Plant symbioses with fungal endophytes: perspectives on conservation and sustainable exploitation of Mediterranean ecosystems

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Summary

Mediterranean ecosystems have evolved under strong environmental changes and disturbances. Some of these actions have involved severe human modifications in water budgets and land usage. The ecosystems of arid zones like those of the Iberian Peninsula, have characteristics that make them exceptional. The features of the physical environment and the climate have shaped organisms to live in a limit environment. Mediterranean ecotones are true sources of stress adaptations in plants and animals. Recent studies have shown that symbioses with endophytes are of crucial importance in
the distribution of plant communities worldwide. These symbioses are in many cases responsible for adaptation to environmental and biological stresses. The use of such plant symbiotic organisms is a new approach which will have a high potential for the sustainable improvement of the overall agricultural production and ecosystems management. We have found that plants adapted to sand dunes and salt marshes in Mediterranean ecosystems harbor a large biodiversity of fungal root endophytes. These fungi can reduce symptoms caused by root pathogens and protect against other environmental stresses. Recently metagenomic studies of desert plants have confirmed this rich mycobiota and evidenced the broad range of ecological functions that endophytes play in nature. Endophytes seem to be a key factor for the functioning of arid ecosystems, and are a potential source of biotechnological tools for the design of new approaches for a sustainable agriculture and ecosystem restoration.

**Keywords:** Endophytes, Habitat-Adapted Symbiosis, Adaptation, Ecotones, Plant Stress, Sustainable Agriculture, Ecosystems Restoration
Mediterranean desertification and Global Warming

Mediterranean ecosystems have evolved under strong environmental changes and disturbances. These changes date back from the Myocene until the present time. They are a «case of study» of human intervention in nature which can be tracked with sedimentological, archaeological and historical records. Some of these human actions, especially in the last 500 years, have involved severe modifications in water budgets and land usage. Landscape has changed dramatically associated with political, socio-economic and demographic events. Since 1960´s social and technological changes in spanish rural life have overexploited water resources leading to desertification (PUIGDEFÀBREGAS and MENDIZABAL, 1998). Soil salinization, damage to fluvial and wetlands have also followed this process. The coastal area, an environment where a great amount of human activities (e.g. tourism, transport infrastructures) have been developed, finds itself in a state of remarkable degradation. Climatic change through global warming and associated drought aggravates this situation, especially when exploiting resources in terrestrial and marine Mediterranean ecosystems. The expected negative impact of this global warming on Mediterranean agriculture is a good example.
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The ecosystems of arid zones like those of the Iberian Peninsula, have characteristics that make them exceptional. The features of the physical environment (e.g. structure and nature of the substrate and the soil, climatology) make the structure, biology and ecology of the organisms (microbes, animals and plants) highly interesting to study life adaptations to a limit environment. At landscape scale we find a rupture in the boundary with the Plateau of Castile. This boundary makes these systems in Levante (eastern spanish Mediterranean) very particular regarding the necessary dose of water required (secular hydraulic architecture) for their functioning. In addition, there is a high variability of soils. Some of these, rich in substrates such as gypsum or dolomites, determine the existence of endemic vegetation. The coastal interface including a well-adapted vegetation, is very important for the protection of agricultural land and other human activities but has been heavily degraded. A quest for suitable management is directed to generate, with emerging technologies, solutions and new opportunities of scientific advance to obtain resources (e.g. organisms, salt or freshwater) in a sustainable way.

The study of arid environments is of great importance in the perspective of the changes due to the anthropic environmental pressure and its global consequences. This kind of stud-
ies have a practical component in the management of limited resources, such as freshwater and its effects on our use of it for activities like operation of the agricultural resources, or management of our residues. The organisms of arid zones, their interactions and systems have received little attention from fields like biotechnology and other emerging activities of industrial interest.

**Ecotones: The frontiers of ecosystems**

Ecotone concept was introduced in Ecology to define frontiers in ecosystems or communities. We will avoid semantic considerations associated with the term and similar ones such as ecocline. An ecotone involves a gradient in which organisms are not as structured as in the communities they edge. Leaving aside succession, some ecotones are clearly identifiable and are dynamic aspects of the landscape (especially in steep gradients) between more homogeneous communities. Our working hypothesis is that they include organisms with interesting adaptations by themselves or by means of symbiosis with microbes (mainly fungi and bacteria).
Mediterranean ecotones: Sources of stress adaptations

Mountains and semi-arid Mediterranean ecosystems are especially vulnerable. In these environments, especially in coastal zones, dramatic ecotones can be found where gradients of environmental parameters (e.g. structure and nature of the substrate and the soil or climatology) affect organisms and shape community boundaries, promoting speciation. A classical example is the richness of Mediterranean vegetation. The vascular flora of Alicante Province (SE Spain, 5,816.5 km²) is ca. 1600 species, which is the whole diversity found in the UK (ca. 245,000 km²) vascular flora. The organisms present in these ecotones, their interactions and the systems themselves constitute an evolutionary laboratory where a lot can be learned to mitigate the negative impacts of human-driven desertification and global warming. Organisms in ecotones are subjected to movement of materials and have to cope with environmental gradients (e.g. temperature, salinity, drought). Adaptations are necessary tools to face life in these gradients.

New evolutionary paradigms: Deconstructing Darwin

Adaptations come through natural selection but symbiosis is increasingly being found as a drive for evolutionary change.
at an ecological level. Horizontal transfer includes not only genes, but very often whole organisms living endosymbiontically within others giving an ecological dimension to the theory for cell evolution (ZILBER-ROSENBERG and ROSENBERG, 2008). The analysis of ecotones with emphasis to adaptation to stress (biotic and abiotic) will generate new cues to be applied in the management of limited resources like freshwater, natural protected areas, habitat restoration and sustainable agriculture and aquaculture. The study of adaptations will include changes in the organisms (microbes, animals and plants) at all levels (ecophysiological, morphological, biochemical and genomic), and the analysis of possible symbiogenetic processes such as the Habitat-Adapted Symbioses (RODRIGUEZ et al., 2008) established between plants and obligate or facultative endophytic microbiota. Many such interactions will provide new resources for sustainable exploitation of Mediterranean ecosystems.

**Fungal endophytes**

The term endophyte (*endon* Gr., within; *phyton*, plant) was coined to refer those organisms inhabiting within the living, functional tissues of plants. Although a great controversy has surrounded the definition of the boundaries of such a broad concept (WILSON, 1995), we accept the practical definition
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given by SIEBER (2002), who defines endophytes as those organisms located within apparently healthy, functional plant tissues at the moment of sample collection. Also for practical reasons we will leave aside from our use of the term endophyte the mycorrhizal fungi, which are in many cases biotrophs with a very specific association with their plant hosts.

Endophytic fungi have been found colonizing all plant species studied, and seem to play a wide range of ecological roles and participate in interactions with host plants, constituting a continuum from mutualism to parasitism (SAIKKONEN et al., 1998; SCHULZ and BOYLE, 2005). SCHULZ et al. (1999) postulated that the endophyte-host interaction is a balanced antagonism which depends on an equilibrium between fungal virulence and plant defenses, and that can change over time and/or environmental conditions. Colonization by endophytic fungi may give benefits to the host plants by means of growth promotion, protection against diseases or pests, assistance in phosphorus uptake, or improve the ecological adaptability of the host by enhancing tolerance to biotic and abiotic stresses (SIEBER, 2002; SCHULZ and BOYLE, 2005).

Endophytic fungi can be divided into at least two classes according to their ecological affinities: i) a relatively small number of fastidious species belonging to the Clavicipitales, with few
hosts within the monocots (BISCHOFF and WHITE JR, 2005); and ii) a large number of non-clavicipitaceous species with a wide host range including both monocots and eudicots (CARR-ROLL, 1988; VAN BAEL et al., 2005). Class 2 endophytes probably represent the majority of microbial symbionts which interact with plants, with a great diversity occurring both at taxonomical and functional levels. In spite of this, the importance of this group of fungi have been largely neglected until recent years, probably due to their facultative mutualism with plants which is often difficult to establish. However, their capacity to grow saprophytically in absence of host plants make them amenable for biotechnological purposes, since they can be isolated and grown in culture media. Unsuitability of some mycorrhizal fungi for mass production is one of the main problems to incorporate these valuable symbionts into mainstream agricultural production (HART and TREVORS, 2005).

**Symbiogenics vs. transgenics: Plant adaptation to habitat through symbiosis**

Sustainable agriculture has currently to cope with serious threats that compromise the food security for a human population under continuous growth, all these exacerbated by climate change. Some of these include the loss of usable land through overuse, deforestation and poor irrigation practices,
which have led to desertification and salinization of soils, specially in drylands (BREITHAUPT, 2003; HELLDÉN and TOTTRUP, 2008). Approaches currently being taken to face this situation come from the development of stress-tolerant crops, e.g. by genetic modification or breeding traits from wild plants. Genetic engineering has been proposed as the solution to these problems through a rapid improvement of crops. Crop genetic modification has generated a great public concern regarding their potential threats to the environmental and public health. As a consequence, legislation of several countries has restricted their use in agriculture. On the other hand, exotic libraries from wild plants for «clever plant breeding» could overcome the problem of narrowed genetic variability of today’s high-yield crops. Plant breeding driven by selection marker has also been a major breakthrough (BREITHAUPT, 2003). However these approaches have met limited success, probably because stress-tolerance involves genetically complex processes and the ecological and evolutionary mechanisms responsible for stress tolerance in plants are poorly defined. New paradigms for sustainable crop improvement are currently arising.

The above approaches do not consider the fact that plants in ecosystems have developed natural symbiotic associa-
tions for at least 400 million years (KRINGS et al., 2007) with a broad diversity of microbial symbionts. The exploitation of plant-fungal symbioses appear as a smart alternative for plant adaptation due to their great quantity, ubiquity and diversity, and broad range of ecological functions they play in natural ecosystems. Recent studies have shown that symbiosis with class 2 endophytes are of crucial importance in the distribution of plant communities worldwide, and are responsible of their adaptation to environments under highly selective pressure (CLAY and HOLAH, 1999; REDMAN et al., 2002; HERRE et al., 2007; MACIÁ-VICENTE et al., 2008a,b; RODRIGUEZ and REDMAN, 2008). These indicate that some endophytes confer tolerance to specific stresses and are responsible of the survival of plants to environments submitted to these particular conditions (REDMAN et al., 1999, 2001, 2002; ARNOLD et al., 2003; RODRIGUEZ et al., 2004; WALLER et al., 2005). A clear example of such adaptation specific from the habitat has been found in native grass species from coastal and geothermal habitats, which respectively require symbiotic fungi for their tolerance to salt or heat (RODRIGUEZ et al., 2008). The stress tolerance conferred by the symbiosis is a habitat-specific phenomenon, which has been defined as Habitat-Adapted Symbiosis (REDMAN et al., 2002; RODRIGUEZ et al., 2004, 2005; MÁRQUEZ et al., 2007; RODRIGUEZ and
REDMAN, 2008), with endophytes from geothermal environments that confer tolerance to heat but not salt, and coastal endophytes conferring tolerance to salt, but not to heat. The same fungal species isolated from plants in habitats devoid of salt or heat stress did not appear to confer tolerance to these stresses. Moreover, fungal endophytes from agricultural crops confer resistance to disease, but no tolerance to salt or heat (RODRIGUEZ et al., 2008). In these studies, the endophytes from agricultural, coastal and geothermal ecosystems were able to colonize tomato and rice, conferring disease, salt and heat tolerance, respectively. Hence these endophytes are capable to transfer their stress tolerance conferment to plants other than their original hosts. Interestingly, these endophytes also conferred drought tolerance irrespective of the habitat of origin (geothermal, coastal or agricultural), related with both a decrease in consumption of water by the plant and the modulation of the sensitivity/generation of reactive oxygen species. Furthermore, it has also been found that endophyte assemblages in a plant species can change in adjacent microhabitats that impose different selective pressures (RODRIGUEZ et al., unpublished). This could provide a «mechanism for plants to make quantum evolutionary changes allowing for habitat expansion and survival in high-stress habitats» (RODRIGUEZ et al., 2005). An interesting arising hypothesis is
whether endophytes of plants growing at the ends of water availability gradients (ecotones) could be used for confer tolerance to other plants along the gradient, which are under drought stress due to climate change (RODRIGUEZ et al., unpublished).

It is currently thought that each plant in natural ecosystems comprises a community of organisms, including class 2 endophytes among others such as mycorrhizae and bacteria. The ability of the endophytic fungi to confer tolerance to stress may provide a new strategy to mitigate the impacts of global climate change on agriculture and natural plant communities (RODRIGUEZ and REDMAN, 2008; RODRIGUEZ et al., 2008). Such symbiotic lifestyles suppose a potential source for the improvement of food crops, through adapting them to situations of increasing desertification and drought on global crop lands. It appears therefore as a sustainable alternative to the use of genetically modified organisms, which on the other hand did not yield the expected results. Genetically modified organisms suppose the introduction of a limited number of genes, in many cases alien to the ecosystems (therefore causing «genetic contamination»). On the contrary, the use of selected endophytes for certain ecosystems/plants entails moving «entire genomes». These genomes native from the
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agroecosystems, and selected over thousands or millions of years are an ecologically sound evolutionary treasure. The characterization of endophytic species that confer tolerance to different stresses, both abiotic and biotic, depending on environmental conditions, as well as the detailed understanding of the mechanisms by which they occur, will be of great relevance in the following years, as environmental problems such as desertification of agro-ecosystems or damage to natural ecosystems aggravate.

Mediterranean ecosystems: «Cases of study»

Dune systems

The seaside is the ecotone most affected by the impact of human activities in the Mediterranean zone. The importance of littoral ecosystems is in relation to their rarity, but mainly to the special adaptive features of the species they contain. Along this line, dune systems are a wonderful case of study. Due to geomorphological factors that help in its formation, physical and chemical characteristics of their substrates, patterns of distribution of their flora and fauna, these systems are unique. A large set of data is available on the biology and ecology of the main plant and animal sand dune species and the conse-
sequences of the different degrees of human disturbance that undergo in these landscapes.

**Arid environments**

Arid, semi-arid zones and deserts, correspond to territories that receive annual rainfall lower than 250 L·m⁻² and in an irregular regime. We could say that most of the Mediterranean basin falls in this precipitation regime. The arid Mediterranean environments mostly have the following physical and ecological characteristics. The southeastern Iberian Peninsula and the northern Africa mostly include sedimentary and metamorphic materials corresponding to the internal and external zones of the Betic System. The river beds are short and of variable volume of flow, unlike the Atlantic ones. This makes the recovery of hydrological resources very difficult. The soils formed in arid and semi-arid regions accumulate an excess of soluble salts, due to the rainfall shortage. This prevents salt solubilization and lixiviation. The accumulation of these compounds goes from calcium and magnesium carbonates, gypsum, up to sodium salts, with increasing soil aridity. The Mediterranean basin is one of more «hot» zones of biodiversity of the planet due to its bioclimatic characteristics and to the historical existence of the diverse cultures in a balanced coexistence with nature. This fact has allowed
rationality in the extraction of resources and a fine grain size of landscaping in the small-medium scale. The Biota of arid zones is characterized by its richness, peculiarities, and high degree of endemicity. Plants from arid environments have a tendency to the formation of authentic islands in a hostile environment, caused by the hard environmental conditions described above.

**Endophytes in Mediterranean vegetation from dunes and saline soils**

Out of the 1.5 million species of fungi estimated on earth, we know as little as 5% of them. The greater part of this broad fungal diversity is somehow associated with plants. Current estimations suggest an occurrence of 5.3 unique fungi per plant host species, out of the ca. 200,000 species of vascular plants (HAWKSWORTH and ROSSMAN, 1997). Biodiversity assessments in unexplored, pristine ecosystems harboring such a great fungal diversity are of fundamental interest by the mere fact of our knowledge, but is also urgently needed for the better understanding on the effect of anthropic and natural disturbances on diversity (SIEBER and GRÜNIG, 2006).

Very little information exists on the abundance, habitat or host preferences of endophytic fungi, and about the ecological
importance of these associations in Mediterranean environments (GIRLANDA et al., 2006). This is especially true for the Iberian Peninsula, in comparison with other biogeographical regions. We have recently studied fungal endophytes of plants from dunes and saline soils (MACIÁ-VICENTE et al. 2008a). Arid locations in the southeast of the Iberian Peninsula have very specific climatic characteristics, including an extended summer drought followed by torrential rains, very irregular distribution of the rainfall, and high temperatures. These climatic characteristics are key factors in soil formation and therefore in the establishment of a vegetation with a high degree of endemism. Plant species of these arid areas grow in soils where they have to withstand water or saline stress, due to climate as well as substrate constraints. These lands can be divided into coastal and inland areas according to their vicinity to sea. Coastal and inland sandy soils both have an eolian origin, but the former are subjected to marine influence. Furthermore, the coastal and inland salt marshes are different in both geomorphology and soil formation. Desertification can become a serious problem where the precipitation regime is particularly erratic, and is combined with anthropogenic pressure exerted over a long time. Desertified Mediterranean ecosystems are very fragile and subject to progressive disturbance of the vegetation cover and to rapid soil erosion. Due
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to the lack of knowledge about root endophytic communities in these environments, their study a valuable information from a basic science level and is of great importance to understand the role of these associations with plants growing in these fragile environments, because they have been shown to alleviate abiotic stresses (BARROW and AALTONEN, 2001; REDMAN et al., 2002). Furthermore, the possibility of finding specific fungal isolates with biocontrol potential or producers of specifically useful secondary metabolites is another bonus to investigate these environments.

We carried out surveys during the years 2002 and 2003 to analyze the diversity of fungal endophytes within roots of 24 plant species, growing at 12 sites comprising soils under either water or salt stress, both in coastal and inland locations in the Alicante Province, at southeastern Spain (Figure 1; MACIÁ-VICENTE et al., 2008a). These studies consisted in the isolation in culture media of culturable fungi isolated from surface sterilized root samples, following standard protocols (Figure 2a). Furthermore, root samples were analyzed under light microscopy to directly observe fungi colonizing the internal root tissues under natural conditions (Figure 2b).

All plant species examined were colonized by endophytic fungi, and displayed a great fungal occurrence and diversity
Figure 1: Images of some of the sites sampled for fungal root endophytes in MACÍÁ-VICENTE et al. (2008a), corresponding to sand dunes and salt marshes, either from coastal or inland locations at the Province of Alicante (southeast Spain). Images of some of the plant species sampled in each environment (sandy or salty soils) are also shown.
Figure 2: (a) Fungal endophytes colonies developing from pieces of surface-sterilized roots plated on culture medium. (b) Endophytic colonization of root tissues by fungi as directly observed by light microscopy. (c) Example of fungal endophytes diversity found within roots of natural vegetation; the image shows some pure cultures of fungal endophytes displaying diverse morphological features.
within their roots (Figure 2c). Surveys yielded a total of 1,830 fungal isolates, which were identified according to morphological and molecular traits (internal transcribed spacer and translation elongation factor 1α gene) into 142 fungal species belonging to 57 genera, and 177 morphospecies grouping sterile mycelia which could not be identified for lack of sporulation. Although this diversity is remarkable, it is probably biased since only a relatively small number of fungi can be isolated in culture media (HAWKSWORTH, 1991). This is clearly evidenced by other studies using culture-independent methods (i.e. analysis of total community DNA), which have shown a fungal diversity of as much as 50 species in roots of a single plant (VANDENKOORNHUYSE et al., 2002). However, the latter techniques do not allow the physical handling of isolates for further studies as the isolation in culture media do, such as plant inoculations and testing of the performance on symbiont plant development in the laboratory. On the other hand, direct isolation of fungi from plant tissues may be appropriate to evaluate the dominant endophytic fungal species (SIEBER, 2002).

Endophyte communities inhabiting roots were strongly dominated by fungal genera such as *Fusarium* and *Phoma* displaying a high intra-genus species diversity, and to a lesser extent
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by fewer species from the genera *Aspergillus*, *Alternaria* and *Acremonium*. These are common taxa found as endophytes in other host plants and biogeographical regions, and in many cases tightly related to plant pathogens. An existing hypothesis is that pathogenic strains may arise in nature from existing non-pathogenic endophytes when potentially new host plants, such as crop species, are introduced into the same habitat (SUMMERELL and LESLIE, 2004). The plant-endophyte associations found were strongly influenced by the specific soil type where they took place, which was translated in an overall difference in species composition between communities from sand dunes and salt marshes. The major contribution to these differences was due to their dominant endophytes: *Fusarium oxysporum* for sand dunes or *Aspergillus fumigatus* and *Alternaria chlamydospora* for salt marshes (Figure 3). No clear differences were found, however, between species composition of endophyte communities from coastal and inland locations, either from sandy or salty soil. The physico-chemical characteristics of a soil seem to have the tendency to select the fungal species more adapted to colonize the plant communities present, while the origin of the soils seems to play a lesser role.

In some instances host preferences by specific isolates could be detected. As an example, within the genus *Fusarium*, the
most frequently found over the surveys, three species presented some kind of preference (but never a complete specificity) for certain plant species. *Fusarium oxysporum* and *F. solani*
occurred more frequently within roots of two plants from the family Leguminosae growing in coastal sandy soils: *Ononis natrix* subsp. *ramosissima* and *Lotus creticus*, respectively. *Fusarium equiseti* showed a preference for *Lygeum spartum* (Poaceae) in salt marshes (Figure 4). Phylogenetic analyses within the genus also revealed that in some cases these host or soil preferences occurred at an intra-specific level, e.g. only some phylogenetically-related isolates of *F. solani* occurred in roots of *L. creticus*, while other isolates from the same species appeared in other plant species or site.

The above presented results represent the first insight into endophytic fungal biodiversity associated to plant roots in southeast Spain. The large array of isolates obtained from a non-previously prospected environment, represent a novel source for the search of beneficial properties conferred to plants through symbiosis, such as enhancement of growth, tolerance to abiotic stresses or protection against fungal diseases or pests.

*Effects of endophytes from Mediterranean vegetation on plant growth and disease*

Fungal endophytes are a valuable source of biotechnological applications, with a special interest for potential management
of natural ecosystems and agroecosystems due to effects on the host plants such as the previously described (e.g. growth promotion or tolerance to stress). A further merit of this group of fungi is their large capacity to produce biologically active secondary metabolites, as compared to other fungal groups, e.g. pathogenic or soil fungi (SCHULZ et al., 2002). One of the reasons for such production of active metabolites could be the continuous liaison of endophytes with plants. This would

Figure 4: Example of plant host preference by a fungal endophyte. In this case, occurrence of the endophyte *Fusarium equiseti* within roots of *Lygeum spartum* was significantly greater than in other plant species (modified from MACIÁ-VICENTE et al., 2008a, courtesy of Blackwell Science).
lead to a continuous «physiological fine tuning» between both partners to keep a balanced antagonism. On the other side, the increasing number of chemical studies on this group of fungi, not as studied as other groups, is leading over the last years to the discovery of novel chemical structures within the endophyte’s secondary metabolites, with an invaluable interest for the pharmaceutical and agricultural industries (TAN and ZOU, 2001; SCHULZ et al., 2002).

Fungal root endophytes obtained from natural vegetation under stress in the above mentioned survey were tested to assess their effects on plant growth and disease (MACIÁ-VICENTE et al., 2008b, MACIÁ-VICENTE et al., 2009). Endophytes were first tested for antifungal activity in dual culture tests vs. two economically important root pathogens of monocots and eudicots: *Gaeumannomyces graminis* var. tritici (Ggt), which causes take-all disease in cereals worldwide,.and *Fusarium oxysporum* f.sp. *radicis-lycopersici* (Forl), which causes root and crown rot in tomato. Dual culture tests consist on assessing the interaction between colonies from the pathogen and the potential biological control agents (e.g. endophytes) on culture media (Figure 5). This *in vitro* bioassay is a method for the fast screening of large arrays of isolates when searching for antifungal secondary metabolites. This approach allowed
us to test a total of 365 endophyte isolates against both pathogens, comprising 66 fungal species within 29 genera, and 37 morphospecies. Out of these, 129 isolates (35%) yielded some kind of inhibition of at least one of the pathogen’s radial growth related to antifungal compounds; 72 isolates (20%) inhibited Ggt, 34 isolates (9%) inhibited Forl, and 23 isolates (6%) were able to inhibit both pathogens.

Figure 5: Dual culture test plates showing interactions between endophytes and the root pathogens *Fusarium oxysporum* f.sp. *radicis-lycopersici* (a) and *Gaeumannomyces graminis* var. *tritici* (b). In both cases release to the medium of antifungal compounds inhibitory to pathogen’s radial growth by endophytes is evidenced by presence of an inhibition zone (arrows).
Among the most inhibitory isolates to pathogens found in dual culture tests, fifteen isolates were selected for further studies on root colonization, and effects on plant growth and disease development. These isolates belonged to *Acremonium blochii*, *A. furcatum*, *Aspergillus fumigatus*, *Cylindrocarpon* sp., *C. destructans*, *Dactylaria* sp., *Fusarium equiseti*, *Phoma herbarum*, *P. leveillei* and a sterile mycelium. They were inoculated into roots of barley plants (a model monocot) under axenic laboratory conditions, both in absence and presence of the pathogen Ggt. All isolates colonized the rhizosphere and very often the root cortex without causing disease symptoms and without affecting plant growth. Eight of the isolates kept their antagonistic effect to the pathogen *in planta*, significantly reducing Ggt symptoms. Six of the isolates were also able to reduce the pathogen populations within barley roots (MACIÁ-VICENTE *et al.*, 2008b). Among these, the most effective were different isolates from *F. equiseti*, which also appeared as the more active colonizers of the barley root systems. As previously shown, this endophyte appeared strongly associated in nature with roots of the Poaceae plant *Lygeum spartum*, but also with roots of other plants from the same family as *Corynephorus canescens* or *Ammophila arenaria* (Figure 4). Two *F. equiseti* isolates were therefore selected to repeat the barley root inoculation experiments with/with-
out Ggt under non-axenic, more practical greenhouse conditions, with plants growing directly in soil instead of using a sterile substrate as previously done (MACÍA-VICENTE et al., 2009). In this case fungi again colonized endophytically barley roots, and were able to efficiently withstand competition with other existing rhizosphere competitors. Although a clear suppressive effect on disease could not be detected, *F. equiseti* reduced the mean root lesion length caused by the pathogen. *Fusarium equiseti* appears hence as a long-term root endophyte through evasion of rhizospheric competition, and confers beneficial effects to the host plant.

**Desert plants: a hot biodiversity spot**

As we have already discussed desert environments are as arid ecosystems challenging to plants. In a recent study (PORRAS-ALFARO et al., 2008), non-culturing techniques (environmental DNA sequencing) revealed that a dominant desert grass from New Mexico (USA) harbored an extremely rich associated mycobiota. A large section of the fungal DNA sequences obtained represented undescribed fungal taxa. Moreover the most surprising finding was that sequences found were homologous to endophytic, coprophilous, mycorrhizal, saprophytic and plant pathogenic fungi. This wide ecological spectrum has led to hypothesize different roles for
each of these categories in the desert ecosystem functioning. This and other studies discuss that mycorrhizae (especially arbuscular fungi) in stressed environments are functionally substituted by endophytes, specially those termed dark septate endophytes (DSE), a polyphyletic group of fungi sharing similar morphological features and which are tightly associated with plant roots. To this respect we have recently found an extremely high presence of DSE in halophytes associated with sand dune environments (MACIÁ-VICENTE et al., unpublished).

Concluding remarks

Colonization of plant roots by endophytic fungi may confer benefits to the host such as protection against abiotic or biotic stresses, or plant growth promotion. The exploitation of these properties is of great relevance at an applied level, either to increase yields of agricultural crops or reforestation activities. Plants adapted to Mediterranean Ecosystems seem to harbor an interesting endophytic mycobiota which could be candidates for these applications and that play an important role in arid ecosystem functioning. Future works aimed in determining the mechanisms involved in plant-endophyte symbiosis and conferred stress tolerance to plants, will be particularly relevant to develop novel strategies for improving
agriculture and mitigating impacts of Climate Change, specially those derived from desertification and soil salinization. The understanding of these phenomena will open new theoretical overviews on the general co-evolution of plants with their associated microbial consortia, and subsequent appearance of symbiosis in nature. This will also be useful to those responsible for ecological monitoring and habitat restoration efforts to take advantage of the broad spectrum of beneficial fungal-plant interactions occurring in specific environmental situations to solve problems in agriculture, reforestation and conservation of natural habitats.

**Acknowledgements**

This work was supported with a grant from CICYT of the Spanish Ministry of Science and Innovation (AGL2008-00716/AGR). We thank Drs. E. Seva (University of Alicante) and R. Rodriguez (U.S. Geological Survey/University of Washington) for useful discussions and supplying information for this article. We also thank Drs. A. de la Torre and M.A. Alonso (University of Alicante) for kindly supplying images of plants and their habitats.
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