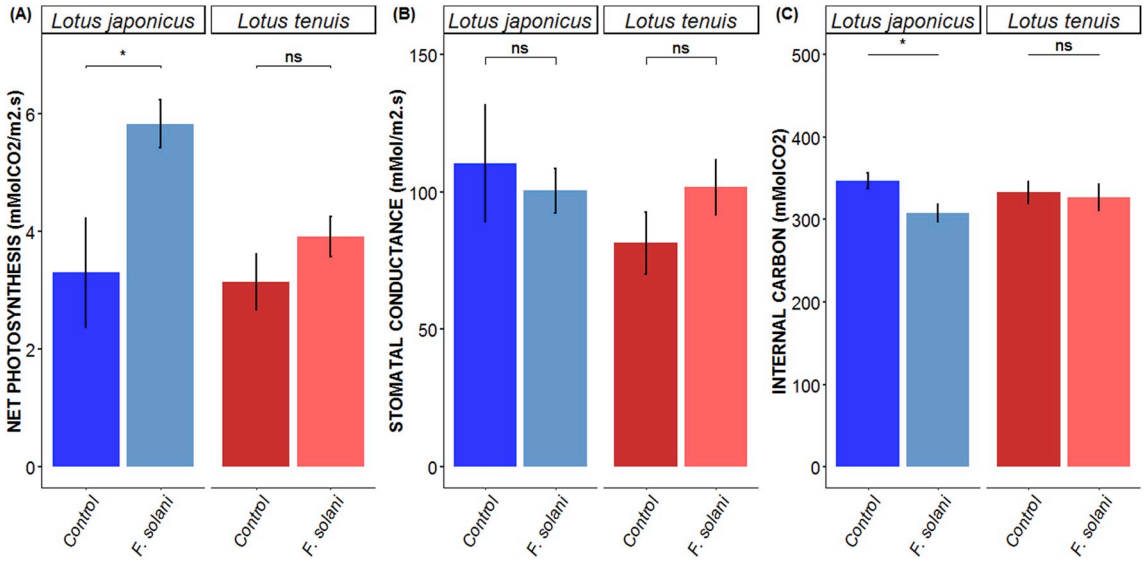

Fig. 4. (A) Net Photosynthesis (PN), (B) Stomatal conductance (GS) and (C) CO_2 in the sub-stomatal cavity (C_i) measured after 37 dpi (days post inoculation) in ten mock and ten *F. solani*-inoculated plants of *L. japonicus* and *L. tenuis*. Statistical differences between controls and *F. solani 142L52B* treatments were assessed using Student's test (mean ± standard deviation, n = 10). * ($p < 0.05$), ** ($p < 0.01$).

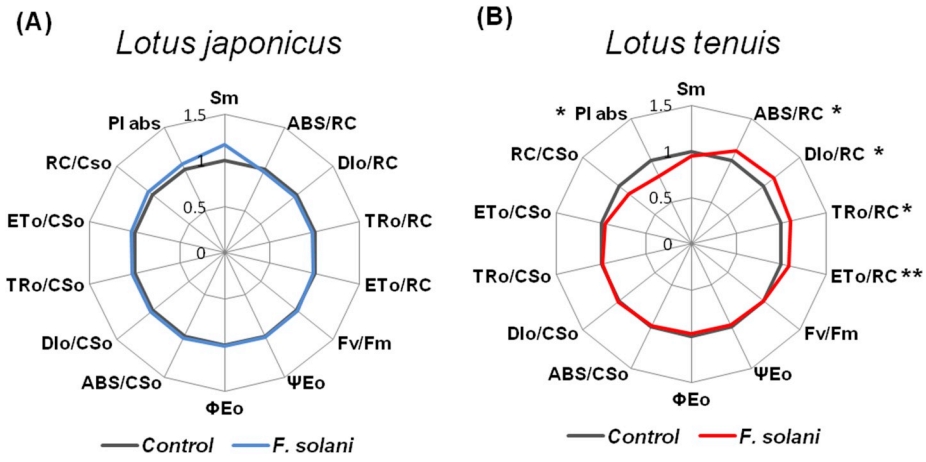

Fig. 5. Ratios of *F. solani 142L52B*/control for each parameter obtained from an *OJIP* analysis of *L. japonicus* (A) and *L. tenuis* (B). Sm: Normalized total complementary area above the *OJIP* transient PIabs: Performance index on the absorption basis, ABS: Absorption flux, DIo: Dissipation flux, TRo: Trapped energy flux, ETo: Electron transport flux, RC: Reaction centre, CSo: Cross section, Fv/Fm (Φ_{Po}): Maximum quantum yield of primary photochemistry, Ψ_{Eo}: Electron transport from *quinone a* to plastoquinone efficiency, Φ_{Eo}: maximum yield of electron transport from *Qa* to plastoquinone primary photochemistry, RC/CSo: active reaction centres per cross section. Statistical differences of the controls compared to *F. solani* treatments of each *OJIP*-derived parameter were assessed using Student's test. * ($p < 0.05$), ** ($p < 0.01$).

japonicus and *Medicago truncatula* (Skiada et al., 2019). To our knowledge, the present work constitutes the first report demonstrating growth promoting effects by an endophytic strain of *F. solani* (*142L52B*) in the model legume *L. japonicus*. Similarly, previous studies on fungal species infecting the native flora in Mediterranean salty-soils of Europe identified the presence of *F. avenaceum* as endophyte of healthy plants of *L. creticus* (Maciá-Vicente et al., 2008). Therefore, it is plausible to think that members of this fungal genus have an important role during plant naturalization in restrictive conditions. In relation to this, our previous studies of the microbiota characterizing the soils from the Flooding Pampa demonstrated that *Fusarium* is the dominant fungal genus in alkaline/saline soils devoted to the promotion of *L. tenuis* (Nieva et al., 2018). The ability of the strains we isolated to tolerate different concentrations of NaCl agrees with the potential existence of a trait allowing them to tolerate these unfavourable abiotic conditions in the soil. In this regard, previous reports have demonstrated that many members of the genus *Fusarium* and other *Deuteromycetes* are tolerant to high NaCl concentrations and restrictive pHs (Tresner and Hayes, 1971; Dumestre et al., 1997). In fact, studies related to the distribution of fungi in alkaline soils have described several *Fusarium* species as alkali-tolerant and alkalophilic (Nagai et al., 1995). Thus, the combination of these abilities (tolerance to salinity and alkalinity) might explain the abundance of *Fusarium* species in restrictive environmental conditions, whereas the growth of other species might be suppressed.

4.2. Plant growth promotion vs. plant growth impairment

We demonstrated in this work that the inoculation of *L. japonicus and L. tenuis* with *F. solani* provokes differential effects on plant growth. In this trend, despite of the phylogenetic proximity between *L. japonicus* and *L. tenuis* (Escaray et al., 2012), differential effects were observed in the biomass of roots and shoots of both plant species. Thus, growth promotion was evidenced in *L. japonicus* as a consequence of the inoculation. Similar effects in plant growth associated to the genus *Fusarium* were reported in other plant species such as *Zea mays* (Yates et al., 1997; Mehmood et al., 2019) and *Hordeum vulgare* (Maciá-Vicente et al., 2009). In turn, an impairment of plant growth was observed in *L. tenuis*-infected plans. However, as common disease-related symptoms such as chlorosis or wilting were not observed, we conceive that *F. solani* could not be considered as a pathogen of this plant species (Agrios, 2005).

We demonstrated in this work that the inoculation of *F. solani 142L52B* in *Lotus* plants is intercellular and limited to the cortex cells of roots, as demonstrated with other plant endophytes of this fungal genus (Schulz et al., 2007). It is of interest to note that there are not reports about *Fusarium* pathogens affecting *L. tenuis* in the Flooding Pampa to date, which reinforces the idea that the mutualism between *Fusarium* and *Lotus* could play an important role in determining diversity and ecosystem properties, as proposed previously for mycorrhizal and *Neotyphodium* endophytes (Van Der Heijden et al., 1998; Rudgers et al., 2004).

4.3. Photosynthetic activity and energetic balance

Plant photosynthetic rates vary during fungal infections. For instance, it has been reported that mycorrhizal colonization causes an increment in the photosynthetic rate in other legumes such as *Glycine max* and *Vicia faba* (Kaschuk et al., 2009). By contrast, a decrease in plant photosynthesis may also occur as a consequence of the fungal colonization of *Neotyphodium lolii* in *Lolium perenne* (Spiering et al., 2006). In the specific case of the interactions established by *Fusarium* species, previous works reported the impairment in Pn, even in symptomless interactions (Lorenzini et al., 1997; Pinto et al., 2000). Under our experimental conditions, *L. japonicus* showed an increment in Pn after *F. solani* inoculation. Importantly, as the Pn value is measured through the rate of CO_2 exchange, this measure correlates with a higher rubisco activity. In addition, this is also in agreement with the reduction in the levels of CO_2 in the sub-stomatal cavity (C_i), which indicate a higher rate of C fixation. Thus, these results indicate that the CO_2 getting into the sub-stomatal cavity is fixed more efficiently in *L. japonicus* after fungal colonization.

The values of Fv/Fm (Φ_{Po}) are used to characterize the plant physiological status during pathogenic interactions (Barón et al., 2016; Pineda et al., 2018). The Fv/Fm values in infected *Lotus* plants were similar to those in mock-inoculated plants, indicating that these interactions could be considered non-stressful for plants. In this trend, the Fv/Fm values observed are in agreement with those obtained in non-stressed plants in our previous studies conducted in two ecotypes of *L. japonicus* (Babuin et al., 2014; Campestre et al., 2016). Besides, the absence of statistically significant differences in additional *OJIP*-derived parameters in *L. japonicus* indicates that the structural components of the photosynthetic activity are not affected by the *F. solani* inoculation in this plant species. The increase in the value of Pn was associated to an increment in the concentration of sugars in *L. japonicus*. Similar effects were reported before as a result of the endophytic interactions between *Lolium perenne-Neotyphodium lolii* (Rasmussen et al., 2008) and *L. esculentum-Leptodontidium orchidicola* (Andrade-Linares et al., 2011). In the case of *L. japonicus*, the amount of fructose and glucose in leaves correlated with the accumulation of its glycolytic intermediates fructose-6-phosphate and glucose-6-phosphate. In addition, the elevated levels in maltose can be attributed to an increased starch degradation rate in order to meet the higher carbon requirements in infected plants. In turn, the increment in the amount of glucose and fructose in roots demonstrates that carbohydrates may be readily transported to the root systems. These results reinforce the assumption that the fungal colonization modified plant primary metabolism in

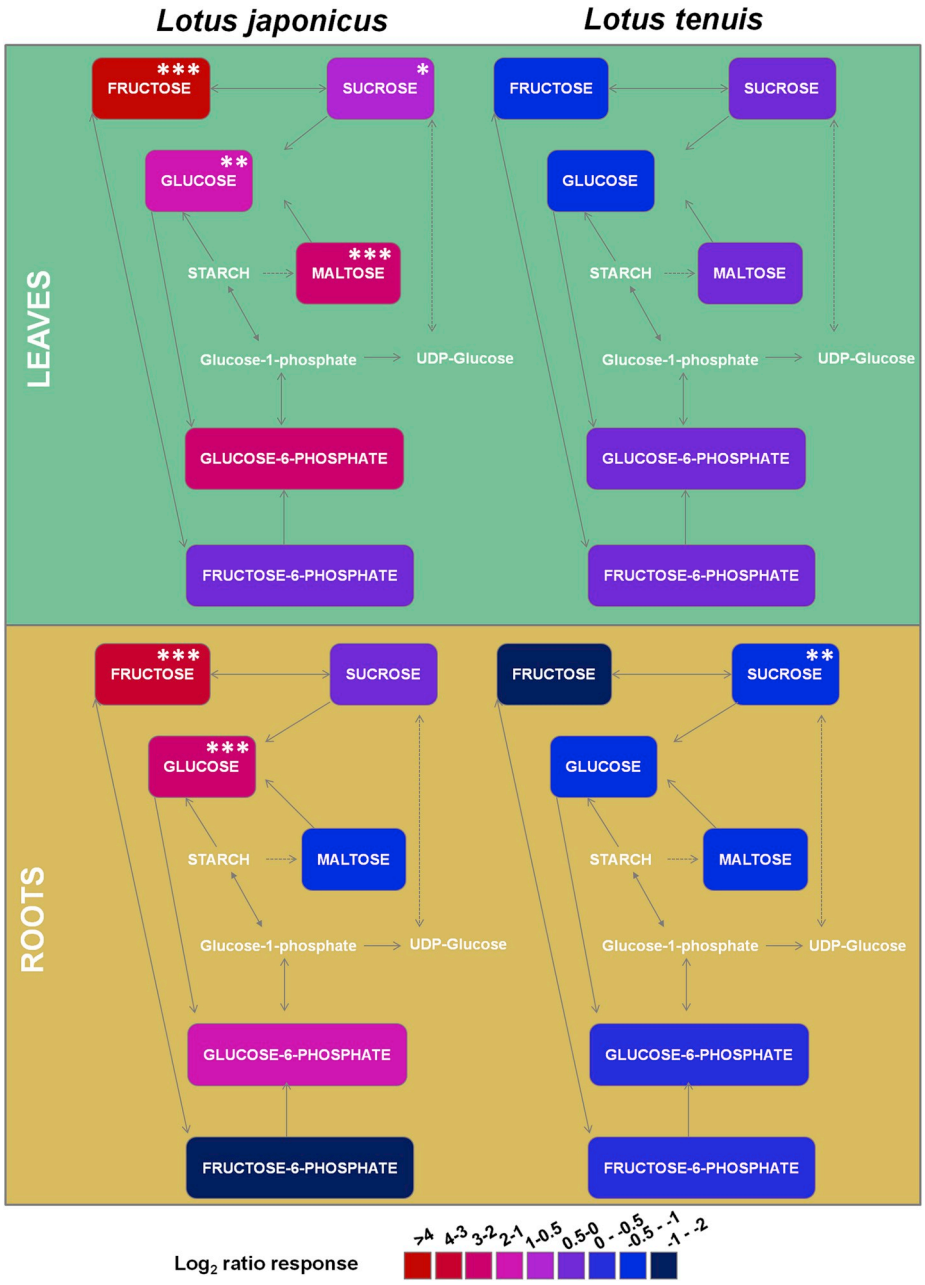

Fig. 6. Log_2-transformed relative sugar concentrations of *F. solani 142L52B* -inoculated versus mock control plants. Sugars were determined by GC-EI/TOF-MS based metabolite profiling in paired leaf and root samples of *L. japonicus* and *L. tenuis*. Log_2-transformed ratios are colour-coded in the range of > 4 (red) to -2 (dark blue) according to the inserted scale. Statistical differences between controls and *F. solani* treatments were assessed using Student's test on ten replications per treatment. * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.0001$). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article).

order to supply carbon needs to the invading fungus.

In turn, the differences observed in PIabs and other *OJIP* parameters in *L. tenuis* indicate an alteration in the energetic status of the plants as a consequence of the inoculation. However, these modifications were not associated to a reduction in the value of Pn. Interestingly, even though this species showed similar photosynthetic rates as *L. japonicus*, the amount of sugars detected in leaves were not modified by the inoculation, whereas the amount of sucrose decreases in roots. A significant reduction in the concentration of fructose, glucose and sucrose was also reported in the interaction between wheat and *F. moniliforme,* which was explained by the consumption of C resources by the endophyte (Bönnighausen et al., 2019). Whether a similar carbohydrate depletion mechanism explains the reduction of sugars and biomass in *L. tenuis* following fungal inoculation needs to be addressed. Overall, differences in the concentration of sugars between both plant species may indicate the existence of distinct strategies for carbohydrate utilization, which could support or limit the establishment of the plant-fungus interaction (Schwachtje et al., 2018). On the other hand, it should be taken into account that sugars also function as signal molecules in several plant processes such as growth and development (Rolland et al., 2006). Thus, hexoses as well as sucrose have been recognized as important signal molecules in source-sink regulation (Roitsch, 1999). Therefore, the differences in the concentration of sugars in both plant species may be a consequence of the existence of complex and source-sink related signalling mechanisms that are differentially triggered during the colonization process.

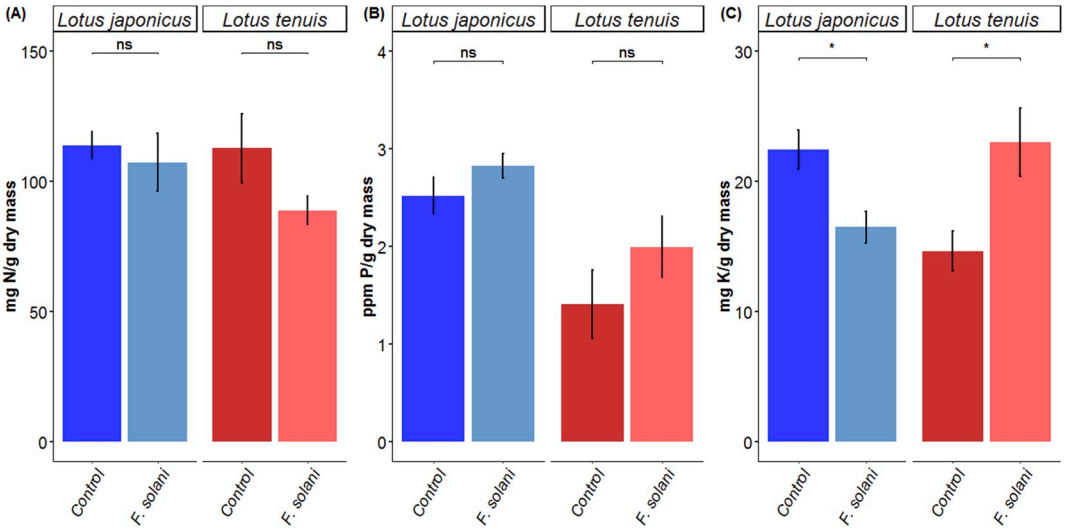

Fig. 7. The *F. solani* effect on macronutrients measured in leaves of *L. japonicus* and *L. tenuis*. Percent of total N (A) P (B) and K (C) in leaves of *L. japonicus* and *L. tenuis* 37 days after inoculation with *F. solani 142L52B*. Statistical differences of the parameters measured were evaluated using Student's test (mean ± standard deviation, n = 10). * ($p < 0.05$).

4.4. Nutritional effects of fungal colonization

It has been widely documented that fungal endophytes can exert nutritional benefits to the host, providing the plant with a better access to important elements such as N, P and K (Bolan, 1991). These effects are explained, at least in part, on the bases of their ability to solubilise inorganic materials. Thus, as *F. solani 142L52B* showed a remarkable phosphate solubilising activity, we speculated that fungal inoculation would favour the uptake of this element. However, our results indicate that the amounts of N and P in roots and leaves of *L. japonicus* and *L. tenuis* are not altered by the inoculation with *F. solani 142L52B*. These results indicate that P facilitation is not directly linked to the increment in the biomass in *L. japonicus*, and that plant growth enhancement in this plant species might be due to a combination of several other alternative mechanisms. In turn, we observed a significant reduction in the contents of K^+ in leaves of *L. japonicus* and an increment of this cation in *L. tenuis*. This effect suggests the existence of an association between plant growth and the transport and/or acquisition of K^+ in infected plants. The mechanisms by which *F. solani* inoculation, plant growth, and K^+ accumulation are linked are currently unclear, but the relationship between this macronutrient and the severity of different plant abiotic and biotic stresses has been described (Shabala and Pottosin, 2014). For instance, the effect of high K levels in crops systems has been related with a reduction in the disease symptoms caused by *Fusarium spp*. (McClellan and Stuart, 1947; Dastur and Bhatt, 1964; Schneider, 1985; Smiley et al., 1972). Moreover, K_2SiO_3 has been proposed as a useful agent to control pathogens (Cherif et al., 1994). Whether the increment of K^+ in *L. tenuis* helps to ameliorate the negative effects of *F. solani* will be explored in our future research.

5. Conclusions

In this study, we analysed the responses of two *Lotus* species to the inoculation by a strain of *F. solani* isolated from restrictive soils and showing a remarkable ability to solubilise phosphate and tolerate saline/alkaline conditions. Our results demonstrated that this fungal strain *is* able to colonize endophytically two *Lotus* species, *L. japonicus* and *L. tenuis*. Plant inoculation has differential consequences in the carbohydrate and photosynthesis status of both plant hosts. We observed growth promotion effects in *L. japonicus*, which was associated to enhancement in photosynthesis. In contrast, *L. tenuis* suffered growth impairment. In this case, even though the photosynthetic rate did not change, the functionality of PSII was affected and the amount of photoassimilates decreased. A better understanding of the mechanisms underlying the establishment of the plant-fungal endophyte interaction will require complementary studies to detect the specific processes and signals that are involved in the switch between mutualism and pathogenesis.

Author contributions

ASN carried out all experiments and data analyses; JMV and SJMC. performed the photosynthetic and physiological measurements; A.G. and ABM carried out data analysis; ASN, AE and JK performed metabolomic analyses; J.K. and O.A.R. supervised and designed the assays. All authors read and wrote the manuscript critically.

Acknowledgments

We thank Ines Fehrle for her technical support of the metabolomics analyses**.** This work was financial supported by the projects PICT 2014 and PICT 2015 funding by Agencia de Promoción Científica y Tecnológica, Argentina.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2019.09.022.

References

Maciá-Vicente, J.G., Rosso, L.C., Ciancio, A., Jansson, H.B., Lopez-Llorca, L.V., 2009. Colonisation of barley roots by endophytic *Fusarium equiseti* and *Pochonia chlamydosporia*: effects on plant growth and disease. Ann. Appl. Biol. 155, 391–401.

Agrios, G.N., 2005. Introduction to Plant Pathology. Elsevier Academic Press Publication.

Andrade-Linares, D.R., Grosch, R., Restrepo, S., Krumbein, A., Franken, P., 2011. Effects of dark septate endophytes on tomato plant performance. Mycorrhiza 413–422.

Antonelli, C.J., Calzadilla, P.I., Escaray, F.J., Babuin, M.F., Campestre, M.P., Rocco, R., Bordenave, C.D., Perea García, A., Nieva, A.S., Llames, M.E., Maguire, V., Melani, G., Sarena, D., Bailleres, M., Carrasco, P., Paolocci, F., Garriz, A., Menendez, A., Ruiz, O.A., 2016. LOTUS spp: biotechnological strategies to improve the bioeconomy of lowlands in the Salado river basin (Argentina). AGROFOR 1, 43–53.

Antonelli, C.J., Calzadilla, P.I., Vilas, J.M., Campestre, M.P., Escaray, F.J., Ruiz, O.A., 2019. Physiological and anatomical traits associated with tolerance to long-term partial submergence stress in the *Lotus* genus: responses of forage species, a model and an interspecific hybrid. J. Agron. Crop Sci. 205, 65–76. https://doi.org/10.1111/jac.12303.

Babuin, M.F., Campestre, M.P., Rocco, R., Bordenave, C.D., Escaray, F.J., Antonelli, C.,

Calzadilla, P., Gárriz, A., Serna, E., Carrasco, P., Ruiz, O.A., Menendez, A., 2014. Response to long-term NaHCO3-derived alkalinity in model Lotus japonicus Ecotypes Gifu B-129 and Miyakojima MG-20: transcriptomic profiling and physiological characterization. PLoS One 9 (5) e97106.
Backhouse, D., Burgess, L.W., Summerell, B.A., 2001. Biogeography of *Fusarium*. In: Fusarium. Paul E. Nelson Memorial Symposium. APS Press, Bethesda, pp. 122–137.
Bacon, C.W., 1993. Abiotic stress tolerances (moisture, nutrients) and photosynthesis in endophyte-infected tall fescue. Agric. Ecosyst. Environ. 44, 123–141.
Barón, M., Pineda, M., Pérez-Bueno, M.L., 2016. Picturing pathogen infection in plants. Z. Naturforschung C 71, 355–368.
Berger, S., Sinha, A.K., Roitsch, T., 2007. Plant physiology meets phytopathology: plant primary metabolism and plant–pathogen interactions. J. Exp. Bot. 58, 4019–4026.
Berrocal-Lobo, M., Molina, A., 2008. *Arabidopsis* defense response against Fusarium oxysporum. Trends Plant Sci. 13, 145–150.
Bolan, N.S., 1991. A critical review on the role of mycorrhizal fungi in the uptake of phosphorus by plants. Plant Soil 134, 189–207.
Bönnighausen, J., Schauer, N., Schäfer, W., Bormann, J., 2019. Metabolic profiling of wheat rachis node infection by Fusarium graminearum–decoding deoxynivalenol-dependent susceptibility. New Phytol. 221 (1), 459–469.
Bremner, J.M., Mulvaney, C.S., 1982. Nitrogen—Total 1. Methods of Soil Analysis. Part 2. Chemical and microbiological properties, pp. 595–624 (methodsofsoilan2).
Burke, D.W., Hall, R., 1991. In: Hall, R. (Ed.), Fusarium Root Rot. Compendium of Bean Diseases. American Phytopathological Society Press, St. Paul, Minn, pp. 9–10.
Calzadilla, P.I., Maiale, S.J., Ruiz, O.A., Escaray, F.J., 2016. Transcriptome response mediated by cold stress in Lotus japonicus. Front. Plant Sci. 7, 374.
Campestre, M.P., Antonelli, C., Calzadilla, P.I., Maiale, S.J., Rodríguez, A.A., Ruiz, O.A., 2016. The alkaline tolerance in Lotus japonicus is associated with mechanisms of iron acquisition and modification of the architectural pattern of the root. J. Plant Physiol. 206, 40–48.
Cherif, M., Menzies, J.G., Ehret, D.L., Bogdanoff, C., Belanger, R.R., 1994. Yield of cucumber infected with Pythium aphanidermatum when grown with soluble silicon. Hortscience 29 (8), 896–897.
Clay, K., 1988. Fungal endophytes of grasses: a defensive mutualism between plants and fungi. Ecology 69, 10–16.
Clay, K., Schardl, C., 2002. Evolutionary origins and ecological consequences of endophyte symbiosis with grasses. Am. Nat. 160, S99–S127.
Dastur, R.H., Bhatt, J.G., 1964. Relation of potassium to Fusarium wilt of flax. Nature 201, 1243.
Dethloff, F., Erban, A., Orf, I., Alpers, J., Fehrle, I., Beine-Golovchuk, O., Schmidt, S., Schwachtje, J., Kopka, J., 2014. Profiling methods to identify cold-regulated primary metabolites using gas chromatography coupled to mass spectrometry. Methods Mol. Biol. 1166, 171–197.
Di Pietro, A., García-Maceira, F.I., Méglecz, E., Roncero, M.I.G., 2001. A MAP kinase of the vascular wilt fungus *Fusarium oxysporum* is essential for root penetration and pathogenesis. Mol. Microbiol. 39, 1140–1152.
Douglas, A.E., 2010. The Symbiotic Habit. Princeton University Press.
Dumestre, A., Chone, T., Portal, J., Gerard, M., Berthelin, J., 1997. Cyanide degradation under alkaline conditions by a strain of *Fusarium solani* isolated from contaminated soils. Appl. Environ. Microbiol. 63, 2729–2734.
Escaray, F.J., Menendez, A.B., Gárriz, A., Pieckenstain, F.L., Estrella, M.J., Castagno, L.N., Ruiz, O.A., 2012. Ecological and agronomic importance of the plant genus *Lotus*. Its application in grassland sustainability and the amelioration of constrained and contaminated soils. Plant Sci. 182, 121–133.
Evans, C.G.T., Herbert, D., Tempest, D.W., 1970. Chapter XIII the continuous cultivation of micro-organisms: 2. construction of a chemostat. In: Methods in Microbiology. vol. 2. Academic Press, pp. 277–327.
Evelin, H., Kapoor, R., Giri, B., 2009. Arbuscular mycorrhizal fungi in alleviation of salt stress: a review. Ann. Bot. 104 (7), 1263–1280.
García, I., Mendoza, R., Pomar, M.C., 2008. Deficit and excess of soil water impact on plant growth of Lotus tenuis by affecting nutrient uptake and arbuscular mycorrhizal symbiosis. Plant Soil 304, 117–131.
Geiser, D.M., Jiménez-Gasco, M.M., Kang, S., Makalowska, I., Veeraraghavan, N., Ward, T.J., Zhang, N., Kuldau, G.A., O'Donnell, K., 2004. FUSARIUM-ID v. 1.0: a DNA sequence database for identifying *Fusarium*. Eur. J. Plant Pathol. 110, 473–479.
Handberg, K., Stougaard, J., 1992. *Lotus japonicus,* an autogamous, diploid legume species for classical and molecular genetics. Plant J. 2, 487–496.
Hummel, J., Strehmel, N., Selbig, J., Walther, D., Kopka, J., 2010. Decision tree supported substructure prediction of metabolites from GC-MS profiles. Metabolomics 6, 322–333.
Imazaki, I., Kadota, I., 2015. Molecular phylogeny and diversity of *Fusarium* endophytes isolated from tomato stems. FEMS Microbiol. Ecol. 91, fiv098.
Kaschuk, G., Kuyper, T.W., Leffelaar, P.A., Hungria, M., Giller, K.E., 2009. Are the rates of photosynthesis stimulated by the carbon sink strength of rhizobial and arbuscular mycorrhizal symbioses? Soil Biol. Biochem. 41, 1233–1244.
Kavroulakis, N., Ntougias, S., Zervakis, G.I., Ehaliotis, C., Haralampidis, K., Papadopoulou, K.K., 2007. Role of ethylene in the protection of tomato plants against soil-borne fungal pathogens conferred by an endophytic *Fusarium solani* strain. J. Exp. Bot. 58, 3853–3864.
Kiers, E., Palmer, T.M., Ives, A.R., Bruno, J.F., Bronstein, J.L., 2010. Mutualisms in a changing world: an evolutionary perspective. Ecol. Lett. 13, 1459–1474.
Kirkbride, J.H., 2006. The scientific name of narrow-leaf trefoil. Crop Sci. 46, 2169–2170.
Kuldau, G.A., Yates, I.E., 2000. Evidence for *Fusarium* endophytes. Microb. Endophytes 85.
Kusari, S., Zühlke, S., Spiteller, M., 2009. An endophytic fungus from *Camptotheca acuminata* that produces camptothecin and analogues. J. Nat. Prod. 72, 2–7.
Lisec, J., Schauer, N., Kopka, J., Willmitzer, L., Fernie, A.R., 2006. Gas chromatography mass spectrometry–based metabolite profiling in plants. Nat. Protoc. 1, 387.
Lorenzini, G., Guidi, L., Nali, C., Ciompi, S., Soldatini, G.F., 1997. Photosynthetic response of tomato plants to vascular wilt diseases. Plant Sci. 124, 143–152.
Luedemann, A., Strassburg, K., Erban, A., Kopka, J., 2008. TagFinder for the quantitative analysis of gas chromatography—mass spectrometry (GC-MS)-based metabolite profiling experiments. Bioinformatics 24, 732–737.
Lyons, P.C., Evans, J.J., Bacon, C.W., 1990. Effects of the fungal endophyte Acremonium coenophialum on nitrogen accumulation and metabolism in tall fescue. Plant Physiol. 92, 726–732.
Maciá-Vicente, J.G., Jansson, H.B., Abdullah, S.K., Descals, E., Salinas, J., Lopez-Llorca, L.V., 2008. Fungal root endophytes from natural vegetation in Mediterranean environments with special reference to *Fusarium spp*. FEMS Microbiol. Ecol. 64, 90–105.
Malinowski, D.P., Belesky, D.P., 1999. Neotyphodium coenophialum-endophyte infection affects the ability of tall fescue to use sparingly available phosphorus. J. Plant Nutr. 22, 835–853.
Marks, S., Clay, K., 1996. Physiological responses of Festuca arundinacea to fungal endophyte infection. New Phytol. 133, 727–733.
Matuo, T., Snyder, W.C., 1973. Use of morphology and mating populations in the identification of formae speciales in *Fusarium solani*. Phytopathology 63, 562–565.
McClellan, W.D., Stuart, N.W., 1947. The influence of nutrition on *Fusarium* basal rot of narcissus and on *Fusarium* yellows of gladiolus. Am. J. Bot. 34, 88–93.
McMullen, M., Jones, R., Gallenberg, D., 1997. Scab of wheat and barley: a re-emerging disease of devastating impact. Plant Dis. 81, 1340–1348.
Mehmood, A., Hussain, A., Irshad, M., Hamayun, M., Iqbal, A., Rahman, H., Tawab, A., Ahmad, A., Ayaz, S., 2019. Cinnamic acid as an inhibitor of growth, flavonoids exudation and endophytic fungus colonization in maize root. Plant Physiol. Biochem. 135, 61–68.
Murphy, J., Riley, J.P., 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta 27, 31–36.
Nagai, K., Sakai, T., Rantiatmodjo, R.M., Suzuki, K., Gams, W., Okada, G., 1995. Studies on the distribution of alkalophilic and alkali-tolerant soil fungi I. Mycoscience 36, 247–256.
Nautiyal, C.S., 1999. An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. FEMS Microbiol. Lett. 170, 265–270.
Nieva, A.S., Bailleres, M.A., Llames, M.E., Taboada, M.A., Ruiz, O.A., Menéndez, A., 2018. Promotion of Lotus tenuis in the Flooding Pampa (Argentina) increases the soil fungal diversity. Fungal Ecol. 33, 80–91.
Nilsson, R.H., Larsson, K.H., Taylor, A.F.S., Bengtsson-Palme, J., Jeppesen, T.S., Schigel, D., Kennedy, P., Picard, K., Glöckner, F.O., Tedersoo, L., Saar, I., Kõljalg, U., Abarenkov, K., 2018. The UNITE database for molecular identification of fungi: handling dark taxa and parallel taxonomic classifications. Nucleic Acids Res. https://doi.org/10.1093/nar/gky1022.
Phillips, J.M., Hayman, D.S., 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc. 55, 158–161.
Pineda, M., Pérez-Bueno, M.L., Barón, M., 2018. Detection of bacterial infection in melon plants by classification methods based on imaging data. Front. Plant Sci. 9, 164.
Pinto, L.S.R.C., Azevedo, J.L., Pereira, J.O., Vieira, M.L.C., Labate, C.A., 2000. Symptomless infection of banana and maize by endophytic fungi impairs photosynthetic efficiency. New Phytol. 147, 609–615.
Rasmussen, S., Parsons, A.J., Fraser, K., Xue, H., Newman, J.A., 2008. Metabolic profiles of Lolium perenne are differentially affected by nitrogen supply, carbohydrate content, and fungal endophyte infection. Plant Physiol. 146, 1440–1453.
Richardson, M.D., Hoveland, C.S., Bacon, C.W., 1993. Photosynthesis and stomatal conductance of symbiotic and nonsymbiotic tall fescue. Crop Sci. 33, 145–149.
Rodriguez, R.J., White Jr., J.F., Arnold, A.E., Redman, A.R.A., 2009. Fungal endophytes: diversity and functional roles. New Phytol. 182, 314–330.
Roitsch, T., 1999. Source-sink regulation by sugar and stress. Curr. Opin. Plant Biol. 198–206.
Rolland, F., Baena-González, E., Sheen, J., 2006. Sugar sensing and signaling in plants: conserved and novel mechanisms. Annu. Rev. Plant Biol. 675–709.
Romberg, M.K., Davis, R.M., 2007. Host range and phylogeny of *Fusarium solani f. sp. eumartii* from potato and tomato in California. Plant Dis. 91, 585–592.
Roncero, M.I.G., Hera, C., Ruiz-Rubio, M., Maceira, F.I.G., Madrid, M.P., Caracuel, Z., Calero, F., Delgado-Jarana, J., Roldán-Rodriguez, R., Martínez, A.L., Velasco, C., Roa, J., Martinez-Urdiroz, M., Córdoba, D., Di Pietro, A., 2003. Fusarium as a model for studying virulence in soil borne plant pathogens. Physiol. Mol. Plant Pathol. 62, 87–98.
Ruan, Y., Kotraiah, V., Straney, D.C., 1995. Flavonoids stimulate spore germination in *Fusarium solani* pathogenic on legumes in a manner sensitive to inhibitors of cAMP-dependent protein kinase. MPMI-Molecular Plant Microbe Interactions 8, 929–938.
Rudgers, J.A., Koslow, J.M., Clay, K., 2004. Endophytic fungi alter relationships between diversity and ecosystem properties. Ecol. Lett. 7, 42–51.
Saikkonen, K., Faeth, S.H., Helander, M., Sullivan, T.J., 1998. Fungal endophytes: a continuum of interactions with host plants. Annu. Rev. Ecol. Systemat. 29, 319–343.
Saikkonen, K., Gundel, P.E., Helander, M., 2013. Chemical ecology mediated by fungal endophytes in grasses. J. Chem. Ecol. 39, 962–968.
Schneider, R.W., 1985. Suppression of Fusarium yellows of celery with potassium, chloride, and nitrate. Phytopathology 75, 40–48.
Schulz, B.J., Boyle, C.J., Sieber, T.N., 2007. Microbial Root Endophytes, vol. 9 Springer Science & Business Media.
Schwachtje, J., Fischer, A., Erban, A., Kopka, J., 2018. Primed primary metabolism in systemic leaves: a functional systems analysis. Sci. Rep. 8, 216.
Shabala, S., Pottosin, I., 2014. Regulation of potassium transport in plants under hostile conditions: implications for abiotic and biotic stress tolerance. Physiol. Plant. 151, 257–279.

Shweta, S., Zuehlke, S., Ramesha, B.T., Priti, V., Kumar, P.M., Ravikanth, G., Spiteller, R., Vasudeva, R., Shaanker, R.U., 2010. Endophytic fungal strains of *Fusarium solani*, from *Apodytes dimidiata* E. Mey. ex Arn (Icacinaceae) produce camptothecin, 10-hydroxycamptothecin and 9-methoxycamptothecin. Phytochemistry 71, 117–122.

Skiada, V., Faccio, A., Kavroulakis, N., Genre, A., Bonfante, P., Papadopoulou, K.K., 2019. Colonization of legumes by an endophytic Fusarium solani strain FsK reveals common features to symbionts or pathogens. Fungal Genet. Biol.

Smiley, R.W., Cook, R.J., Papendick, R.I., 1972. *Fusarium* foot rot of wheat and peas as influenced by soil applications of anhydrous ammonia and ammonia potassium azide solutions. Phytopathology 62, 86.

Spiering, M.J., Greer, D.H., Schmid, J.A.N., 2006. Effects of the fungal endophyte, Neotyphodium lolii, on net photosynthesis and growth rates of perennial ryegrass (*Lolium perenne*) are independent of in planta endophyte concentration. Ann. Bot. 98, 379–387.

Tayung, K., Barik, B.P., Jha, D.K., Deka, D.C., 2011. Identification and characterization of antimicrobial metabolite from an endophytic fungus, *Fusarium solani* isolated from bark of Himalayan yew. Mycosphere 2, 203–213.

Tresner, H.D., Hayes, J.A., 1971. Sodium chloride tolerance of terrestrial fungi. Appl. Microbiol. 22, 210–213.

Van Der Heijden, M.G., Klironomos, J.N., Ursic, M., Moutoglis, P., Streitwolf-Engel, R., Boller, T., Wiemken, A., Sanders, I.R., 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature 396, 69.

Vilas, J.M., Romero, F.M., Rossi, F.R., Marina, M., Maiale, S.J., Calzadilla, P.I., Pieckenstain, F.L., Ruiz, O.A., Gárriz, A., 2018. Modulation of plant and bacterial polyamine metabolism during the compatible interaction between tomato and Pseudomonas syringae. J. Plant Physiol. 231, 281–290.

White, T.J., Bruns, T., Lee, S.J.W.T., Taylor, J.L., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR Protoc.: Methods Appl. 18, 315–322.

Xu, M., Gessner, G., Groth, I., Lange, C., Christner, A., Bruhn, T., Deng, Z., Li, X., Heinemann, H., Grabley, S., Bringmann, G., Sattler, I., Lin, W., 2007. Shearinines D–K, new indole triterpenoids from an endophytic Penicillium sp. (strain HKI0459) with blocking activity on large-conductance calcium-activated potassium channels. Tetrahedron 63, 435–444.

Yates, I.E., Bacon, C.W., Hinton, D.M., 1997. Effects of endophytic infection by *Fusarium moniliforme* on corn growth and cellular morphology. Plant Dis. 81, 723–728.