# Life of Microbes Inside the Plant: Beneficial Fungal Endophytes and Mycorrhizal Fungi

(Article begins on next page)

07 January 2023

1  **Life of microbes inside the plant: beneficial fungal endophytes and mycorrhizal**

2  **fungi**

3

4  Luisa Lanfranco and Valentina Fiorilli

5

6  Department of Life Sciences and Systems Biology, University of Turin, Italy

7  Viale Mattioli 25 10125, Torino, Italy

8

9

10

11  Number of Words: ~~6328~~

12  Number of Figures: 2

13

14  Corresponding Author: Luisa Lanfranco

15  E-mail: luisa.lanfranco@unito.it

16

17

18

19  Running Title: Beneficial fungi living in plants

20

21

22  Key words: endophytes; mycorrhizal fungi; plant microbiota; symbiosis; mycobiota

23

24

25

26

**Abstract**

Fungi are notably diverse and proliferate in different environments (soil, air, water) but, remarkably, the highest numbers of fungi are found in the soil or associated with plants. Fungal endophytes, the ones living within plant tissues, are important members of the plant microbiota, but the nature of the relationship established with the host can be variable. The majority of endophytes do not affect the plant at all; some are pathogenic and some may even be beneficial, influencing plant growth and health by modulating different biological processes.

In this chapter we will consider fungal endophytes with a recognized beneficial impact on plants, and on a special group of endophytes, the mycorrhizal fungi. We will describe the interaction at the cellular level, highlight the functional significance of the associations and the impact of these fungal symbionts on the host plant, and illustrate potential applications.

The plant-endophyte alliance may increase plant phenotypic plasticity, which is perhaps an advantage in fluctuating environments such as those emerging from global climate change. Understanding the complex interactions between fungal endophytes and plants is therefore of relevance for the development of tailored microbial inocula to improve crop production and quality under varying environmental conditions. This appears to be a promising means of developing more environmentally friendly agricultural crop production systems and increasing global food security.

## Introduction

### The plant microbiota

Plants are not alone: in many ecosystems, plants are greatly outnumbered by microorganisms such as bacteria and fungi that may live in close proximity or even in strict association with plant hosts (Vandenkoornhuyse et al., 2015; Müller et al., 2016). Complex microbial communities thrive on the outer surfaces as well as in internal tissues of plants; this microbial component, referred as the plant microbiota (all microorganisms) or plant microbiome (all microbial genomes), may influence plant growth and health by modulating a wide range of biological processes (Trivedi et al., 2020). Pioneering reports on the plant microbiota focused on the characterization of bacterial assemblages in the model species *Arabidopsis thaliana* (Bulgarelli et al., 2012), but more recent papers also consider fungi so that the term plant *mycobiota* is also currently used (Bonfante et al., 2019). High through-put sequencing techniques applied to environmental samples have revealed that fungi proliferate in many different environments (soil, air, water) but, remarkably, the greatest numbers of fungi are found in the soil or associated with plants, at an estimated fungal / plant species ratio of 17 / 1 (Nilsson et al., 2019).

Living together, which has been described as a symbiosis (Martin and Schwab, 2012), implies different possible types of interactions ranging from parasitic or pathogenic, where one partner gains benefit at the expense of the other, to mutualistic, where reciprocal benefits occur. The knowledge of the nature of these interactions and on how these fungal symbionts can influence plant physiology and metabolism is of fundamental relevance because it is recognized nowadays that plants likely host a still overlooked reservoir of beneficial fungi that may contribute to support their survival and growth under biotic and abiotic stresses. Some of these plant-fungal associations may function as key elements in the development of sustainable programs of crop production and environmental protection which, in the context of global climate global change, represent emerging challenges to modern societies.

In this chapter we focus on fungal endophytes with a recognized beneficial impact on plants and on a special group of endophytes, the mycorrhizal fungi. Mycorrhizal fungi are root endophytes which form specialized plant-fungal interfaces for resource exchange. In addition, they are phylogenetically distinct from most groups of endophytes (Smith and Read 2008; Porras-Alfaro and Bayman, 2011).

92    We will describe here the interaction at the cellular level, emphasize the functional
93    significance of the associations and the impact of the fungal symbionts on the host plant,
94    and illustrate potential applications.
95
96    **Fungal endophytes of plants**
97    Notwithstanding the knowledge of plant-associated microbes has been significantly
98    advanced by molecular high-throughput sequencing techniques (Nilsson et al., 2019), the
99    study of plant microbiota has very ancient roots. Anton de Bary, in 1886, coined the term
100   *endophytes* to define microorganisms, normally bacteria and fungi, that live within plant
101   tissues (Wilson 1995; Martin and Schwab, 2012). The term endophyte therefore refers to
102   the location, *i.e.* inside plant tissues, irrespective of being inside the cells or among the
103   cells, and does not specify the nature of the relationship established with the host plant.
104   Indeed, the majority of endophytes do not affect the plant at all; some are pathogenic and
105   others may even be beneficial. The outcome of the association may depend on the
106   partners involved, and the functional roles can also vary during the life cycle of the
107   microbe or in response to plant or environmental factors (Porras-Alfaro & Bayman
108   2011).
109   It has been shown that each plant species has a highly variable and in some cases
110   impressively rich community of fungal endophytes - up to 90 species have been
111   described within a single leaf of a tropical tree (Bayman 2006). Plant organs of a single
112   plant also often differ greatly in the dominant taxa of endophytes present, but we are far
113   from having a global picture of their functional significance.
114   Considering this heterogeneity, we will here give examples of some of the most well
115   characterized fungal endophytes known to have a positive impact on the host plant. They
116   include the shoot grass endophytes of the genus *Epichloë spp.* (Ascomycota,
117   Sordariomycetes, Clavicipitaceae), the well known biocontrol agents belonging to
118   *Trichoderma* spp. (Ascomycota, Sordariomycetes, Hypocreaceae), the generalist root
119   endophyte *Serendipita indica* (Basidiomycota, Sebacinales) and the recently described
120   root/systemic endophyte *Colletotrichum tofieldiae* (Ascomycota, Sordariomycetes,
121   Glomerellaceae) identified in natural samples of the model species *Arabidopsis thaliana*.
122
123   ***Epichloë* spp.**
124   The genus *Epichloë,* belonging to the family Clavicipitaceae within Ascomycota, forms
125   symbiotic relationships with many grasses of the Pooideae subfamily (Schardl et al.,

4

126    2004). Phylogenetic analysis places *Epichloë* species within a group of endophytic and
127    plant pathogenic fungi, with a common ancestor probably derived from an animal
128    pathogen (Spatafora et al., 2007).

129    *Epichloë* endophytes grow asymptomatically in the intercellular spaces of the aerial
130    tissues of the host plant. Notably, the expansion of hyphae occurs in synchrony with the
131    growth of the newly developing leaf, while the root is essentially uncolonized (Herd et al.,
132    1997; Tan et al., 2001). These endophytes, in most cases, are exclusively seed-
133    transmitted from previously infected hosts (Schardl et al., 2004), a mode of transmission
134    that represents another signature with ecological relevance of the intimate plant-fungus
135    association.

136    The nature of this symbiosis has been described as 'defensive mutualism' (Bastias et al.,
137    2017): *Epichloë* endophytes are indeed able to produce several alkaloids, bioactive
138    secondary metabolites that are detrimental for herbivores (Schardl et al., 2004; 2013).
139    Genomic studies and chemical analyses have been instrumental in deciphering the
140    alkaloid biosynthetic pathways (Schardl et al., 2013; Young et al., 2015); it is now well
141    established that alkaloid profiles depend not only on the specific fungal strain but also on
142    the host plant phenology and on environmental factors (Saikkonen *et al*., 2013; Ball *et al*.,
143    1995; Justus et al., 1997). Moreover, the quali- and quantitative alkaloid profile (i.e.
144    chemical structure and amount of specific compounds) is a key determinant in the
145    defense efficiency against a given attacker.

146    In the late 70s, *Epichloë* endophytes were considered undesirable, as they were found to
147    lead to serious health problems in livestock (Bacon *et al*., 1977; Fletcher and Harvey,
148    1981). Later, however, since the yield of endophyte-free grasses was lower compared to
149    endophyte-infected grasses, a major effort was undertaken to select livestock-friendly
150    endophytes that still produced alkaloids with insecticidal and nematocidal activities
151    (Cagnano et al., 2019) and artificially infected grass cultivars were developed for the
152    market (Johnson et al., 2013).

153    In order to gain a deeper understanding of the biological system, the *Epichloë festucae* -
154    ryegrass *(Lolium perenne)* model was characterized at the cellular and molecular levels.
155    The benefits provided by *E. festucae* were found to extend far beyond protection against
156    herbivores (the only issue that has been mechanistically explained). Improvement in
157    pathogen resistance (Tian et al., 2008), drought tolerance and enhanced growth (Hahn et
158    al., 2008), and better ability to compete with other plant species (Quigley 2000) have also
159    been reported.

160 To gain insight into the molecular changes induced by endophyte infection, a comparison
161 of the gene expression profiles of perennial ryegrass infected or not with *E. festucae* was
162 carried out (Dupont et al., 2015). Endophyte infection triggered a strong reprogramming
163 of host genes, in particular favouring secondary metabolism at a cost to primary
164 metabolism. This is similar to what found in plant pathogenic interactions but in stark
165 contrast to what has been observed in response to fungal colonization by mycorrhizal
166 fungi where only a modest host gene modulation occurs.

167 A transcriptomic analysis of the *E. festucae* - *L. perenne* interaction also was carried out
168 with a focus on the developmental stages of different host tissues (Schmid *et al*., 2017).
169 Notably, fungal genes annotated as encoding small secreted proteins (SSP), which can
170 function to control colonization of host tissue in pathogenic and mutalistic symbioses
171 (Toruño *et al*., 2016; Plett *et al*., 2011; Kloppholz *et al*., 2011), were highly expressed in
172 hyphae within emerging leaves. By contrast, genes involved in synthesizing anti-
173 herbivore compounds are highly expressed in the fungal mycelium of mature plant
174 tissues, in agreement with the alkaloid profiles. But *E. festucae*-infected plants can have a
175 second line of defence: endophyte colonization induces a systemic effect in the plant.
176 young as well as mature plant tissues display the activation of genes mainly involved in
177 hormone biosynthesis and signalling and in stress and pathogen resistance (Schmid *et al*.,
178 2017; Dupont *et al*., 2015). Considering, in particular, defence against chewing insects, it
179 has been proposed that *Epichloe* endophytes can use not only alkaloids but also can
180 activate a level of intrinsic immunity in the plant by promoting innate defense responses
181 (Bastias *et al*., 2017). These data suggest that *E. festucae* is able to trigger an activated
182 status (Martinez-Medina *et al*., 2016), in analogy to a mechanism that has been proposed
183 for mycorrhizal fungi (Pozo and Azcón-Aguilar, 2007). This activated status could be the
184 basis of the increased resilience of the host plants to a broad variety of stresses (Fig. 1).

185

186 ***Trichoderma* spp.**
187 Filamentous fungi belonging to the genus *Trichoderma* thrive in the rhizosphere (the soil
188 adjacent to and influenced by plant roots), and are able to colonize the root system from
189 early seedling developmental stages (Harman *et al*., 2004). As early as 90 years ago,
190 *Trichoderma* species were recognized as pathogens of other soil fungi (Weindling,
191 1932). Since then, the knowledge of *Trichoderma* biology has evolved continuously,
192 leading to the successful development and marketing of *Trichoderma*-based products to
193 be used in agriculture as biocontrol agents to control plant pathogenic fungi (Fraceto *et al*.

194  2018).

195  Studies focused mainly on *Trichoderma harzianum* have demonstrated that parasitism by

196  fungi, even beneficial ones, relies on the production of many hydrolytic enzymes, which

197  act in synergy with fungitoxic metabolites (Lorito *et al*., 2010). However, these may not

198  be the primary mechanisms of biocontrol, since root colonization also stimulates plant

199  defense mechanisms initiated by extensive physiological and metabolic changes

200  mediated mainly by jasmonic acid and another plant hormone, ethylene (Contreras-

201  Cornejo *et al*., 2015; Martínez-Medina *et al*., 2017). A strain of *T. harzianum* also has

202  been found to produce a nitrogen heterocyclic compound called harzianic acid, which has

203  various biological properties including plant growth promotion, antimicrobial activity

204  and the ability to chelate soil iron, facilitating its solubilisation and, as a consequence, its

205  availability to plants (Vinale *et al*., 2013). Thus, the multiple benefits provided to the

206  host plant encompass not only protection from pathogens, but also improvement of plant

207  growth and vigor and enhanced defense responses against various stresses (Fig. 1).

208  Collectively, these reasons explain why the new generations of *T. harzianum*

209  formulations are therefore described as plant protectants and biostimulants of crop yield

210  and quality.

211

212  ***Serendipita indica***

213  Another plant endophyte that has been well characterized in recent decades is *Serendipita*

214  *indica* (syn. *Piriformospora indica;* Varma *et al*., 1999), a filamentous fungus belonging

215  to Sebacinales (Basidiomycota). It is worth noting that the order Sebacinales include

216  endophytes that associate with roots (*i.e. S. indica, S. vermifera* and *S. herbamans*),

217  mycorrhizal fungi forming symbioses with orchids, as well as saprotrophs, but not

218  pathogenic fungi. The root associated Sebacinales likely evolved from saprotrophic

219  ancestors (Garnica *et al*., 2016) and are described as ubiquitously distributed generalists.

220  In particular, *S. indica* colonizes the intercellular spaces of plant roots and the

221  intracellular regions of epidermal and cortical cells, but never the central cylinder of the

222  root. In early colonization stages the fungus is surrounded by a plant-derived plasma

223  membrane, indicating that the colonized cells are viable. Later, it switches to a host cell

224  death-associated phase, where thin fungal hyphae are found in dead or dying cells,

225  although massive necrotic areas are never observed (Deshmukh *et al*., 2006; Fesel and

226  Zuccaro, 2016).

227 Benefits for the host plant include increased biomass, possibly as a consequence of
228 enhanced assimilation of mineral nutrients such as phosphate (Yadav *et al*., 2010), and a
229 higher tolerance against biotic and abiotic stresses (Sherameti *et al*., 2008; Fig. 1). The *S.*
230 *indica-Arabidopsis thaliana* interaction has emerged as a model to study the systemic
231 effect leading to an increase tolerance to leaf pathogens. In that specific case, the
232 protection against the powdery mildew *Golovinomyces orontii* was demonstrated to be
233 mediated by the jasmonate hormonal signaling pathways (Stein *et al*., 2008).

234

235 *Colletotrichum tofieldiae*
236 Another intriguing endophyte, the recently characterized *Colletotrichum tofieldiae*, was
237 originally isolated from natural populations of asymptomatic *A. thaliana* plants in Spain
238 (García *et al*., 2013). It was also detected in trees and orchids across the Eurasian
239 continent (Damm *et al*., 2009; Tao *et al*., 2013) suggesting a very broad host range. The
240 fungus colonizes the root and occasionally spreads into the aerial parts of the plant
241 through the central cylinder, reminiscent of a pathogenic association; indeed *C. tofieldiae*
242 is closely related to pathogenic *Colletotrichum* species. *C. tofieldiae* was shown to
243 promote plant growth as a consequence of improved mineral nutrition, thanks to
244 endophyte-mediated transfer of phosphorus. Remarkably, phosphate transfer only occurs
245 under low phosphorus conditions (Hiruma *et al*., 2016), which is similar to what is
246 observed in the arbuscular mycorrhizal symbiosis (Fig. 1). This result has opened a
247 window on how non-mycorrhizal plants cope with low nutrient availability, since *A.*
248 *thaliana*, like most Brassicaceae, has lost the ability to form mycorrhizal symbioses
249 during evolution.
250 The comparison between the genomes of *C. tofieldiae* and its pathogenic relative, *C.*
251 *incanum*, and the between the transcriptomes of both fungi and the host plant
252 *Arabidopsis,* was instrumental in highlighting the genomic traits related to the transition
253 from pathogenicity to mutualism (Hacquard *et al*., 2016). The *C. tofieldiae* genome has a
254 narrowed repertoire of genes encoding secreted proteins, chitin-binding and secondary
255 metabolism-related proteins compared to that of *C. incanum*. Moreover, *C. tofieldiae*-
256 colonized plants show limited activation of pathogenicity-related genes compared to *C.*
257 *incanum*-infected plants. It is worth noting that *A. thaliana* roots induced defence genes
258 when colonized by *C. tofieldiae* under conditions of high phosphorus concentrations
259 (Hacquard et al., 2016). This finding pointed for the first time to an interdependency
260 between plant immunity and plant nutrition. A similar activation of plant defence-related

8

261      genes mirrored by alterations in the root-inhabiting fungal community was observed in
262      maize plants grown under high phosphorus concentrations (Yu *et al*., 2018). Lowering
263      plant defences under low phosphorus availability therefore appears to be a strategy to
264      favor the recruitment of beneficial soil microbes that can help to overcome the nutritional
265      stress.

266

267      Fungal endophytes are thus ubiquitous and taxonomically very diverse organisms.
268      Phylogenetic analyses and data from whole genome sequencing, although still limited to
269      only a few taxa, have given important insights into the evolutionary origin of
270      endophytism. As mentioned before, some of endophytes, such as *Epichloë* and *C.*
271      *tofieldiae,* are clearly derived from fungi with a pathogenic life style, whereas the root-
272      associated Sebacinales (i.e. *S. indica*) are most probably derived from a saprotrophic
273      ancestor (Lahrmann *et al*., 2015; Fesel and Zuccaro, 2016). The genomic signature of
274      this origin is a reduced repertoire in *S. indica* of genes involved in secondary metabolite
275      production compared to that found in the genomes of *Epichloë spp.* and *C. tofieldiae.*
276      Despite these differences, colonization of the roots of *A. thaliana* by *S. indica* or *C.*
277      *tofieldiae* shares common features: both fungi initially penetrate the epidermis by
278      undifferentiated hyphae and spread in the cortical tissue intercellularly and
279      intracellularly; at later stages of both interactions cell death, maily limited to the
280      epidermal layer, is observed. Moreover, in both cases, to restrict fungal growth inside the
281      root and keep a well-balanced endophytic interaction, an intact plant immune system is
282      required (Fesel and Zuccaro, 2016). Further comparative analysis of additional
283      interactions will help to define commonalities and unicities.

284

285      A further level of complexity in the plant-fungal endophyte interaction is the presence
286      within the microbial symbiont of endobacteria (Bonfante et al., 2019) and viruses (Bao
287      and Roossinck, 2013). Notably, these bacteria can influence several aspects of the fungal
288      biology including the outcome of plant-fungal symbioses. One example from the
289      prokaryotic world is *Rhizobium radiobacter* strain F4, which was detected inside *S.*
290      *indica*; the endobacterium improves the fitness of *P. indica* and contributes to the
291      beneficial effects exerted by the fungal endophyte on the host plant (Guo *et al*., 2017).
292      Fungal viruses, also called mycoviruses, also are emerging as an abundant component of
293      the microbiota of fungal endophytes (Bao and Roossinck, 2013) that may have an impact
294      on fungal fitness and, as a consequence, on the plant host, in a tripartite interaction. A

9

295 historic case is the virus that makes the fungal pathogen *Cryphonectria parasitica* less
296 virulent for the host plant, which led to the identification of a powerful biocontrol agent
297 (Choi and Nuss, 1992). Another example refers to thermotolerance: a mycovirus, living
298 inside the fungal endophyte *Curvularia protuberate* was shown to confer heat tolerance
299 to the fungus and to the host plant *Dichanthelium lanuginosum,* allowing their growth in
300 the geothermal soils of Yellowstone National Park (Márquez et al., 2007). The precise
301 molecular mechanisms at the basis of the phenomenon are not known but osmolytes and
302 reactive oxygen species were differentially accumulated in plants colonized with the
303 virus-infected endophyte.
304 From these few examples it is clear that plants can host a taxonomically broad spectrum
305 of fungal endophytes; this heterogeneity inevitably mirrors functional diversity
306 potentially leading to a range of significance for the host plant growth and health.
307 Digging into this world is indispensable to expanding our knowledge on endophytism
308 and its many beneficial applications to agriculture.
309
310 **Mycorrhizal fungi: ancient allies of plants**
311 Under the word mycorrhizas - literally fungus-root interaction - we include several types
312 of interactions which have originated over 400 million years of co-evolution between
313 plants and fungi (Genre *et al*., 2020). About 85% of vascular and non-vascular plants
314 develop mycorrhizas (Brundrett and Tedersoo, 2018). This number and their scattered
315 geographical distribution are clear indication of their evolutionary and ecological success
316 (Davison *et al*., 2015). Four main mycorrhizal types are recognized - ectomycorrhiza,
317 arbuscular, orchid and ericoid mycorrhizas - that feature specific morphological traits and
318 different combinations of partners (Fig. 2).
319 Ectomycorrhizas (ECM) are established between soil-borne fungi (predominantly
320 belonging to Basidiomycota and Ascomycota) and shrubs and forest trees including
321 woody species with commercial value, such as poplar, birch, oak, pine and spruce
322 (Wiensczyk *et al*., 2002). In ECM the young root tips are enwrapped by fungal hyphae
323 that generate a pseudoparenchymatous tissue known as the 'sheathing mantle'. The
324 mantle's inner hyphae further develop between epidermal and cortical root cells, leading
325 to the so-called 'Hartig net', but intraradical hyphae never penetrate the cell lumen (Fig.
326 2). Together with the extraradical mycelium, these fungal structures are directly involved
327 in the mobilization, absorption, and translocation of mineral nutrients and water from soil
328 to roots. ECM has a massive potential in forest management, including reforestation,

329  phytostabilization and arboriculture activities. The promotion of abiotic stress tolerance
330  provided by ECM to the host plants may also have a positive impact on the likelihood of
331  plant adaptation to climate changes (Bennet and Classen, 2020).
332  Furthermore, a growing interest in ECM fungi lies in the production of worldwide-
333  appreciated fruitbodies such as boletes, chanterelles and truffles (Danell *et al.*, 1997;
334  Mello *et al.*, 2012; Murat *et al.*, 2015).
335  The other mycorrhizal types are all endomycorrhizas, since they are characterized by the
336  occurrence of fungal intracellular structures (Fig. 2). In the ericoid mycorrhizas (ERM),
337  some fungi belonging to Leotiomycetes (Ascomycota) or some Basidiomycota penetrate
338  the epidermal cell walls of Ericaceae (heather) roots and form intracellular hyphal coils.
339  The ERM fungi help the host species to colonize low mineral and acid soil (i.e.
340  heathland), since they play a key role in releasing soil nutrients through the efficient
341  decomposition of a wide variety of complex and recalcitrant organic substrates (Smith
342  and Reads, 2008; Perotto *et al.*, 2018).
343  In orchid mycorrhizas (ORM), plants belonging to the Orchidaceae family establish with
344  fungal taxa from several similar groups of Basidiomycota in a peculiar symbiotic
345  interaction (Dearnaley *et al.*, 2012). In the early stages of their development, orchids
346  show a mycoheterotrophic phase, since instead of fixing carbon themselves via
347  photosynthesis, they rely on their mycorrhizal partners to obtain carbohydrates, likely
348  extracted by the fungus from soil organic matter (Merckx 2012). ORM fungi colonize the
349  functional cells of the host by forming dense hyphal coils called pelotons (Peterson *et al.*,
350  2004; Fig. 2). In the adult phase several orchids switch exclusively to photosynthesis;
351  however, at maturity some of them continue to obtain carbon from mycorrhizal fungi or
352  utilize both strategies (Dearnaley *et al.*, 2016; Merckx 2012). Beside ensuring the orchids'
353  livelihood, ORM fungi also play a pivotal role in orchid propagation and conservation
354  (Batty et al., 2002).
355  The arbuscular mycorrhizal (AM) symbiosis is established between a small group of soil
356  fungi that belong to the Glomeromycotina subphylum (Spatafora *et al.*, 2016) and an
357  extremely wide range of land plants, including the majority of staple crops. Among
358  mycorrhizal types, the AM symbiosis is therefore the dominant association (72%),
359  followed by ORM (10%), ECM (3%) and ERM (1%) (Brundrett and Tedersoo, 2018).
360  AM fungi are characterized by peculiar features such as multinucleate hyphae and spores
361  and the apparent absence of a sexual cycle (Kamel *et al.*, 2016; Lanfranco *et al.*, 2016;
362  Corradi and Brachmann, 2017). They are also obligate biotrophs, that is, uncultivable in

11

363 the absence of host plants, with very large genomes extremely rich in transposable
364 genetic elements (Chen *et al.*, 2018; Kobayashi *et al.*, 2018; Morin *et al.*, 2019; Sun *et al.*,
365 2019; Venice *et al.*, 2019). A further level of complexity is given by their microbiota,
366 which include endobacteria (Bonfante and Desirò, 2017) and viruses (Ikeda *et al.*, 2012;
367 Turina *et al.*, 2018).
368
369 Although mycorrhizal fungi are highly diverse in terms of their evolutionary history, the
370 genome sequencing data (see the database MycoCosm) has highlighted some symbiotic
371 signatures (Genre *et al.*, 2020). A common trait found in the genome of all mycorrhizal
372 fungi is the presence of several genes encoding small secreted proteins (SSP) that may
373 serve to manipulate host plant metabolism and enhance colonization. A few such proteins
374 have been characterized and indeed shown to interfere with plant defence responses
375 (Plett *et al.*, 2011; Kloppholz *et al.*, 2011). A limited repertoire of genes encoding plant
376 cell wall-degrading carbohydrate-active enzymes (CAZymes) characterizes the genomes
377 of AM and ECM fungi (Kohler *et al.*, 2015; Martin *et al.*, 2016), which are evolutionarily
378 oldest. The low ability to attack plant cell wall polysaccharidic components has been
379 interpreted as a strategy to limit the production of degradation products that are well
380 known activate plant defence responses. These common features may have originated by
381 processes of convergent evolution, possibly related to adaptation to a lifestyle dependent
382 upon living host tissues as food sources (Genre *et al.*, 2020).
383 The current knowledge on mycorrhizal symbioses, especially with regard to cellular and
384 molecular aspects, mainly refers to the AM symbiosis because it has been investigated
385 more due to its wide host range and geographical distribution. For this reason, in the
386 following paragraphs we will more deeply consider some aspects of this mycorrhizal
387 association.
388
389 **The AM symbiosis: from the origin to the present**
390 The origin and the evolution of mycorrhizal symbioses have been deciphered in the light
391 of palentological and, more recently, phylogenomic data (Strullu-Derrien *et al.,* 2018;
392 Genre *et al.*, 2020). These data identify the AM symbiosis as the most ancient
393 mycorrhizal type. The co-evolution of land plants with AM fungi is witnessed by fossil
394 records of early land plants from the Rhynie chert dated 407-million-year-old, where
395 arbuscule-like structures were identified in the rhizomes of extinct plants such as
396 *Aglaophyton majus* (Remy *et al.*, 1994; Strullu-Derrien et al., 2018). To support the

397 hypothesis that the evolutionary history of land plants and AM fungi are inextricably
398 linked, phylogenetic analyses indicate that plant genes required for AM formation are
399 present in the genomes of the closest algal relatives to land plants (Delaux *et al.*, 2015).
400 The occurrence of AM symbiosis in liverworts, that are the earliest diverging group
401 among living plants, clearly shows that this mutualistic association predates the
402 development of true roots (Brundrett, 2002; Field *et al.*, 2012) and suggests that AM
403 fungi assisted the early land plants to cope with nutrient acquisition from a new
404 environment, the harsh and primeval soil (Pirozynski and Malloch, 1975).

405 Nutrient transfer appears to be a dominant function, as AM fungi provide access to
406 phosphorus, which is poorly mobile in soil, and also, to a lesser extent, nitrogen, sulfur
407 and other essential nutrients (Wang *et al.*, 2017). As a consequence, under limiting
408 nutrient availability, mycorrhizal plants often display a growth effect compared to non-
409 mycorrhizal plants. But the AM symbiosis is multifunctional, as the benefits at at the
410 level of the entire plant extend beyond improved mineral nutrition. AM fungi offer plant
411 protection against pathogens (Pozo and Azcon Aguilar, 2007; Cameron *et al.*; Fiorilli *et*
412 *al.*, 2018; Miozzi *et al.*, 2020), positively influence plant water potential (Porcel & Ruiz-
413 Lozano, 2004) and can enhance drought tolerance (Ruiz-Lozano *et al.*, 2015; Chitarra *et*
414 *al.*, 2016; Bahadur, *et al.*, 2019; Fracasso *et al.*, 2020; Balestrini *et al.*, 2020). These
415 benefits are not only a mere consequence of a better nutritional status, but involve several
416 metabolic and physiological changes at local and systemic levels (Fiorilli *et al.*, 2018;
417 Miozzi *et al.*, 2020; Fracasso *et al.*, 2020). The AM symbiosis acts therefore as a natural
418 mechanism against biotic and abiotic stresses.

419 In addition, AM fungi confer several ecosystem benefits in natural and agricultural
420 environments (van der Heijden *et al.*, 2015). They are the main drivers of carbon
421 sequestration in rooting zone (Johnson *et al.*, 2016), and, thanks to their hyphal network,
422 they improves soil quality by promoting particle aggregation, thereby limiting erosion by
423 wind and water, and they decrease the risks of ground water contamination (Chen *et al.*,
424 2018).

425 AM fungi have a major role in the composition of plant communities (van der Heijden *et*
426 *al.*, 2015). Moreover, each fungal mycelium can colonize several host plants of the same
427 or different species, leading to the formation of the so-called common mycorrhizal
428 networks (CMNs). A strongly interconnected plant community can potentially gain
429 stability, because weaker individuals might profit from the mineral nutrient supply of the
430 CMN at the expense of older plants, a phenomenon known as facilitation. These multiple

13

431  benefits of AM fungi translate into significant ecological services not only in natural
432  contexts, but also in agricultural sites. In the current context of growing concerns about
433  environmental issues, AM fungi are proposed as crucial actors of ecosystem functioning
434  and in the so-called Microbial Revolution, *i.e.* the route to increase, through the
435  exploitation of the plant microbiota, of sustainable crop productivity (Baez-Rogelio *et*
436  *al*., 2017).
437
438  **How to establish a mutualistic AM relationship**
439  The establishment of a functional AM requires a fine coordination at the cellular and
440  metabolic levels of both partners; a number of signaling molecules and genes play a role
441  in this inter-kingdom communication (MacLean *et al*., 2017; Lanfranco *et al*., 2018;
442  Pimprikar and Gutjahr, 2018). Under conditions of limiting inorganic phosphorus, host
443  plant roots actively exude strigolactones, a class of compounds (Waters *et al*., 2017) that
444  are perceived by AM fungi and promote spore germination, hyphal branching, nuclear
445  divisions and respiratory metabolism (Besserer *et al*., 2008; Salvioli *et al*., 2016). In turn,
446  the fungal partners release structural carbohydrate-derived molecules, including lipo-
447  chito-oligosaccharides (Maillet *et al*., 2011) and short chitin tetra- and pentamers (Genre
448  *et al*., 2013), which are perceived by the root surface epidermal cells, where they trigger
449  the activation of the so-called common symbiosis signaling pathway - the same pathway
450  involved in the nitrogen fixing legume symbiosis (Oldroyd, 2013; MacLean *et al*., 2017),
451  which includes several evolutionarily conserved genes needed for fungal accommodation.
452  The CSSP induces the expression of a range of symbiosis-supporting genes (Schmitz and
453  Harrison, 2014; Hohnjec *et al*., 2015) among which are factors that coordinate the
454  expression of a set of downstream genes involved in specific plant symbiotic functions
455  such as phosphate uptake and lipid synthesis and transport (Pimprikar and Gutjahr, 2018;
456  Hartmann *et al*., 2019). Notably, a recent comparative survey of hundreds of plant
457  genomes and transcriptomes showed that a core set of CSSP members and downstream
458  genes are conserved in all plant lineages hosting intracellular symbionts including AM,
459  ORM and ERM, as well as nitrogen-fixing symbioses, but these genes have been lost in
460  plant lineages hosting extracellular symbionts (including ECM) or those that are not
461  symbiotic (Radhakrishnan *et al*., 2020).
462  At a cellular level, fungal hyphae contact the surface cells of host roots through swollen
463  adhesion structures called hyphopodia. Penetrating hyphae develop inside the root cortex,
464  taking an intercellular and/or intracellular route. Once the inner root cortex is reached,

14

465  hyphae penetrate single cells, where they give rise to highly branched structures called
466  arbuscules (Gutjahr & Parniske, 2013; Lanfranco et al., 2018; Fig. 2). Intracellular
467  hyphae, including large and fine branches of arbuscules, are surrounded by the perifungal
468  membrane, a plant derived specialized membrane, which functions to host the fungal
469  structures and separate them from the plant cell contents (Bonfante, 2018). Arbuscules
470  are believed to be the main sites for nutrient transfer between partners. Different studies
471  based on functional genomics analyses have provided evidence of these nutritional
472  exchanges between partners (Javot et al., 2007; Willmann et al., 2013, Rich et al., 2017;
473  Roth and Paszkowski, 2017; Wang et al., 2017; Keymer and Gutjahr, 2018). Mycorrhiza-
474  specific phosphate transporters, located at the membrane surrounding arbuscules and
475  responsible of the uptake of inorganic phosphorus ions released by the fungus, have been
476  pinpointed in different host species (Harrison et al., 2002; Paszkowski et al., 2002),
477  providing a mechanicistic basis for the well known benefit of phosphorus tranfer. Only
478  recently, a nitrate transporter from rice (OsNPF4.5), exclusively expressed in the cells
479  containing arbuscules, was shown to play a key role in mycorrhizal nitrate acquisition;
480  the up-regulation of related genes in maize and sorghum suggests that this symbiotic
481  route of nitrogen uptake might be conserved in cereal species (Wang et al., 2020).
482  On the fungal side, a six-carbon sugar transporter possibly involved in the uptake of
483  compounds from the host has been characterized (Helber et al., 2011). However, one of
484  the most important discoveries in the biology of AM fungi was obtained from genomic
485  analyses that showed that they lack genes encoding for the synthesis of fats (Wewer et
486  al., 2014). Their dependence on a living host for these molecules was then supported by
487  several other studies showing that AM fungi rely on lipids received from the host plant
488  (Jang et al., 2017; Luginbuehl, et al., 2017; Keymer et al., 2017). This finding has shed
489  light on the obligate nutritional nature of AM fungi and has suggested new strategies for
490  large scale production of AM inocula (Kameoka et al., 2019).
491  There is now great interest to decipher whether AM fungi prefer carbohydrates or fats
492  (Rich et al., 2017) derived from the host and to clarify the forces controlling the
493  exchange of nutrients in this mutualistic association. Toby Kiers has proposed the
494  "biological market" theory in which one partner preferentially allocates resources in
495  direct proportion to the relative amount of benefits it receives from the other partner
496  (Kiers et al., 2011; Werner et al., 2015). However, this simple mechanism does not
497  always apply and the issue deserves further investigations (Walder and van der Heijden,
498  2015; Kiers et al., 2016).

15

499

**Genetic variation among the partners affects the outcome of the symbiosis**

500      With the increasing availability of genomic resources, natural or induced (*via*
501      domestication) genetic variation has become a promising field of investigation that will
502      be instrumental to further explore the genetic determinants controlling the establishment
503      and functioning of mycorrhizal symbioses. It is well known that the outcome of the AM
504      symbiosis can be strongly influenced by the specific genetic makeup of the plant and the
505      fungal partners (Lanfranco *et al*., 2018). Despite the low morphological variation, AM
506      fungi show a high level of genetic variability and high functional diversity, even
507      considering isolates belonging to the same species (Koch *et al*., 2017). On the other hand,
508      responsiveness to the AM symbiosis can differ among plant cultivars of the same species
509      and, in addition, it is affected by soil nutritional status (Sawers *et al*., 2010; Chu *et al*.,
510      2013), pointing out a complex genotype-environment interaction. Sawers and colleagues
511      (2017) investigated AM-responsiveness in several American maize lines and identified a
512      correlation bewteen the performance of the symbiosis (in terms of shoot phosphorus
513      content and dry weight) with the amount of associated hyphae outside the root. The plant
514      molecular determinants at the basis of the fungal performance are still unknown; it has
515      been hypothesized that it could be related to the amount of carbohydrates and lipids
516      released by the plant.

517      Plant genetic variation also determines the root colonization level. In this context,
518      Lehnert *et al*. (2017) monitored root colonization on 94 bread wheat genotypes colonized
519      by a mixed inoculum of three AM fungal species. Genomic regions containing genes
520      related to defence and cell wall metabolism have been reported to be associated with the
521      low colonization level detected in some genotypes (Lehnert *et al*., 2017).

522      It has also been suggested that domestication, which was likely carried out under highly
523      fertilized conditions, may have decreased the ability of plants to be colonized by and to
524      respond positively to AM fungi (Lehmann *et al*., 2012). To address this issue, Stefani and
525      colleagues (2020) recently surveyed the AM fungal communities associated to different
526      durum wheat varieties released at different times in the history of durum wheat breeding.
527      Using deep 18S rDNA sequencing under field conditions, low variation in the AM fungal
528      diversity associated with the different cultivars was found. It has been hypothesized that
529      the genetic variation among plant genotypes seems to be too narrow to select for specific
530      plant-AM fungal associations; thus, in this case breeding has only marginally altered the

532 symbiotic signalling systems and the core of genes involved in mycorrhization (Stefani *et*
533 *al*., 2020).

534 In another interesting work, Martın-Robles *et al*. (2018) compared the AM-
535 responsiveness in different crops and in their wild progenitors. They showed that growth
536 benefits exerted by the AM symbiosis were dependent on inorganic phosphorus
537 availability; while wild progenitors positively responded to the AM symbiosis
538 irrespective of availability, in domesticated plants the growth effect became negligible
539 when phosphorus availability increased. These data suggest that domestication selected
540 for AM independence at high phosphorus concentrations, which might support the
541 possibility to increase yield in the absence of the fungus-associated carbon drain.

542 We envisage that further investigations on natural variations on both the fungus and the
543 plant side will have an impact on fundamental as well as on applied research.

544

545 **AM translational research: pitfalls and successes**
546 Due to the several benefits that mycorrhizal fungi can provide, ranging from the level of
547 the individual host plant to the entire ecosystem, these fungi are of high relevance for
548 applied purposes. The impressively wide host range, which includes major crops, implies
549 a large potential for AM fungi, especially in agroecosystems (Berruti *et al*., 2016; Chen
550 *et al*., 2018). However, results of the benefits provided by AMF to host plants obtained in
551 glasshouses may not directly be relevant in the field since, under these conditions,
552 several complex variables contribute to shape the outcome of the symbiosis. A very
553 important issue to consider is how benefit is defined; one parameter, often used under
554 controlled conditions, is the mycorrhizal growth response, MGR, the difference in
555 growth between colonized and non colonized plants at a defined level of resource supply
556 (Janos 2007; Johnson *et al*., 2015). However, under field conditions, crop yield may
557 represent a more interesting parameter. As commented by Ryan & Graham (2018) in a
558 field meta analysis study, a benefit in terms of increased yield is not often evident,
559 especially when cereals are considered. But to measure the activity of AM on crop
560 productivity in field conditions is not easy, mainly due to the need to have non
561 mycorrhizal (control) plants for comparison. Despite some inconsistencies, there are
562 many crops for which AM fungi have been clearly shown to increase yield, including
563 cassava (Ceballos *et al*., 2013; Rodriguez and Sanders, 2015), potato (Hijri, 2016) and
564 chickpea (Rocha *et al*., 2019). Moreover, increased temporal productivity stability (Yang
565 *et al*., 2014), enhanced yield quality (Lehmann *et al*., 2014; Bona *et al*., 2017; Torres *et*

17

566 *al*., 2018) and higher tolerance to biotic stresses (Fiorilli *et al*., 2018) have also been
567 observed (Fig. 1). Remarkably, even in the absence of a yield increase, the reduction in
568 the amount of water and fertilizer required to achieve the same yield, will influence
569 profitability and reduce the environmental impact. As advocated by Rillig *et al*. (2019)
570 all these aspects have to be considered: following the concept of agroecology, based on
571 the enhancement of ecosystem services to improve sustainable agricultural production
572 (Bender *et al*., 2016; DeClerck *et al*., 2016), the development of production systems,
573 where all the potential benefits provided by mycorrhizal symbionts as well as by other
574 beneficial soil microbes is maximized, needs to be pursued in the future.
575
576 **Conclusions and perspectives**
577 Plants represent a preferential ecological niche for many fungi which may provide
578 several benefits to the host (Fig. 1). If mycorrhizal symbiosis -at least the AM- has
579 received great attention, the world of plant endophytes is still overlooked. Based on the
580 current data on the description of novel fungi and the discovery of new metabolites,
581 endophytes seem to be an extremely valuable resource for bioprospecting.
582 There is also a clear need to integrate endophytic and mycorrhizal fungal research, as
583 important insights on how the host plant can recognize the symbiont, manage its
584 accommodation and the establishment of a beneficial interaction can be identified
585 (Hilbert *et al*., 2020). In many mycorrhizal studies, endophytes are ignored or reported as
586 contaminants, but we can envisage complex interactions with functional significance
587 (Lace *et a*l., 2015). Studying the plant microbiota as a whole system will be the next
588 challenge to gain a more comprehensive knowledge of the functions of the fungal
589 symbionts living inside a plant (van der Heijden and Hartmann 2016; Thomashow et al.,
590 2019).
591 Mycorrhizal fungi and endophytes enable plant hosts to survive in hostile environments
592 and can play a key role in improving plant responses to climate change. The development
593 of tailored microbial inocula for specific crops and environmental conditions appears to
594 be a promising contribution to solve urgent societal needs, an improvement of
595 environment-friendly agricultural crop production systems, and an increase of global
596 food security.
597
598
599

18

**References**

Bacon CW, Porter JK, Robbins JD, Luttrell ES. 1977. *Epichloe typhina* from toxic tall fescue grasses. *Applied and Environmental Microbiology* 34: 576-581.

Baez-Rogelio A, Morales-García YE, Quintero-Hernández V, Muñoz-Rojas, J. 2017. Next generation of microbial inoculants for agriculture and bioremediation. *Microbial Biotechnology* 10: 19-21. https://doi.org/10.1111/1751-7915.12448

Bahadur A, Batool A, Nasir F, Jiang S, et al. 2019. Mechanistic insights into arbuscular mycorrhizal fungi-mediated drought stress tolerance in plants. *International Journal of Molecular Sciences* 20: 4199.

Balestrini R, Brunetti C, Chitarra W, Nerva L. 2020. Photosynthetic traits and nitrogen uptake in crops: which is the role of arbuscular mycorrhizal fungi? *Plants* 9: 1105.

Ball OJ, Prestidge RA, Sprosen JM. 1995. Interrelationships between *Acremonium lolii,* peramine, and lolitrem B in perennial ryegrass. *Applied Environmental Microbiology* 61: 1527-1533.

Bao X, Roossinck MJ. 2013. Multiplexed interactions: viruses of endophytic fungi. *Advances in Virus Research* 86, 37-58. doi:10.1016/B978-0-12-394315-6.00002-7.

Bastias DA, Martínez-Ghersa MA, Ballaré CL, Gundel PE (2017) *Epichloë* fungal endophytes and plant defenses: not just alkaloids. *Trends in Plant Science* 22 (11): 940-948.

Batty AL, Dixon KW, Brundrett MC, Sivasithamparam K. 2002. Orchid Conservation and Mycorrhizal Associations. In: Sivasithamparama K., Dixon K.W., Barrett R.L. (eds) Microorganisms in Plant Conservation and Biodiversity. Springer, Dordrecht

Bayman P. 2006. Diversity, scale and variation of endophytic fungi in leaves of tropical plants. In Microbial Ecology of Aerial Plant Surfaces, ed. MJ Bailey, AK Lilley, TM Timms-Wilson, pp. 37-50. Oxfordshire, UK: CABI

Bender SF, Wagg C, van der Heijden MG. 2016. An underground revolution: biodiversity and soil ecological engineering for agricultural sustainability. *Trends in Ecology and Evolution* 31: 440-452.

Bennet AE, Classen AT. 2020. Climate change influences mycorrhizal fungal-plant interactions, but conclusions are limited by geographical study bias. *Ecology* 101(4), e02978.

Berbee ML, Strullu-Derrien C, Delaux P-M, Strother PK, Kenrick P, Selosse M-A, Taylor JW. 2020. Genomic and fossil windows into the secret lives of the most ancient fungi. *Nature Review Microbiology* 18: 717-730.

Berruti A, Lumini E, Balestrini R, Bianciotto V. 2016. Arbuscular mycorrhizal fungi as natural biofertilizers: let's benefit from past successes. *Frontiers in Microbiology* 6: 1559. https://doi.org/10.3389/fmicb.2015.01559

20

655

656  Besserer A, Becard G, Roux C, Jauneau A, et al. 2008. GR24, a synthetic analogue of
657  strigolactones, stimulates mitosis and growth of the arbuscular mycorrhizal fungus
658  *Gigaspora rosea* by boosting its energetic metabolism. *Plant Physiology* 148: 402-413.
659

660  Bona E, Cantamessa S, Massa N, Manassero P, et al. 2017. Arbuscular mycorrhizal fungi
661  and plant growth-promoting pseudomonads improve yield, quality and nutritional value
662  of tomato: a field study. *Mycorrhiza* 27: 1-11.
663

664  Bonfante P. 2018. The future has roots in the past: the ideas and scientists that shaped
665  mycorrhizal research. *New Phytologist* 220: 982-995.
666

667  Bonfante P, Francesco Venice F, Lanfranco L. 2019. The mycobiota: fungi take their
668  place between plants and bacteria. *Current Opinion in Microbiology* 49:18-25.
669

670  Bradford M, Schwab E. 2012. Symbiosis: "Living together" in Chaos December 2012;
671  Studies in history of biology 4: 7-25.
672

673  Brundrett MC, Tedersoo L. 2018. Evolutionary history of mycorrhizal symbioses and
674  global      host      plant      diversity.      *New      Phytologist*, *220*:      1108-1115.
675  https://doi.org/10.1111/nph.14976
676

677  Brundrett MC. 2002. Coevolution of roots and mycorrhizas of land plants. *New
678  Phytologist* 154: 275-304.
679

680  Bulgarelli D, Rott M, Schlaeppi K, Loren Ver, van Themaat E, et al. 2012. Revealing
681  structure and assembly cues for Arabidopsis root-inhabiting bacterial microbiota. *Nature*
682  488(7409): 91-5.
683

684  Bulgarelli D, Schlaeppi K, Spaepen S, Loren Ver, van Themaat E, et al. 2013. Structure
685  and functions of the bacterial microbiota of plants. *Annual Review of Plant Biology* 64:
686  807-838.
687

688  Cagnano G, Roulund N, Jensen CS, Forte FP, Asp T, et al. 2019. Large scale screening
689  of *Epichloë* endophytes infecting schedonorus pratensis and other forage grasses reveals
690  a relation between microsatellite-based haplotypes and loline alkaloid levels. *Frontiers in
691  Plant Science* 10:765. doi: 10.3389/fpls.2019.00765
692

693  Cameron DD, Neal AL, van Wees SC et al. 2013. Mycorrhiza-induced resistance: more
694  than the sum of its parts? *Trends in Plant Science* 18: 539-545.
695

696  Ceballos I, Ruiz M, Fernández C, Peña R, et al. 2013. The in vitro mass-produced model
697  mycorrhizal fungus, *Rhizophagus irregularis*, significantly increases yields of the
698  globally   important   food   security   crop   cassava. PloS   one, 8(8),   e70633.
699  https://doi.org/10.1371/journal.pone.0070633
700

701  Chen ECH, Morin E, Beaudet D, et al. 2018. High intraspecific genome diversity in the
702  model arbuscular mycorrhizal symbiont *Rhizophagus irregularis. New Phytologist*
703  220:1161-1171. doi:10.1111/nph.14989
704

21

705  Chen M, Arato M, Borghi L, Nouri E, et al. 2018. Beneficial services of arbuscular
706  mycorrhizal fungi - from ecology to application. *Frontiers in Plant Science* 9: 1270.
707  https://doi.org/10.3389/fpls.2018.01270
708
709  Chitarra W, Pagliarani C, Maserti B, Lumini E, et al. 2016. Insights on the impact of
710  arbuscular mycorrhizal symbiosis on tomato tolerance to water stress. *Plant Physiology*
711  171:1009–1023. doi: 10.1104/pp.16.00307.
712
713  Choi GH, Nuss DL. 1992. Hypovirulence of chestnut blight fungus conferred by an
714  infectious viral cDNA. *Science* 257: 800-803.
715
716  Christensen MJ, Voisey CR 2007. The biology of the endophyte/grass partnership. Pages
717  123-133 in: Proceedings of the 6th International Symposium on Fungal Endophytes of
718  Grasses, A. J. Popay and E. R. Thom, eds. New Zealand Grassland Association,
719  Palmerston North, New Zealand.
720
721  Chu Q, Wang X, Yang Y, Chen F et al. 2013. Mycorrhizal responsiveness of maize (*Zea*
722  *mays* L.) genotypes as related to releasing date andavailable P content in soil. *Mycorrhiza*
723  23: 497-505.
724
725  Coleman-Derr D, Desgarennes D, Fonseca-Garcia C, Gross S, Clingenpeel S, et al. 2016.
726  Plant compartment and biogeography affect microbiome composition in cultivated and
727  native *Agave* species. *New Phytologist* 2016, 209:798-811.
728
729  Contreras-Cornejo HA, López-Bucio JS, Méndez-Bravo A, Macías-Rodríguez L, Ramos-
730  Vega M, et al. 2015. Mitogen-activated protein kinase 6 and ethylene and auxin signaling
731  pathways are involved in Arabidopsis root-system architecture alterations by
732  *Trichoderma atroviride*. *Molecular Plant-Microbe Interactions* 28: 701-710. doi:
733  10.1094/MPMI-01-15-0005-R
734
735  Corradi N, Brachmann A. 2017. Fungal mating in the most widespread plant symbionts?
736  *Trends in Plant Science* 22: 175-183.
737
738  Damm U, Woudenberg JHC, Cannon PF, Crous PW: *Colletotrichum* species with curved
739  conidia from herbaceous hosts. *Fungal Diversity* 2009, 39:45.
740
741  Danell E, Camacho, FJ. 1997. Successful cultivation of the golden chanterelle. *Nature*
742  385, 303.
743
744  Davison J, Moora M, Öpik, M, Adholeya A et al. 2015. Fungal symbiont. Global
745  assessment of arbuscular mycorrhizal fungus diversity reveals very low
746  endemism. *Science* 349(6251): 970-973. https://doi.org/10.1126/science.aab1161.
747
748  Dearnaley JD, Cameron DD. 2017. Nitrogen transport in the orchid mycorrhizal
749  symbiosis - further evidence for a mutualistic association. *New Phytologist* 213(1): 10-
750  12. https://doi.org/10.1111/nph.14357.
751
752  Dearnaley JDW, Martos F, Selosse M. 2012. Orchid mycorrhizas: molecular ecology,
753  physiology, evolution and conservation  aspects. In: Esser K (ed.).  The Mycota Volume
754  IX – Fungal  Associations, 2nd ed., pp. 207–230. Berlin: Springer Verlag. *(13)*.

22

755
756 DeClerck FAJ, Jones SK, Attwood S, Bossio D, et al. 2016. Agricultural ecosystems and
757 their services: the vanguard of sustainability? *Current Opinion in Environmental*
758 *Sustainability* 23: 92-99.
759
760 Delaux PM, Radhakrishnan GV, Jayaraman D, Cheema J, et al. 2015. Algal ancestor of
761 land plants was preadapted for symbiosis. *Proceedings of the National Academy of*
762 *Sciences of the United States of America*.112: 13390-13395.
763
764 Deshmukh S, Hückelhoven R, Schäfer P, Imani J, Sharma M, et al. 2006. The root
765 endophytic fungus *Piriformospora indica* requires host cell death for proliferation during
766 mutualistic symbiosis with barley. *Proceedings of the National Academy of Sciences of*
767 *the United States of America* USA 103: 18450-18457.
768
769 Dupont PY, Eaton CJ, Wargent JJ, Fechtner S, et al. 2015. Fungal endophyte infection of
770 ryegrass reprograms host metabolism and alters development. *New Phytologist* 208:
771 1227-1240.
772
773 Fesel PH, Zuccaro A. 2016. Dissecting endophytic lifestyle along the
774 parasitism/mutualism continuum in Arabidopsis. *Current Opinion in Microbiology*,
775 32:103-112.
776
777 Field KJ, Cameron DD, Leake JR, Tille S. 2012. Contrasting arbuscular mycorrhizal
778 responses of vascular and non-vascular plants to a simulated Palaeozoic $CO_2$ decline.
779 *Nature Communications* 3: 835.
780
781 Fiorilli V, Vannini C, Ortolani F, Garcia-Seco D, et al. 2018. Omics approaches revealed
782 how arbuscular mycorrhizal symbiosis enhances yield and resistance to leaf pathogen in
783 wheat. *Scientific Reports 8:* 9625. https://doi.org/10.1038/s41598-018-27622-8.
784
785 Fletcher LR, Harvey IC. 1981. An association of a lolium endophyte with ryegrass
786 staggers. *New Zealand Veterinary Journal* 29, 185-186. doi: 10.1080/00480169.1981.
787 34839
788
789 Fracasso A, Telò L, Lanfranco L, Bonfante P, et al. 2020. Physiological beneficial effect
790 of *Rhizophagus intraradices* inoculation on tomato plant yield under water deficit
791 conditions. *Agronomy* 10: 71.
792
793 Fraceto LF, Maruyama CR, Guilger M, Mishra S, Chetan K, et al. 2018. Trichoderma
794 harzianum-based novel formulations: potential applications for management of Next-Gen
795 agricultural challenges. *Journal of Chemical Technology and Biotechnology* 93: 2056-
796 2063.
797
798 García E, Alonso Á, Platas G, Sacristá S. 2013. The endophytic mycobiota of
799 *Arabidopsis thaliana*. *Fungal Diversity* 60:71- 89.
800
801 Garnica S, Riess K, Schön ME, Oberwinkler F, et al. 2016. Divergence times and
802 phylogenetic patterns of Sebacinales, a highly diverse and widespread fungal lineage.
803 *Plos One*, 11:e0149531.
804

805 Genre A, Chabaud M, Balzergue C, Puech-Pagès et al. 2013. Short-chain chitin
806 oligomers from arbuscular mycorrhizal fungi trigger nuclear $Ca^{2+}$ spiking in *Medicago*
807 *truncatula* roots and their production is enhanced by strigolactone. *New Phytologist* 198:
808 190-202.

810 Genre A, Lanfranco L, Perotto S, Bonfante, P. 2020. Unique and common traits in
811 mycorrhizal symbioses. *Nature Reviews Microbiology*, 18(11): 649-660.

813 Guether M, Balestrini R, Hannah M, He J, et al. 2009. Genome-wide reprogramming of
814 regulatory networks, transport, cell wall and membrane biogenesis during arbuscular
815 mycorrhizal symbiosis in *Lotus japonicus*. *New Phytologist* 182: 200-212.

817 Guimil S, Chang HS, Zhu T, Sesma A, Osbourn A, et al. 2005. Comparative
818 transcriptomics of rice reveals an ancient pattern of response to microbial colonization.
819 *Proceedings of the National Academy of Sciences of the United States of America* 102:
820 8066-8070.

822 Guo H, Glaeser SP, Alabid I, Imani J, et al. 2017. The abundance of endofungal
823 bacterium *Rhizobium radiobacter* (syn. *Agrobacterium tumefaciens*) increases in its
824 fungal host *Piriformospora indica* during the tripartite sebacinalean symbiosis with
825 higher plants. *Frontiers Microbiology* 8:629.

827 Hacquard S, Kracher B, Hiruma K, Münch PC, et al. 2016. Survival trade-offs in plant
828 roots during colonization by closely related beneficial and pathogenic fungi. *Nature*
829 *Communication* 7:11362.

831 Hahn H., McManus MT, Warnstorff K, Monahan BJ, et al. 2008. *Neotyphodium* fungal
832 endophytes confer physiological protection to perennial ryegrass (*Lolium perenne* L.)
833 subjected to a water deficit. *Environmental and Experimental Botany* 63: 183-199.

835 Harman GE, Howell CR, Viterbo A, Chet I, Lorito M. 2004. *Trichoderma* species -
836 opportunistic, avirulent plant symbionts. *Nature Review Microbiology* 2: 43-56. doi:
837 10.1038/nrmicro797

839 Harrison MJ, Dewbre GR, Liu J. 2002. A phosphate transporter from Medicago
840 truncatula involved in the acquisition of phosphate released by arbuscular mycorrhizal
841 fungi. *The Plant Cell* 14: 2413–2429.

843 Hartmann RM, Schaepe S, Nübel D, Petersen AC, et al. 2019. Insights into the complex
844 role of GRAS transcription factors in the arbuscular mycorrhiza symbiosis. *Scientific*
845 *Reports* 9: 3360. https://doi.org/10.1038/s41598-019-40214-4

847 Hassing B, Winter D, Becker Y, Mesarich CH, Eaton CJ, Scott B (2019) Analysis of
848 *Epichloë festucae* small secreted proteins in the interaction with *Lolium perenne*. *PLoS*
849 *ONE* 14: e0209463.

851 Hawksworth DL, Lücking R. 2017 Fungal diversity revisited: 2.2 to 3.8 million species.
852 *Microbiology Spectrum* 5.
853

854 Helber N, Wippel K, Sauer N, Schaarschmidt S, et al. 2011. A versatile monosaccharide
855 transporter that operates in the arbuscular mycorrhizal fungus *Glomus sp.* is crucial for
856 the symbiotic relationship with plants. *The Plant Cell* 23: 3812-3823.

858 Herd, S., Christensen, M. J., Saunders, K., Scott, D. B., and Schmid, J. 1997.
859 Quantitative assessment of *in planta* distribution of metabolic activity and gene
860 expression of an endophytic fungus. *Microbiology* 143: 267-275.

862 Hijri M. 2016. Analysis of a large dataset of mycorrhiza inoculation field trials on potato
863 shows highly significant increases in yield. *Mycorrhiza* 26: 209-214.

865 Hilbert M, Novero M, Rovenich H, Mari S, Grimm C, et al. 2020. MLO differentially
866 regulates barley root colonization by beneficial endophytic and mycorrhizal Fungi.
867 *Frontiers in Plant Science* 16:1678.

869 Hiruma K, Gerlach N, Sacristán S, Nakano RT, et al. 2016. Root endophyte
870 *Colletotrichum tofieldiae* confers plant fitness benefits that are phosphate status
871 dependent. *Cell* 165:464-474.

873 Hohnjec N, Czaja-Hasse LF, Hogekamp C, Küster, H. 2015. Pre-announcement of
874 symbiotic guests: transcriptional reprogramming by mycorrhizal
875 lipochitooligosaccharides shows a strict co-dependency on the GRAS transcription
876 factors NSP1 and RAM1. *BMC Genomics 16*: 994.

878 Ikeda Y, Shimura H, Kitahara R, Masuta C, Ezawa T. 2012. A novel virus-like double-
879 stranded RNA in an obligate biotroph arbuscular mycorrhizal fungus: a hidden player in
880 mycorrhizal symbiosis. *Molecular Plant-Microbe Interactions* 25: 1005-1012.

882 Janos DP. 2007. Plant responsiveness to mycorrhizas differs from dependence upon
883 mycorrhizas. *Mycorrhiza* 17: 75-91.

885 Javot H, Penmetsa RV, Terzaghi N, Cook DR, et al. 2007. A *Medicago truncatula*
886 phosphate transporter indispensable for the arbuscular mycorrhizal symbiosis.
887 *Proceedings of the National Academy of Sciences of the United States of America* 104:
888 1720-1725.

890 Jiang Y, Wang W, Xie Q, Liu N, et al. 2017. Plants transfer lipids to sustain colonization
891 by mutualistic mycorrhizal and parasitic fungi. *Science* 356: 1172-1175.
892 https://doi.org/10.1126/science.aam9970.

894 Johnson DS, Warren RS, Deegan LA, Mozdzer TJ. 2016. Saltmarsh plant responses to
895 eutrophication. *Ecological applications: a publication of the Ecological Society of*
896 *America* 26: 2647-2659. https://doi.org/10.1002/eap.1402

898 Johnson NC, Wilson GWT, Wilson JA, Miller RM, et al. 2015. Mycorrhizal phenotypes
899 and the law of the minimum. *New Phytologist* 205: 1473–1484. 10.1111/nph.13172

901 Johnson LJ, De Bonth ACM, Briggs LR, Caradus JR., et al. 2013. The exploitation of
902 epichloae endophytes for agricultural benefit. *Fungal Diversity* 60: 171-188. doi:
903 10.1007/s13225-013-

904
905 Johnson LJ, De Bonth ACM, Briggs LR, Caradus JR, et al. 2013. The exploitation of
906 epichloae endophytes for agricultural benefit. *Fungal Diversity* 60: 171-188. doi:
907 10.1007/s13225-013-
908
909 Justus M, Witte L, Hartmann T. 1997. Levels and tissue distribution of loline alkaloids in
910 endophyte-infected *Festuca pratensis*. *Phytochemistry* 44, 51–57.
911
912 Kamel L, Keller-Pearson M, Roux C, Anè J-M. 2016. Biology and evolution of
913 arbuscular mycorrhizal symbiosis in the light of genomics. *New Phytologist,* 213: 531-
914 536.
915
916 Kameoka H, Tsutsui I, Saito K, Kikuchi Y, et al. 2019. Stimulation of asymbiotic
917 sporulation in arbuscular mycorrhizal fungi by fatty acids. *Nature Microbiology 4*: 1654-
918 1660. https://doi.org/10.1038/s41564-019-0485-7
919
920 Keymer A, Gutjahr C. 2018. Cross-kingdom lipid transfer in arbuscular mycorrhiza
921 symbiosis and beyond. *Current Opinion in Plant Biology* 44: 137-144.
922
923 Kloppholz S, Kuhn H, Requena N. 2011. A secreted fungal effector of *Glomus*
924 *intraradices* promotes symbiotic biotrophy. *Current Biology* 21: 1204-1209.
925
926 Kiers ET, West SA, Wyatt GA, Gardner A, et al. 2016. Misconceptions on the
927 application of biological market theory to the mycorrhizal symbiosis. *Nature Plants 2*:
928 16063. https://doi.org/10.1038/nplants.2016.63
929
930 Kiers ET, Duhamel M, Beesetty Y, Mensah JA, et al. 2011. Reciprocal rewards stabilize
931 cooperation in the mycorrhizal symbiosis. *Science* 333: 880-882.
932
933 Kobayashi Y, Maeda T, Yamaguchi K, Kameoka H, et al. 2018. The genome of
934 *Rhizophagus clarus* HR1 reveals a common genetic basis for auxotrophy among
935 arbuscular mycorrhizal fungi. *BMC genomics 19*: 465. https://doi.org/10.1186/s12864-
936 018-4853-0
937
938 Koch AM, Antunes PM, Maherali H, Hart MM, Klironomos JN. 2017. Evolutionary
939 asymmetry in the arbuscular mycorrhizal symbiosis: conservatism in fungal morphology
940 does not predict host plant growth. *New Phytologist* 214: 1330-1337.
941
942 Kohler A, Kuo A, Nagy LG, Morin E, Barry KW, et al. 2015. Convergent losses of
943 decay mechanisms and rapid turnover of symbiosis genes in mycorrhizal mutualists.
944 *Nature Genetics* 47: 410-415.
945
946 Lace B, Genre A, Woo S, Faccio A, et al. 2015. Gate crashing arbuscular mycorrhizas: in
947 vivo imaging shows the extensive colonization of both symbionts by *Trichoderma*
948 *atroviride*. *Environmental Microbiology Reports*: 64-77. https://doi.org/10.1111/1758-
949 2229.12221
950
951 Lahrmann U, Strehmel N, Langen G, Frerigmann H, et al. 2015. Mutualistic root
952 endophytism is not associated with the reduction of saprotrophic traits and requires a non
953 compromised plant innate immunity. *New Phytologist* 207: 841-57.

954
955 Lanfranco L, Bonfante P, Genre A. 2016. The mutualistic interaction between plants and
956 arbuscular mycorrhizal fungi. *Microbiology Spectrum* 4: FUNK-0012- 2016.
957
958 Lanfranco L, Fiorilli V, Gutjahr C. 2018. Partner communication and role of nutrients in
959 the arbuscular mycorrhizal symbiosis. *New Phytologist* 220: 1031-1046.
960 https://doi.org/10.1111/nph.15230
961
962 Lehmann A, Barto EK, Powell JR, Rillig MC. 2012. Mycorrhizal responsiveness trends
963 in annual crop plants and their wild relatives – a meta-analysis on studies from 1981 to
964 2010. *Plant and Soil* 355: 231-250.
965
966 Lehmann A, Veresoglou SD, Leifheit EF, Rillig MC. 2014. Arbuscular mycorrhizal
967 influence on zinc nutrition in crop plants – a meta-analysis. *Soil Biology and
968 Biochemistry* 69: 123-131.
969
970 Lehnert H, Serfling A, Enders M, Friedt W, et al. 2017. Genetics of mycorrhizal
971 symbiosis in winter wheat (*Triticum aestivum*). *New Phytologist* 215: 779-791.
972
973 Lorito M, Woo SL, Harman GE, Monte E. 2010. Translational research on *Trichoderma*:
974 from 'omics to the field. *Annual Review of Phytopathology* 48: 395-417.
975
976 Luginbuehl LH, Menard GN, Kurup S, Van Erp H, et al. 2017. Fatty acids in arbuscular
977 mycorrhizal fungi are synthesized by the host plant. *Science* 356: 1175-1178.
978
979 MacLean AM, Bravo A, Harrison MJ. 2017. Plant signalling and metabolic pathways
980 enabling arbuscular mycorrhizal symbiosis. *The Plant Cell* 29: 2319-2335.
981
982 Maillet F, Poinsot V, Andre O, Puech-Pages V, et al. 2011. Fungal
983 lipochitooligosaccharide symbiotic signals in arbuscular mycorrhiza. *Nature* 469: 58-63.
984
985 Márquez LM, Redman RS, Rodriguez RJ, Roossinck MJ. 2007. A virus in a fungus in a
986 plant: three-way symbiosis required for thermal tolerance. *Science* 315: 513-515.
987 doi:10.1126/science.1136237.
988
989 Martin B, Schwab E. 2012 Current usage of symbiosis and associated terminology.
990
991 Martin F, Kohler A, Murat C, Veneault-Fourrey C, Hibbett DS. 2016. Unearthing the
992 roots of ectomycorrhizal symbioses. *Nature Review Microbiology* 14: 760-773.
993
994 Martinez-Medina A, Flors V, Heil M, Mauch-Mani B, et al. 2016. Recognizing Plant
995 Defense Priming. *Trends in Plant Science*, 21(10) 818-822.
996 https://doi.org/10.1016/j.tplants.2016.07.009
997
998 Martínez-Medina A, Van Wees SCM, Pieterse CMJ. 2017. Airborne signals by
999 *Trichoderma* fungi stimulate iron uptake responses in roots resulting in priming of
1000 jasmonic acid-dependent defences in shoots of *Arabidopsis thaliana* and *Solanum
1001 lycopersicum*. *Plant Cell Environment* 40, 2691-2705. doi: 10.1111/pce.13016
1002

1003 Martín-Robles N, Morente-López J, Freschet G, Poorter H, Roumet C, et al. 2018. Root
1004 traits of herbaceous crops: Pre-adaptation to cultivation or evolution under
1005 domestication? *Functional Ecology* 33(2): 273-285. ff10.1111/1365-2435.13231ff. ffhal-
1006 0230965

1008 Mello A. 2012. in Edible ectomycorrhizal mushrooms. *Soil Biology* Vol. 34 (eds
1009 Zambonelli, A. & Bonito, G. M.) 73-81. Springer.

1011 Merckx V.S.F.T. 2012 Mycoheterotrophy: the biology of plants living on fungi. Springer
1012 New York, Dec 6, 2012 - Science.

1014 Miozzi L, Vaira AM, Brilli F, Casarin V, et al. 2020. Arbuscular mycorrhizal symbiosis
1015 primes tolerance to *Cucumber Mosaic Virus* in tomato. *Viruses*, *12:* 675.
1016 https://doi.org/10.3390/v12060675

1018 Morin E, Miyauchi S, San Clemente H, Chen ECH, et al. 2019. Comparative genomics
1019 of *Rhizophagus irregularis*, *R. cerebriforme*, *R. diaphanus* and *Gigaspora*
1020 *rosea* highlights specific genetic features in Glomeromycotina. *New*
1021 *Phytologist* 222: 1584-1598.

1023 Müller DB, Vogel, C, Bai Y, Vorholt, JA. 2016. The plant microbiota: systems-level
1024 insights and perspectives. *Annual Review of Genetics* 50: 211-234.

1026 Murat C. 2015. Forty years of inoculating seedlings with truffle fungi: past and future
1027 perspectives. *Mycorrhiza* 25: 77–81.

1029 Nilsson RH, Anslan S, Bahram M, Wurzbacher C, Baldrian P, et al. 2019. Mycobiome
1030 diversity: high-throughput sequencing and identification of fungi. *Nature Review*
1031 *Microbiology* 17:95-109.

1033 Oldroyd GE. 2013. Speak, friend, and enter: signalling systems that promote beneficial
1034 symbiotic associations in plants. *Nature Reviews Microbiology* 11: 252–263.

1036 Paszkowski U, Boller T. 2002. The growth defect of *lrt1*, a maize mutant lacking lateral
1037 roots, can be complemented by symbiotic fungi or high phosphate nutrition. *Planta* 214:
1038 584-590.

1040 Perotto S, Daghino S. Martino E. 2018. Ericoid mycorrhizal fungi and their genomes:
1041 another side to the mycorrhizal symbiosis? *New Phytologist* 220: 1141-1147.

1043 Peterson RL, Massicotte HB, Melville LH. Mycorrhizas: anatomy and cell biology
1044 (CABI Publishing, 2004).

1046 Pimprikar P, Gutjahr C. 2018. Transcriptional regulation of arbuscular mycorrhiza
1047 development. *Plant and Cell Physiology* 59: 673–679.

1049 Pirozynski KA, Malloch DW. 1975. The origin of land plants: a matter of mycotrophism.
1050 *BioSystems* 6: 153-164.
1051

Plett JM, Kemppainen M, Kale SD, Kohler A, et al. 2011. A secreted effector protein of *Laccaria bicolor* is required for symbiosis development. *Current Biology* 21: 1197-1203.

Porcel R, Ruiz-Lozano JM. 2004. Arbuscular mycorrhizal influence on leaf water potential, solute accumulation, and oxidative stress in soybean plants subjected to drought stress. *Journal of Experimental Botany* 55:1743-1750. doi: 10.1093/jxb/erh188.

Porras-Alfaro A, Bayman P. 2011. Hidden fungi, emergent properties: endophytes and microbiomes. *Annual Review Phytophatology* 49: 291-315.

Pozo MJ, Azcón-Aguilar, C. 2007. Unraveling mycorrhiza-induced resistance. *Current Opinion in Plant Biology* 10: 393-398.

Quigley, PE. 2000. Effects of *Neotyphodium lolii* infection and sowing rate of perennial ryegrass (*Lolium perenne*) on the dynamics of ryegrass/ subterranean clover (*Trifolium subterraneum*) swards. *Australian Journal Agricultural Research* 51:47-56.

Radhakrishnan GV, Keller J, Rich MK, et al. 2020. An ancestral signalling pathway is conserved in intracellular symbioses-forming plant lineages. *Nature Plants* 6: 280–289. https://doi.org/10.1038/s41477-020-0613-7

Remy W, Taylor TN, Hass H. Kerp, H. 1994. Four hundred-million-year-old vesicular arbuscular mycorrhizae. *Proceedings of the National Academy of Sciences of the United States of America* 91: 11841-11843.

Rich MK, Nouri E, Courty P-E, Reinhardt D. 2017. Diet of arbuscular mycorrhizal fungi: bread and butter? *Trends in Plant Science* 22: 652-660.

Rillig MC, Aguilar-Trigueros CA, Camenzind T, Cavagnaro TR. et al. 2019. Why farmers should manage the arbuscular mycorrhizal symbiosis. *New Phytologist 222*: 1171-1175.

Rocha I, Duarte I, Ma Y, Souza-Alonso P, et al. 2019. Seed coating with arbuscular mycorrhizal fungi for improved field production of chickpea. *Agronomy 9*: 471.

Rodriguez A, Sanders IR. 2015. The role of community and population ecology in applying mycorrhizal fungi for improved food security. *The ISME Journal 9*: 1053-1061. https://doi.org/10.1038/ismej.2014.207

Roth R, Paszkowski U. 2017. Plant carbon nourishment of arbuscular mycorrhizal fungi. *Current Opinion in Plant Biology* 39: 50-56.

Ruiz-Lozano JM, Azcón R, Gomez M. 1995. Effects of arbuscular-mycorrhizal glomus species on drought tolerance: physiological and nutritional plant responses. *Applied Environmental Microbiology* 61: 456-460. doi: 10.1128/AEM.61.2.456-460.1995.

Ryan MH, Graham JH. 2002. Is there a role for arbuscular mycorrhizal fungi in production agriculture? *Plant and Soil* 244: 263-271.

1101 Saikkonen, K. Pedro G, Halander M. 2013 Chemical ecology mediated by fungal
1102 endophytes in grasses. *Journal of Chemical Ecology* 39: 962-968.
1103
1104 Salvioli A, Ghignone S, Novero M, Navazio L, 2016. Symbiosis with an endobacterium
1105 increases the fitness of a mycorrhizal fungus, raising its bioenergetics potential. *The
1106 ISME Journal* 10: 130-144.
1107
1108 Sawers RJH, Svane SF, Quan C, Gronlund M, et al. 2017. Phosphorus acquisition
1109 efficiency in arbuscular mycorrhizal maize is correlated with the abundance of root-
1110 external hyphae and the accumulation of transcripts encoding PHT1 phosphate
1111 transporters. *New phytologist* 214: 632-643. https://doi.org/10.1111/nph.14403.
1112
1113 Schardl CL. 1996. *Epichloë* species: fungal symbionts of grasses. *Annual Review of
1114 Phytopathology* 34:109-130.
1115
1116 Schardl CL, Leuchtmann A, Spiering MJ 2004. Symbioses of grasses with seedborne
1117 fungal endophytes. *The Annual Review of Plant Biology* 55: 315-340.
1118
1119 Schardl, C. L., Young, C. A., Hesse, U., Amyotte, S. G., et al. 2013. Plant-symbiotic
1120 fungi as chemical engineers: Multi-genome analysis of the clavicipitaceae reveals
1121 dynamics of alkaloid loci. *PLoS Genetic* 9: e1003323
1122
1123 Schardl CL, Young CA, Pan J, Florea S, et al. 2013. Currencies of mutualisms: sources
1124 of alkaloid genes in vertically transmitted epichloae. *Toxins 5*: 1064-1088.
1125 https://doi.org/10.3390/toxins5061064
1126
1127 Schmid J, Day R, Zhang N, Dupont PY., et al. 2017. Host tissue environment directs
1128 activities of an *Epichlöe* endophyte, while it induces systemic hormone and defense
1129 responses in its native perennial ryegrass host. *Molecular Plant Microbe Interaction* 30:
1130 138-149. doi: 10.1094/MPMI-10-16-0215-R
1131
1132 Schmitz AM, Harrison MJ. 2014. Signaling events during initiation of arbuscular
1133 mycorrhizal symbiosis. *Journal of Integrative Plant Biology 56*: 250–261.
1134 https://doi.org/10.1111/jipb.12155.
1135
1136 Sherameti I, Tripathi S, Varma A, Oelmüller R. 2008. The root-colonizing endophyte
1137 *Pirifomospora indica* confers drought tolerance in *Arabidopsis* by stimulating the
1138 expression of drought stress–related genes in leaves. *Molecular Plant Microbe
1139 Interaction* 21: 799-807.
1140
1141 Smith SE, Read D. Mycorrhizal Symbiosis, 3rd edn (Academic Press, 2008).
1142
1143 Spatafora JW, Chang Y, Benny GL, Lazarus K, et al. 2016. A phylum-level phylogenetic
1144 classification of zygomycete fungi based on genome scale data. *Mycologia* 108: 1028-
1145 1046.
1146
1147 Spatafora JW, Sung GH, Sung JM, Hywel-Jones NL, et al. 2007. Phylogenetic evidence
1148 for an animal pathogen origin of ergot and the grass endophytes. *Molecular Ecology*
1149 16:1701-1711.
1150

1151    Stefani F, Dupont S, Laterrière M, Knox R, et al. 2020. Similar arbuscular mycorrhizal
1152    fungal communities in 31 durum wheat cultivars (*Triticum turgidum* L. var. durum)
1153    under field conditions in Eastern Canada. *Frontiers in Plant Science* 11: 1206.
1154    doi:10.3389/fpls.2020.01206
1155
1156    Stein E, Molitor A, Kogel KH, Waller F. 2008. Systemic resistance in Arabidopsis
1157    conferred by the mycorrhizal fungus *Piriformospora indica* requires jasmonic acid
1158    signaling and the cytoplasmic function of NPR1. *Plant Cell Physiology* 49:1747-51.
1159
1160    Strullu-Derrien C, Selosse MA, Kenrick P, Martin FM. 2018. The origin and evolution of
1161    mycorrhizal symbioses: from palaeomycology to phylogenomics. *New Phytologist* 220:
1162    1012-1030.
1163
1164    Sun X, Chen W, Ivanov S, MacLean AM, et al. 2019. Genome and evolution of the
1165    arbuscular mycorrhizal fungus *Diversispora epigaea* (formerly *Glomus versiforme*) and
1166    its bacterial endosymbionts. *New Phytologist* 221: 1556-1573.
1167
1168    Tan YY, Spiering MJ, Scott V, Lane GA et al. 2001. *In planta* regulation of extension of
1169    an endophytic fungus and maintenance of high metabolic rates in its mycelium in the
1170    absence of apical extension. *Applied and Environmental Microbiology* 67: 5377-5383.
1171
1172    Tao G, Liu ZY, Liu F, Gao YH, et al. 2013. Endophytic *Colletotrichum* species from
1173    *Bletilla ochracea* (Orchidaceae), with descriptions of seven new species. *Fungal
1174    Diversity* 61:139-164.
1175
1176    Thomashow LS, LeTourneau MK, Kwak Y-S, Weller DM. 2019. The soil-borne legacy
1177    in the age of the holobiont. *Microbial Biotechnology* 12: 51-54.
1178
1179    Tian P, Nan Z, Li C, Spangenberg G. 2008. Effect of the endophyte *Neotyphodium lolii*
1180    on susceptibility and host physiological response of perennial ryegrass to fungal
1181    pathogens. *European Journal of Plant Pathology* 122: 593-602.
1182
1183    Torres N, Antolin MC, Goicoechea N. 2018. Arbuscular mycorrhizal symbiosis as a
1184    promising resource for improving berry quality in grapevines under changing
1185    environments. *Frontiers in Plant Science* 9: 18.
1186
1187    Toruño TY, Stergiopoulos I, Coaker G. 2016. Plant-pathogen effectors: cellular probes
1188    interfering with plant defenses in spatial and temporal manners. *Annual Review of
1189    Phytopathology* 54: 419-441. doi:10.1146/annurev-phyto-080615-100204
1190
1191    Trivedi P, Leach JE, Tringe SG, Sa T, Singh BK. 2020. Plant-microbiome interactions:
1192    from community assembly to plant health. *Nature Reviews Microbiology*
1193    https://doi.org/10.1038/s41579-020-0412-1
1194
1195    Turina M, Ghignone S, Astolfi N, Silvestri A, Bonfante P, Lanfranco L. 2018. The
1196    virome of the arbuscular mycorrhizal fungus *Gigaspora margarita* reveals the first report
1197    of DNA fragments corresponding to replicating nonretroviral RNA viruses in fungi.
1198    *Environmental Microbiology* 20: 2012-2025.
1199

31

1200 van der Heijden MG, Hartmann M. 2016. Networking in the plant microbiome. *PLoS*
1201 *Biology* 14 :e1002378.
1202
1203 van der Heijden MG, Walder F. 2016. Reply to 'Misconceptions on the application of
1204 biological market theory to the mycorrhizal symbiosis'. *Nature Plants 2*: 16062.
1205 https://doi.org/10.1038/nplants.2016.62
1206
1207 van der Heijden MGA, Martin FM, Selosse MA, Sanders IR. 2015. Mycorrhizal ecology
1208 and evolution: the past, the present, and the future. *New Phytologist* 205: 1406-1423. doi:
1209 10.1111/nph.13288
1210
1211 Vandenkoornhuyse P, Quaiser A, Duhamel M, Le Van A, Dufresne A. 2015. The
1212 importance of the microbiome of the plant holobiont. *New Phytologist* 206: 1196-1206.
1213
1214 Varma A, Savita V, Sudha, Sahay N, Butehorn B, Franken P. 1999. *Piriformospora*
1215 *indica*, a cultivable plant-growth-promoting root endophyte. *Applied and Environmental*
1216 *Microbiology* 65: 2741–2744.
1217
1218 Venice F, Ghignone S, Salvioli di Fossalunga A, Amselem J, et al. 2019. At the nexus of
1219 three kingdoms: the genome of the mycorrhizal fungus *Gigaspora margarita* provides
1220 insights into plant, endobacterial and fungal interactions. *Environmental Microbiology* 22,
1221 122-141.
1222
1223 Vinale F, Nigro M, Sivasithamparam K, Flematti G, Ghisalberti EL, et al. 2013.
1224 Harzianic acid: a novel siderophore from *Trichoderma harzianum*. *FEMS Microbiology*
1225 *Letters 347*(2):123-9.
1226
1227 Walder F, van der Heijden MGA. 2015. Regulation of resource exchange in the
1228 arbuscular mycorrhizal symbiosis. *Nature Plants* 1: 15159.
1229
1230 Wang W, Shi J, Xie Q, Jiang Y, Yu N, et al. 2017. Nutrient exchange and regulation in
1231 arbuscular mycorrhizal symbiosis. *Molecular Plant* 10: 1147-1158.
1232
1233 Waters MT, Gutjahr C, Bennett T, Nelson DC. 2017. Strigolactone signaling and
1234 evolution. *Annual Review of Plant Biology* 68: 291-322.
1235
1236 Wei X, Chen J, Zhang C, Pan D. 2016. A new *Oidiodendron maius* strain isolated
1237 from *Rhododendron fortunei* and its effects on nitrogen uptake and plant
1238 growth. *Frontiers in Microbiology* 7: 1327.
1239
1240 Weindling R. 1932. *Trichoderma lignorum* as a parasite of other soil fungi.
1241 *Phytopathology* 22: 837-45.
1242
1243 Werner GD, Kiers, ET. 2015. Partner selection in the mycorrhizal mutualism. *New*
1244 *Phytologist 205*: 1437-1442. https://doi.org/10.1111/nph.13113
1245
1246 Wewer V, Brands M, Dörmann P. 2014. Fatty acid synthesis and lipid metabolism in the
1247 obligate biotrophic fungus *Rhizophagus irregularis* during mycorrhization of *Lotus*
1248 *japonicus*. *The Plant Journal* 79: 398-412.
1249

1250 Wiensczyk A, Gamiet S, Durall D, Jones M, et al. 2002. Ectomycorrhizae and forestry in
1251 British Columbia: a summary of current research and conservation strategies. Br
1252 Columbia *Journal Ecosystem Managment* 2: 1-19.
1253
1254 Willmann M, Gerlach N, Buer B, Polatajko A, et al. 2013. Mycorrhizal phosphate uptake
1255 pathway in maize: vital for growth and cob development on nutrient poor agricultural
1256 and greenhouse soils. *Frontiers in Plant Science* 4: 533.
1257
1258 Wilson D. 1995. Endophyte: the evolution of a term, and clarification of its use and
1259 definition. Oikos 73: 274-276.
1260
1261 Yadav V, Kumar M, Deep DK, Kumar H, Sharma R, et al. 2010. A phosphate transporter
1262 from the root endophytic fungus *Piriformospora indica* plays a role in phosphate
1263 transport to the host plant. *Journal of Biological Chemistry* 285: 26532-26544.
1264
1265 Yang GW, Liu N, Lu WJ, Wang S, et al. 2014.The interaction between arbuscular
1266 mycorrhizal fungi and soil phosphorus availability influences plant community
1267 productivity and ecosystem stability. *Journal of Ecology* 102: 1072-1082.
1268
1269 Young CA, Schardl CL, Panaccione DG, Florea S, et al. 2015. Genetics, genomics and
1270 evolution of ergot alkaloid diversity. *Toxins* 7: 1273-1302.
1271 https://doi.org/10.3390/toxins7041273.
1272
1273 Yu P, Wang C, Baldauf JA, Tai H, et al. 2018. Root type and soil phosphate determine
1274 the taxonomic landscape of colonizing fungi and the transcriptome of field-grown maize
1275 roots. The *New phytologist* 217: 1240–1253. https://doi.org/10.1111/nph.14893
1276
1277
1278

**Figure legends**

**Figure 1. Beneficial effects of plant fungal endophytes and mycorrhizal fungi.** The plant-associated endophytes *Epichloë* (light pink), *Trichoderma* (yellow), *Serendipita indica* (turquoise), *Colletotrichum tofieldiae* (blue) and the AM fungi (purple) colonize different plant organs (dashed lines). They can provide several benefits to the host plant directly, via nutrient acquisition, or indirectly, through the modulation of plant hormones and the induction of plant defences to respond to pathogen attack. Each microorganism is represented by dot with corresponding color code.

**Figure 2. Scheme of the main cellular features of ectomycorrhizas, arbuscular, orchid and ericoid mycorrhizas** (from Genre *et al*., 2020). In arbuscular mycorrhizas, fungal hyphae contact epidermal cells through a flattened structure, the hyphopodium. Penetrating hyphae then develop *via* inter- and intracellular structures to reach the inner cortical cells, where they form highly branched tree-like structures called arbuscules. Fungal hyphae are always separated from the plant cell cytoplasm by an extracellular interface (green) surrounded by a membrane that develops from an extension of the host plasma membrane (orange). This interface is thought to be the main site of nutrient exchange. In ericoid mycorrhizas, hyphae penetrate the thick surface cell walls of ericaceae roots and produce dense hyphal coils inside each epidermal cell that also are surrounded by an interface compartment (green) and an extension of the plant membrane. In orchid mycorrhizas, the best described colonization process is in protocorms, the post-embryonic developmental phase after seed germination. Hyphae penetrate epidermal hair cells and reach the cortical tissue, where they form large hyphal coils, called 'pelotons', within a membrane-delimited symbiotic interface. In ectomycorrhizas, fungal hyphae form a tissue known as the 'sheathing mantle', which engulfs the whole root tip. Inner hyphae further develop between epidermal cells, reaching different depths into the cortical tissue depending on the host plant. These intraradical hyphae, called the Hartig net, never penetrate the cell lumen, and form an intercellular interface (green).