Arbuscular mycorrhizal fungal communities associated with date palms in a traditional and a modern experimental plantation and with desert plants in the adjacent natural habitats in Southern Arabia

UNI BASEL

Inauguraldissertation
zur
Erlangung der Würde eines Doktors der Philosophie
vorgelegt der
Philosophisch-Naturwissenschaftlichen Fakultät
der Universität Basel

Dissertazione di dottorato di Ricerca
in
Biologia e Biotecnologia dei Funghi
Scuola di Dottorato in Scienza e Alta Tecnologia
Università Degli Studi Di Torino

von

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Basel, April 2008

Genehmigt von der Philosophisch-Naturwissenschaftlichen Fakultät

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Preface

In 1993, I started to study soil sciences at the Sultan Qaboos University, Oman. Since then, I have committed myself to participate in the scientific efforts for finding sustainable solutions for problems facing agricultural and natural ecosystems in my country. Years later, during my graduate studies, I realized the potential benefits of AM fungi on the fitness of plants living under extreme environmental conditions. I became convinced that for a better management of these fungi, there is a need for a deeper understanding of their biodiversity in such ecosystems. In 2003, I initiated a pioneering project: the exploration of AM fungal diversity in the ecosystems of Oman.

Table of contents

Table of contents

Summary

The ubiquitous symbiosis between plants and arbuscular mycorrhizal (AM) fungi is multifunctional. In this symbiosis, plants exchange photosynthates for phosphorus (P) and other mineral nutrients, and they gain increased resistance to soil borne diseases, drought and extreme temperature. All of these benefits might be crucial for plants growing in extreme environments.

The aim of this thesis was to shed light on the diversity and dynamics of AM fungal communities in Southern Arabia, known for its particularly arid conditions and low fertility of soils.

AM fungal communities in two agricultural sites were compared with those in adjacent natural habitats. The agricultural sites were cultivated with date palms (*Phoenix dactylifera*) and managed according to "traditional" and "modern" farming systems. The natural sites contained native plant species (among those *Zygophyllum hamiense, Salvadora persica, Prosopis cineraria* and *Heliotropium kotschyi*). Soil was sampled from the rhizosphere of plants and from these samples, AM fungal spores were isolated and morphologically identified. Furthermore, "trap cultures" were established in the green house, using the soil samples from the field as AM fungal inocula.

The results showed that the AM fungal community composition at the agricultural sites differed from that at the natural habitats. Agricultural sites had a much higher AM fungal spore abundance, species richness and inoculum potential supposedly due to the land-use change from natural to agricultural with irrigation and fertilizer application.

A molecular approach was used to identify the AM fungi colonizing the roots of the date palms at the two agricultural sites. Nine phylogenetic taxa were revealed, eight of which could be

attributed to the *Glomus* group A, the most diverse group in the Glomeromycota, and one to the *Scutellospora* group that occurred at the traditional agriculture site only. Two of the nine taxa could be associated to AM fungal species already described. These were *Glomus sinuosum* and *Glomus proliferum.* Three phylotype groups were associated with AM fungal sequences previously detected in environmental samples. The other 4 phylotype groups were not associated with any of the sequences in the GenBank nor in large database of the Botanical Institute and, therefore, we assume that they are new to science. The communities of these fungi were found to differ between the two agricultural sites and consisted of both site-specialist and site-generalist groups. This was in accordance with spore morphospecies differences found between the two sites. The composition of the detected phylotypes was quite unique because it lacked certain groups commonly occurring in most habitats around the world investigated so far.

Trap cultures inoculated with rhizosphere soils of date palms growing on a modern agricultural plantation showed an AM fungal community consisting of *Glomus aurantium, Glomus intraradices*, *Diversispora spurca*, *Acaulospora* sp. and five different *Glomus* phylotypes which presumably new to science.

Based on morphological identification of AM fungal spores, a total of 36 morphospecies were detected at the five sites investigated in Southern Arabia. Twenty two of them belonged to the genus *Glomus*, six to *Scutellospora*, four to *Acaulospora*, two to *Archaeospora* and one to each genus of *Paraglomus* and *Ambispora.* This is a quite high richness considering that so far only around 200 AM fungal species have been described worldwide in the phylum Glomeromycota.

The composition of AM fungal communities detected in this study was compared with communities found in other habitats of the world to seek for biogeographical patterns. It was found that the agricultural sites in the present study have a composition most similar to those

found at sites with sandy soils around the world. The natural sites, however, seem to maintain a unique species composition, which might have emerged due to unique local biotic and abiotic environmental factors of Southern Arabia.

To my knowledge, this is the first report on AM fungal communities in Arabian Peninsula and the first molecular investigation ever on AM fungi associated with date palm, a socio-economically important plant in many dry lands of the world. On a global scale, I believe that this work is a significant contribution to the knowledge on diversity, phylogeny and ecology of AM fungi.

Chapter 1: General introduction

1.1 The past and the present of AM fungi

Spores and hyphae strongly resembling today's arbuscular mycorrhizal (AM) fungi were found in 460-million-years old dolomite Ordovician rocks (Redecker et al., 2000a). This is the earliest known evidence for the existence of fungi. When plants first colonized the land, the most serious problem they faced was likely to have been acquiring phosphate ions which are known to be very poorly mobile in soils because they form insoluble compounds with most of the dominant cations in soils. Hence, plant roots in soil rapidly become surrounded by a depletion zone and uptake is then strongly limited by the rate of diffusion. Therefore, the symbiosis was a key factor in the colonization of land by plants (Nicolson, 1967; Pirozynski and Malloch, 1975).

Now, AM fungi are associated with about two-thirds of modern plant species (Fitter and Moyersoen, 1996), making this symbiosis the most ubiquitous and abundant terrestrial symbiosis. Several mycorrhizal types have evolved more recently (for example, ectomycorrhizas, ericoid and orchid mycorrhizas) all involving different fungi and different plant species compared to the ancestral AM symbiosis.

The first described AM fungus was from the genus *Glomus* (Tulasne and Tulasne, 1844). Since then, the history of AM fungal taxonomy has shown continuous changes. Recently, one of the general gaps regarding knowledge about AM fungal taxonomy and phylogeny was closed. Based on comprehensive small subunite (SSU) rRNA analyses, Schüßler et al. (2001) showed that the AM fungi can be separated in a monophyletic clade, which probably shares common ancestry

with the Ascomycota-Basidiomycota clade. They recognized a new fungal phylum, the Glomeromycota (Fig. 1.1), based on natural relationships for AM and related fungi. The phylum Glomeromycota currently consists of four orders, 10 families and 13 genera (Table 1.1).

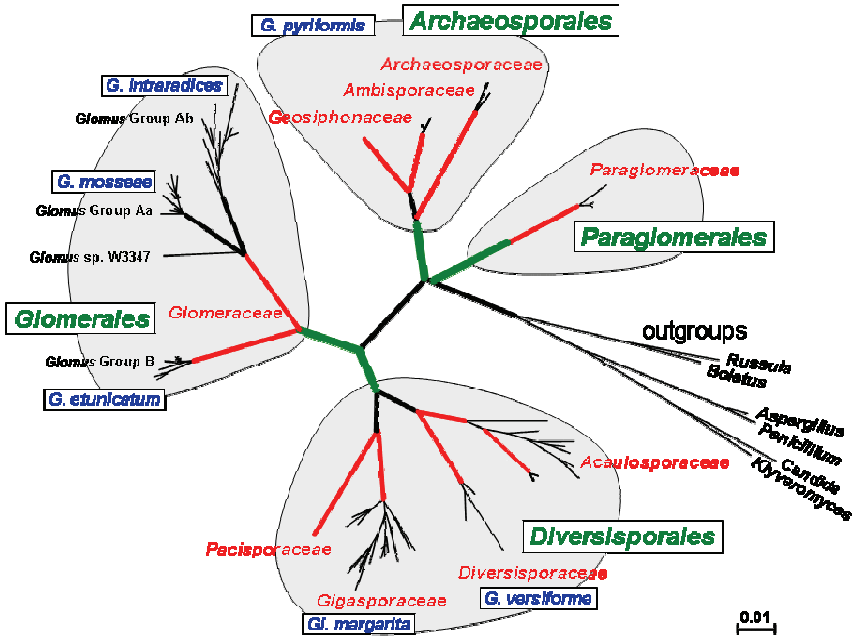

Fig. 1.1 The latest tree (updated 2008) of higher taxa within the Glomeromycota. Some model species are shown in blue.

From A. Schüßler's website: www.lrzmuenchen.de/~schuessler/amphylo/

Orders	Families	Genera
Glomerales	Glomeraceae	*Glomus*
Diversisporales	Gigasporaceae	*Gigaspora & Scutellospora*
	Acaulosporaceae	*Acaulospora & Kuklospora*
	Entrophosporaceae	*Entrophospora*
	Pacisporaceae	*Pacispora*
	Diversisporaceae	*Diversispora*
Paraglomerales	Paraglomeraceae	*Paraglomus*
Archaeosporales	Geosiphonaceae	*Geosiphon*
	Ambisporaceae	*Ambispora*
	Archaeosporaceae	*Archaeospora & Intraspora*

Table 1.1 Orders, families and genera of Glomeromycota. Table was modified from A. Schüßler's website.

1.2 The importance of AM fungi in natural and agricultural ecosystems

Symbiosis between plants and AM fungi is multifunctional (Newsham et al., 1995). In this symbiosis, plants exchange photosynthates not only for P and other mineral nutrients, but also for increased resistance to disease, drought and extreme temperatures (Smith and Read, 1996). Plants associated with AM fungi often show higher stomatal conductance and transpiration (Augé, 2004). This suggests that AM root systems obtain water of low activity more effectively. The moisture characteristics of a soil depend on the size and distribution of its pores. Because mycorrhizal fungi are effective in stabilizing soil structure through the production of glomalin, AM fungal colonization of a soil might affect its moisture retention characteristics (Wright and Upadhyaya, 1998) and, hence, the behavior of plants, growing in the soil, particularly when it is dry.

1.2.1 Roles of AM fungi in desert ecosystems

The diversity of AM fungal communities was investigated in many deserts of the world. In desert environments, AM fungal colonization was found to vary with the availability of water (Staffeldt and Vogt, 1975) and with the composition of the plant community (Hirrell et al., 1978).

In general, the species richness of AM fungi in deserts seems to be low. Stutz et al. (2000) reported a range of 7 to14 species at 13 sampling sites in two arid regions and semi-arid grass lands in North America and in the Namib Desert in Africa. Twelve AM fungal species were reported to be associated with 10 sites in the Namib Desert (Jacobson, 1997a). A similar number of species was reported from 3 sites in an arid region of Namibia (Uhlmann et al., 2006). (See Table A of the appendix for more detailed view about AM fungal diversity in desert habitats

around the world). Regardless of the generally low richness, AM fungal communities seem to play an important role in the survival of vegetation under the extreme environments of the deserts. Kiran Bala et al. (1989) reported >50% infection by AM fungi in 17 tree species of the Indian desert (Mathur and Vyas, 1995), suggesting an active AM fungal community. Cui and Nobel (1992) observed that due to AM fungi there was an improved hydraulic activity, an increase in CO_2, water and nutrient uptake in the desert succulents *Agave deserti, Ferocactus acanthodes* and *Opuntia ficus-indica*. AM fungi are also known to restore soil productivity by enriching soil organic carbon, as observed in *Prosopis juliflora* inoculated with *G. caledonium* (Mathur and Vyas, 1995). Beneficial effects of certain AM fungal species on *Moringa concanensis* were reported in conservation studies of this endangered multipurpose tree species in the Indian desert (Panwar and Vyas, 2002). During the slow succession process, a characteristic of arid habitats, it was hypothesized that mycorrhizal plant species gradually replace the non mycorrhizal plants (Reeves et al., 1979) due to the competitive edge that the former possess. There might even be certain AM fungal species which are indigenous to desert habitats. *Glomus deserticola,* for example, was found to be indigenous to many desert soils (Trappe, 1981).

1.2.2 AM fungi in agricultural ecosystems

The AM fungal functioning under agricultural systems might be influenced by many factors, including the levels of disturbance, fertilization and crop rotation. While these fungi were found to be an important part of sustainable agricultural systems (Bethlenfalvay and Schuepp, 1994; Jeffries and Barea, 1994), in regularly disturbed agricultural systems, delayed establishment of AM symbiosis was observed and thought to limit plant growth (Kuyper et al., 2004). Unless P

fertilization is added at the start, such a disturbance could result in limited P uptake by seedlings, as demonstrated for maize (Miller, 2000) or low nodulation as demonstrated for soybean (Goss and De Varennes, 2002). Crop rotation effects on AM fungal functioning have been observed. Harinikumar and Bagyaraj (1988) reported a 13% reduction in AM fungal colonization after one year cropping with a non-mycorrhizal crop. Especially in climates with an extended dry vegetationless season, inoculum insufficiency after a long bare fallow may result in low uptake of P and Zn and in plants with nutrient deficiency symptoms that have been described as long-fallow disorder. Overcoming this disorder by using an AM fungal host as cover crop was observed to be possible (Thompson, 1996).

AM fungi were used as inocula in agricultural practices and despite of all the challenges that such a biotechnology may face (Feldmann and Grotkass, 2002), cases of success have been reported in introducing these fungi under greenhouse conditions (Miller et al., 1986), in nurseries (Nemec, 1987) and in the field (Thompson, 1994). To benefit from AM associations in agriculture, emphasis has to be on practices that promote the occurrence and functioning of AM fungi. It has been shown that conventional agriculture, relying on tillage and external inputs, is often known to lead to a lower AM fungal species diversity compared to the natural lands (Helgason et al., 1998; Boddington and Dodd, 2000; Oehl et al., 2003).

1.3 The importance of the AM fungal community's richness

All the functions of AM fungi seem to be mutually incompatible (Fitter, 2005). Acquiring phosphate ions for example, requires an extensive extra radical mycelium, deployed far from the

root beyond the depletion zone, whereas binding roots to soil implies that the extra radical mycelium develops close to the root, and protection from pathogens involves the internal mycelium (Fig. 1.2). A fungus good at one of these functions might not be the best at another. Jakobson et al. (1992) and Smith et al. (2000) demonstrated differences in mycelial growth and phosphorus acquiring strategies among species of AM fungi. Variation in resource acquisition patterns should mean that an increased diversity of AM fungi will more efficiently extract resources from the soil. Furthermore, the fungal community has to respond to environmental biotic and abiotic factors and it is reasonable to assume that different fungal taxa respond distinctively to soil pH, temperature, soil moisture, disturbance and other factors and therefore, biotic and abiotic niche differentiation was suggested (Fitter, 2005).

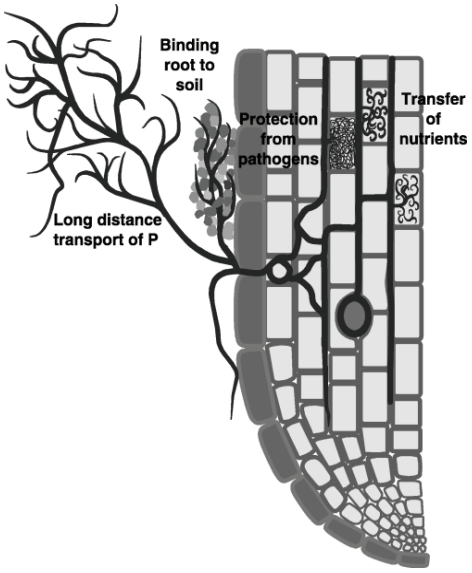

Fig. 1.2 An illustration of the suggested incompatible morphological requirements of different mycorrhizal functions. The extra radical mycelium remote from the root is necessary for the P transport. Improving water relations necessitates maintaining the root-soil bond and hence the water pathway as soil dries, and involves extra radical mycelial development in the rhizosphere. Protection from pathogens and P transfer both depend on the intra radical mycelium. Diagram from Fitter (2006).

The AM fungal community appears to extend its influence to the level of plant populations and communities. This influence was demonstrated by van der Heijden et al. (1998a) who, interestingly, found that increasing the number of introduced AM fungal species in an artificial system led to increases in both plant productivity and diversity. The proposed mechanism for this effect was based on other experiments of the same authors who demonstrated that AM fungal composition have the potential to determine plant community structure through the differential effects of these fungi on plant growth (van der Heijden et al., 1998b). Read (1998), suggested that the relationship between fungal richness and plant diversity is due to a greater probability of "functionally compatible" plant-fungus combinations.

1.4 Factors controlling AM fungal composition

1.4.1 Effects of host plant

Host specificity and the influence of plant species composition have also been demonstrated in natural ecosystems, using both spore production (Eom et al., 2000) and AM fungal DNA (Husband, 2002; Sýkorová et al., 2007). Using an artificial system, Burrows and Pfleger (2002) found that richer plant communities supported greater AM fungal spore production and spore richness. Assessing AM fungal DNA, Johnson et al. (2003) found that the composition of artificial plant assemblages had a significant impact on AM genetic diversity.

Such an influence of plant community on AM fungal composition might partially be due to the roots exudates. Plant roots exude not only carbohydrates, to be used as the energy source for AM fungi, but also a wide variety of other organic compounds including amino acids, nucleotides,

phenol aldehydes, and esters (Koske and Gemma, 1992). Root exudates from host plants have been shown to influence both spore germination and hyphal growth in some species of AM fungi (Gianinazzi-Pearson et al., 1989). It was suggested as well that infection with AM fungi is regulated by the anatomical features of the root (Bonfante-Fasolo and Vian, 1989). Recently, it was elegantly demonstrated that the host plant prepares and organizes AM infection of the root, and both a plant–fungal signaling mechanisms are involved in the process of colonization (Genre et al., 2005).

1.4.2 Effects of abiotic conditions

The distribution of some AM fungal species was demonstrated to be dependent on soil type, host species and some on specific plant–soil combinations (Johnson et al., 1992). Some AM fungal species were reported to be able to access organic phosphorus (Koide and Kabir, 2000) and organic nitrogen (Hawkins et al., 2000; Hodge et al., 2001) and therefore, the characteristics of soil organic matter may play a greater role in determining AM fungal species composition than previously thought. Many of the abiotic influences on the AM fungal communities are indirect effects of the plant community, via organic matter deposition. Differences in pH, nutrient status and phenolic content of the soil organic horizon, which all may impact fungal growth, are mainly due to inputs from the plant community (Hobbie, 1992; Wardle, 2002). Disturbance was already mentioned to be a strong factor influencing AM fungal community (Helgason et al., 1998; Boddington and Dodd, 2000; Oehl et al., 2003). More abiotic factors will be discussed in relationship to that of the Southern Arabia in the following four Chapters of this thesis.

1.5 Evaluating taxonomic diversity of AM fungal communities

The taxonomy of AM fungi has traditionally been based on the morphology of the spores. Families and genera were mainly distinguished by the hyphal attachment and the mode of formation of the spore, whereas spores are identified mainly based on the substructure their walls (Gerdemann and Trappe, 1974; Morton, 1988; Schenck and Perez, 1990; Walker, 1992). Using the morphological taxonomy of AM fungi, numerous studies on ecology and composition of AM fungal communities in different parts of the world were conducted (Table A, Appendix). Morphological taxonomy was used in this thesis to explore the diversity of AM fungi in both natural and agricultural sites of Southern Arabia.

Based on morphological taxonomy, the Glomeromycota was viewed as a species-poor group, with around 150 species recognized (Morton and Benny 1990). With other new species identified since then, the number raised to around 200 species (A. Schüßler's website: www.lrz-muenchen.de/~schuessler/amphylo/). Since a small number of AM fungal species can associate with around 250 000 plant species, it is self-evident that these fungi are not host specific. Indeed, it has been suggested that theoretically, mutualists, in contrast to species in antagonistic relationships, should have broad host ranges, because there is a benefit in being able to acquire carbon from as many hosts as possible (Law and Lewis, 1983). However, molecular evidence has now started to challenge that view.

Several molecular approaches have been used to develop tools that would allow identification of AM fungi colonizing plant roots, independent from spore formation. Most authors used approaches based on the existing variabilities within ribosomal DNA to identify AM fungi. Provided a sufficient database of sequences and knowledge of phylogeny, this approach allows

designing specific PCR primers to target species, genera or any other level of taxonomy. Simon et al. (1992) conducted the first pioneering studies of molecular phylogeny and detection of AM fungi. The authors designed the primer VANS1, based on only three 18S subunit sequences from the Glomeromycota, and a set of other primers specific for subgroups. As more sequences became available from the Glomeromycotan taxa, it became clear that the VANS1 primer did not amplify all AM fungi (Clapp et al., 1999; Schüßler et al., 2001). Using the group specific PCR primers for a portion of the 18S rDNA designed by Simon et al. (1993b), Clapp et al. (1995) performed the first molecular study of a field population of AM fungi. While their PCR results for *Acaulospora* and *Scutellospora* species were in agreement with the spores present, there was a difference between strong root colonization by *Glomus* and absence of sporulation. Moreover, concurrent colonization of the same five cm root length by all three genera was demonstrated. These results underlined the need to apply molecular methods in order to obtain data on AM fungal populations in roots.

Other authors targeted different parts of the ribosomal genes, e.g. the large subunit (Kjøller and Rosendahl, 2000; Van Tuinen et al., 1998) or the Internal Transcribed Spacers (Redecker, 2000). van Tuinen et al. (1998) employed a nested PCR procedure to avoid the problem of PCR inhibitors present in many root samples. These authors also found that root fragments of one cm length were colonized by more than one fungus. In this study, a complex pattern of fungal species interactions was detected. The colonization by the two species from the Gigasporaceae was significantly enhanced in combination with certain others, suggesting interactions among species.

Helgason et al. (1998, 1999) used a variable portion of the 18S small subunit that was amplified in a single step from roots with the PCR primer AM1. This primer discriminates most AM fungal

and plant DNAs. The initial screening of the PCR products was performed by a RFLP analysis, before they were sequenced. Using the primer AM1 in combination with NS31 (a universal eukaryotic primer), Helgason et al. (1998) reported that the AM fungal species diversity in cultivated soils was strikingly lower than a seminatural woodland. The authors attributed the differences in AM fungal species composition to soil disturbance by ploughing in the cultivated field sites. Helgason et al., (1999) demonstrated by using the same combination of primers that AM fungal communities in roots of bluebell (*Hyacynthoides non-scripta*) in the woodland site was characterized by complex seasonal patterns and a significant influence of the plant type. A similar approach was used here to investigate the AM fungal community associated with agricultural and natural plants in Southern Arabia (Chapter 2 of this thesis).

A set of five primers targeted at major phylogenetic groups within the Glomeromycota (Fig. 1.3) was designed to amplify parts of the 18S ribosomal subunit, the ITS and the 5.8S subunit (Redecker, 2000; Redecker et al., 2003). The advantage of these primers is that they amplify *Archaeospora* and *Paraglomus* which had not been detectable with previously designed primers. This approach was successfully used to study the AM fungal communities in dry habitats of Namibia (Uhlmann et al., 2005), to investigate the AM fungal communities in different land uses in Central Europe (Hijri et al., 2006) and to demonstrate pattern of host specificity in cooccurring plants in Swiss upper mountains (Sýkorová et al., 2007). It was also used in the present thesis (Chapters 2 and 4) because of its amplification efficiency especially from field roots.

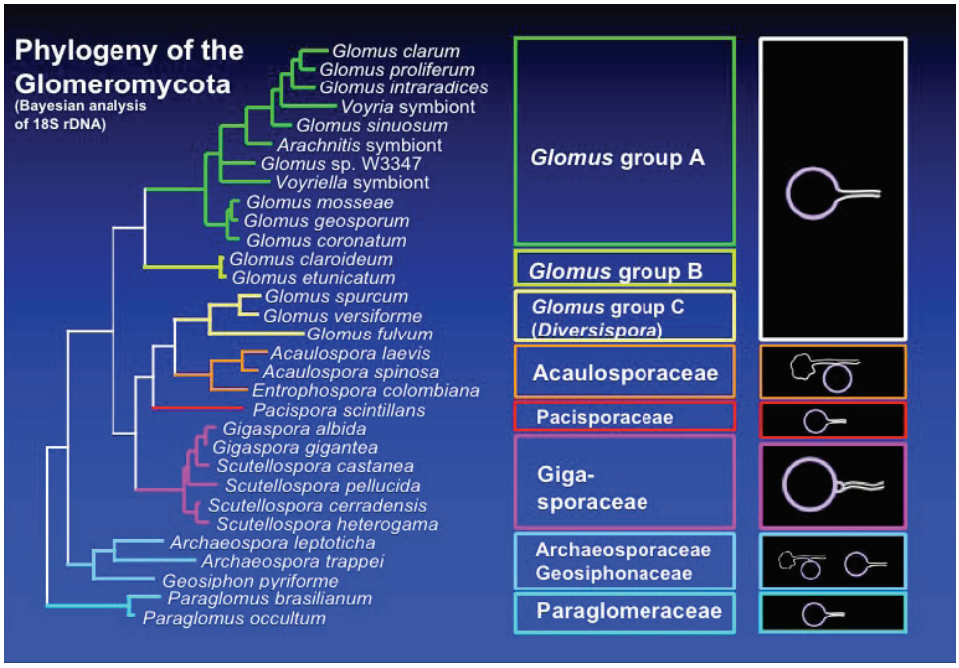

Fig. 1.3 The main Glomeromycotan groups which were targeted by the primers designed by Redecker (2000) and Redecker et al., (2003). Phylogenetic tree by Dirk Redecker.

Some of the diversity encountered in these studies maybe within rather than among individuals (Rosendahl and Stukenbrock, 2004). What might be described as single morphospecies, can contain extensive genetic variation (Lanfranco et al., 1999). There is no easy correlation between sequence identity and species identity, as each spore harbours many sequence types. There is no phylogenetic species concept for the Glomeromycotan members and several aspects of their genetics continue to be a mystery (Redecker, 2002).

Nevertheless, the existing molecular tools described above were and still very useful. They brought a solution for a major problem of ecological studies on AM fungal communities which was the inability to directly identify AM fungi colonizing plant's roots. Molecular studies concluded that many phylotypes of AM fungi occur in any community and even on the roots of one species, with sometimes as many as 20 associated with a single plant (see Öpik et al. 2006

for review). Interestingly, these studies concluded as well that many of those types are not ones known from the culture collections and might represent new taxa which are specialized on certain ecosystem, while others are clearly widespread taxa that are found almost ubiquitously in other habitats.

1.6 Environmental settings of Southern Arabia and the relevance of AM fungi

Natural vegetation in Southern Arabia (Fig.1.4) faces various environmental challenges. Despite occasional heavy rain, most of the area is characterized as hyper-arid today (Fisher and Membery, 1998). The annual rainfall generally occurs during the winter. The precipitation is ≈100 mm per year (Jones et al., 1988). More than half of the region has maximum summer temperatures which exceed 50°C (Glennie and Singhvi, 2002). Natural vegetation that survives these conditions is described in Ghazanfar and Fisher (1998).

Agriculture in Southern Arabia as well faces environmental challenges. These are the infertile nature of the soils, in addition to soil salinity, drought, light soil structure (MAF. 1989, 1991). High P fixation where phosphorus fertilization is not available to plants is another problem (Cookson, 1996). It is known that inorganic P added to the soil may undergo complex exchanges between various soil P pools (Stevenson, 1986), making most of the applied P unavailable for plant uptake. This is a common problem in arid lands where many soils have extremely high P fixation capacities. Cookson (1996) found that in some agricultural areas of Southern Arabia, 23-76% of phosphorus fertilization was fixed and was not available to the plants.

As already discussed, AM fungi were found to play an important role in the survival of natural vegetation in desert ecosystems. They are also an important part of sustainable agricultural systems. In a situation where soil P is limiting, the AM fungal community may be especially beneficial for the plant (Smith and Read, 1997). Thus, in the environmental conditions of Arabia with the low soil fertility and where P is limiting, AM fungal communities might be of great relevance. Nevertheless, Arabia is paradoxically still one of the least studied areas with respect to AM fungal communities. There are just two known AM fungal morphospecies reported so far (Malibari et al, 1988; Khaliel, 1989). Therefore, some basic background knowledge on the native biodiversity of these fungi in the target habitat is crucial and a pre-requisite for agriculture and ecosystem planning. In addition, knowing the diversity of these fungi in such a unique habitat would contribute significantly to the understanding of the global ecology and biogeography of such an important symbiosis.

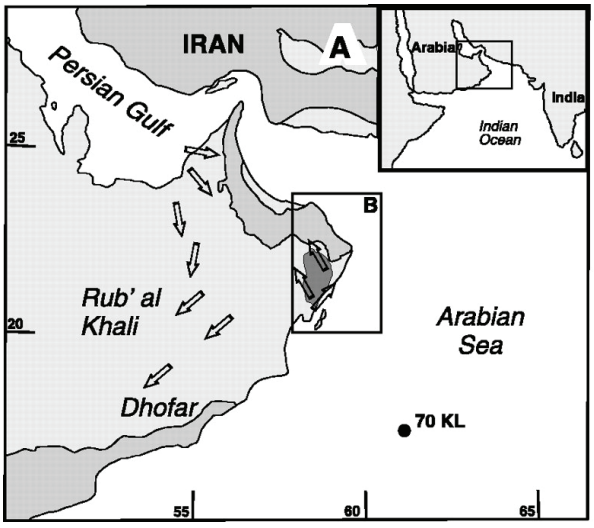

Fig. 1.4 Map of the area in Southern Arabia where the present study was conducted (frame B). Map from Preusser et al. (2002).

1.6.1 AM fungi associated with date palms

AM fungi associated with date palm (*Phoenix dactylifera*), were a main focal point of this thesis. It is the main food crop in Southern Arabia and some other dry areas of the world, cultivated since prehistoric times. Date palm is widely believed to be indigenous to the countries around the Arabic Gulf. According to Food and Agriculture Organization of the United Nations (FAO), the world harvested area with date palms has increased 4.3 fold from 263,665 ha in 1970 to 1,130,803 ha in 2005. This increase in the harvested area led to a yield increase of only 3.7 fold from 1,881,730 to 6,924,975 million tons (FAO statistics, 2006).

AM fungi were found to promote the growth of date palm seedlings, especially on nutrient poor soils (Al-Whaibi and Khaliel, 1994). However, the AM fungal communities associated with date palm in the whole world have not been well investigated. To our knowledge, the only study which addressed the composition of AM fungal communities associated with this tree in field conditions was conducted in North Africa (Bouamri et al., 2006). Based on the classical identification method by spore morphology, this study reported 10 AM fungal morphospecies.

1.6.2 Land-use change impact on native AM fungi

In Southern Arabia, date palms were traditionally cultivated in oases with a natural source of water. However, the dramatic growth of the economy in Arabia in the last few decades has revolutionized the status of agriculture in that arid area. Applying new technologies to trace the sources of underground water has led to discoveries of new water sources. Electrical pumps were used in a large scale to extract underground water for agricultural purposes. Due to the partial overcoming of the lack of water which is the main natural obstacle for agriculture in many arid lands, there was and still a tendency to a large scale introduction of agriculture in many desert

habitats under modern irrigation systems and conventional agricultural practices. Consequently, the areas cultivated with date palm had increased in the GCC countries (Arabia Peninsula without Yemen) from 56,340 ha in 1970 to 390,300 ha in 2005 (FAO Statistics, 2006), an increase of 693%.

In Southern Arabia in the Sultanate of Oman, date production increased by 93% in the years between 1991 and 2001, making it the world's highest increase for that period (FAO, 2002). This dramatic rise in production is paralleled with an expansion in land-use change from natural to agricultural. The effect of such a dramatic land-use change on native AM fungal communities of the converted lands is not known. Furthermore, in the face of such a dramatic land-use change, one crucial issue to be understood is the response of the native AM fungal communities to the alterations in their habitats.

1.7 Objectives of the thesis

The general aim of this thesis was to shed light on the diversity and dynamics of the AM fungi in Southern Arabia, one of the globally least known areas in this respect. This investigation was focused on a natural and an agricultural ecosystem. The work specifically is divided into three parts (Fig. 1.5) as follows:

1. Evaluation of the taxonomic diversity of AM fungal communities associated with natural vegetation (*Zygophyllum hamiense, Salvadora persica, Prosopis cineraria* and *Heliotropium kotschyi*) and agricultural crops (*Phoenix dactylifera* and *Mangifera indica*) using both morphological and molecular approaches (Chapter 2).

2. Evaluation of the impact of a change from natural sites to agricultural land-use on the composition, abundance, species richness and inoculum potential of the native AM fungal communities (Chapter 3).
3. Molecular identification and comparison of the AM fungal communities associated with *Phoenix dactylifera* grown under two different management systems (Chapter 4).

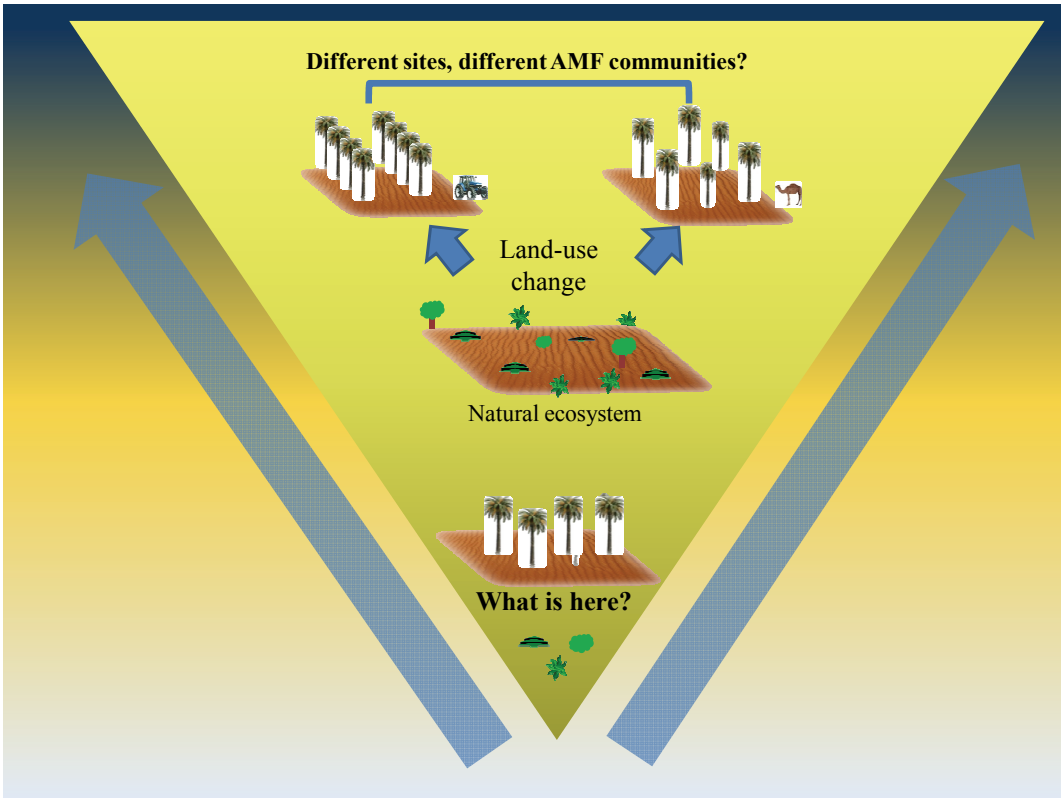

Fig. 1.5 A representation of the aims of the thesis. It is organized through different levels from the bottom to the top of the graph. At the basic level, it was important to survey the AM fungi present. A more sophisticated experimental design was applied to obtain preliminary data on how the introduction of agriculture can influence the already existing AM fungal community. A molecular approach was then applied to check whether the two introduced agricultural sites have the same or different AM fungal communities.

Chapter 2: AM fungi of Southern Arabia: a glimpse on their diversity

2.1 Abstract

The AM fungi associated with the rhizospheres of some plants in Southern Arabia were investigated. *Phoenix dactylifera* and *Mangifera indica* both cultivated at the same site in addition to naturally growing *Zygophyllum hamiense* and *Salvadora persica* in nearby sand dunes were chosen. The first step in this work was to view the presence of AM fungi in the roots of studied plants through staining with cotton blue and observing under light microscope. Spores were isolated and the AM fungal species present were identified. Soil and roots from the rhizospheres were used as inocula for a trap culture system. A molecular approach was used to analyse the AM fungal community in the roots of the trap culture plants. Morphologically, 15 AM fungal species were found to be associated with the studied plants. Sequences associated with *Glomus aurantium, Glomus intraradices*, *Diversispora spurca* and *Acaulospora* sp. and five different unknown *Glomus* entities were detected.

2.2 Introduction

This introductory chapter presents the first step in our progress to study the biodiversity of AM fungi of Southern Arabia. A project road map for the work of this chapter is shown in Fig. 2.1. It includes the use of both morphological and molecular approaches. Four plant species were selected. Among them were date palm *(Phoenix dactylifera*) and mango (*Mangifera indica*) (Fig.2.2). These two have an important agricultural and socio-economical value in Southern Arabia. The other two plants were *Zygophyllum hamiense* and *Salvadora persica* (Fig. 2.3) representing a part of the natural growing vegetation at the margin of a sand dune habitat, known as Wahiba Sands. The name of the habitat has been recently changed to Al-Sharquia Sands. However, the former name is still used here because all the previous scientific work referred to this habitat as Wahiba Sands.

Since the occurrence of PCR inhibiting compounds in the field roots has often been reported, a trap culture technique (Fig. 2.4) was used in this study to explore the molecular diversity of AM fungi. Using molecular tools, the trapped taxa are in general easier to identify than those which occur in the field roots.

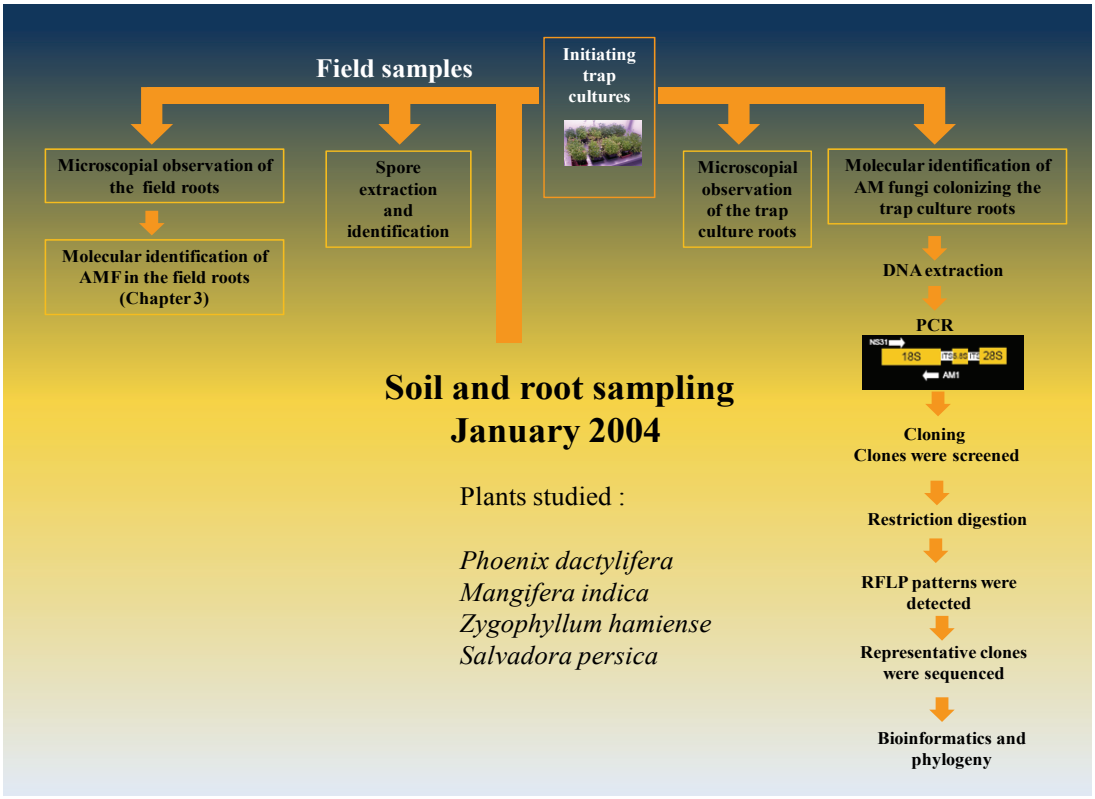

Fig. 2.1 The roadmap of the work presented in this chapter. It includes the morphological and molecular approaches used to explore the diversity of AM fungi in the target habitat. Soils sampled in 2004 were used as inocula for trap culture system. The identity of the AM fungi colonizing the roots of the trap plants was identified by molecular methods. One part of the sampled soils was used to extract the AM fungal spores and identify them morphologically. The field roots were stained and observed under a light microscope. The AM fungi colonizing the roots of *Phoenix dactylifera* in the field were also identified by molecular methods (see Chapter 4).

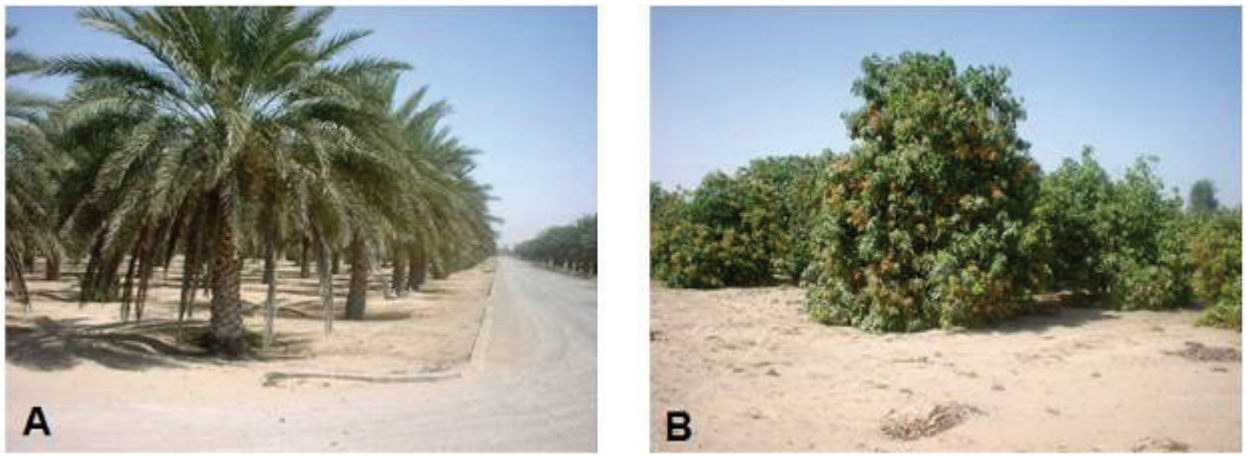

Fig. 2.2 The agricultural station where samples were collected. (A) *Phoenix dactylifera* and (B) *Mangifera indica.* Photos were taken during the sampling trip in January 2004

Fig. 2.3 Natural habitats where the soil samples were collected. **(A)** Part of the natural vegetation growing on the edge of the Wahiba Sands. **(B)** Trees of *Prosopis cineraria*, as part of the natural vegetation. In the back of the vegetation, the mega sand dunes rise. **(C)** Both *Salvadora persica* (S) and *Zygophyllum hamiense* (Z). **(D)** *Salvadora persica* **(E)** *Zygophyllum hamiense* **(F)** Part of the natural vegetation growing on the top of the sand dunes. Photos were taken during the sampling trip January 2004.

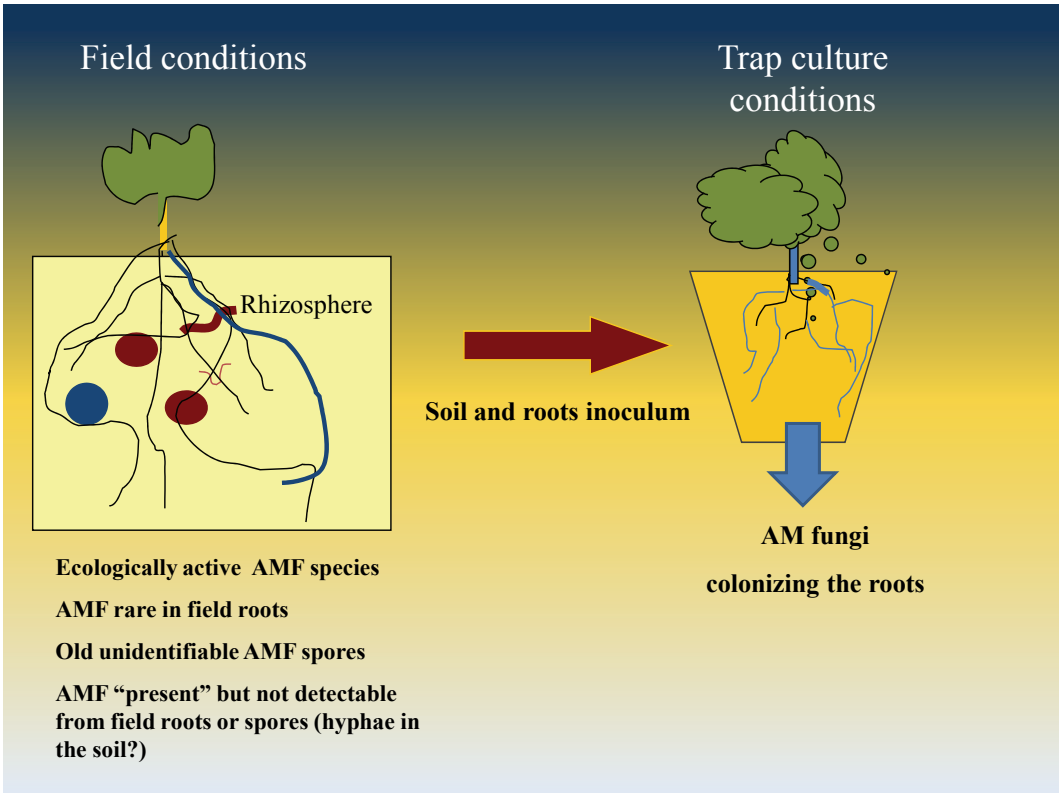

Fig. 2.4 AM fungal communities are present in different forms in the rhizosphere and in trap culture systems. Only AM fungal entities colonizing the trap culture roots were identified in the work of this chapter. Trap culture systems are often used in the studies of mycorrhizal diversity. They are based on the use of field soils as inocula for "trap plants" under green house conditions. The aims of this chapter were:

1. To check the mycorrhization status of the field roots of the studied plants by staining and observing them under light microscope.
2. To morphologically identify the AM fungal spores isolated directly from the field soils.
3. To use molecular methods to characterise the AM fungi colonising the trap culture roots.

2.3 Materials and methods

2.3.1 Sampling sites

The natural site

The natural site is located about 10 km west of the agricultural site. It is on the eastern margin of the Wahiba Sands which are spectacular sand dunes in the South-Eastern Arabia Peninsula, (approximately 20' 45" and 22' 30" north and 58' 30" and 59' 10" east). This habitat is characterized by a low level of biodiversity due to the extreme environmental conditions where shortage of water is a key factor (Munton, 1988). Beside some of the vegetation which appears after the rare rainy season, the two sampled plants in the present study are among the few plant species permanently growing in the area.

The agricultural site

The management of this site has followed the modern way of farming since the establishments of the farm in 1990, i.e., synthetic pesticides were used rarely and chemical fertilizers (mainly NPK) have been added annually in reasonable amounts. For more details about this site, see the modern site description in materials and methods section of Chapter 3.

2.3.2 Sampling

Sampling took place on January 2004. Each of the four plants was sampled in five replicates. At the agricultural site, soil cores were sampled in the depth of 0-30 cm whereas for the natural plants, soils around to the plant root systems were collected. Roots were washed with tap water and kept cool inside microcentrifuge tubes during the whole period of transportation. Soils were air dried and packed in plastic bags. Subsamples were used as inoculum for the trap culture system and for the spore extraction.

2.3.3 AM fungal trap culture

For each of the five replicate samples of each studied plant, one trap culture pot was established. In total there were 20 pots, all equipped with drainage mat and filled with 3 kg of autoclaved commercial sand. The mycorrhizal inocula which consisted of soil and roots were placed on the surface of the substrate in the pots. Each pot received 100 g of inoculum. The inoculum was then covered with a layer of autoclaved commercial sand. *Trifolium pratense* seeds were randomly planted in every pot. This trap plant species was chosen because it is well known as AM fungal host plant and frequently used for trap culture systems. The trap cultures were kept in a greenhouse for 8 months from May until December 2004.

2.3.4 Microscopy and mycorrhizal parameters evaluation

Morphological characteristics of the colonizing AM fungi where recorded in both the roots coming from the field and from the trap culture system. Roots were incubated overnight in room temperature in 0.1% cotton blue (w: v) in lactic acid. Segments then were destained in lactic acid and observed under a light microscope. The morphology and anatomy of the AM fungi in the colonized roots were recorded.

2.3.5 AM fungal spores isolation and identification

AM fungal spores occurring in the field soil samples were extracted by wet sieving and sucrose density gradient centrifugation (Daniels and Skipper, 1982). Spores were extracted from three soil samples (25g each) from the rhizosphere of each *Phoenix dactylifera*, and *Mangifera indica*, and two samples from each *Zygophyllum hamiense* and *Salvadora persica.* For details about this extraction method, see the materials and methods section of Chapter 3**.** Identification was based on current species morphological descriptions and an identification manual (Schenck and Perez,

1990; International Culture Collection of Arbuscular and Vesicular-Arbuscular Endomycorrhizal Fungi [http://invam.caf.wvu.edu/Myc_Info/Taxonomy/species.htm]).

2.3.6 DNA extraction from roots colonized by AM fungi

DNA was extracted from approximately 5cm of fresh root fragments of each trap culture plant using DNeasy Plant Mini Kit (QIAGEN) according to the manufacturer's instructions.

2.3.7 PCR amplification

Partial SSU DNA fragments were amplified (550 bp) using a universal eukaryotic primer NS31 (Simon *et al*. 1992) and an AM fungal specific primer AM1 (Helgason *et al*. 1998). The PCR reaction was performed in the presence of 0.2mM dNTPs, 10 pmols of each primer and the manufacturer's reaction buffer. PCR was carried out for 30 cycles on a GeneAmp PCR system 2700 (Applied Biosystems). Checking the PCR product in gel electrophoresis confirmed the success of the amplification from some of the sampled roots. The PCR product was purified using QIAquick purification kit (QIAGEN) according to the manufacturer's instructions and was then checked in a 2% agarose gel using a low DNA low mass ladder (Invitrogen) as a marker. The band intensity (Fig.2.8) was used to estimate the concentrations of the purified PCR products to optimize the vector/PCR product ratio for cloning as recommended by the pGEM-T Vector system I (Promega).

The AM1 primer became known to exclude a number of fungal types from previously unrecognized groups, the Archaeosporaceae and Paraglomaceae which were discovered by Morton and Redecker (2001). Therefore, primer combinations of ARCH1311A/B-ITS4i and PARA1313-ITS4i (Redecker, 2000), which were designed to target Archaeosporaceae and

Paraglomaceae respectively, were used on the roots of 3 different trap cultures of *Phoenix dactylifera.* The full approach of the nested PCR is described in the methodology of Chapter 4.

2.3.8 Cloning

The resulting product was cloned into a pGEM-T Vector system I (Promega) and transformed into *Escherichia coli* (strain: XL10-StrataGen). To obtain PCR products for restriction fragment length polymorphism (RFLP) analysis, positive transformants were selected by picking the bacterial colonies with a toothpick into 10µl of H_2O and then 15µl of the PCR mix was added to each sample tube. Amplification was performed with the vector primers SP6 and T7.

2.3.9 Restriction analysis

The positive clones products were digested with restrictions enzymes *Hinf* I and HSP 92 II. 4ul of each PCR product was mixed with 16ul of digestion mix (2.0ul buffer 10x, 0.2ul BSA, 13.3ul H_2O and 0.5ul restriction enzyme) for 3 h at 37^oC. Fragment patterns were analyzed on agarose gels containing 1.5 % agarose (BDH Laboratory Supplies, England) and 1.5% high resolution agarose (Sigma)

2.3.10 Sequencing

Clones were selected for sequencing on the basis of the HSP 92II and *Hinf*I RFLP types. One clone from each RFLP type found in each root sample was sequenced.

2.3.11 Phylogenetic analysis

The Glomeromycotan origin of the sequences was initially tested by BLAST. Those sequences in GenBank which show high similarity to the blasted sequences were obtained. Using PAUP*4b10

(Swofford, 2001), sequences from the present study were aligned to previously published ones (Fig. 2.9 and Fig. 2.10). Phylogenetic trees were obtained by distance analysis using the neighbor joining algorithm in PAUP*4b10 using the Kimura two-parameter model and a gamma shape parameter=0.5. In the phylogenetic trees, sequence phylotypes were defined in a conservative manner as consistently separated monophyletic groups.

2.4 Results

2.4.1 The mycorrhizal structures in field roots

AM fungal structures were observed in the original roots coming from the field (Fig. 2.5) mainly in the roots from agricultural plants *Phoenix dactylifera* and *Mangifera indica* which were found to be colonized. Estimation of the mycorrhization levels was not possible due to the difficulty in assessing the mycorrhizal structures, especially the arbuscules.

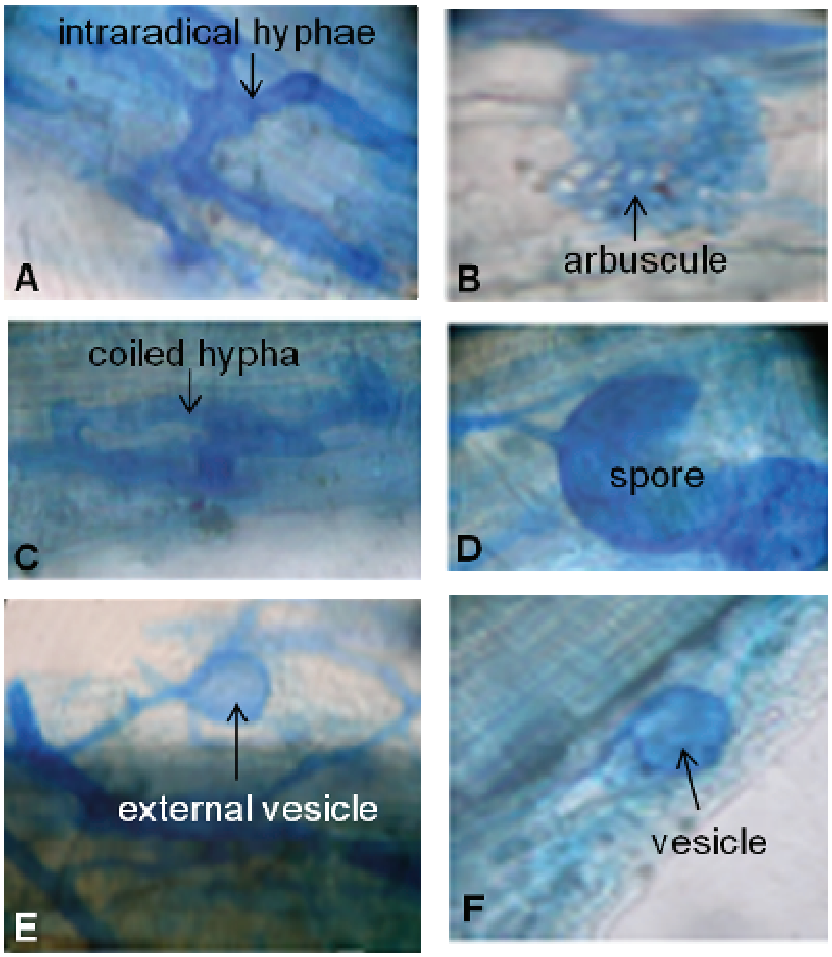

Fig. 2.5 Some of the morphological structure of AM fungi in the field roots as recorded using the light microscope.

2.4.2 The mycorrhizal structures in trap culture roots

The AM fungal structures in the roots of the trap plants were easier to observe than the field roots (Fig. 2.6). All the trap plants without any exception were found to be colonized by AM fungi.

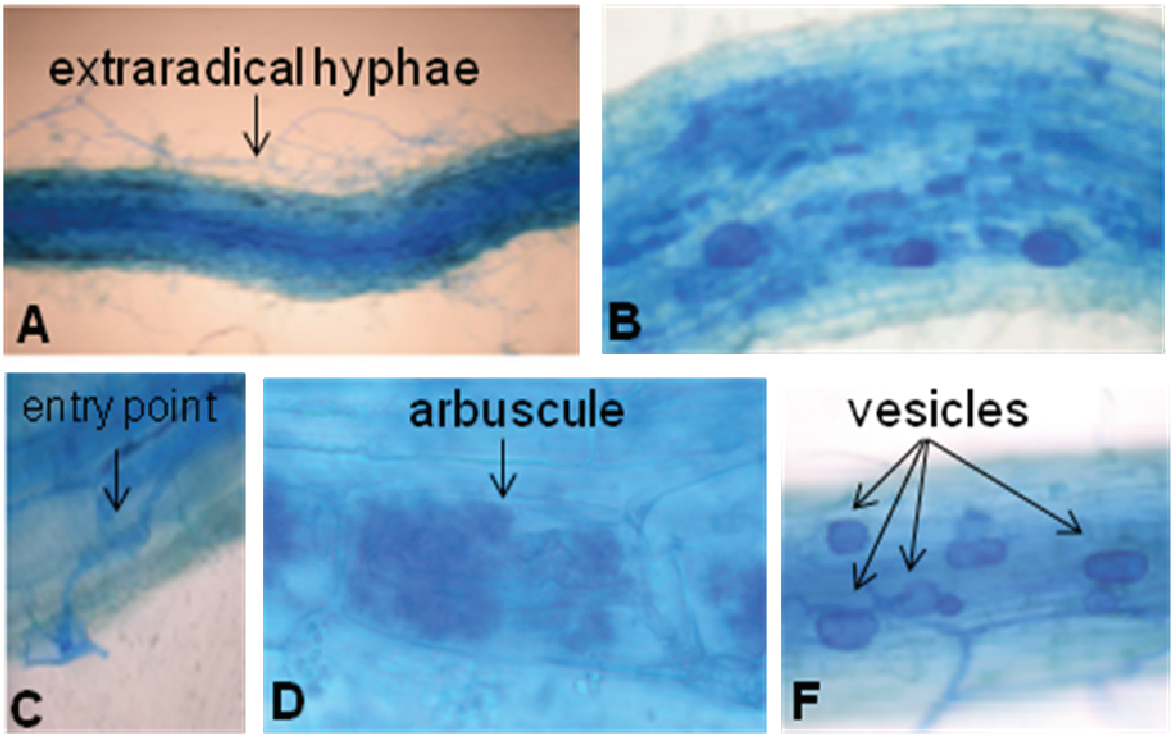

Fig. 2.6 Infection patterns of the AM fungi in the roots of the trap cultures as recorded using the light microscope.

2.4.3 Morphological diversity

Spores extracted from the field were identified morphologically. Fifteen morpho-species were recorded in the rhizospheres of the four studied plant species (Table 2.1). Eight of the species belonged to the genus *Glomus*, three to *Acaulospora* two to *Scutellospora* and two to *Archaeospora*. The morphologies of some of the identified spores are shown in Fig. 2.7. Many of the extracted spores were difficult to be identified morphologically, due to their degraded wall layers which are their main identification key. The global distribution of the detected species and the available information on the host plant and/or the type of land use are given in Table A of the appendix with all the other detected species in this thesis.

Plant's rhizosphere	Agricultural plants		Natural plants	
	P.dactylifera	*M. indica*	*Z. hamiense*	*S persica*
Glomus eburneum		X		
Glomus aureum		X		X
Glomus intraradices		?		?
Glomus hoi		X		
Glomus constrictum	X	?		X
Glomus macrocarpum			X	
Glomus invermaium			?	
Glomus sinuosum	X			
Acaulospora sp.			X	X
Acaulospora scrobiculata			X	
Acaulospora morrowiae	X			
Scutellospora biornata			X	
Scutellospora persica	X	X		
Archaeospora trappei	X			
Archaeospora sp.				X

Table 2.1 Fifteen morphospecies detected in the field soils of the 4 studied plants. The question mark indicates uncertainty in the species identification.

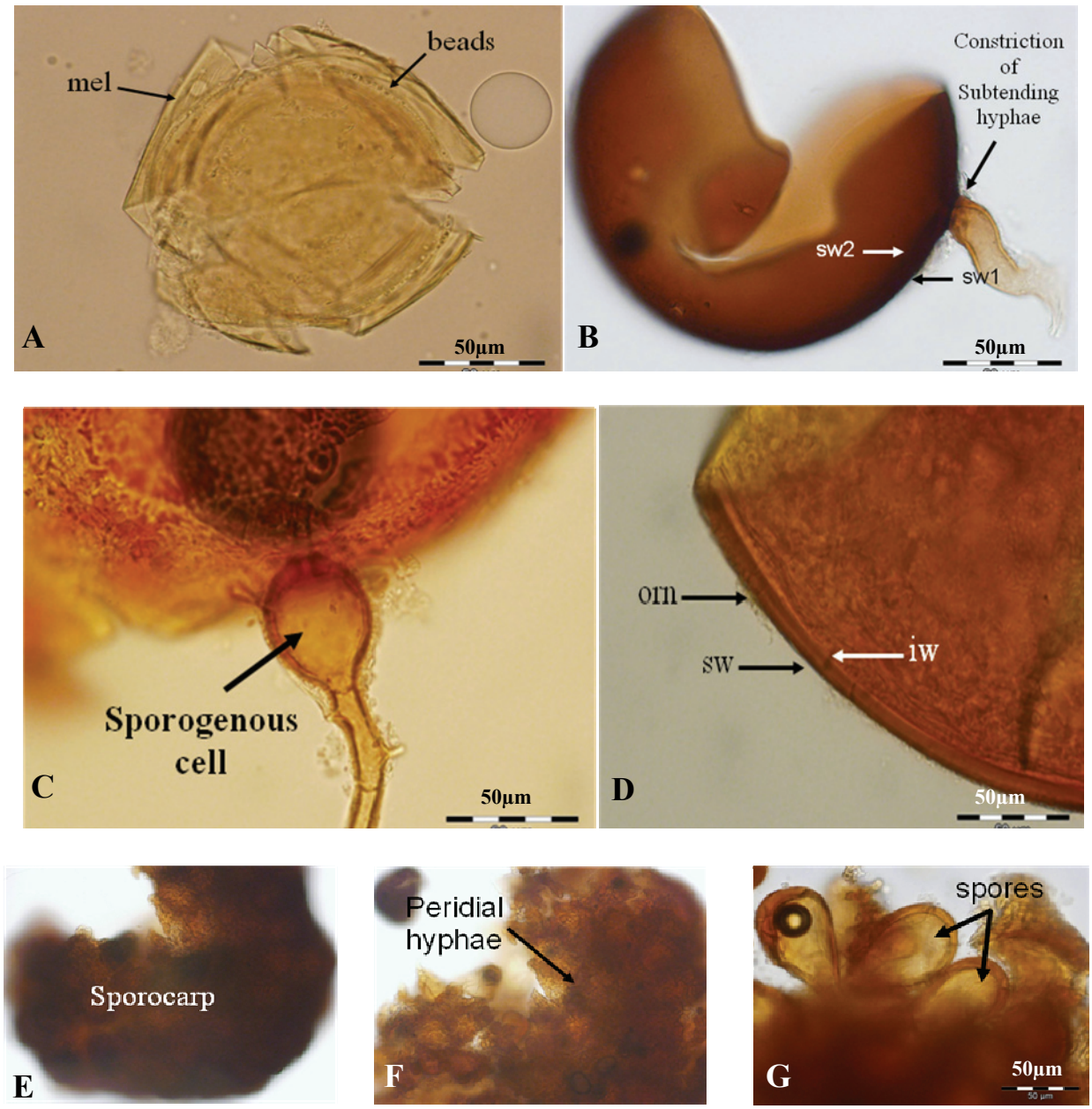

Fig. 2.7 (A) Old spore of *Acaulospora morrowiae* with 'beaded' inner wall; innermost sublayer staining in Melzer's reagent (mel). **(B)** *Glomus constrictum* spore isolated from the rhizosphere of *Phoenix dactylifera* L and *Salvadora persica* L. **(C-D)** Old spore of *Scutellospora sp*. closely resembling *S. persica* with one inner wall group (iw) and a fine ornament (orn) on the outer surface of the spore wall (sw). This species was isolated from the rhizosphere of both *Phoenix dactylifera* and *Mangifera indica*. **(E-G)** Sporocarp and spores of *Glomus sinuosum* found in the rhizosphere of *Phoenix dactylifera*.

2.4.4 Molecular diversity

The molecular approach used in this analysis was based on an AM fungal-specific PCR strategy targeting the 18S rDNA (Helgason et al., 1998). In addition, two pair of primers designed specifically to target Archaeosporaceae and Paraglomaceae, which are most likely to be missed by AM1 primer, were used. BLAST search results indicated that the obtained sequences belong to Glomeromycota. The phylogenetic analysis based on the sequences and the closest matches among sequences found by a BLAST search of GenBank indicate that the sequences which were amplified by the AM1-NS31 primer combination, belonged to different 18S rDNA phylotype groups of the genera *Glomus*, *Diversispora* and *Acaulospora.*

The study was performed using roots from trap-culture plants. Four originated from four trap culture samples of *Phoenix dactylifera*, two from *Zygophyllum hamiense* and one from *Mangifera indica* yielded clonable PCR products (Fig. 2.8). The obtained sequences were aligned with related sequences from the public database (Fig. 2.9 and Fig. 2.10). The trap culture samples of *Phoenix dactylifera* showed the presence of 9 phylogenetic groups (Fig. 2.11 and Fig. 2.12). Among them 7, belonged to the genus *Glomus* (the most diverse group in the Glomeromycota), one belonged to the genus *Diversispora* (DIVE 1) and another one to the genus *Acaulospora* (ACA 1). Only one root sample from the trap culture of *Mangifera indica* yielded a clonable PCR product, which resulted in one sequence cluster with another sequence of *Phoenix dactylifera* (GLOM-1). A root sample from the *Zygophyllum hamiense* trap culture yielded a sequence associated with *Glomus* genus (GLOM-3) and another root sample for the same plant yielded another sequence associated with the genus *Diversispora* (DIVE1) which also contained a sequence related to *Phoenix dactylifera.* In the case of *Salvadora persica,* the PCR product was not clonable despite two attempts.

The analysis of three different trap culture roots from *Phoenix dactylifera* using the additional primers targeting Archaeosporaceae and Paraglomaceae yielded only one band which was obtained through the ARCH1311A/B-ITS4i primer combination. The resulting sequence was associated with *Glomus aurantium (*Fig. 2.12)

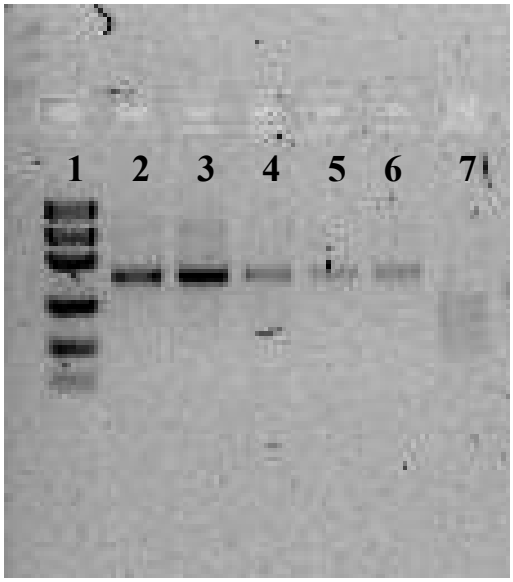

Fig. 2.8 PCR products from trap culture roots colonized by AM fungi present in the rhizosphere of *Phoenix dactylifera* (2-3) *Zygophyllum hamiense* (4 and 6) *Salvadora persica.* The product in in lane 5 could not be cloned. Lane 1 shows the low DNA mass ladder marker used to estimate the size of the amplified fragments and the concentration of the PCR amplification product in order to determine the best vector/product ratio for the cloning step. Lane 7 represents the negative control PCR where water was added instead of any DNA. The primers combination used was AM1 and NS31.

Fig. 2.9 The most variable part of the alignment (using PAUP*4b10) of the sequences used to calculate the phylogenetic tree (Fig. 2.11). The alignment includes the sequences obtained using the primer combination AM1-NS31 and related sequences from the public database.

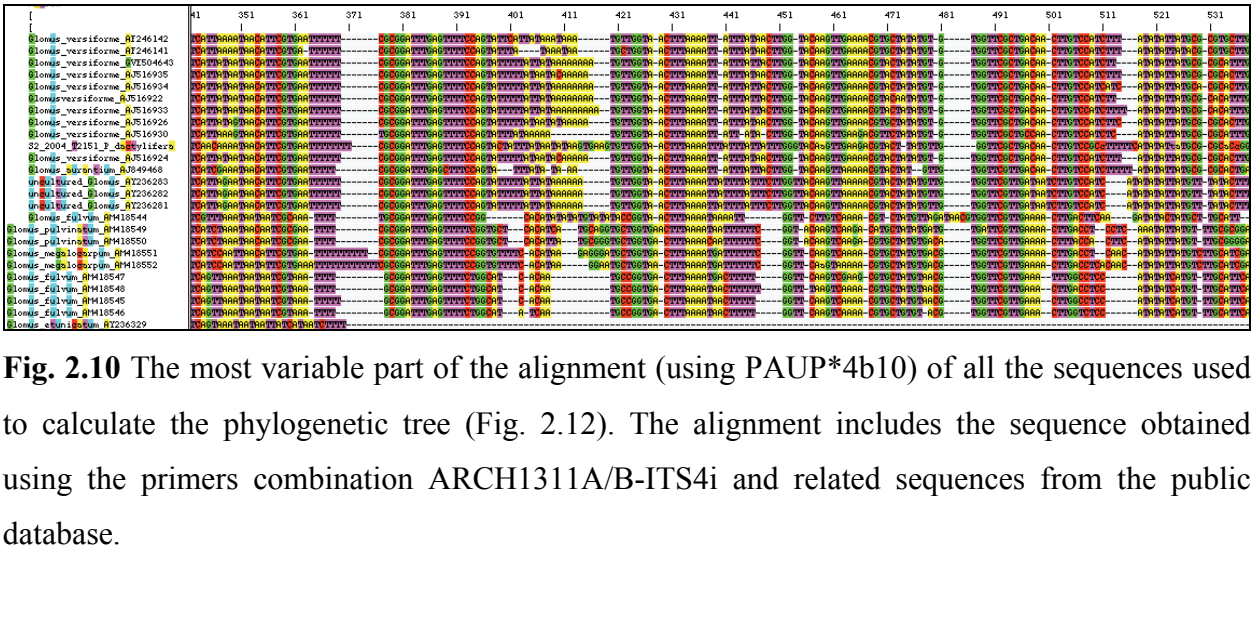

Fig. 2.10 The most variable part of the alignment (using PAUP*4b10) of all the sequences used to calculate the phylogenetic tree (Fig. 2.12). The alignment includes the sequence obtained using the primers combination ARCH1311A/B-ITS4i and related sequences from the public database.

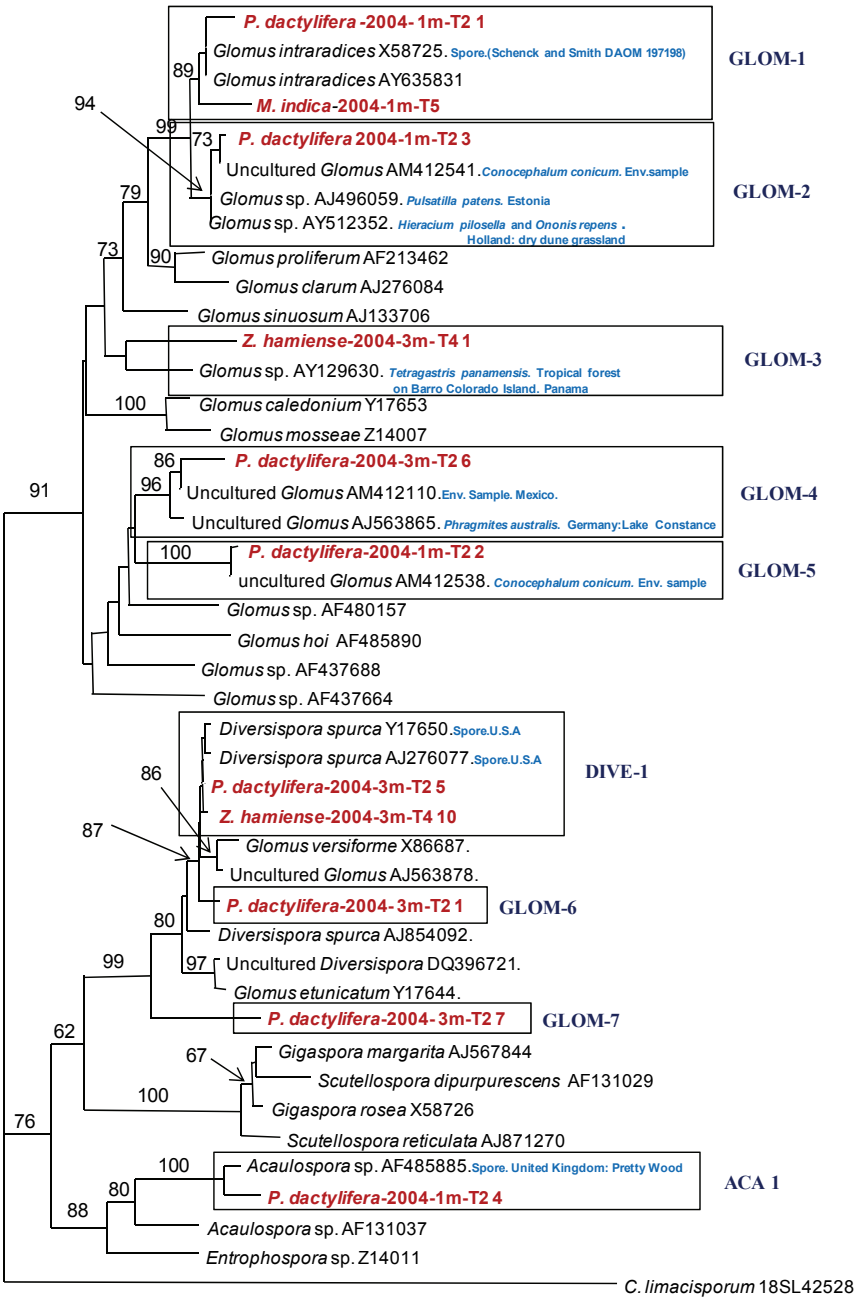

Fig. 2.11 Phylogenetic relationships of sequences obtained in this study with other Glomeromycotan sequences obtained from GenBank. The rooted phylogenetic tree was constructed using PAUP*4.0b10 and the neighbour joining algorithm based on 511 characters from the 18S rDNA. A PCR-RFLP approach was performed using a universal eukaryotic primer NS31 (Simon *et al.* 1992) and the primer AM1 (Helgason *et al.* 1998). The numbers above the branches are neighbour-joining bootstrap values from 1,000 replications. Sequences obtained in present study are shown in red and boldface, with the plant species in which the sequence was found.

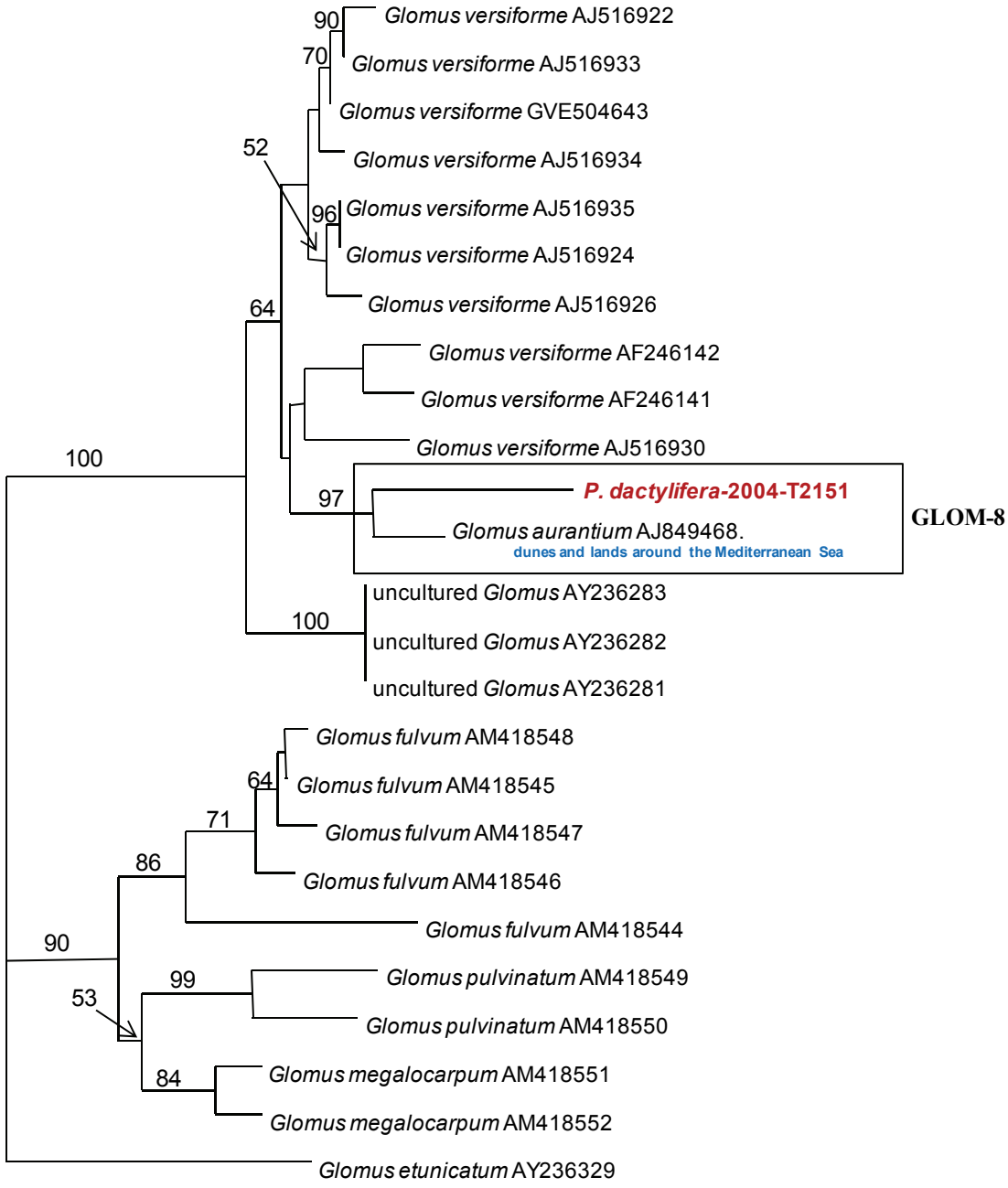

Fig. 2.12 Phylogenetic relationships of sequences obtained in the present study with other Glomeromycotan sequences obtained from the GenBank. The rooted phylogenetic tree was constructed using PAUP*4.0b10 and the neighbour joining algorithm based on 538 characters from the 18S rDNA and the ITS1. A PCR-RFLP approach was performed using ARCH1311A/B-ITS4i primers (Redecker, 2000) in the second nested PCR (see methodology in Chapter 3). The numbers above the branches are neighbour-joining bootstrap values from 1,000 replications. The sequence obtained in present study is shown in red boldface.

2.5 Discussion

2.5.1 Morphological diversity

Morphologically, 15 different AM fungal morphospecies belonging to four different glomeromycotan genera were identified from the two different habitats with four different host plants (Table 2.1). This number represents 7% of the globally known AM fungal species which are around 200. These 15 species are all newly reported in Southern Arabia. Arabian Peninsula as a whole, as have been previously reported, known to have just *G. mosseae* and *G. fasciculatum* (Malibari et al, 1988; Khaliel, 1989). Therefore, this investigation added 15 new species. More than half of the detected species (8 out of the 15) belong to the genus *Glomus.* This is in agreement with the dominance of small-spored *Glomus* species in the arid environments (Stutz et al., 2000). Most of these species seem to be present in most parts of the world (Table A, Appendix). *Scutellospora biornata* might be an exception. To our knowledge, it has been detected only in South America so far. In the present study a species resembling *Scutellospora biornata* was detected in the rhizosphere of *Zygophyllum hamiense*.

Acaulospora scrobiculata, which was suggested to highly prefer sand dunes (Blaszkowski, http://www.agro.ar.szczecin.pl/~jblaszkowski) was also found in the sand dunes of this study.

Clearly there is a considerable presence of the species found in the present study in other arid, semi-arid, sand dunes and desert habitats (Table A, Appendix).

The morphological diversity of AM fungi in the studied habitats is believed to be underestimated and the actual AM fungal species number might be higher. This underestimation might be due to low number of soil samples analysed, and the presence of decayed spores which are difficult to identify.

2.5.2 Molecular diversity

Ten phylotypes were found in the study site most of them belong to *Glomus*, one to *Diversispora* and one to *Acaulospora* (Fig. 2.11 and Fig. 2.12). The phylogenetic analysis of all the sequences showed that only 3 out of the 10 revealed phylotypes could be assigned to named AM fungi. These are: GLOM-1 associated with *Glomus intraradices*; a Diversispora group which contains sequences associated to *Diversispora spurca* (DIVE1 in Fig. 2.11) and GLOM-8 which is associated with *Glomus aurantium* (Fig. 2.12). The presence of sequences related to *Glomus intraradices* in the present study (Fig. 2.11) supports the hypothesis that this species is present in almost every continent (Morton, 1990b).

Interestingly, non-target amplification by the ARCH1311A/B-ITS4i primer combination revealed a sequence phylogenetically associated to *Glomus aurantium* (Fig. 2.12). As morphospecies, *Glomus aurantium* was discovered in a trap culture of rhizosphere soil of *Cenothera drummondi* colonizing dunes of the Mediterranean Sea adjacent to Tel-Aviv (Blaszkowski et al., 2004). Later, spores of *Glomus aurantium* were isolated from trap cultures established from soils collected under *A. arenaria* growing in Spain, and seven trap cultures containing rhizosphere soil and root mixtures taken from under *A. arenaria* growing in dunes adjacent to Calambrone, Italy.(http://www.agro.ar.szczecin.pl/~jblaszkowski). The spores of this species were not found in ca. 3000 soil samples coming from dune and non-dune soils of northern Europe (Blaszkowski, 2003). The finding of a sequence from the study habitat which is clearly associated with *Glomus aurantium* supports the suggestion of Blaszkowski et al., (2004) that this fungus prefer the warm sites.

A *Glomus* sp. sequence (AY512352) which originated from a dry dune grassland in Holland clustered with a sequence of the present study (GLOM2 in Fig. 2.11). This is a nother example of

the presence of a sequence coming from a sandy and dry environment. All the rest of sequences produced in this study were clustering with other sequences associated with "uncultured" and to "unknown" glomeromycotan species coming from Estonia, Mexico, Panama, USA, and UK. The increase of such unknown species in GenBank is due to the increasing use of molecular tools to analyse the AM fungal communities in the roots or in soils.

In this study, a part of AM fungi community present in both the agricultural site and the natural sand dunes of Southern Arabia were revealed. Molecular identification of the trap culture roots did not reflect the spore population found in the field. This might be because most of the species detected morphologically in the present study still do not have corresponding sequences in GenBank. The species of the genus *Glomus* were dominant among the other glomeromycotan morphospecies and phylotypes.

Chapter 3: Impact of land-use change on the biodiversity of native AM fungi: a community shift

3.1 Abstract

The main aim of this study was to evaluate the effect of land-use change of a hyper arid site in Southern Arabia on the diversity of AM fungi. Rhizosphere soils of the native plant species (*Zygophyllum hamiense, Salvadora persica, Prosopis cineraria* and *Heliotropium kotschyi* in addition to the natural vegetation growing between these plants) were sampled. The agricultural ecosystems were represented by two sites cultivated with date palms (*Phoenix dactylifera*) situated close to a natural site and managed by two management systems (traditional and modern). AM fungal spores found in the field and those which sporulated in green house trap cultures were identified morphologically. AM fungal inoculum potential was assessed through the evaluation of colonization in the roots of *Sorghum bicolor* inoculated with soils coming from the rhizospheres of different plants growing in the different sites.

The results showed that AM fungal community composition in the agricultural sites shifted from that in the natural site. The agricultural sites had higher AM fungal spore abundance, species richness and inoculum potential. In total, 25 AM fungal species were found; 18 of them belong to the genus *Glomus*, four to *Scutellospora*, and one species from each genus of *Acaulospora*, *Paraglomus* and *Ambispora.* The study showed an uncommon pattern of a dramatic increase of AM fungal diversity due to land-use change. This pattern might be due to the reduction of the extreme abiotic environmental stresses factors through the agricultural practices.

Keywords: Arabia, dry lands, AM fungi, land-use change.

3.2 Introduction

The expansion of human population along with the access to technology have led to a change on the earth's biota and communities, including species introductions, species extinctions and fragmentation of the natural habitats through land-use change (Vitousek et al., 1997). Through its dramatic alteration of the ecosystem functions, land-use change was nominated to be the main cause of biodiversity loss in the 21st century (Sala et al., 2000).

Arid and semiarid lands cover about one third of the Earth's terrestrial land, and unlike the common idea that they are hostile places, they are in fact, often heavily used by man. The extreme environments of arid lands have led to evolution of considerable biodiversity and unique adaptation, some of which are potentially valuable in human terms (Huenneke and Nobel 1996). This unique biodiversity of the arid lands is not immune from biodiversity loss (Sala et al., 2000). While land-use change of the desert ecosystems through introduction of agriculture could have an obvious strong negative effect on the biodiversity of macro organisms, there are uncertainties about its effect on microbial diversity.

There is a lack of explicit investigation on the effect of land-use change on the diversity of AM fungi in hyper arid regions. In general, and under different ecosystems, experiments evaluating these responses to the land-use change showed contradictory results.

It was hypothesized that generally, in cases where the soil receives more water through irrigation and additional nutrients, the introduced ecosystem can have higher AM fungal species richness and spore abundance (Koske et al 1997; Li et al, 2007). However, in other cases, it was shown that disturbance resulting from agriculture decreases AM fungal species richness and infectivity

(Helgason et al. 1998; Douds and Millner 1999; Oehl et al., 2003). It seems that there is no simple correlation between management and AM fungal diversity and that there are many factors that could have effects on the diversity of these fungi in the introduced agricultural systems.

The purpose of this study was to provide a description of the effect of land-use change from natural to agricultural on the native AM fungi in Southern Arabia.

In this study, date palm was chosen as a model for the introduced agriculture. We have compared the AM fungal community structure in the rhizosphere of introduced date palms cultivated under two agricultural systems (traditional and modern), with that of the surrounding native desert vegetation (*Zygophyllum hamiense, Salvadora persica* and *Prosopis cineraria*) and with a successional vegetation on a modern agricultural site where all the natural vegetation was mechanically removed. Another site occurring in an interdune area has been chosen as a control site for the study. The data gathered included AM fungal spore density, species richness, and inoculum potential of the AM fungal communities.

3.3 Materials and methods

3.3.1 Study sites

All the study sites (Fig. 3.1) were located in Southern Arabia and all samples were taken in August 2006 as follows:

Natural site

A site with native vegetation not influenced by human activities was selected (Fig. 3.2). The vegetation consisted of three perennial plant species. They were *Salvadora persica, Zygophyllum hamiense* and *Prosopis cineraria*. The areas between the plants were covered with more than one kind of vegetation (Fig. 3.2 D and E). Social indigenous knowledge was helpful to locate this site. According to this knowledge, before agriculture was introduced, the agricultural lands used to look like this native site.

Traditional agricultural site

This site (Fig. 3.3 A) was cultivated mainly with date palms. The management has followed the local traditional way of farming since the establishment of the farm in 1992. The only source of fertilization was the manure produced on the same farm. Irrigation was based on the traditional flooding irrigation system (Al-Marshudi, 2001) where water flows from a natural source of few kilometers away from the farm through narrow channels called *Aflaj* (http://whc.unesco.org/en/list/1207/). The inter-plant area of the farm was cultivated mainly with sorghum. In order to prepare the land for the sorghum cultivation, the soil was tilled annually using the tractor. No synthetic pesticides or chemical fertilizers were ever used. The source of most of the date palm seedlings was the same cultivated area, whereas some other seedlings of popular varieties were brought from other geographical areas in the interior part of Oman around

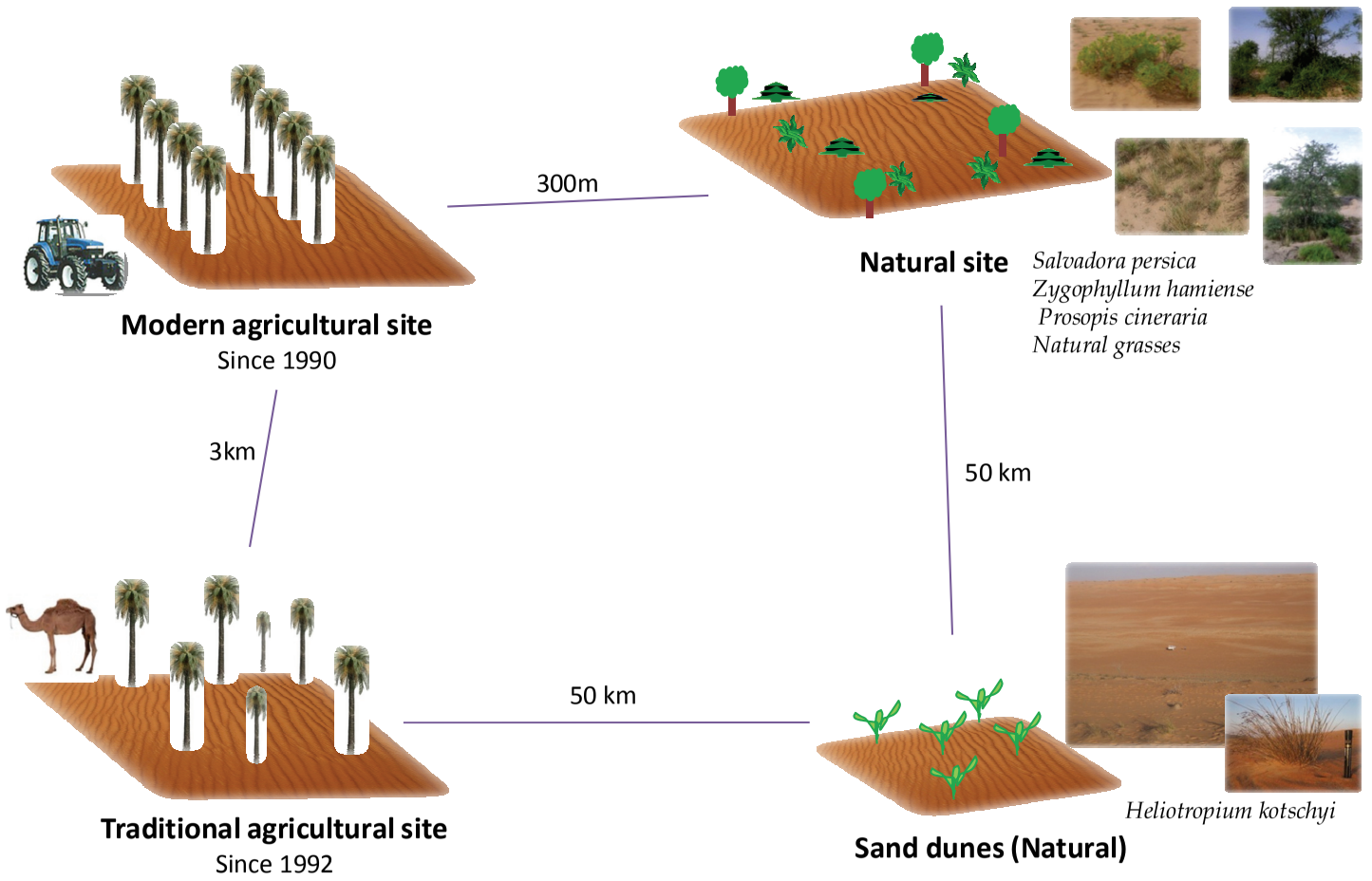

Fig. 3.1 The four study sites representing the natural land, the modern and the traditional agricultural sits in addition to the sand dunes.

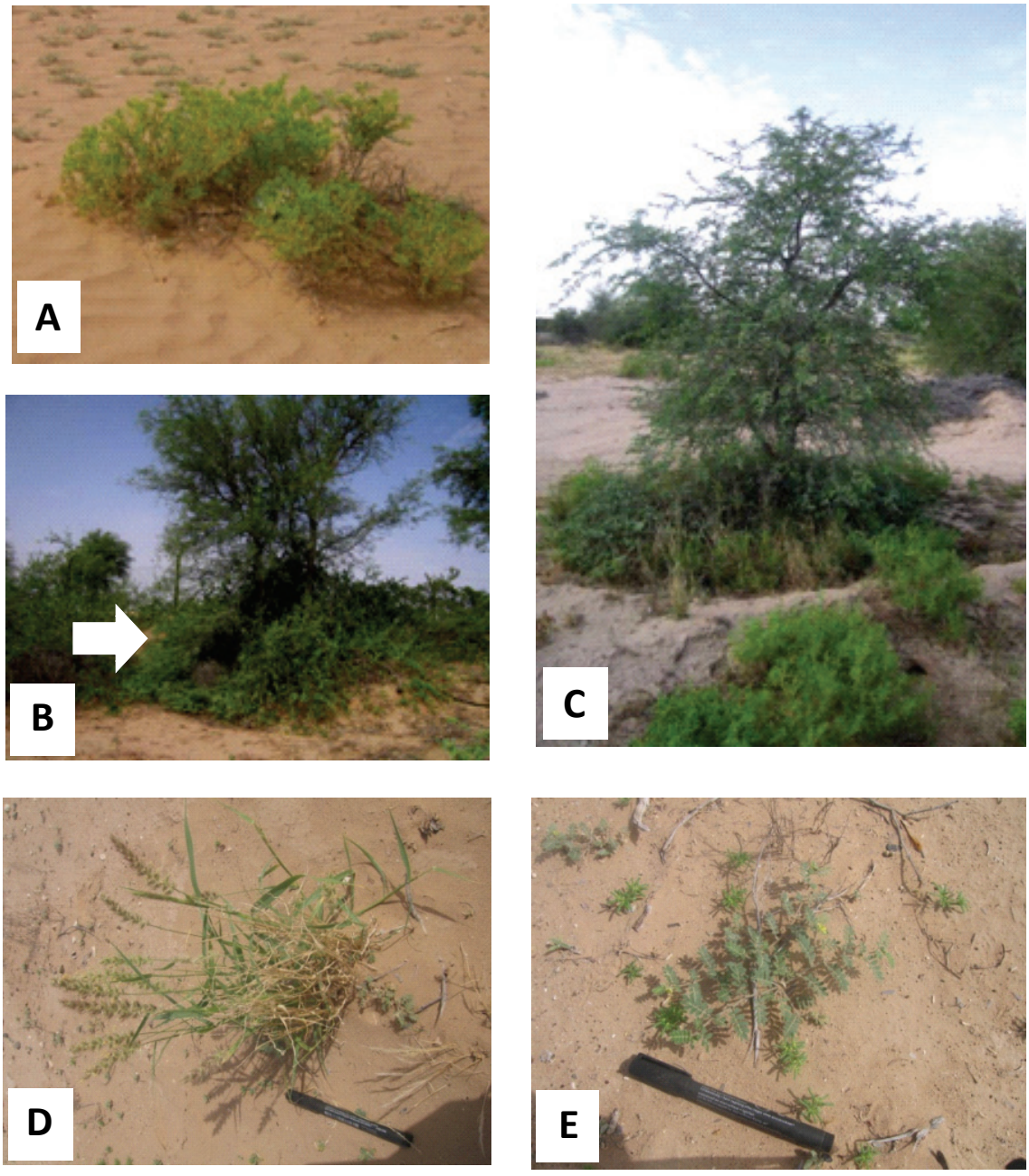

Fig. 3.2 The native vegetation of the natural site. **(A)** *Zygophyllum hamiense*. **(B)** *Salvadora persica,* where the arrow indicates **(C)** A tree of *Prosopis cineraria*. **(D and E)** Natural vegetation growing between the perennial plant species. The pen was included to demonstrate the scale.

300 km northwest. Soil traces from the original land usually are attached to the roots of the imported seedlings.

Modern agriculture site

This site (Fig. 3.3 B) was situated 3 km away from the traditional agriculture site. It was located inside an agricultural experimental station and cultivated exclusively with date palms. The management followed the modern way of farming since the establishments of the farm in 1990, i.e., synthetic pesticides were used rarely and chemical fertilizers (mainly NPK) were added annually in reasonable amounts. A drip irrigation system was being used. The area between the date palms was not cultivated with any other plants and lost its native vegetation apparently because of the mechanical and human activities. The date palms were originally produced through the tissue culture (micropropagation) technique in The Tissue Culture Laboratory of the Ministry of Agriculture of Oman located 300 km North West of the study site. The seedlings passed a period of weaning in a shadow house in the same laboratory where they were transferred to an unsterilized mixture of soil and organic matter. Then they were transferred to be planted in the study site.

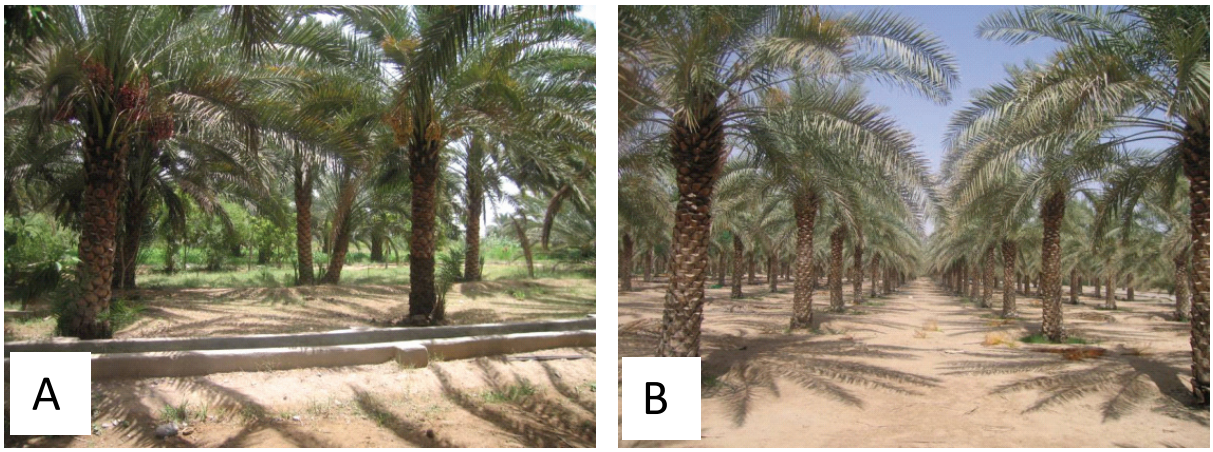

Fig. 3.3 The two agricultural sites. **(A)** The traditional site and **(B)** the modern site.

3.3.2 An extinct Holocene lake site

A natural site occurring outside the studied habitat was chosen to be a control site for the study. The location of sampling (Fig. 3.4) was the bottom of an extinct Holocene lake (Radies et al., 2005). Soil samples of the rhizosphere of one of the native plants *Heliotropium kotschyi* (Fig. 3.4 D), were collected at this site.

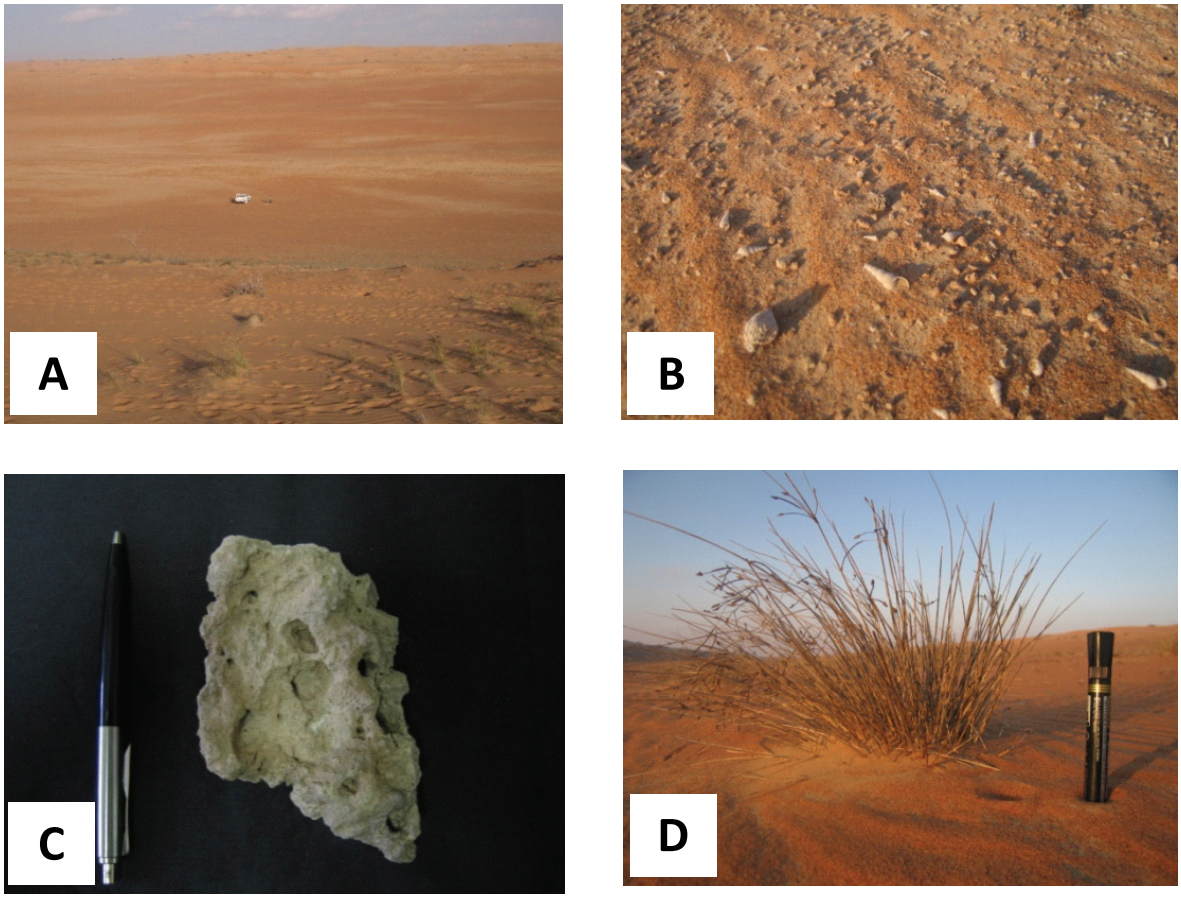

Fig. 3.4 The Holocene extinct lake. **(A)** A view of the location of the extinct lake from of the surrounding dunes. The white spot in the middle is our car. **(B)** The surface of the extinct lake as appears from around 50 cm elevation. Diverse trace fossils were exposed on the present day surface. **(C)** A rock found in the bottom of the lake. Most probably it is a calcium carbonate precipitation (Dr.Frank Preusser, personal communication). **(D)** *Heliotropium kotschyi* growing at the site. The pen was included to deomonstrate a scale.

3.3.3 Soil sampling

Samples were collected on August 2006. For each of the four sites, four replicate plots were randomly chosen. Each plot size was ≈ 200 m^2. From each plant in each plot four plants were selected for a pooled sample (Fig. 3.6)

In the case of the natural vegetation (*Salvadora persica, Zygophyllum hamiense* and *Prosopis cineraria*) and the date palms at the two agricultural sites, rhizosphere soils were sampled by making a soil cross section of 30 cm diameter and a depth of 30 cm, 20 cm away from the stem of the trees to have an access to the roots. Soils collected represent vertical cross sections of the root zones. The moist soils samples from the agricultural sites were air dried and then transported to the lab in Basel.

Rhizosphere soils of the inter-plant vegetation at the natural site, the successional vegetatione in the modern agricultural site and *Heliotropium kotschyi* in the sand dunes were sampled by collecting around 2 kg of the soil around the their root systems. This sampling method was chosen because the shallow root system did not allow making the soil cross section. In addition, in the sand dunes where soil has no texture, such a cross section in the soil was not possible. A graphical representation of the sampling strategy is shown in Fig. 3.5.

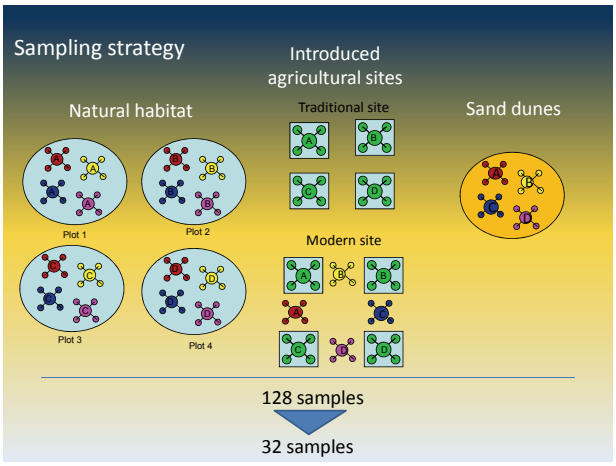

Fig. 3.5 A representation of the sampling collection strategy in the four sites. Each circle with a letter inside represents a pooled sample from four subsamples (represented by four smaller circles attached to it) corresponding to the same plant species. Diagram is not to scale.

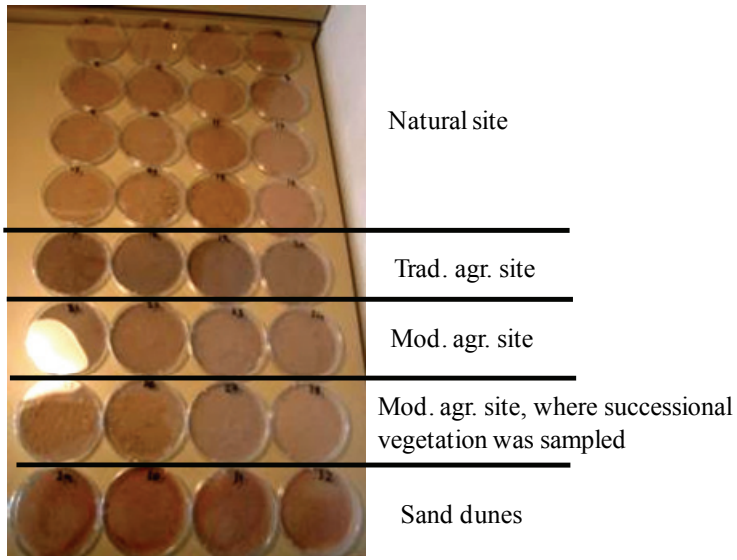

Fig. 3.6 The soil samples of the different sites in the present study in petri dishes. The colors of the soils of different sites appear different. The soils from the agricultural lands have darker colors than the soils from the natural site. This is most probably due to the addition of the organic matter in the agricultural land especially the traditional site. The red colour of soils from sand dunes is attributed to the presence of iron oxides.

3.3.4 Trap culturing the AM fungi

For each pooled soil sample representing plants in each of the four replicate plots at each field site, two trap culture pots (0.85L) were established (Fig. 3.7). Pots were filled with 0.45 kg of an autoclaved substrate consisting of Terragreen (American aluminium oxide, oil dry US special, type III R, <0.125 mm; Lobbe Umwelttechnik, Iserlohn, Germany). The mycorrhizal inocula (50 g of soil sample) were spread as a layer on the surface of the substrate in the pots and covered with a thin layer of the substrate. The two trap cultures were the following:

A. Trapping system with various plants

Three different trap culture plants (*Plantago lanceolata, Hieracium pilosella* and *Allium porrum*) were randomly planted in every pot (Fig. 3.8 A). Using more than one trap plant species was expected to increase the resolution of the species richness of AM fungi in the field soils. This is based on the assumption that different plants might trap different AM fungi. All the three species are known to be AM fungi host plants and routinely used for trap cultures. The trap cultures were kept in a greenhouse for 8 months and were irrigated with an automatic irrigation system (Tropf-Blumat; Weninger GmbH, Telfs, Austria).

B. Trapping system with *Sorghum bicolor*

Five 2-week-old AM fungi-free plantlets of *Sorghum bicolor* were randomly planted in every pot (Fig. 3.8 B). The roots of the trapping plant were used to evaluate the AM fungal colonization levels as an indicator for the inoculum potential of the present AM fungal communities. *Sorghum bicolor* was chosen because it is one of the crops cultivated in the study area of Southern Arabia,

therefore, evaluating its response to mycorrhizal communities in the natural and agricultural sites is useful to predict the functional change in these communities due to the land-use change.

3.3.5 AM fungal spore isolation and identification

AM fungal spores occurring in the field soil samples and those propagated in the trap cultures were extracted by wet sieving and sucrose density gradient centrifugation (Daniels and Skipper, 1982). Fifteen g of air-dried field soil or 30 cm^3 of harvested trap culture substrate were passed through 1,000-, 500-, 125-, and 32-µm sieves. The 500-µm sieve was checked for large spores, spore clusters, and sporocarps whereas the contents of the 125- and 32-µm sieves were layered onto a water-sucrose solution (70% [wt/vol]) gradient and centrifuged at 900 x *g* for 2 min. The resulting supernatant was washed with tap water for 2 minutes in a 32-µm sieve, and transferred to petri dishes. Spores, spore clusters, and sporocarps obtained from all sieves were mounted on slides with polyvinyl-lactic acid-glycerol (Koske and Tessier, 1983) or polyvinyl-lactic acid-glycerol mixed 1:1 (vol/vol) with Melzer's reagent (Brundrett et al., 1994). The spores were examined under a light microscope (Zeiss; Axioplan) at a magnification of up to x400. Identifications were based on current species morphological descriptions and an identification manual (Schenck and Perez, 1990; International Culture Collection of Arbuscular and Vesicular-Arbuscular Endomycorrhizal Fungi) The presence/absence of spores from all AM fungal species was determined for each sample and expressed as the number of AM fungal spores per 15 gram of soil for the field site samples.

3.3.6 Inoculum potential evaluation

The parameters of mycorrhizal colonization (Trouvelot et al. 1986) for each replicate of each sample of the trap culture system were evaluated. The evaluation was based on 30 roots (1cm-

segments) of each replication. These roots were incubated overnight at room temperature in 0.1% cotton blue (w: v) in lactic acid. Segments then were destained in lactic acid and observed under a light microscope for the quantification assay. Inoculum potential was defined as the percentage of mycorrhization (M%) according to the method used by Trouvelot et al. (1986).

3.3.7 Statistical analysis

Significance of differences in AM fungal spore abundance, species richness and inoculum potential between the samples coming from and within different sites was tested using Fisher`s least significant difference (LSD) at $p < 0.05$ after one-way analysis of variance (ANOVA). The correlation between the AM fungal spore abundance and species richness and inoculums potential was calculated through a simple regression test. A dendrogram was obtained by cluster analysis displaying the similarity of AM fungal species composition across the host plants in all sites. Ward's clustering method and squared Euclidean distance metric were used. All the statistical tests were performed using Statgraphics (Version 3.1).

Sites	Plants from study sites Rhizosphere soils were sampled August 2006		Trap plants in green house (pot numbers)							
			Plantago lanculata *Hieracium pilosella* *Allium porrum*				*Sorghum bicolor*			
Natural site	*Zygophyllum hamiense*		1	2	3	4	33	34	35	36
	Salvadora persica		5	6	7	8	37	38	39	40
	Prosopis cineraria		9	10	11	12	41	42	43	44
	Inter-plant area		13	14	15	16	45	46	47	48
Agricultural sites	Date palm (traditional site)		17	18	19	20	49	50	51	52
	Date palm (modern site)		21	22	23	24	53	54	55	56
	Successional veget.(modern site)		25	26	27	28	57	58	59	60
Sand dunes	*Heliotropium kotschyi*		29	30	31	32	61	62	63	64

Fig. 3.7 The different trap cultures of the study. Four replicates of the soil samples of each sampled plant were used to establish trap culture with a mixture of trap plants and *S. bicolor*, respectively.

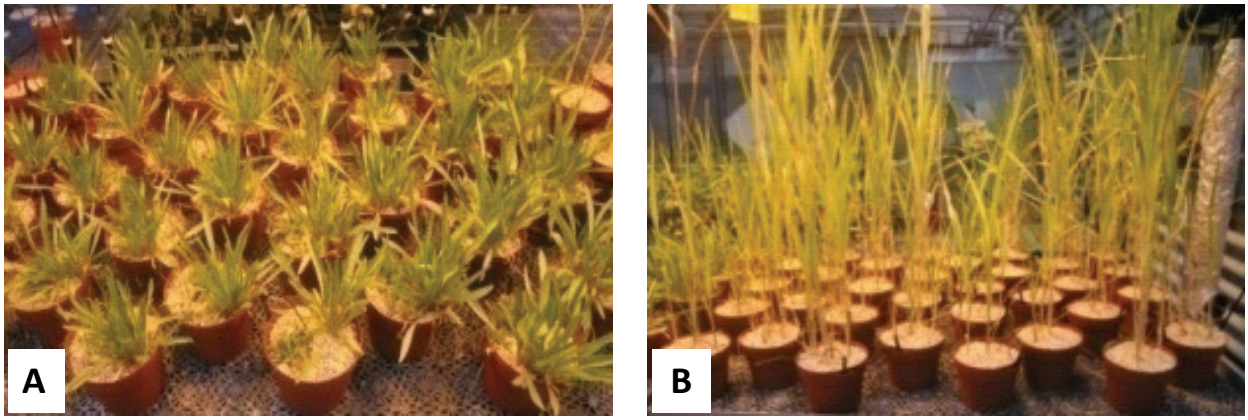

Fig. 3.8 The trap culture pots in the green house. **(A)** Pots with a mixture of plants (*Plantago lanceolata, Hieracium pilosella* and *Allium porrum*) **(B)** Pots with *Sorghum bicolor*.

3.4 Results

Twenty five taxa of AM fungi were detected from 32 soil samples originating from the natural site, adjacent and nearby agricultural sites and sand dunes. Eighteen of these species belonged to the genus *Glomus*, 4 to *Scutellospora*, and 1 species from each genus of *Acaulospora*, *Paraglomus* and *Ambispora* (Table 3.1). Among the 25 identified species, only 11 species of *Glomus* and 1 species of *Scutellospora* were identified to the genus level. The most frequent species in the field was *Glomus aggregatum*, with 26 occurrences in the 32 samples while the least frequent was *Glomus* sp. OMA8 with one rare occurrence (Fig. 3.10). However, the most frequent species in the trap cultures was *Glomus eburneum* with 29 occurrences in the 32 trap culture pots (Fig. 3.10)

As shown in Table 3.1, there were 4 AM fungal species (*Gl. sinuosum, Glomus* sp. OMA6, *Sc. calospora* and *Scutellospora.* sp. OMA10) which were found exclusively in both agricultural sites. Another 6 AM fungal species (*Gl. microaggregatum, Gl.* sp. OMA9, *Sc. fulgida, Sc. gregaria, Ac. spinosa* and *Gl. etunicatum*) were found exclusively in only one of the agricultural sites but not the other. *Gl. aggregatum, Glomus* sp. OMA3, *Gl. eburneum* and *Gl. microcarpum* were found both in the natural and in the agricultural lands. However, *Gl. macrocarpum and Glomus* sp.OMA5 (which is an un described species), *Ambispora gerdemannii*, *Glomus* sp.OMA7, *Glomus* sp.OMA8, *Glomus* sp.OMA 11 and *Glomus* sp. OMA12 were detected only in the natural site and not in any of the two agricultural sites.

3.4.1 Changes in the composition of AM fungal communities

The AM fungal species detected in the natural site and in the two different agricultural sites clearly form two different clusters in the dendrogram (Fig. 3.9), suggesting that the AM fungal

communities in the agricultural sites differ from that on the natural site. The AM fungal communities associated with successional vegetation and the sand dunes were closer to that of the natural site than the agricultural one.

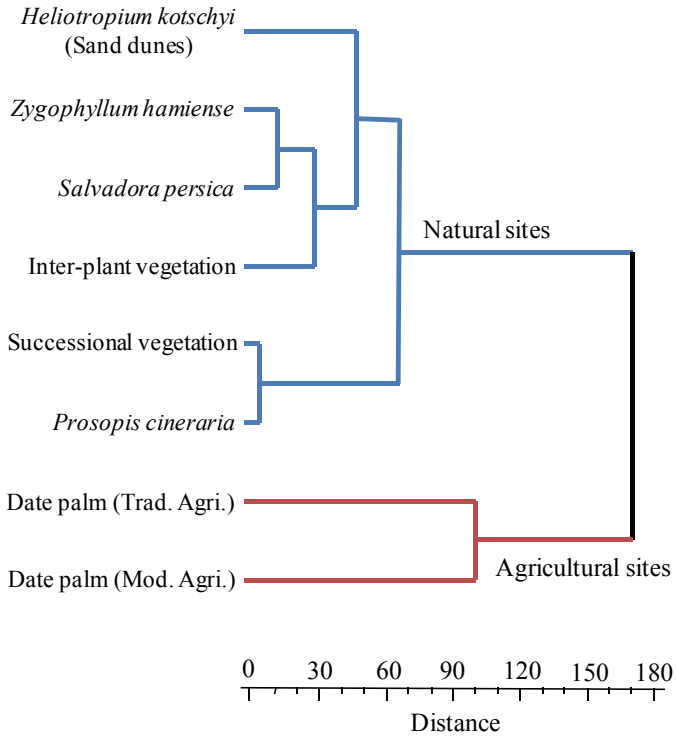

Fig. 3.9 Dendrogram of cluster analysis based on the similarity of AM fungal species composition across the host plants in all sites. Two main clades were formed suggesting the presence of two different communities occurring in natural and agricultural sites. Ward's clustering method and squared Euclidean distance metric were used.

3.4.2 Changes in the species abundance

The spore abundance in the field was by far higher in the agricultural sites (mean=70.5) than the natural site (mean=16) and the successional vegetation site (mean=8) (Fig 3.11 A). The spore abundance related to the successional vegetation was not statistically different from those found

the natural site (Fig. 3.11 A). No AM fungal spores were found in the rhizosphere of *Heliotropium kotschyi* growing in the sand dunes (Fig. 3.11 A).

The spore abundance also varied among different host plants in the same site (Fig. 3.12 A). The highest mean of spore abundance in the natural site was 25.7, corresponding to the inter-plant area. This abundance was significantly higher than the that corresponding to both *Zygophyllum hamiense* (mean=9.75), and *Salvadora persica* (mean=7), but not to *Prosopis cineraria* (mean=21.5). The two agricultural sites also differed in spore abundance (58.75 and 82.25 at traditional site and modern site respectively).

3.4.3 Changes in the species richness

The AM fungal species richness represents the number of species found in both the field soils and in the trap culture system (Table 3.1). The agricultural sites were found to have significantly higher species richness (mean= 9.75) than the natural land (mean= 4.88) and than successional vegetation (mean=4.25) (Fig. 3.11 B). The spore richness related the successional vegetation was not statistically different from those related to the natural site (Fig. 3.11 B). The lowest number of species was found associated to *Heliotropium kotschyi* growing in the sand dunes (mean=1) this is statistically lower than the agricultural sites, natural site and successional vegetation (Fig.11.3 B).

The species richness did not vary greatly among different host plants in the same site (Fig. 3.12 B). The highest mean of species richness in the natural site corresponded to *Prosopis cineraria* (mean=6). This richness was significantly higher than the richness corresponding to both *Zygophyllum hamiense* (mean=4.5) and *Salvadora persica* (mean=4), but not to inter-plant area (mean=5) (Fig. 3.12 B). The two agricultural sites, unlike their species abundance, did not have

significantly different species richness, and the means were 10.25 and 9.25 species at the traditional and modern sites, respectively (Fig. 3.12 B).

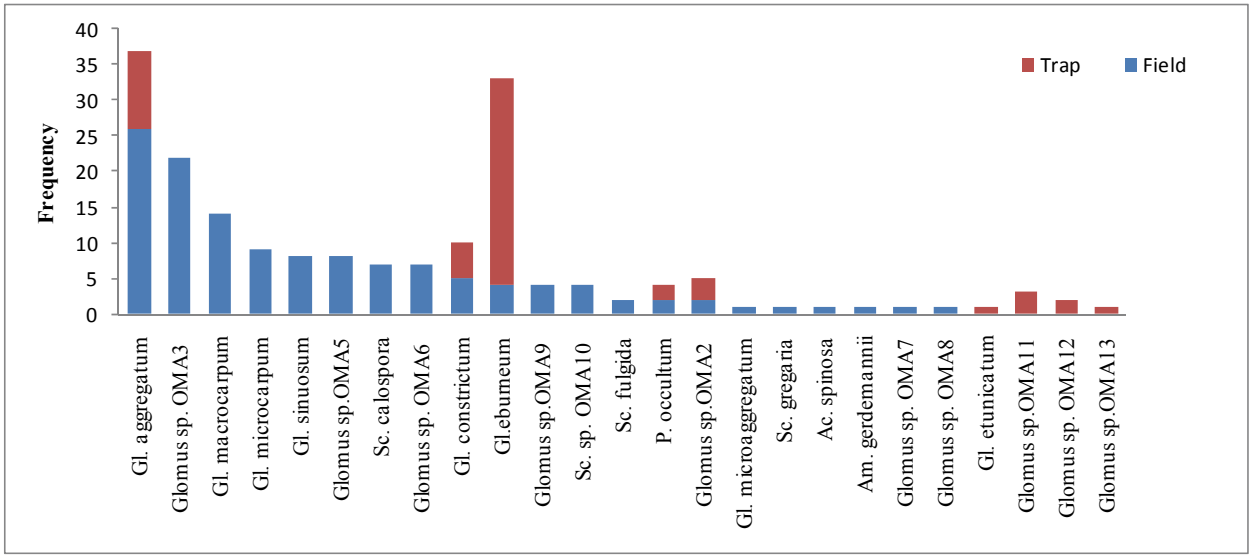

Fig. 3.10 The frequency of species occurrence in the field and in the trap culture samples.

3.4.4 Changes in the inoculum potential

The inoculum potential was higher in the agricultural sites (mean=52.79) than both the natural site (mean= 22.91) and than successional vegetation (mean=35.66). The inoculum potential of AM fungal community associated to the successional vegetation, however, was not significantly different from those related to the natural site (Fig. 3.11 C). The lowest inoculum potential was found to be associated with *Heliotropium kotschyi* growing in the sand dunes (mean=2.74) which was significantly lower than the agricultural sites, natural site and successional vegetation (Fig. 3.11 C).

The AM fungal inoculum potential was not different among different host plants in the natural site (Fig. 3.12 C). The two agricultural sites, however, did maintain statistically different

inoculum potential with higher value in the traditional site (mean 62.6) than the modern site (mean=45.43) (Fig. 3.12 C).

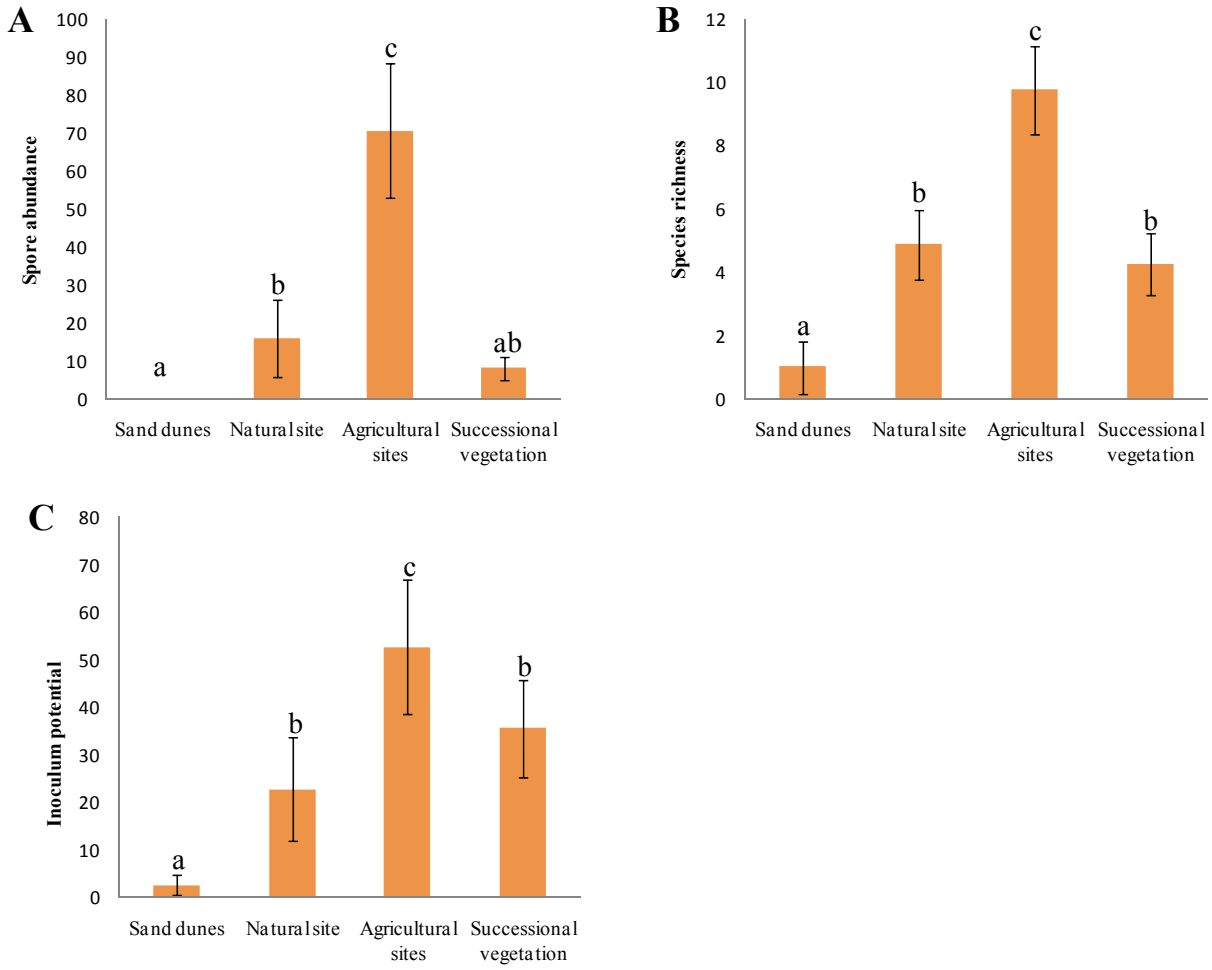

Fig. 3.11 Comparison of the effect of the land-use type on **(A)** AM fungal spore abundance in the field **(B)** Species richness in the field and the trap cultures and **(C)** the inoculum potential. Values are reported as means (±SD). Non-significant differences between the means are indicated by similar letters above their error bars as determined by Fisher's least significant difference (LSD) at the 5% level after one-way ANOVA.

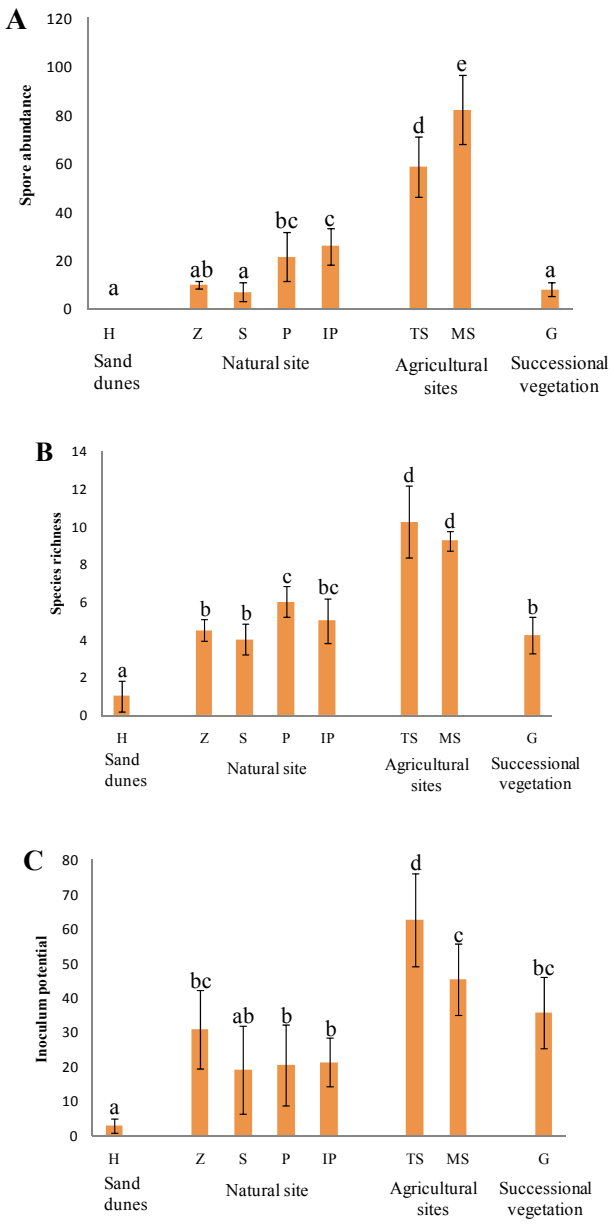

Fig. 3.12 Comparison of the effect of the type of vegetation in different land-use types on **(A)** AM fungal spore abundance in the field **(B)** Species richness in the field and the trap cultures and **(C)** inoculum potential. Plant species are H: *Heliotropium kotschyi,* Z: *Zygophyllum hamiense,* S: *Salvadora persica,* P: *Prosopis cineraria,* IP: inter-plant vegetation, G: successional vegetation, TS: traditional site of date palm, MS: modern site of date palm. Values are reported as means (±SD). Non-significant differences between the means are indicated by the same letters above their error bars as determined by Fisher's least significant difference (LSD) at the 5% level after one-way ANOVA.

3.4.5 Correlations between spore abundance and species richness and inoculum potential

The results of the simple regression analysis revealed a significant positive correlation between the AM fungal spore abundance in the field and the AM fungal inoculum potential (Fig. 3.13 A) (correlation coefficient=0.676, r^2 =45.68, P=0.0001). AM fungal species richness found both in field soils and trap cultures had as well a positive correlation with the AM fungal inoculum potential (correlation coefficient=0.729, r^2 =53.09, P= 0.0001) (Fig. 3.13 B).

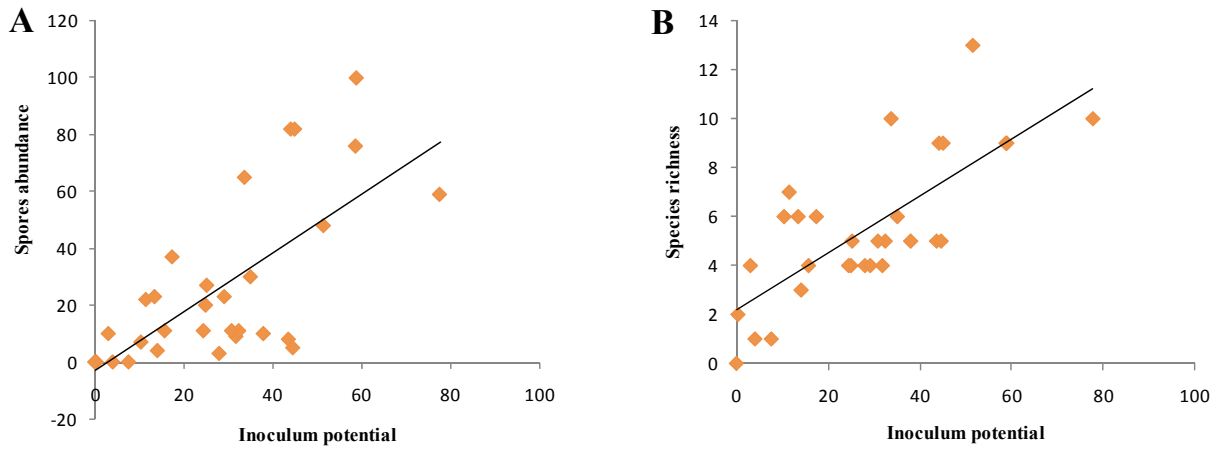

Fig. 3.13 The relationship between the inoculum potential and **(A)** field spore abundance (correlation coefficient=0.676, r^2 =45.68, P=0.0001) and **(B)** field and trap culture species richness (correlation coefficient=0.729, r^2 =53.09, P=0.0001)

AM fungal species	Sand dunes	Natural site				Successional vegetation	Agricultural sites	
		Z. hamiense	*S. persica*	*P. cineria*	Inter-plant		Date palm (Traditional site)	Date palm (Modern site)
Glomus sinuosum							A, B, C, D	A, B, C, D
Glomus sp. OMA6							a, b, c, d	a, b, d
Scutellospora calospora							a, b, d	a, b, c, d
Scutellospora sp. OMA10							a, b, c	a
Glomus microaggregatum								b
Glomus sp. OMA9								a, b, c, d
Scutellospora fulgida							a, c	
Scutellospora gregaria							a	
Acaulospora spinosa							b	
*Glomus etunicatum**							c*	
Glomus sp. OMA13*								b*
Glomus sp. OMA2*				d*			a (rare), d*	a (rare), c*
*Glomus constrictum**						d (rare)	a*,b*,c*	a, B, c, d c*,d*
Resembling *Glomus aggregatum**	c*	a, b, c, d	b, c, d	a, b, c, d, a*,b*,c*,d*	A, b, d a*	a, b, c, d	a, b, c, d a*,b*,d*	a, b, c, d a*,c*
Glomus sp. OMA3		c, d	c, d	a, b, c, d	a, c, d	a, b, c, d	a, b, c, d	b, c, d
*Glomus eburneum**	a*,b*,c*	a*, b*, c*, d*	b a*,b*,c*,d*	A, b, D, a*,b*,c*,d*	A*,b*,c*,d*	a*,b*,d*	a*,b*,c*,d*	b*,c*,d*
Glomus microcarpum		a, b, d	d	c	a, d	d (rare)		c (rare)
*Paraglomus occultum**				a, b			a*,d*	
Glomus sp.OMA5 (NEW sp.)		b, c, d	a, b	a	D	d		
Glomus macrocarpum			a, b, c, d	c, d	a, b, c, d	a, b, c, d		
Ambispora gerdemannii				c				
Glomus sp.OMA7					b			
Glomus sp.OMA8					c (rare)			
Glomus sp.OMA 11*		a*,b*	a*					
Glomus sp. OMA12*				b*		a*		
Species richness	2	6	7	10	8	8	14	13

Table 3.1 The AM fungal species detected in the present study and their distributions among the different habitats and plant species. Four replicates (a, b, c, d) per site. Upper case letters show species dominating the spore populations at corresponding replicate field sample. The star * sign indicates the AM fungal species found in the trap cultures. Glomus sp.OMA6 resembling *Glomus liquidambaris*; *Scutellospora* sp.OMA10 resembling *Scutellospora aurigloba*; Glomus sp.OMA2 resembling *Glomus versiforme*; *Glomus* sp.OMA3 resembling *Glomus invermaium*; *Glomus* sp.OMA9 resembling *Glomus glomerulatum*; *Glomus* sp.OMA5 resembling *Glomus multicaule*; *Glomus* sp.OMA7 resembling *Glomus tortuosum*; *Glomus* sp.OMA8 resembling *Glomus rubiforme* (= *Glomus* sp. MEX1; Bashan et al. 2007); *Glomus* sp.OMA5 is a new species, *Glomus* sp.OMA3 & G sp.OMA9 & G sp.OMA8 might be new species; others have not yet been clearly attributable to resembling known species.

3.4.6 Soil chemical and physical properties

	P (mg kg^{-1})			% N	K (cmol kg^{-1})	Mg (cmol kg^{-1})	Ca (cmol kg^{-1})	Na (cmol kg^{-1})
	Total	Sodium bicarbonate[a]	Active					
Natural sites	119	41.4	47.4	< 0.1	0.7	2.6	7.2	1.4
Agricultural site (Trad.)	194	71	93	0.18	0.35	5.7	8.6	1.9
Agricultural site (Mod.)	166.6	93	41.2	0.1	0.25	2.8	7.8	1.4
Sand dunes	101.4	22.6	34.8	< 0.1	0.22	0.42	2.3	1.1

	pH		Salinity (µS)	% Organic matter	% C	% H	% sand	% silt	% clay	Soil texture
	H_2O	KCl								
Natural sites	8.2	8.1	1638	1.455	4.1	0.27	70.7	25.4	4.3	sandy loam
Agricultural site (Trad.)	8.4	7.9	482	3.657	5.4	0.49	67.2	24.8	8.1	sandy loam
Agricultural site (Mod.)	8.5	7.9	235	2.559	4.8	0.3	60.5	33.6	5.9	sandy loam
Sand dunes	9.1	8.5	108	0.452	2.2	< 0.1	90.7	9.3	0	sand

Table 3.2 Soils chemical and physical properties. [a] It was analyzed using the method given by Chapman and Pratt (1961).

3.5 Discussion

The results suggest that there was a high impact of land-use change on the community of AM fungi in the studied ecosystem. The agricultural sites had higher AM fungal spore abundance, species richness and AM fungal inoculum potential. The AM fungal community composition in the natural site also differed from that in the two agricultural sites. This change occured in a realatively short time (15 and 17 years for traditional and agricultural sites, respectively) (Fig.3.14).

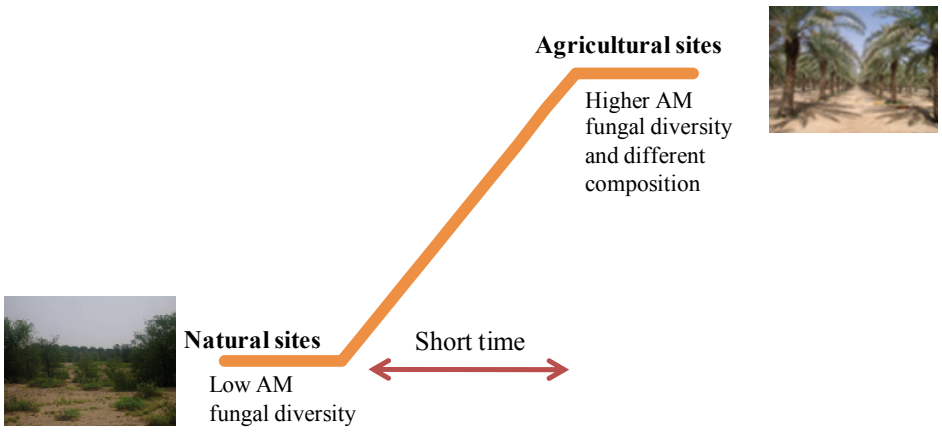

Fig. 3.14 The effect of land-use change from natural to agricultural on the AM fungal communities of the study sites.

Regardless of the mechanical disturbance of the uncultivated land occurring between the date palms in the modern agricultural site, the successional vegetation had similar AM fungal spore abundance (Fig. 3.11 A), species richness (Fig. 3.11 B) and inoculum potential (Fig. 3.11 C) as the natural vegetation. It is an indication that the propagules of the native AM fungi at least in the rhizospheres of the successional vegetation were not affected by the mechanical disturbance and the shift in the AM fungal communities seems to be due to the direct agricultural practices.

The degree of mycorrhization on the roots of the *Sorghum bicolor* is an indirect estimation of the vitality and activity of the AM fungal community present in the field. The higher inoculum potential of the AM fungal communities of the agricultural sites (Fig. 3.11 C) suggests a shift in the activity of this community due to the land-use change. The change in the abundance and the richness of this community (Fig. 11.3 A and B) was possibly responsible for such an increase in the inoculum potential. This suggestion is supported by the strong positive correlation between the AM fungal spore abundance and species richness and the AM fungal inoculum potential (Fig. 3.13).

Agricultural practices often lead to a lower AM fungal species diversity compared to the natural sites (Helgason et al., 1998; Boddington and Dodd, 2000, Oehl et al., 2003). In a long term experiment, it was found that these practices caused an almost complete dissappearance of AM fungal population (Plenchette, 1989).

In fact, in the present study, some of the AM fungal species occurring in the natural site were not detected in the agricultural sites nor recovered in the trap cultures (*Gl. macrocarpum* and *Glomus.* sp.OMA5, *Ambispora gerdemannii*, *Glomus* sp.OMA7, *Glomus* sp.OMA8, *Glomus* sp.OMA11 and *Glomus* sp. OMA12). These species might have been sensitive to habitat alteration which led to their disappearance or to a decrease of their abundance below detection level.

However, the general species richness was higher in the agricultural sites than the natural site.

The results of the present study represent a contradiction to the commonly-believed idea that land-use change reduces the AM fungal species richness. Recently, Li et al (2007) reported observations similar to the present study. They found that the Shannon diversity index in an agricultural site was higher than that corresponding to a natural site. The agricultural site of their

study was a 30 years-old hand tilled and low-input managed field while the natural site was composed of grasses and bushes with a few trees. The experiment was conducted in Southwest China where mean annual rainfall and temperature is 629 mm and 21.9 ºC respectively.

3.5.1 The possible drivers for the community shift

In the case of the present study at least, such a trend of AM fungal richness increasing might be attributed partially to the particularity of the studied ecosystem. The natural site is exposed to the extreme environmental factors of Southern Arabia (see section 1.6 of Chapter 1).

Introducing agriculture in such a site represents a dramatic change in the environmental conditions and that will consequently lead to a dramatic shift in the AM fungal community. The following environmental factors or the interactions between them may be the cause for such a dramatic shift in the community of AM fungi in the studied site:

1. **Soil moisture alteration**

   Soil moisture is a limiting factor in dry lands. The natural site of the present study is characterized as hyper arid region. One of the main dramatic changes on the soil when the site was converted to agricultural ecosystem was to increase the availability of water through permanent irrigation over years of the agriculture establishment. The structure of AM fungal communities along drought gradients has not been well studied. However, moisture and substrate stability were found to determine AM fungal community distribution and structure in an arid land (Jacobson, 1997). The long dry season in the study site might have affected the spore production due to overall limitation of the mycorrhizal status.

2. **Soil chemical alteration**

The soil properties of the natural and agricultural sites suggest that there is an alteration of the soil chemical properties (Table 3.2). There is an increase in all the nutrient levels including phosphorus (P) which is known to have a particular effect on the AM fungal status in the soil. High soil P levels limit formation of AM fungi (Menge et al., 1978; Sanders, 1975). Consequently the elevated soil P level could decrease the size of the AM fungal community. The increase in soil P supply could also change the community structure. This might be attributed to the variation in sensitivity to P among AM fungal species (Sylvia and Schenck, 1983). A negative impact of cumulative P fertilization on AM fungal inoculum potential and effectiveness together with a change in AM fungal species composition was observed (Thomson et al., 1986; Johnson, 1993; Gryndler and Lipavsky, 1995).

Paradoxically, there is some evidence that in a situation where soil P is very low, the mycorrhization and spore abundance will increase due to moderate increase of P (Bolan et al., 1984; Koide and Li, 1990; Xavier and Germida 1997). In some cases, the rates of colonization were found to decrease only when the bicarbonate-soluble P level exceeded 140 ppm (Amijee et al. 1989) or 133 ppm (Abbott and Robson 1977; 1978). The development of the mycorrhizal association was found to be highest at soil P levels of 50 ppm (Schubert and Hayman, 1986). In the present study, the bicarbonate-extracted P levels in the two agricultural sites were 71 and 93 ppm for the traditional and the modern agricultural sites respectively, (Table 3.2). Both P concentrations did not exceed the reported P level which might suppress the mycorrhization development. In the contrary, the P levels in the agricultural sites may have been raised in the present study to a degree

which might promote the mycorrhizal association compared to the lower level of P of 41.4 ppm in the natural site. It might be possible that the enhanced mycorrhization status of the plants led to a higher production of spores of different species occurred as an inculum before the agriculture was introduced.

3. **Soil physical alteration**

Another factor which might contribute to the AM fungal community shift is the soil physical alteration. While soil physical alteration affects negatively the abundance of certain species, it can encourage the abundance of other species (Douds et al., 1995, Jansa et al, 2002). It was found as well that different soil layers were shown to contain different AM fungal communities (Oehl et al, 2005). Alteration of the deep soil layers due to preparing land for cultivation and for date palms sowing, might have caused a redistribution of the community present in different layers. Oehl et al, (2005) found that *Scutellospora* species occur more abundantly with increasing soil depth (50-70 cm). Interestingly, the two *Scutellospora* species detected in the present study were found exclusively in the agricultural sites where soil layers were disturbed. It might be hypothesized then that the presence of some of the species on the top soils are due to mechanical lifting of the deep soil AM fungal community to the top soils.

4. **Soil temperature**

Although it was not measured in the different sites of this experiment, soil temperature in Southern Arabia is considered to be hyperthermic (United States Department of Agriculture, 1997). The natural site has higher exposure to direct sun compared to that in

the agricultural site. This is because of the scattered natural vegetation with their small sizes and vegetative parts.

The date palms, however, have larger vegetative parts and they were planted relatively close to each other, which provides enough shadow to cover the rhizosphere during most of the day. Hence, I assume that the AM fungal community in the natural site was exposed to higher soil temperature than the community on the agricultural sites. Temperature is known to have a negative impact on AM fungal activity. It was shown that root colonization by AM fungi often decreases when the temperature exceeds 30°C (Bowen 1987), while in other cases, soil temperatures above 40°C generally suppress the AM fungi (Bendavid-Val et al. 1997). Germination of the AM fungal spores can also be affected by temperature. Above 34°C, temperature caused a reduction in the germination of *Scutellospora coralloidea* and *Scutellospora heterogama* (Schenck et al. 1975*)*. Numbers of arbuscules in the roots of soybean were found to decrease at tempreatures above 30°C, while temperature above 34°C caused a reduction in the production of external hyphae outside the roots (Schenck and Schröder, 1974*)*. Colonization of *Anacardium occidentale* roots by *Glomus intraradices* was as well found to decline at temperatures above 30°C and was severely reduced at 38°C (Haugen and Smith, 1992). All examples above of a negative effect of high temperature on AM fungal activities and spore germination might suggest that temperature was a cause of the lower AM fungal abundance, richness and inoculum potential in the natural site with respect to the agricultural one.

5. **Introduction of exotic AM fungal species**

There were four AM fungal species (*Gl. sinuosum, Glomus* sp. OMA6, *Sc. calospora* and *Scutellospora* sp. OMA10) which were found exclusively in both agricultural sites. Agricultural inputs might have introduced these species in the traditional and modern agricultural sites. Seedlings of the date palm in the traditional agricultural site were brought from another geographical area due to the abundance of preferable date palm varieties grown in the interior part of Oman. Soil traces from the original land usually are attached to the roots of the imported seedlings. In the modern agricultural site, the source of the date palms is the same geographical area as the trees of the traditional site. They were tissue-cultured and then passed a "weaning" period where they had been transplanted into unsterilized soils. This process of transplanting the seedlings might be a source for exotic AM fungal species which were unintentionally introduced into the rhizosphere of the agricultural site. These exotic introduced species might use the new habitat with its low indigenous AM fungal diversity as an open ecological niche that can easy to be colonized, leading to an increase in the AM fungal species richness

6. **Changes of the host plant**

Another possible reason for the community shift might be the introduction of a new host plant (date palm). Host specificity and influence of plant species community on AM fungal composition have been demonstrated in natural ecosystems (Eom et al., 2000). With the widespread root network of date palms, part of the dormant mycorrhizal community might found the new host's roots as a good source of carbon. Consequently higher spore abundance and richness were detected in the agricultural sites.

This study was targeting a habitat with unique environmental settings. Such a habitat offered a new perspective to look at the response of AM fungal community to land-use change.

The agricultural sites had a different community structure with higher spore abundance, species richness and AM fungal inoculum potential. Many factors can be responsible for this community shift. More detailed future studies of the effects of single factors such as irrigation, fertilization, physical alteration, temperature etc., need to be designed to elucidate the influence of these factors and the interaction between them on the native AM fungal communities. Controlled experiments are also needed to determine how alterations of community structure in agricultural ecosystems have an impact upon the functionality of mycorrhizal associations, including effects on plant productivity.

Chapter 4: Molecular diversity of AM fungi associated with date palm: a closer look

4.1 Abstract

The AM fungal community associated with date palm cultivated in sandy loam alkaline soils of Southern Arabia was investigated. Roots from two sites under modern and traditional agricultural systems were sampled. The modern agriculture site was sampled in 2004 and in 2006, whereas the traditional agriculture site was sampled only in 2006. Specific amplifications of the nuclear-encoded 18S ribosomal RNA gene fragments of the AM fungi were used to determin their phylogenetic identity. This was achieved by PCR followed by cloning, RFLP digestion, sequencing and bioinformatics.

The overall diversity revealed from the two sites consisted of 9 phylogenetic taxa 8 of which belonged to the *Glomus* group A (the most diverse group in the Glomeromycota). One *Scutellospora* group was detected in the traditional agriculture site. Out of the 9 taxa revealed only 2 can be associated to named species of AM fungi. These are *Glomus sinuosum* and *Glomus proliferum.* Three phylotype groups are associated with AM fungal environmental sequences. The other 4 phylotype groups are not associated with any of the sequences in GenBank nor to our own database, and therefore we assume that they are new to science. The communities of these fungi were found to be differentiated between the two agriculture sites and consisted of both site-specialist and site-generalist groups.

Keywords: Date palm, Arabia, AM fungi, biodiversity, molecular identification.

4.2 Introduction

On the global scale, to our knowledge, the only study which dealt with AM fungal communities associated with date palm under field conditions came from North Africa (Bouamri et al. 2006). Based on the classical identification method by spore morphology, this study reported ten AM fungal morphospecies. The diversity of the AM fungal spores, however, was found not to necessarily reflect the species composition or abundance (Clapp et al. 1995). The AM fungi colonizing the roots in natural ecosystem are likely to be functionally active, and their identification is crucial for the understanding of the ecology of this symbiosis. The exact identification of the AM fungal community within roots requires the use of PCR-based techniques. These techniques have been used to analyze the composition of AM fungal communities colonizing the roots in the fields around the globe (see Öpik et al. 2006 for review).

The aim of this study was to characterize the molecular diversity of the AM fungal communities colonizing the roots of date palm, an ecologically, economically and socially important food crop in many arid parts of the world. The study also aims to compare these communities in two different agriculture sites which have different histories of establishment and management.

4.3 Materials and methods

4.3.1 Study sites

Traditional agriculture site

See the site description in the materials and methods section of Chapter 3.

Modern agriculture site

See the site description in the materials and methods section of Chapter 3.

4.3.2 Samples collection

In August 2006, the two sites were sampled on the same day. Each site was divided into four plots. In every plot roots were collected from the rhizosphere of four trees. These four subsamples were then pooled. Thus, four pooled samples were obtained from each site. Roots were washed with tap water and kept inside microcentrifuge tubes in a cooling box during the whole period of transportation. DNA was extracted from the roots as soon as the samples arrived to the laboratory. In January 2004 only the modern agriculture site was sampled by taking root samples from the rhizosphere of 5 different date palms. They were treated in the same way for transportation and DNA extraction.

4.3.3 DNA extraction and polymerase chain reaction

Five cm of the roots were first ground in liquid nitrogen within a 1.5 ml microcentrifuge tube using a pellet pestle. A DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) was used according to the manufacturer's instructions to extract the total DNA. A volume of 30µl of elution buffer was used to elute the DNA. DNA extracts were diluted 1:10, 1:50 or 1:100 in TE buffer and used as template for the first PCR reaction. PCR was performed in a nested procedure as described by Redecker (2000) and Redecker et al., (2003) using Taq polymerase from Amersham (Basel, Switzerland), 2 mM $MgCl_2$, 0.5 µM primers and 0.13 mM of each desoxynucleotide. The first

PCR was performed using the universal eukaryote primers NS5 and ITS4 (White et al., 1990). The PCR was carried out as follows: 3 min at 94°C, followed by 30 cycles (45 sec at 94°C, 50 sec at 51°C and 1 min 30 sec at 72°C) and a final extension phase of 10 min at 72°C. Dilutions of 1:100 of the PCR products in TE buffer were used as a template for the second PCR. Primer sequences and target AM fungal clades were: ACAU1661 (TGA GAC TCT CGG ATCGGG, Acaulosporaceae), ARCH1311AB (equimolar mixture of TGC TAA ATA GCTAGG CTG C and TGC TAA ATA GCC AGG CTG T; *Archaeospora*/*Paraglomus*), GIGA1313 (CTA AAT AGT CAG GCT AWT CTT, Gigasporaceae), GLOM1310 (AGCTAG GYC TAA CAT TGT TA, *Glomus* group A), LETC1677 (CGG TGA GTA GCAATA TTC G, *Glomus* group B), PARA1313 (CTA AAT AGC CAG GCT GTT CTC, *Paraglomus*), GIGA5.8R (ACT GAC CCT CAA GCA KGT, Gigasporaceae), GLOM5.8R (TCC GTT GTT GAA AGT GAT, *Glomus* group A), ITS4i (TTG ATATGC TTA AGT TCA GCG). The priming sites of the primers used are shown on Fig. 4.1.

For the second PCR, a hot start at 61°C was performed to minimize the possibility of non-specific amplification. The cycling parameters of the second PCR step were the same as the first ones except that the annealing temperature was raised to 61 °C. The second PCR products were then run on agarose gels (2%:1% NuSieve/SeaKem, Cambrex Bio Science, Rockland, ME, USA) in Tris/Acetate buffer at 120V for 25 min. In the cases no second PCR product was observed, the amplification process was repeated using combinations of different dilutions of the template DNA and different dilutions of first PCR products.

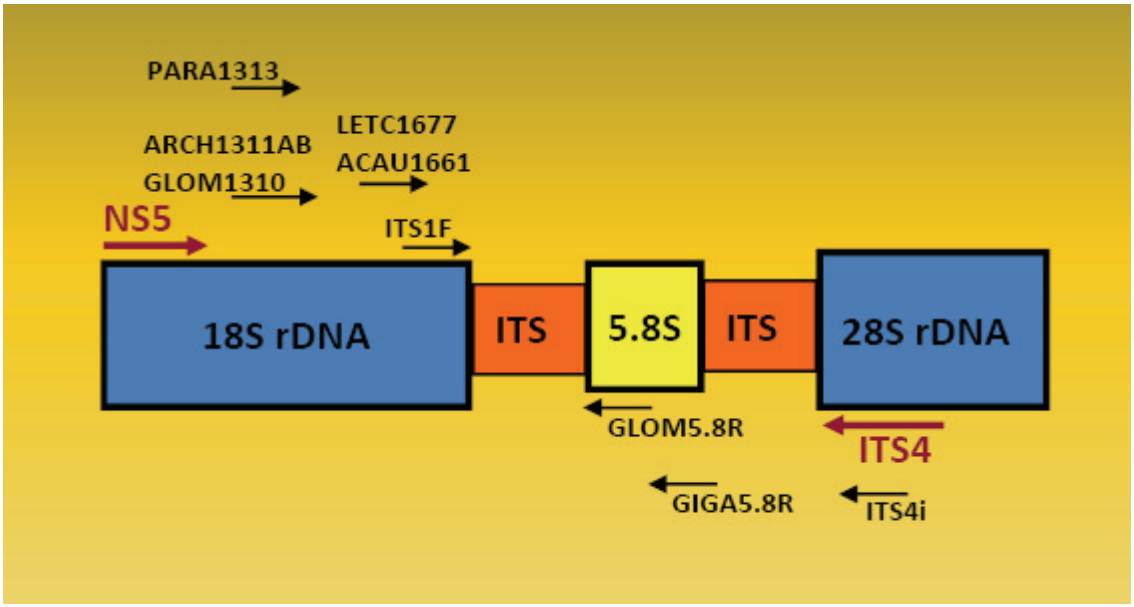

Fig. 4.1 Ribosomal RNA genes with annealing sites of the primers. Diagram is not to scale.

4.3.4 Cloning, RFLP analysis and sequencing

Positive PCR reactions were purified using the High Pure Kit from Hoffman LaRoche (Basel, Switzerland) and cloned using the pGEM-T vector (Promega/Catalys, Wallisellen, Switzerland). Thirty four positive clones of each PCR product were chosen randomly and the inserts were re-amplified. Digestion reactions of three hours were performed using the restriction enzymes *Hin*fI and *Mbo*I. Products of the reaction were revealed by agarose gels as described above. The sizes of the bands and the classification of different RFLP patterns in addition to their occurrences in different samples are presented in Table 4.2. Representative clones of restriction types were then re-amplified, purified using the High Pure Kit and sequenced in both directions. The BigDye Terminator Cycle Sequencing Kit (ABI, Foster City, CA, USA) was used for labeling. Samples were run on an ABI 310 capillary sequencer.

4.3.5 Sequence analyses

The Glomeromycotan origin of the sequences was initially tested by BLAST search. Those sequences in GenBank which showed high similarity to the blasted sequence were downloaded. Using PAUP*4b10 (Swofford, 2001), sequences from the present study were aligned to previously published sequences in addition to yet unpublished sequences from our own database (Fig. 4.2). Phylogenetic trees were primarily obtained by distance analysis (using the neighbor joining algorithm) in PAUP*4b10 using the Kimura two-parameter model and a gamma shape parameter=0.5. Results were verified by performing maximum likelihood analyses based on parameters estimated in Modeltest 3.5 (Posada, 2004). In the resulting phylogenetic trees sequence phylotypes were defined in a conservative manner as consistently separated monophyletic groups. The sequence phylotypes were named based on the numerical system used to define groups detected by the group of Basel.

4.3.6 Statistical analysis

A cluster analysis based on the similarity of AM fungal phylotypes composition across the host plants in the two agriculture sites was performed. Ward's was used as clustering method and squared Euclidean as a distance metric. Cluster analysis was performed using the Statgraphics program (Version 3.1).

4.4 Results

4.4.1 The detected groups are members of *Glomus* group A and *Scutellospora*

The primer combinations GLOM1310-GLOM5.8R and GLOM1310-ITS4i were used successfully. These primers were designed to target *Glomus* group A (as defined by Schwarzott et al., 2001). In addition, the primer combination GIGA-GIGA5.8R which was designed to target members of the *Gigasporaceae* was successful in one of the samples originating from the traditional agriculture site. All the other primer combinations targeted at other groups of Glomeromycota, did not yield PCR products. Clones were selected for sequencing on the basis of their RFLP patterns (Table 4.1). In the samples of 2006, 263 clones were classified into 19 different RFLP patterns and 33 representatives were sequenced. In the samples of 2004, 43 clones were classified into 10 different RFLP patterns (results are not shown) and 16 samples were sequenced. At least one clone representing each RFLP pattern found in each root sample was sequenced. A total of 49 clones were sequenced and analyzed phylogenetically in this study. Details about the number of screened clones and the occurrence of the different RFLP patterns among the different root samples of 2006 in both sites are shown in Table 4.2

The phylogenetic tree (Fig. 4.3) constructed in this study contains sequences obtained from the two sites in 2006 and one site in 2004. It reveals that there are at least 9 phylogenetic taxa colonizing the roots of the date palms. Eight of the 9 revealed taxa belong to the *Glomus* group A. There is one *Scutellospora* phylotype detected in one sample from the traditional agriculture site. Out of the 9 taxa, only 2 (GLOM-A32 and GLOM-A34) can be associated to arbuscular mycorrhizal fungal known species. Sequence types clustering in the GLOM-A32 group are associated with both *Glomus sinuosum* and a *Glomus clarum* sequence at the same time. There

are also some sequences from semi arid areas of Namibia clustering in this group. GLOM-A34 is a sequence type which is related to *Glomus proliferum.* GLOM-A35 and GLOM-A39 are sister groups of AM fungal environmental sequences. GLOM-A36, GLOM-A37, GLOM-A38 and the *Scutellospora* are not closely related to neither sequences in GenBank nor in our own database, and hence we assume that they are new to science. They might be unique to the studied habitat. No members from Diversisporaceae, Archaeosporaceae, Acaulosporaceae or Paraglomeraceae were detected in this study.

4.4.2 Site differentiation of AM fungal phylotypes

The cluster analysis (Fig.6.4) only those taxa colonizing the roots sampled in the two field sites at the same time (2006 sampling) were compared. It demonstrates the existence of site specialist AM fungal groups by the obvious separation of the two sites.

Table 4.1: RFLP banding patterns and their occurrence in the different field sites. The sizes of the bands of each RFLP type are listed in bp.

		RFLP type	*Hin*fI				*Mbo*I				
			Band A	Band B	Band C	Sum	Band A	Band B	Band C	Band D	Sum
Mod. site and trad. site 2006 (primers GLOM1310-GLOM5.8R)		A	300	235	70	605	350	125	85	65	625
		B	250	230	140	620	470	125	–	–	595
		C	360	240	–	600	370	126	100	–	596
		D	250	200	119	569	465	120	–	–	585
		E	305	145	100	550	340	116	–	–	456
		F	550	–	–	550	237	114	–	–	351
		G	313	246	–	559	344	166	–	–	510
		H	360	240	–	600	475	125	–	–	600
		I	239	149	–	388	404	148	109	–	661
		J	242	242	143	627	470	126	–	–	596
		K	360	156	109	625	370	126	100	–	596
		L	360	200	–	560	370	126	100	–	596
		M	255	143	–	398	370	126	100	–	596
		N	250	230	140	620	470	125	–	–	595
		O	300	235	70	605	306	101	–	–	407
Trad site 2006 Primers GIGA1313-GIGA5.8R		P	385	242	–	627	416	149	134	–	699
		Q	385	242	–	627	416	262		–	678
		R	420	242	–	662	416	149	134	–	699
		S	292	262	–	554	416	262	134	–	812
Modern site 2004	Primers GLOM1310-GLOM5.8R	T	359	236	–	595	373	162	–	–	535
Modern site 2004	Primers GLOM1310-ITS4i	V	468	358	260	1086	395	228	127	–	750
Modern site 2004	Primers NS5-GLOM5.8R	W	550	370	260	1180	1000 (undigested)		–	–	1000
		X	550	260	210	1020	1000 (undigested)		–	–	1000
		Y	550	–	–	550	1000 (undigested)		–	–	1000
		Z	450	400	260	1110	1000 (undigested)		–		1000
		A1	700	350	–	1050	680		120	–	800
		B1	500	–	–	500	–		–	–	–
		C1	680	310	–	990	680		–	–	680
		D1?	660	290	–	950	680		–	–	680

	Primers	Sample	Number of screened clones	Positive clones	Number of RFLP patterns	Sequenced clones	RFLP pattern types and their corresponding phylotypes																				
							A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	Total
							GLOM-A35	GLOM-A33 GLOM-A35	GLOM-A32 GLOM-A35	GLOM-A39	GLOM-A35	GLOM-A35	GLOM-A35	GLOM-A32	GLOM-A32	GLOM-A32	GLOM-A32	GLOM-A32	GLOM-A32	GLOM-A33	GLOM-A35	Scutel. group	Scutel. Group	Scutel. group	Scutel. group	
Tradition agri. Site 2006	GLOM1310-GLOM5.8R	1	34	32	2	4	6	25	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	31
	GIGA1313-GIGA5.8R		39	35	4	7	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	29	2	1	1	33
	GLOM1310-GLOM5.8R	2	34	32	3	5	28	–	–	3	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	32
		3	34	34	4	6	29	–	–	–	2	1	2	–	–	–	–	–	–	–	–	–	–	–	–	34
Modern agri. site 2006	GLOM1310-GLOM5.8R	1	34	34	3	3	–	–	32	–	–	–	–	1	1	–	–	–	–	–	–	–	–	–	–	34
		2	34	34	5	4	–	–	30	–	–	–	–	–	–	1	1	1	1	–	–	–	–	–	–	34
		3	34	34	1	1	–	–	34	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	34
		4	34	31	3	3	–	22	8	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	31

Table 4.2 RFLP types, the corresponding phylotypes and the occurance in the root samples of date palm roots which were samples in 2006. The value numbers indicate the number of screened clones which have an insert with RFLP type. The last column is the total number of the clones which gave a readable RFLP pattern.

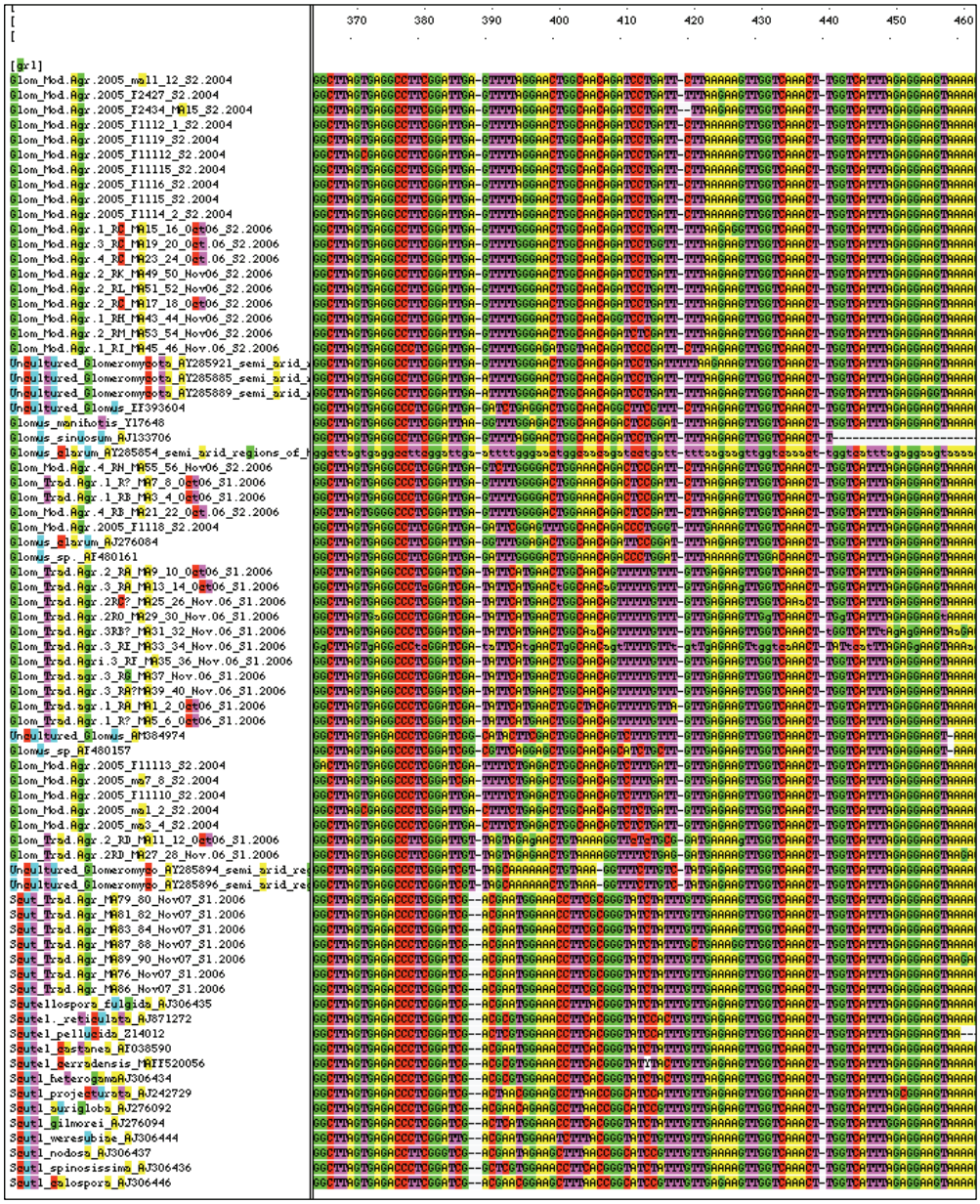

Fig. 4.2 The part of the 18s rDNA alignment (using PAUP*4b10) which has the highest variability among the sequences used to build the phylogenetic tree in this study (Fig. 4.3). The alignment includes the sequences obtained in the present study in addition to all those in GenBank which show high similarity to them.

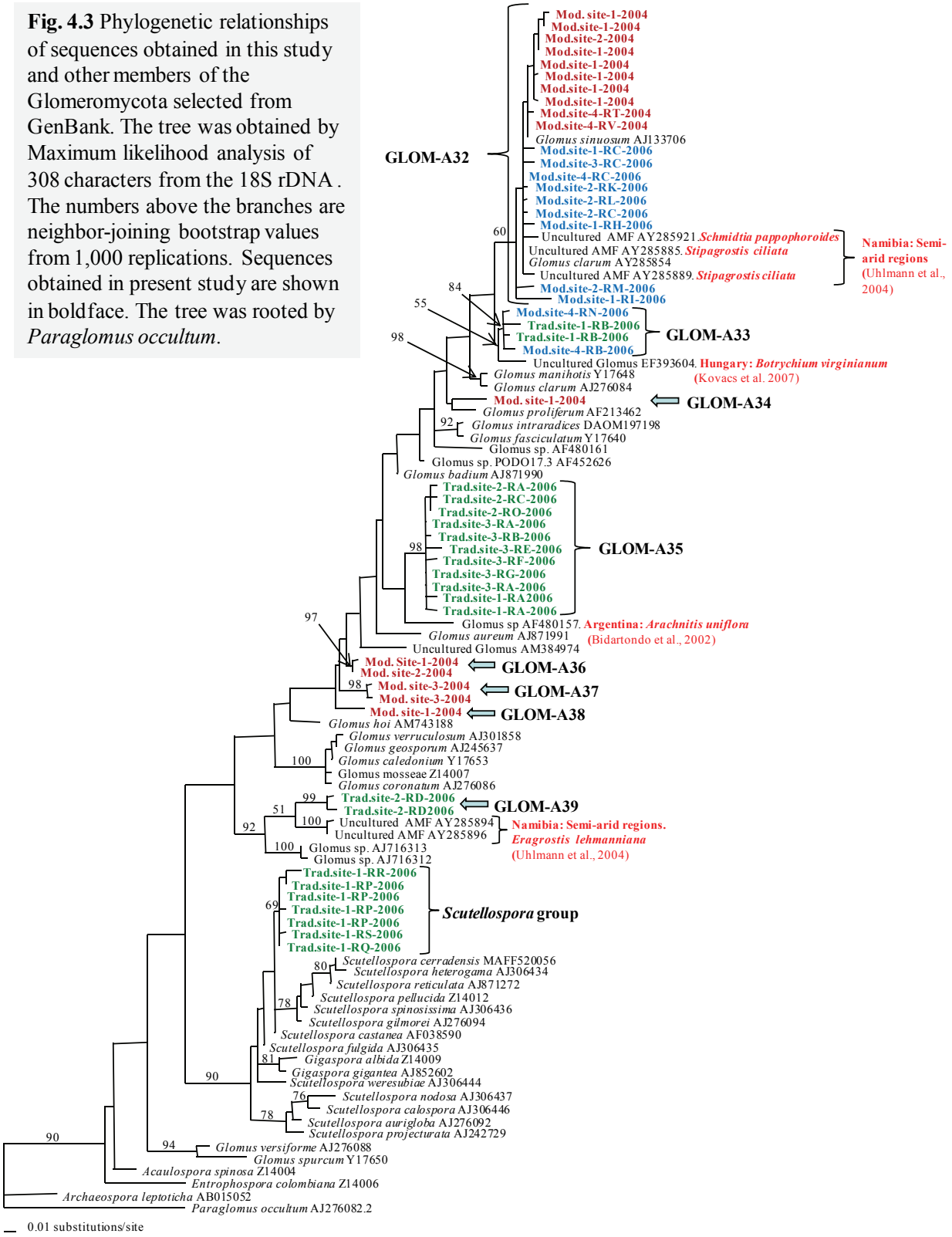

Fig. 4.3 Phylogenetic relationships of sequences obtained in this study and other members of the Glomeromycota selected from GenBank. The tree was obtained by Maximum likelihood analysis of 308 characters from the 18S rDNA. The numbers above the branches are neighbor-joining bootstrap values from 1,000 replications. Sequences obtained in present study are shown in boldface. The tree was rooted by *Paraglomus occultum*.

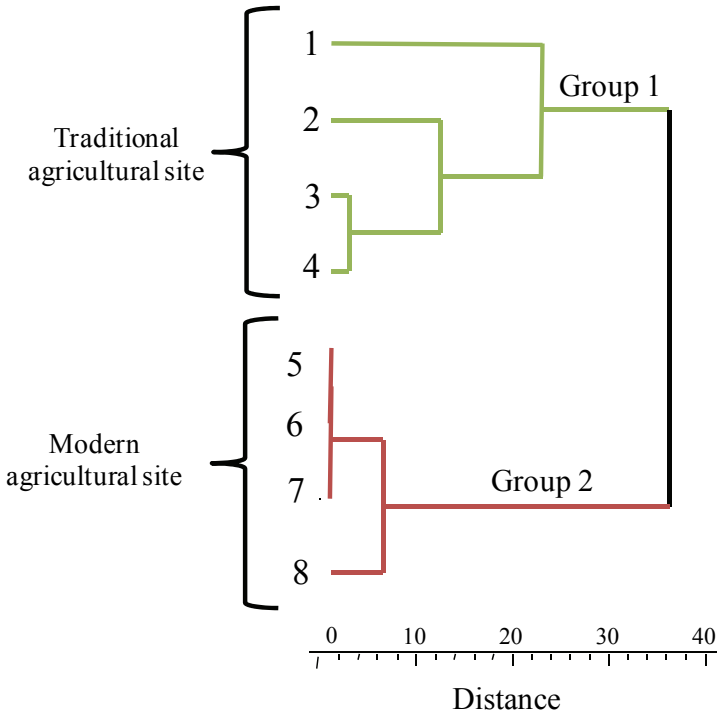

Fig. 4.4 Dendrogram of a cluster analysis based on the similarity of AM fungal composition across all the 8 root samples of both agricultural sites. Two main groups were formed suggesting the presence of two different communities occurring in natural and agricultural sites. Ward's clustering method and squared Euclidean distance metric were used.

4.5 Discussion

4.5.1 AM fungal community associated with date palm

Data from two sites with different management histories sampled in 2006 in addition to data from the modern agriculture site sampled in 2004 were all put together to better estimate the overall AM fungal diversity associated with date palm. The total number of taxa detected was 9. This is in accordance with earlier field studies which showed that single plants can be associated with several AM fungal taxa. Molecular techniques have been used to investigate AM fungal communities colonizing the roots in the field in different ecosystems around the world. In these studies, the number of taxa detected in the roots of one plant species per site ranged between 2 associated with *Taxus baccata* in Germany (Wubet et al., 2003) and 22 associated with *Tetragastris panamensis* growing in tropica rainforest in Panama (Husband et al., 2002a). In a literature survey of studies using the SSU rDNA -the same gene used as in the present study- to identify the AM fungal community within the roots, Öpik et al. (2006) found that the number of taxa detected per host plant species was significantly dependent on the habitat type. They found that the average number of taxa associated with host plant species ranged from 5.2 in habitats under "strong anthropogenic" influence to 5.6 in temperate forests, 8.3 in temperate grasslands and 18.2 in tropical forests. The 9 taxa detected in the present study are associated with roots of a plant grown in an agricultural system in a hyper-arid environment with alkaline soils. This number of taxa is closer to 8.3, the average number of taxa detected in the temperate grasslands. We are not aware of any similar study dealing with AM fungal communities associated with date palm, nor with any other plant growing in the ecosystems of Arabia. This makes it difficult to compare our results with other similar studies. In general, the number of the AM fugal taxa

associated with date palm does not seem to be exceptionally different from those associated with different plants in different ecosystems.

4.5.2 Sequences associated to *Glomus sinuosum* and *Glomus proliferum*

Out of the 9 AM fungal taxa recorded in this study, only 2 can be associated with known and named species. In the GLOM-A32 group (Fig. 4.3), our sequences clustered with a sequence of *Glomus sinuosum* isolated from Maryland in the USA. Interestingly, the same group contained sequences obtained from roots and rhizosphere of different plants growing in a semi-dry region of Namibia (Uhlmann et al., 2004).

One of these sequences was obtained from a spore of *Glomus clarum* (AY285854) which is surprising as *Glomus sinuosum* and *Glomus clarum* are easy to distinguish morphologically. Other sequences from *Glomus clarum* in the database were not closely related to *Glomus sinuosum.* If this discrepancy is not due to an error in assigning AY285854 to *Glomus clarum,* the most likely explanation would be that a species fitting the morphological description of *Glomus clarum* cannot be distinguished from *Glomus sinuosum* using 18s DNA sequences. We also found sporocarps of *Glomus sinuosum* in the same field (see Table 3.1, Chapter 3) but no *Glomus clarum*-like spores in the site of the study. Therefore, all available evidence appears to support assigning GLOM-A32 to *Glomus sinuosum.*

Phylogenetically, *Glomus sinuosum*-associated sequences obtained from different regions of the rDNA of AM fungi were found within the roots of different plant species growing in different habitats around the world. *Glomus sinuosum* associated sequences were detected in the roots of:

- *Prunus africana* in Afromontane a forest in Ethiopia (altitudinal range of 1500-2700m, an average annual temperature between 14-20ºC, annual rainfall of 700-1100 mm) (Wubet et al., 2003)
- *Hieracium pilosella* growing in an unmanaged grassland on the North coast of Zeeland in Denmark (Rosendahl and Stukenbrock, 2004)
- *Faramea occidentalis* and *Tetragastris panamensis* in a tropical forest in Panama (mean annual rainfall 2.6m) (Husband et al., 2002)
- *Potentilla erecta* in Thuringia, Germany (950-1099 mm annual rainfall and 6.0-7.0 mean annual temperature) in a mountain meadow where soil P was not detectable, but not in another meadow where soil P was 76.28 mg/kg (Börstler et al., 2006).

Morphologically, *Glomus sinuosum* is one of the easiest AM fungal species to identify due the unique structure of its sporocarps (see Fig. 2.7 E-G, Chapter 2) so its existence is unlikely to be missed in morphological studies. This species was found in a semi-arid soils of monsoonal southern India (annual rainfall ranges between 500-700 mm), in the rhizosphere of *Cymbopogon caesius* dominating ungrazed grasslands and in scrub jungles at slopes dominated by *Acacia* species (Muthukumar and Udaiyan, 2002). It was reported as well in the arid part of northwest China, in meadow steppe, desert steppe, steppe desert and typical desert (Shi et al. 2007). Low-input agricultural practices did not seem to affect the presence of *Glomus sinuosum* in Southwest China (mean annual rainfall and temperature is 629 mm and 21.9 C respectively) and it was found in the natural land composed of grasses and bushes with a few trees and from 30 years old converted land to a hand tilling and low-input managed field cultivated with sweet potato, peanut, onion and sorghum, and from old field fallowed from the cultivated land (Li et al., 2007). However in Southern Brazil, at 1425 m above sea level (described as humid mesothermic

climate according to Köppen classification), *Glomus sinuosum* was isolated from native grassland but neither in conventional nor in organic apple orchards (Purin et al. 2006). Similarly, in Central Europe (mean annual rainfall and temperature are 650-850 mm and c. 9.5 ºC respectively) in a study comparing the AM fungal community in five different field sites representing three different agriculture land-use systems, sporocarps of *Glomus sinuosum* were found in an extensively managed grassland but they were absent in another extensively managed grassland site with a continuous slight surface erosion, in a vineyard and in an intensively-managed mono-cropped maize field (Oehl et al., 2005). However, in a submediterranean climate of Tuscany, Italy, (mean annual rainfall and temperature are 930 mm and 15 ºC respectively) *Glomus sinuosum* was found in adjacent three different land use types including long-term intensive maize monoculture, unmanaged forest and native grassland (Bedini et al., 2007).

In these studies, it seems from the available data that *Glomus sinuosum* prefers low-disturbed habitats. The finding of this species in the modern agriculture site of the present study is in agreement with the observation of the low-disturbance preference of *Glomus sinuosum*. In the traditional agriculture site, the soil was tilled annually using the mechanical tractor in order to prepare the land between the date palms for cultivation. In addition, the nutrient level of the soil P is higher in the traditional agriculture site. In the modern agriculture site, however, the area between the date palms was not cultivated and therefore, the rhizosphere of the date palms was not disturbed mechanically in the last 16 years of cultivation.

The other sequence which had an association with a sequence of known species was GLOM-A34 (Fig. 4.3). It had an association to *Glomus proliferum* which was relatively recently described (Declerck et al., 2000). The authors found this species in a single site in Guadeloupe (altitude of 250 m and mean annual rainfall of 3500 mm). It was isolated from the rhizosphere of *Clausena*

excavata at the margin of a banana plantation. The occurrence of *Glomus proliferum* both in roots and as spores has been rarely reported, and hence, it is difficult to compare its global ecology with the finding of the present study.

Glomus proliferum as a morphospecies did not co-occur with *Glomus sinuosum* in any of the above mentioned studies. In the study of AM fungal diversity associated with date palm (Bouamri et al., 2006), no spores of *Glomus sinuosum* nor of *Glomus proliferum* were detected. However, two sequences closely related to these two species co-occurred in the roots of *Botrychium ternatum* in Korea (Lee et al., 2004).

4.5.3 Environmental sequences

Three taxa were related to other environmental Glomeromycotan phylotypes**.** GLOM-A33 (Fig. 4.3) was associated with a sequence coming from a sandy and alkaline soil **(**Kovacs et al. 2007) which are two characteristics as well of the soil in the present study. GLOM-A35 (Fig. 4.3) was associated with an AM fungal sequence obtained from the roots of the non-photosynthetic plant *Arachnitis uniflora* which was found to be epiparasitic plant specialized on arbuscular mycorrhizal fungi (Bidartondo et al., 2002). GLOM-A39 (Fig. 4.3) group was another group -in addition to GLOM-A32- which was associated with sequences obtained from roots and rhizosphere of different plants growing in a semi-dry region of Namibia (Uhlmann et al., 2004).

4.5.4 New sequence types

Four taxa (GLOM-A36, GLOM-A37, GLOM-A38 and the *Scutellospora* group) (Fig. 4.3) have no closely related sequences in GenBank, and hence cannot be related to taxonomically described and named fungi nor to environmental sequences. We hypothesize that the groups which are not associated to any other sequences in Genbank and those which have the closest

relative sequences to be found in semi-arid lands to be entities specialized to the dry habitats. This hypothesis is in agreement with the observation that there are some groups which are specific to certain habitats (Öpik et al, 2006).

4.5.5 The missing AM fungal groups

A noteworthy finding of this study is that none of our sequences is related to the *Glomus intraradices*/ *fasciculatum* group, the globally most frequently detected *Glomus* groups nor to *Glomus mosseae* which is often found in temperate arable fields, tropical forests, grasslands and wetlands. It is worth mentioning however, that a sequence related to *Glomus intraradices* was detected in the roots of trap culture plants setup to trap the AM fungi in the rhizosphere of date palms in the modern agriculture site in the sampling of 2004 (see Fig. 2.11 Chapter 2). However, we have no evidence that the same taxon was symbiotically active and colonized the roots of date palm in the field. Glomeromycotan groups other than *Glomus* group A and Gigasporaceae were not detected although in our two study sites spores belonging to the genera *Paraglomus* and *Acaulospora* were identified morphologically (Table 3.1, Chapter 3). The absence of these groups in the field roots cannot be attributed to PCR problems because the PCR positive controls used in the present study worked.

4.5.6 Fungal community differentiation between the two sites

One of the aims of this study was to examine whether the two agricultural sites are associated with different AM fungal communities. Relatively few molecular studies have explored these communities associated with one plant species growing in different agricultural systems a. Some of these studies showed community differentiation between two sites (Wubet et al., 2003; Börstler et al., 2006; Hijri et al., 2006). In the present study, the community differentiation

observed (Fig. 4.4) might be due to the differences in the agricultural practices in the two sites. The chemical soil properties of the two sites (Table 3.2, Chapter 3) are different. It has been shown that agricultural practices (Boddington and Dodd, 2000), including soil fertilization (Johnson, 1993), can affect the formation of mycorrhiza. The occurrence of site-specific taxa in these two sites might imply that in different agricultural systems, date palm might be associated with site-specific taxa, and to test that a larger-scale survey is needed.

4.5.7 Soil characteristics

Regardless of the many years of agricultural inputs in the two studied fields, the soil nutrient content has remained low to medium (Table 3.2, Chapter 3). Although the types of agricultural practices in the two sites appear different from each other, they both did not seem to be intensive enough to raise the content of nutrients to high levels perhaps because of the inherited low soil fertility (MAF, 1989, 1991) and the reasonable amount of the inputs added. The levels of sodium bicarbonate extracted P in the soils of the two agricultural sites (Table 3.2, Chapter 3) seems to be within the range where AM fungi have been found to be symbiotically active (see the Discussion section of Chapter 3).

This report is the first on the molecular diversity of an AM fungal communities associated with date palm and from the whole Arabian Peninsula. Although the richness of the AM fungal taxa associated with this tree in two agricultural sites does not seem to be exceptionally different from the richness associated with different plants in other ecosystems, the composition of this community suggests some degree of uniqueness. We believe that this study is contributing to a better understanding of the global biodiversity of such an important symbiosis through exploring one of the least known habitats in this respect.

Chapter 5: General discussion

5.1 Common trends of diversity revealed by using molecular and morphological approaches

Only *Glomus sinuosum* and *Glomus intraradices* were detected as morphospecies in soil and as phylotypes inside roots. Although the morphological and molecular approaches used in this project revealed different AM fungal communities, they showed the following common diversity trends:

- A high dominance of the genus *Glomus* (Fig. 5.1): This is in accordance to the findings that under arid and semiarid environments, morphospecies and phylotypes belong to the genus *Glomus* were the dominant (Stutz et al. 2000; Uhlmann et al. 2006).
- The presence of a high number of taxa which cannot be identified to the species level:

  Out of 27 morphospecies (Table 2.1; Table 3.1), only 14 where identified to species level with high identification confidence, 12 resembled some known species and one seems to be an un-described species. Phylogenetically, the identities of most phylotypes were not clear. In trap culture roots, only three phylotypes where associated with known AM fungi *(Glomus intraradices, Diversispora spurca* and *Glomus aurantium).* Four phylotypes were similar to previously reported environmental sequences and two had no closely related sequences in the Genbank (Fig. 2.11). In the date palm field roots, only two sequences were associated to known species *(Glomus sinuosum* and *Glomus proliferum)* while three phylotypes were associated with AM fungal environmental sequences and four with no sequences in GenBank (Fig. 4.3)

- Both morphological and molecular approaches suggested that the AM fungal communities in the two date palm sites were differentiated between the two sites (Fig. 3.9; Fig. 4.6). Some groups were specialised in each site in addition to some groups present in both sites.

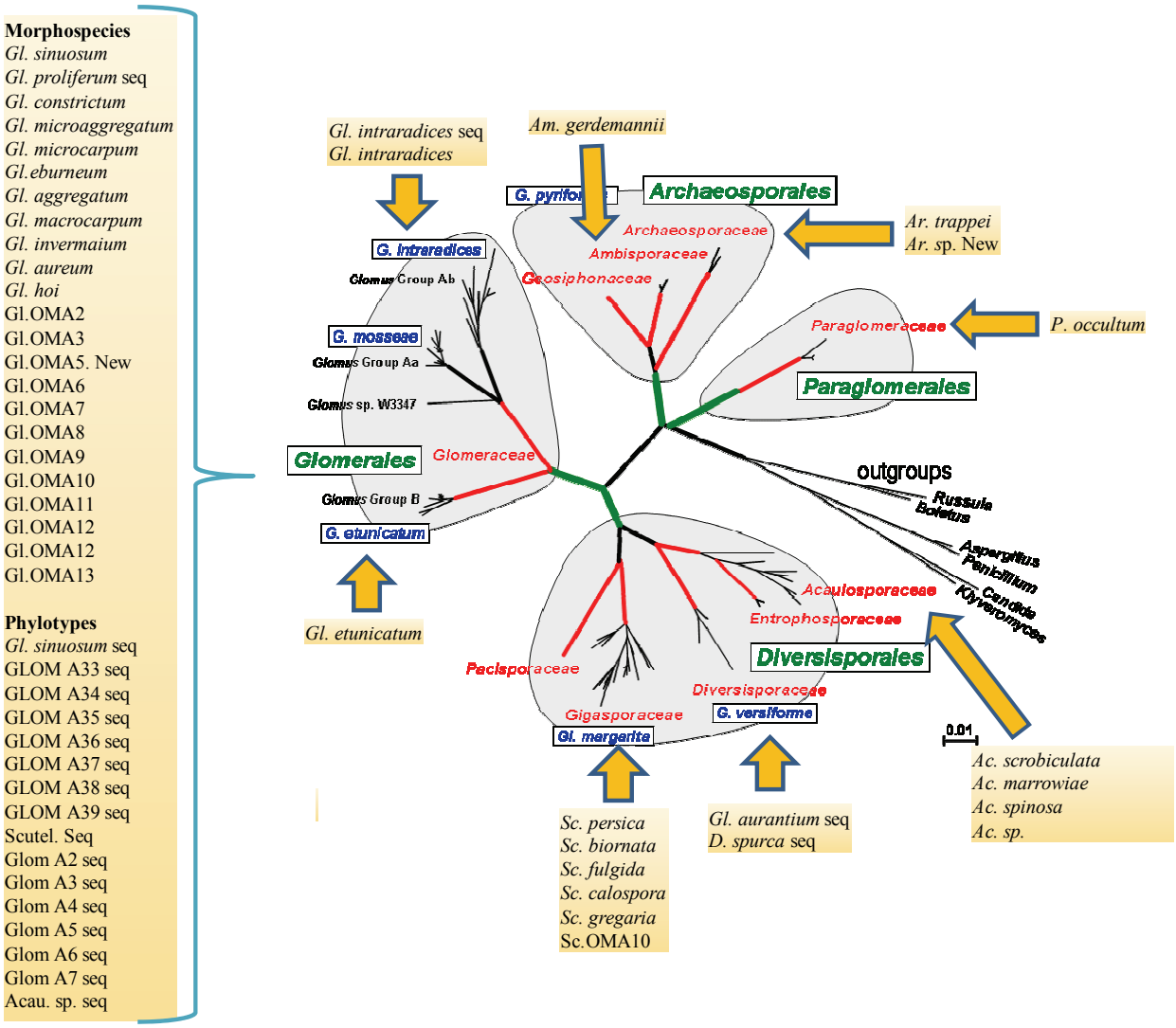

Fig. 5.1 The affiliation of different AM fungal species detected in the present study. Phylogenetic tree from A. Schüßler's website: www.lrz-muenchen.de/~schuessler/amphylo/

5.2 Richness and composition of AM fungi in Southern Arabia

5.3.1 AM fungal richness

Based on morphological identification of AM fungal spores, a total of 36 morphospecies were detected at five sites in Southern Arabia. Altogether, 31 species were detected in the field samples directly, and five additional ones were found upon trap culturing for eight months with a consortium of three AM fungal trap plant species. This is a high total richness given that so far only around 200 AM fungal species have been described worldwide for the phylum *Glomeromycota* (see: http://www.lrz-muenchen.de/~schuessler/amphylo/amphylo_species.html). In natural sites, 15 species were associated with the vegetation in the marginal sand dunes (Table 2.1, Chapter 2), 13 with natural site adjacent to the modern agriculture site and only two with the vegetation in a Holocene extinct lake (Table 3.1, Chapter 3). The detected species richness seems to be in the range of richness in other similar dry habitats. Stutz et al. (2000) reported a range of 7 to 14 species at 13 sampling sites in two arid regions and semi-arid grass lands in North America and in Namib Desert in Africa. Jacobson (1997a) reported 12 AM fungal species in soil samples of 10 sites in the Namib Desert. Uhlmann et al. (2006) reported, as well, 12 species from 3 sites in an arid region of Namibia.

In agricultural sites, 13 and 14 morphospecies were associated with date palm growing in modern and traditional sites, respectively (Table 3.1, Chapter 3). This is higher than the richness range (4 to 7 species) found to be associated with date palm in ten different sites in an arid zone Southwest of Morocco but closer to the overall richness of 10 species (Bouarmi et al. 2006). An exceptionally high richness of 42 species was reported by Li et al. (2007) in an agricultural land in Southwest China (Mean annual temperature is 21.9ºC, the highest up to 43ºC. Mean annual rainfall is 629 mm). The species richness range of 8 to 13 was found in lands with monocropping

in Central Europe (Oehl et al., 2003). Other studies on species richness in European and North American arable lands are in the same range found in Central Europe (Douds and Millner, 1999; Franke-Snyder et al., 2001; Kurle and Pfleger, 1996; Land and Schönbeck, 1991).

The molecular techniques were used to target AM fungal entities colonizing roots coming from two different sources. One source was the trap culture plants established from rhizosphere soils of date palm, mango and *Zygophyllum hamiense*, all sampled in 2004 (Chapter 2). The other source of roots was date palm cultivated in two agricultural sites and sampled in 2006 (Chapter 4). The roots of the trap culture plants were investigated using a different pair of primers (AM1-NS31) than those used to investigate the field roots. Both molecular approaches, interestingly, revealed the presence of very similar number of taxa (9 and 10 from field and trap culture roots, respectively). Moreover, the composition of communities in both roots was exceedingly dominated by taxa which belong to the genus *Glomus* (8 out of 9 and 8 out of 10 taxa in fields and trap cultures, respectively).

It was already discussed in chapter 4 that the average number of taxa associated with host plant species was found to have a range from 5.2 in habitats under "strong anthropogenic" influence to 5.6 in temperate forests, 8.3 in temperate grasslands and 18.2 in tropical forests (Öpik et al., 2006). With the exception of taxa number associated with tropical forests, the 9 to 10 taxa present in the our studied agricultural sites seem in the range of average number of taxa associated with different plants in different ecosystems.

5.3.2 AM fungal composition

Biogeography is a science which studies all patterns of geographic variation in nature -from genes to entire communities and ecosystems- elements that vary across geographical gradients including those of area, isolation latitude, depth and elevation (Lomolino et al., 2006).

There is a known old theory that microorganisms have no biogeography and they do not exhibit a taxa-area relationship (Beijerinck, 1913). However, recent evidence has challenged that view and showed that microbes can exhibit a taxa-area relationship (Horner-Devine et al. 2004). Öpik et al. (2006) used 26 published studies on the molecular identification of AM fungi colonizing roots of different plants to compare the diversity and composition of AM fungal communities in different environments around the world. Interestingly, they found that AM fungal communities exhibit different compositions in broadly defined habitat types like tropical forests, temperate forests and habitats under anthropogenic influence. Investigating AM fungal spores composition Stutz et al. (2000) proposed that pattern of taxonomic range of AM fungi in arid regions lands appears to exist. We hypothesized that the AM fungal communities in Southern Arabia should show a more similarity to another AM fungal community inhabit an environment which has some degree of similarities to Southern Arabia. To test this hypothesis, a cluster analysis (Fig. 5.3) was performed. The analysis included those taxa which were found in the present study and which were identified to a species level with highest identification confidence. These species were *Gl. sinuosum, Gl. etunicatum, Gl. constrictum, Gl. microaggregatum, Gl. microcarpum, Gl.eburneum, Gl. macrocarpum, Sc. fulgida, Sc. gregaria, Sc. calospora, Ac. spinosa, P. occultum, Am. gerdemannii and Ac. morrowiae.* Phylotypes detected in the present study were excluded from the analysis in order to avoid the bias which might result from the non presence of

enough molecular phylotypes associated with the natural vegetation investigated by the present study.

The selection of different published studies to construct a database of the cluster analysis was based on two methods. First, the search through the species distribution database of Department of Plant Pathology, University of Agriculture, Poland (http://www.agro.ar.szczecin.pl/~jblaszkowski/), a world wide known source of information on AM fungal taxonomy (Author: Prof. Janusz Blaszkowski**).** Second, and to complement the first searching method, the name of each fungus was searched on the Web of Science. The total number of the studies used in the comparison was 87, representing different broadly-defined habitats and land-uses (deserts, agricultural lands, natural lands and sandy soils) in the six continents (Table A, Appendix).

The cluster analysis (Fig.5.2) indicates that the AM fungal community detected in the agricultural lands of Southern Arabia was closely related to those AM fungal communities which occur in sandy soils of different parts of the world. This might be due to some similar environmental factors controlling the composition of AM fungal communities in sandy soils.

The communities of AM fungi present in the natural sites, however, seem to be unique and do not resemble a community of any of the broadly-defined habitats. Many studies provide evidence that the distribution of AM fungal spores in soil can be affected by biotic and abiotic factors, such as ecosystem type, soil pH, soil moisture, total soil C and N, temperature and disturbance regime (McGraw and Hendrix 1984; Koske 1987; Gibson and Hetrick 1988; Johnson et al. 1991, 1992; Boddington and Dodd 2000; Carvalho et al., 2003).

Therefore, the proposed different community composition present in the studied natural sites of Southern Arabia might have emerged due to unique biotic and abiotic environmental factors

(Fisher and Membery, 1998; Glennie and Singhvi, 2002; Ghazanfar and Fisher,1998) affecting these sites.

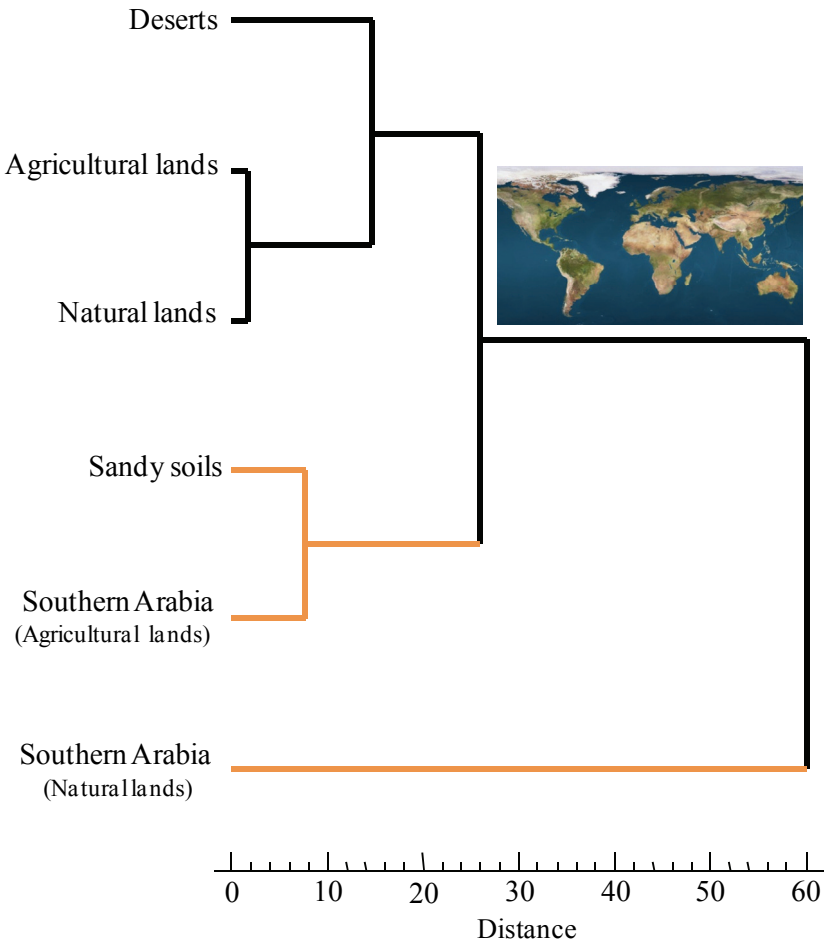

Fig. 5.2 Cluster analysis based on the similarity of AM fungal species composition across the natural and agricultural ecosystems, deserts and sandy soils around the world. The differentiation between the AM fungal communities in natural and agricultural sites in our study was already recognized and discussed in Chapter 3. Ward's clustering method and squared Euclidean distance metric were used.

These results show the need for a more elaborate study in order to compare the AM fungal community of the studied region of Southern Arabia to that in different habitats of the world.

Such a study needs more information on the AM fungal communities, both in study sites of Southern Arabia and in other parts of the world.

5.3 Overall contributions of this work to science

To my knowledge, this is the first comprehensive report on AM fungal communities in Arabian Peninsula, which was in this respect; known only through two studies reported a very low AM fungal diversity (Malibari et al, 1988; Khaliel, 1989). It has been shown, however, through the studies presented in this thesis that natural and agricultural sites of Southern Arabia were associated with a wide range of AM fungal species. It is, as well, the first molecular investigation ever on AM fungi associated with date palm, a socio-economically important plant in many dry lands of the world. An important finding was that the agricultural sites were associated with a higher AM fungal abundance, richness and inoculum potential than their surrounding natural sites suggesting a shifting impact of agricultural practices on native AM fungal communities (Fig. 5.3).

The composition of AM fungal communities detected in the present study was compared to that found in broadly-defined habitats of the world to seek for biogeographical patterns. It was found that agricultural sites have a composition most similar to those found in sandy soils of different parts of the world. Natural sites, however, seem to maintain a unique species composition, which might have emerged due to unique local biotic and abiotic environmental factors.

I believe that this scientific work was crucially needed to fill a gap in our knowledge about AM fungi in Southern Arabia, and consequently, contributes to the knowledge on global diversity, phylogeny and ecology of the AM fungi.

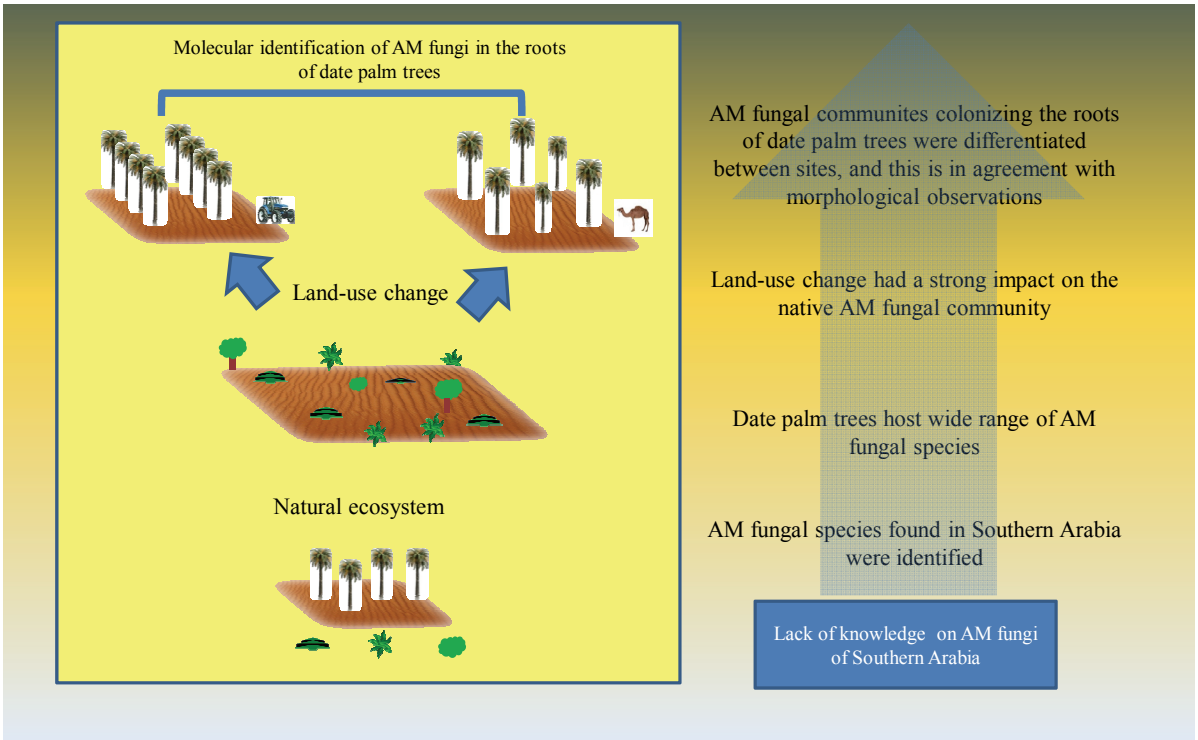

Fig. 5.3 The main achieved goals of the thesis.

5.4 Future perspective

- **Interaction of date palm with native AM fungal community**

Through the work of this thesis, one of the questions which surfaced was: when date palms are being introduced to natural sites, how do they interact with the already existing native AM fungal communities? This interaction might include preferences toward particular AM fungal species (Husband et al., 2002; Šmilauerová et al., 2007) which might lead to their higher abundance in the field. Other species which would not be able to associate with the introduced date palms might be pushed toward local ecological extinction (Estes et al. 1989). We hypothesize that date palm itself is responsible -at least partially- for AM fungal community shift described in Chapter 3, through its interaction with the native AM fungi. To test this hypothesis, the diversity of AM fungi associated with date palm seedlings will be compared with that associated with plants in the natural and agricultural sites (Table 3.1, Chapter 3) and to the AM fungal species produced in trap cultures consisted of a consortium of three trap plant species (Fig. 3.8 A) inoculated with the same soils as date palm seedlings. See the experimental design in appendix B.

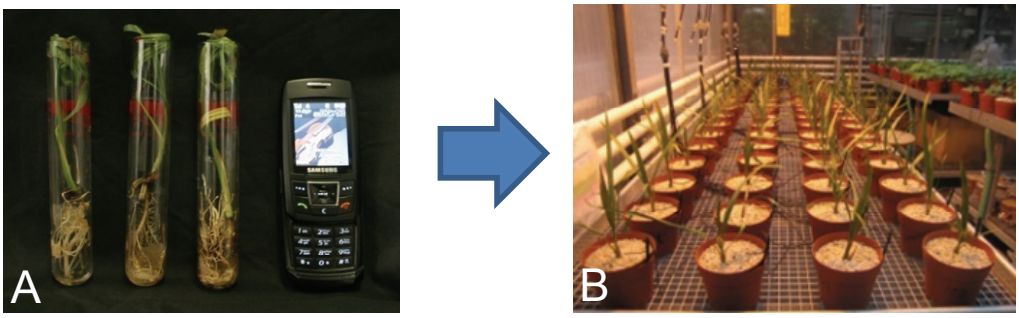

Fig. 5.4 Introduction of date palm plantlets into natural and agricultural soils under green house conditions. **(A)** Plantlets produced through tissue culture technique. **(B)** Plantlets transferred in pots containing the AM fungal inocula.

Appendix A: Global distribution of AM fungal species

Table A: Twenty three morphospecies detected in the present study and their global distribution. The country or the geological location of their occurrences is given in boldface followed by the available information on the host plant and/or the type of habitat. AM fungal species were followed by the plant species which were associated with (H: *Heliotropium kotschyi,* Z: *Zygophyllum hamiense,* S: *Salvadora persica,* P: *Prosopis cineraria,* I: inter-plant vegetation, V: successional vegetation, DP(T): Traditional site of date palm, DP(M): Modern site of date palm), (S) indicates that the plants was growing in the sand dunes).This survey is not intended to be a complete assessment of all the studies reporting the presence of these species. However, it seeks examples of their occurrences in agricultural and natural sites in the six continents.

AM fungal morphospecies	Global distribution

Gl. etunicatum
 	
DP(T)
	• **Brazil:** the states of Bahia and Sergipe, associated with citrus (Weber and Deoliveira, 1994)
	• **Cameroon:** undisturbed soil (Musoko et al.,1994)
	• **China:** desert plants (Shi et al., 2007)
	• **Central Europe**: the border between France, Switzerland and Germany in semi natural and agricultural lands (Oehl et al., 2005)
	• **Germany:** a wet fallow agricultural habitat (Kuehn, 1992)
	• **Iran:** wheat fields in the Golestan Province **(**Sadravi, 2006)
	• **Israel:** (Blaszkowski et al., 2001)
	• **Namibia** :
	  ○ a desert under different plant species colonizing a dune (Stutz et al., 2000)
	  ○ a semi natural land in a semi-arid region (Uhlmann et al., 2004)
	• **Pakistan**: wheat fields (Nasim and Bajwa, 2003)
	• **Poland:** both agricultural and natural lands (Blaszkowski, 1990)
	• **U.S.A:**
	  ○ sandy soil (Koske and Tews, 1987)
	  ○ a common species associated with vineyards in Northern California (Cheng and Baumgartner, 2004)

	○ Chihuahuan and Sonoran Deserts and semi-arid grasslands (Stutz et al., 2000)
Gl. microaggregatum DP(M)	• **Brazil:** trapped from soils collected from maize field (Carrenho et al., 2001) • **China:** ○ associated with *R. repens* in the Pearl River Delta area Southern China (Chen et al., 2008) ○ present the highest relative abundance in desert ephemerals in Southern China (Shi et al., 2007) ○ One of the most common species in a hot and arid region in Southern China (Li and Zhao, 2005) • **India**: Western Ghats (Muthukumar et al., 1994) • **Namibia**: Namib desert (Stutz et al., 2000) • **Pakistan**: wheat fields (Nasim and Bajwa, 2003) • **U.S.A:** ○ an area planted with landscape trees and shrubs at an experimental site in Phoenix, AZ (Whitcomb and Stutz, 2007) ○ Chihuahuan and Sonoran Deserts and semi-arid grasslands (Stutz et al., 2000) ○ Hawaian dunes (Koske, 1988) ○ maritime and lacustrine sand dunes of North America and Hawai (Koske et al., 1986)
Gl. microcarpum Z, S, P, I, V, DP(M)	• **West of U.S.A and Canada:** *Festuca* (Molina et al., 1978) • **France:** described as a first species of the genus *Glomus*. Found in the province Touraine (Tulasne and Tulasne, 1844) • **India**: sandy beach soils of Madras coast (Mohankumar et al., 1988) • **Iran:** wheat fields in the Golestan Province **(**Sadravi, 2006) • **Israel** (Blaszkowski et al., 2001) • **Italy:** dunes (Puppi and Riess, 1987) • **Australia:** (Hayman and Stovold 1979) • **Poland**: in ca. 40 sites, including both cultivated natural vegetation (Blaszkowski, 1993) • **U.S.A**: citrus (Menge et al., 1977) • **UK:** frequently in Bristol area (Godfrey,1957)
Sc. fulgida	• **Argentina**: cultivated *Triticum aestivum* (Schalamuk et al., 2006)

DP(M)	• **China** (Gai et al., 2006)
	• **Portugal , Italy and Oman**: trap culture of plants colonizing maritime dunes (www.agro.ar.szczecin.pl/~jblaszkowski)
	• **U.S.A.:**
	  ○ Virginia: *A. breviligulata* fern colonizing maritime dunes of the Seashore in the State Park (Koske and Walker, 1986)
	  ○ Florida: *U. paniculata* and *Panicum* sp. growing in soils of a beach replenishment site (Silvia and Will, 1988)

Sc. gregaria DP(T)	• **India:** had high spore abundance in the coastal sand dunes of southwest coast (Beena et al., 2001) • **Japan:** costal dunes plants (Abe et al., 1994) • **Korea:** sand dunes plants (Kim et al., 1993) • **Senegal:** semi-arid area (Diallo et al., 1999)
Sc. calospora DP(T+M)	• **Australia** (Hall and Abbott, 1984) • **Brazil:** sand dunes plants (Trufem et al., 1989) • **Canada:** sandy soils and plants of maritime sand dunes and shores (Dalpé, 1989) • **Iran:** wheat fields in the Golestan Province **(**Sadravi, 2006) • **Italy:** maritime sand dunes (Giovannetti, 1985) • **Scotland***:* this species has originally been described from spores isolated from a cultivated soil of Scotland (Nicolson and Gerdemann, 1968). • **U.S.A.**: sand dunes (Gemma and Koske, 1997)
Ac. spinosa DP(T)	• **Argentina:** plants in a native mountain grassland in Central Argentina (Lugo, 1999) • **Brazil:** the states of Bahia and Sergipe, associated with citrus (Weber and Deoliveira, 1994) • **Canada:** sandy soils and plants of maritime sand dunes and shores (Dalpé, 1989) • **China**: one of the most common species in a hot and arid region in Southern China (Li and Zhao, 2005) • **Costa Rica**: tropical forest (Lovelock and Ewel, 2005) • **Germany:** a wet fallow agricultural habitat (Kuehn, 1992) • **India**: plants growing on fairly established iron ore mine dumps in Goa (Rodrigues, 2000).

	• **Namibia:** semi natural land in a semi-arid region (Uhlmann et al., 2004)

P. occultum
 	
P, DP(T)
	• **Brazil:**
	  o field soils and nursery conditions (Trindade et al., 2006)
	  o maize (Carrenho et al., 2001)
	• **Iran:** wheat fields in the Golestan Province **(**Sadravi, 2006)
	• **Namibia:** semi arid region (Uhlmann et al., 2004)
	• **Poland:** both agricultural and natural lands (Blaszkowski 1990)
	• **Spain:** a dry and hot region of Alicante province (East Spain). Associated with *Pistacea lentiscus*, one of the most representative shrub species from Mediterranean regions (Ferrol, 2004)
	• **U.S.A.:** a common species associated with vineyards in Northern California (Cheng and Baumgartner, 2004)

Am. gerdemannii
 	
P
	• **Australia** (Morton and Redecker, 2001)
	• **Brazil:** associated with *Araucaria angustifolia* (Moreira-Souza et al., 2003)
	• **Namibia:** semi natural land in a semi-arid region (Uhlmann et al., 2004)
	• **Poland**: in 19 samples of roots and rhizosphere soils of 14 species of uncultivated plants. None of the almost 1500 root and soil mixtures coming from cultivated sites of Poland contained spores of this fungus (www.agro.ar.szczecin.pl/~jblaszkowski)
	• **U.S.A.**:
	  o This species was described from mountains of Oregon on volcanic soil in stressed and pioneer sites (Rose et al., 1979)
	  o Desert habitat (Allen and MacMahon, 1985)
	  o Soybean (An et al., 1990)
	  o Tobacco (An et al., 1993)
	  o Mown grassland (Bever et al., 1996)
	  o Truf grass (Koske et al., 1977)

Glomus eburneum H(S), Z, S, P, I, V, DP(T+M)	• **Iran:** wheat fields in the Golestan Province **(**Sadravi, 2006) • **Namibia:** a desert under different plant species colonizing a dune (Stutz et al., 2000) • **U.S.A:**

	○ South Eastern Arizona, trapped from the soil and root fragments from a semiarid giant sacaton, *Sporobolus wrightii* (Kennedy et al., 1999) ○ In an area planted with landscape trees and shrubs at an experimental site in Phoenix, AZ (Whitcomb and Stutz, 2007)
Resembling *Glomus aureum* M, S(S)	• **Central Europe:** described as a new species from extensively managed grass land of upper Rhine valley of France, Germany, Switzerland and in Umbria, Italy (Oehl et al., 2003) • **Switzerland:** in agricultural soils (Oehl et al., 2004)
Resembling *Glomus aggregatum* H(S), Z, S, P, I, V, DP(T+M)	• **China:** Agricultural lands in Sichuan Province (Wang et al., 2008) • **Poland** ○ many plants colonizing the Baltic Sea coast sandy soils of the banks of the Odra river (Blaszkowski, 1991) ○ cultivated plants (Blaszkowski, 1993b) ○ this fungus has been the third species in frequency of occurrence in soils of the Bledowska Desert (Blaszkowski et al., 2002b) ○ soils of the Tuchola Forests (Tadych and Blaszkowski, 2000b). • **U.S.A:** ○ dunes of the eastern coast of North America (Friese and Koske, 1991) ○ San Miguel Island (Koske and Halvorson, 1989)
Resembling *Glomus intraradices* M, S(S)	• **Canada** (Dalpé, 1989) • **China** (Gai et al., 2006) • **India** (Mohankumar et al., 1988) • **Iran:** wheat fields in the Golestan Province **(**Sadravi, 2006) • **Israel** (Blaszkowski and Czerniawska, 2006) • **Namibia:** a desert under different plant species colonizing a dune (Stutz et al., 2000) • **Poland**: different ecosystems including sand dunes (www.agro.ar.szczecin.pl/~jblaszkowski) • **Switzerland** (Jansa et al., 2002; Oehl et al., 2005) • **Turkey and Cyprus**: (www.agro.ar.szczecin.pl/~jblaszkowski)

	• **U.S.A.:**
	  ○ Florida: associated with roots of many plant species (Schenck and Smith, 1981, 1982)
	  ○ California (Bethlenfalvay et al., 1984; Koske and Halvorson, 1989)
	  ○ Kentucky (An et al., 1993)
	  ○ Texas (Stutz and Morton, 1996)
	  ○ Chihuahuan and Sonoran deserts and semi-arid grasslands (Stutz et al., 2000)

Resembling *Glomus hoi* M	• **Central Canada:** sugar maple roots (Moutoglis et al.,1995) • **China**: desert plant (Shi et al., 2007) • **Iceland**: sandy soils (Greipsson et al., 2002) • **Pacific Northwest**: in trap cultures from of plants transplanted from sand dunes, forests and roadsides in British Colombia, Canada, Oregon and Washington (Berch and Trappe, 1985) • **Finland** : the most frequently identified (Vestberg, 1995) • **Namibia:** in a semi-arid region (Uhlmann et al., 2004) • **India**: some tree species of Mudumalai forest area and arabica coffee (Sumana et al., 2002)
Glomus constrictum V, DP(T+M), S(S), M	• **Brazil**: dune soils on the island of Santa Catarina (Stürmer and Bellei, 1994) **China**: desert plants (Shi et al., 2007) • **Canada:** sandy soils and plants of maritime sand dunes and shores (Dalpé, 1989) • **Germany:** a wet fallow agricultural habitat (Kuehn, 1992) • **India***: Acacia nilotica* plants in arid and semiarid region of Rajasthan (Kaushal, 2004) • **Iran**: wheat fields in the Golestan Province **(**Sadravi, 2006; Zanganeh and Blaszkowki, 2001) • **Israel**: cultivated land (Blaszkowski et al. 2001) • **Italy**: both in cultivated and uncultivated lands (Bedini et al., 2007) • **Mexico and Guadeloupe** (Trappe, 1977) • **Morocco**: date palm (*Phoenix dactylifera)* (Bouamri et al., 2006) • **Namibia:** from semi natural land in a semi-arid region (Uhlmann et al., 2004) • **Poland**: cultivated and uncultivated lands (Blaszkowski,

	1993b)
	• **U.S.A:**
	  o California: cultivated land (Haas and Menge, 1990)
	  o Hawaii: dune plants (Koske, 1988)
	• **Spain:** a dry and hot region in Alicante province (East Spain). Associated with *Pistacea lentiscus*, one of the most representative shrub species from Mediterranean regions (Ferrol, 2004)

Glomus macrocarpum S, P, I, V, Z(S)	• **Australia** (Hall and Abbott, 1984) • **Brazil:** trapped from soils collected from maize field (Carrenho et al., 2002) • **Canada:** sandy soils and plants of maritime sand dunes and shores (Dalpé, 1989) • **China**: desert plants (Shi et al., 2007) • **Germany:** a wet fallow agricultural habitat (Kuehn, 1992) • **India***: Acacia nilotica* plants in arid and semiarid region of Rajasthan (Kaushal, 2004) • **Morocco**: date palm (*Phoenix dactylifera*) (Bouamri et al., 2006) • **Poland**: Tuchola Forests (Tadych and Blaszkowski, 2000) • **U.S.A.:** Wisconsin: sandy soils (Koske and Tews, 1987)
Resembling *Glomus invermaium* Z(S)	• **Brazil:** cultivated soils (Purin et al., 2006) • **China**: desert plants (Shi et al., 2007) • **New Zealand:** (Hall, 1977) • **Spain**: semiarid lands (Diaz and Honrubia, 1993)

Glomus sinuosum
 	
DP(T+M)
	• **Brazil:** un cultivated soils (Purin et al., 2006)
	• **China**:
	  o a meadow steppe, desert steppe, steppe desert and typical desert (Shi et al., 2007)
	  o Adjacent cultivated land, old field and a never cultivated field (Li et al., 2007)
	• **India**:
	  o a semi-arid soils of monsoonal southern under *Cymbopogon caesius* dominating ungrazed grasslands and in scrub jungles at slopes dominated by *Acacia* species (Muthukumar and Udaiyan, 2002)

	• **Iran:** wheat fields in the Golestan Province **(**Sadravi, 2006) • **Italy**: both in cultivated and uncultivated lands (Bedini et al., 2007) **Korea**: in cultivated land (Eom et al., 1992) • **Mexico**: in petroleum contaminated soils (Franco-Ramirez et al., 2007) • **U.S.A:** o Florida: associated with citrus and bahia grass (Nicolson and Schenck, 1979) o Hawaii: sand dunes plants (Koske, 1988)
Resembling *Acaulospora scrobiculata* Z(S)	In the maritime sand dunes of: • **Australia** (Koske, 1975) • **Brazil** (Stürmer and Bellei, 1994) • **Canada** (Dalpé, 1989) • **Italy** (Paccioni and Puppi, 1988) • **Spain, Greece and Israel**: (www.agro.ar.szczecin.pl/~jblaszkowski) • **U.S.A.** (Friese and Koske, 1991) In lacustrine dunes of: • **U.S.A**. (Koske and Tews, 1987) In non-dune soils: • **Argentina:** plants in a native mountain grassland in Central Argentina (Lugo et al., 1999) • **Brazil:** the states of Bahia and Sergipe, associated with citrus (Weber and Deoliveira, 1994) • **China** (Zhang et al., 1992) • **Costa Rica:** in a tropical forest (Lovelock and Ewel, 2005) • **Finland** (Vestberg, 1995) • **Germany:** in a wet fallow agricultural habitat (Kuehn, 1992) • **India**: Western Ghats (Muthukumar et al., 1994) • **Japan**: humus-rich Ando soils (Saito and Vergas, 1991) • **Korea:** sand dunes plants (Kim et al., 1993) • **Mexico** (Trappe, 1977) • **U.S.A.** : apple rootstock plantings (Miller et al., 1985)
Acaulospora morrowiae	• **Brazil:** o cultivated soils (Purin et al., 2006).

DP(M)	○ in the states of Bahia and Sergipe, associated with citrus (Weber and Deoliveira, 1994) • **Colombia**: native grasses (Schenck et al., 1984) • **Poland**: ○ a forest (Blaszkowski, 1990) ○ maritime dunes in Slowinski National Park (Tadych and Blaszkowski, 2000a) ○ inland dunes of the Bledowska Desert (Blaszkowski et al., 2002) • **U.S.A**.: West Virginia in high aluminum, low pH soils (Morton, 1986) • **Costa Rica:** a tropical forest (Lovelock and Ewel, 2005)
Resembling *Scutellospora biornata* Z(S)	• **Argentina**: central mountains grasslands (Lugo et al., 1999) • **Brazil:** naturally established citrus plants (Carrenho, 1998) • **Colombia:** described as new species from llanos Orientales (Spain et al., 1989)
Resembling *Scutellospra persica* DP(M), M	• **Brazil:** trapped from soils collected from maize field (Carrenho et al., 2002) • **Greece** (Blaszkowski and Tadych, 1997) • **Italy**: dunes (Puppi and Riess, 1987) • **Poland:** dunes of Swinoujscie (Blaszkowski and Tadych, 1997) and the Slowinski National Park (Tadych and Blaszkowski, 2000) • **U.S.A.:** a barrier dune in New Jersey (Koske and Walker, 1985) and in the eastern coast (Bergen and Koske, 1984)
Resembling *Archaeospora trappeii* DP(M)	• **Australia, Brazil, Cuba, Japan, Namibia, South Africa, Scotland** (Morton and Redecker, 2001) • **Central Europe**: in a small area located in the borders of Germany, Switzerland and France in a trap culture of grassland and arable land but not in the field of these sites (Oehl et al., 2003) • **Iceland:** plants in sandy soil (Greipsson et al., 2002) • **Israel:** avocado (Blaszkowski et al., 2001) • **Namibia**: Namib desert (Stutz et al., 2000) • **Poland:** ○ dune of the Bledowska Desert (Blaszkowski et al., 2002a) ○ Slowinski National Park (Tadych and

	Blaszkowski, 2000a) o Vistula Bark (Blaszkowski et al., 2002b) o soils of The Tuchola Forests (Tadych and Blaszkowski 2000b) • **Taiwan:** bamboo vegetation (Wu and Chen, 1986) • **U.S.A**: o mown grassland (Bever et al., 1996) o field crops in monoculture on a newly cleared woodland site (Schenck and Kinloch, 1980) o Chihuahuan and Sonoran Deserts and semi-arid grasslands (Stutz et al., 2000)

Appendix B: Experimental design

A greenhouse pot experiment was initiated to test our hypothesis. Mycorrhiza-free plantlets of date palms (Fig. 5.4 A) were obtained from the tissue culture lab belonging to Ministry of Agriculture in Oman. They were transported to laboratory in Basel were they passed 4 weeks of weaning process in which they were transplanted into sterilized "terra green" under control relative humidity of 80% and temperature between 20-30ºC . After the weaning period, the plantlets were transplanted to the green house (Fig. 5.4 B) using the same procedure, materials, amounts and type of inocula used for the trap other trap culture plants (Fig. 3.7). A set of control pots were also included. These control pots were inoculated with autoclaved soil collected from rhizosphere of *Zygophyllum hamiense* growing in the natural site and suspension of soil bacteria extracted from the same soil before it was autoclaved. The bacteria suspension was obtained by fine filtration (LS 141/2; Schleicher & Schuell, Feldbach, Switzerland) of a soil suspension (50 g of air-dried soil suspended in 1 liter of water).

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Acknowledgments

I am indebted to wonderful people who made my experiences through the PhD journey so meaningful and enjoyable.

I would like to express my deep gratitude and appreciation to Prof. Paola Bonfante in the University of Torino and to Prof. Andres Wiemken in the University of Basel for giving me the chance to conduct my PhD in their departments. They surrounded me with their generous support, assistance and inspiring counsel and provided me with the freedom to explore the Arabian habitats. Profound gratitude to Dr. Dirk Redecker in the University of Basel for his valuable advices and supervision concerning the molecular and phylogenetical work in addition to his critical revision of this thesis. My thanks and appreciation to Dr. Fritz Oehl in the University of Basel for his help in the morphological identification of the AM fungal species and for his critical revision for parts of this thesis.

In the University of Torino, heartfelt thanks to To Dr. Marta Vallino, Dr. Erica Lumini and Dr. Valeria Bianciotto for their generous support on the molecular techniques. To Prof. Luisa Lanfranco for being member of my PhD examining committee. To Mara Novero for her guidance on the evaluation of the mycorrhizal colonization parameters. To Maria Teresa Della Beffa for the help and advices in setting up and maintaining the trap culture system. To Dr.Massimiliano Del Pero for the molecular technical advices. To Dr.Alessandra Pachi in the International School of Advanced Studies of the University of Torino (ISASUT) for her formal efforts during the whole period of the PhD.

In the University of Basel, I would also like to express my gratitude and appreciation to Prof. Thomas Boller for his support and for the helpful scientific comments and suggestions. To Zuzana Sýkorová, Susanne Appoloni, Boris Börstler and Odile Thiéry for the sharing of their valuable scientific experiences in a daily basis. To Kurt Ineichen for the help and advices in setting up and maintaining the trap culture system and in his help during the weaning process of the date palm seedlings. To Sally Koegel and Jens Schuetzek for their time and effort spent in the isolation of the AM fungal spores. To Sarah Symanczik for her challenging trials to set up mono-spore cultures for some isolates obtained in this study. To Dr. Mathimaran Natarajan for his advices on the statistical analysis program Statgraphics. To Vaclav Mandak and Giacomo Busco

for all the technical help. To Erika Roth for all the formal work. To Heidi Strohm from the Geographical Institute for the soil chemical and physical analysis.
In the University of Bern, I would like to thank Dr. Frank Preusser and Prof. Albert Matter in the Geological Institute for their useful information on the geology of the study sites.
In Oman, I would like to express my gratefulness to the Ministry of Agriculture for accepting my "study leave" request to conduct this research. I would also like to say a special "thank you" to Dr. Ahmad Al-Bakri the General Director of the Agricultural Research for all his unlimited logistical and formal support and for providing the facilities of the directorate during the sampling periods. To the colleagues Eng.Hamood Al-Hashmi, Eng.Ahmad Al-Hashmi and Eng.Khalid Al-Hasmi for their support during the sampling trips and to Obayed Al-Amri for his valuable knowledge on the natural plants local names and distribution and for his skillful driving through the mega dunes of Wahiba Sands in the sampling trip to reach the ancient extinct lake. To Dr. Ishaq Al-Ruqishi the director of The Tissue Culture Lab in Oman for providing the date palm seedlings to be used in a running green house experiment in Basel. To Muneer al-Yahyay from the Soil and Water section for analyzing the soil chemical properties of the studied sites.

To Richard Splivallo, Valeria Siciliano and Caroline Gutjahr, wherever they are now, for the real joy to be in their company.
To all the scientists and colleagues in Torino, Basel, Oman for their positive interaction through my PhD span.
The PhD work was supported by grants from the University of Torino, University of Basel, The Swiss National Science Foundation (SNSF) and a short-mission grant from the European COST383 action**.** I would like to thank them all for their support.

Finally, I would like to express my enduring gratitude, appreciation and love to my parents Asilah and Nasser and to my family for the unlimited support and love during the period of being away from home for the sack of this study.

Curriculum Vitae

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1999- 2002	MSc (Department of Soil and Water Sciences) College of Agriculture, Sultan Qaboos University, Oman.

International conferences

Conference	Participation type	Place	Period
Arbuscular Mycorrhiza Research in Europe: The Dawning of A new Millennium	Poster 1: Assessment of Arbuscular Mycorrhizal Inoculum Potential to Enhance Growth and Nutrient Uptake of *Lycopersicon esculentum* in Nutrient Deficient Soils. Coauthors: Peter Cookson, Abdulqader Al-Shafiea and Mike Deadman Poster 2: Multidisciplinary Approach to explore Arbuscular Mycorrhizal Fungi Biodiversity and Habitat Relationships in the Southeast Arabian Peninsula (Oman). Coauthors: Peter Cookson, Abdulqader Al-Shafiea and Mike Deadman	University of Pisa, Italy	10-12 October 2002
The International Conference on Soil and Ground Water Contamination and Cleanup in Arid Regions	Presentation: Are Mycorrhizal Inoculated Plants More Efficient in Up taking Nitrogen than Uninoculated Ones?"	Sultan Qaboos University, Muscat, Oman	20-22 January 2003.
Achievements and Future Landscape for Arbuscular Mycorrhiza Research	Poster: Diversity of arbuscular mycorrhizal fungi from desert habitats. Co-Authors: Erica LuminiMarta Vallino, Dirk Redecker , Fritz Oehl, Valeria Bianciotto, Andres Wiemken and Paola Bonfante.	Dijon, France	2 -4 June 2005
Feeding the Needs of Tomorrow- Plant sciences from Basics to application	Poster: Biodiversity of Arbuscular mycorrhizal fungi associated with desert plants . Co-Authors: Dirk Redecker, Fritz Oehl, Marta Vallino, Andres Wiemken and Paola Bonfante	Zurich, Switzerland	31 March 2006
RHIZOSPHERE 2 International Conference	Poster: Molecular Biodiversity of the Arbuscular Mycorrhizal Fungi Associated with Phoenix dactylifera L. From Southern Arabia Co-Authors: Dirk Redecker ,Andres Wiemken and Paola Bonfante	Montpellier, France	26-31 August 2007
Plant reproductive systems: from the molecular basis of reproduction to pollination ecology	Poster: Molecular Biodiversity of the Arbuscular Mycorrhizal Fungi Associated with Phoenix dactylifera L. From Southern Arabia Co-Authors: Dirk Redecker ,Andres Wiemken and Paola Bonfante	Zurich, Switzerland	26th October, 2007

Teaching experiences during the PhD program

Course	Location	Period	Course Coordinator	Details
Molecular and morphological identification of AM fungi	University of Torino	November 2005- Januery 2006	Prof. Paola Bonfante	A program for an undergraduate student. It focused on the theoretical and practical aspects of molecular and morphological identification of AM fungi present as spores or those colonizing the roots.
Plant biology Practical course – photosynthesis	University of Basel,	Summer semester 2006	Dr. Dirk Redecker	4-hours weekly undergraduate laboratory program. Students learn the following skills in the subject of photosynthesis: extracting and quantifying chlorophyll a and b, extracting the chloroplasts and observe it under microscope, demonstrate the Hill reaction and calculate the electron transport rate, evaluate the effect of different inhibitors on Hill reaction, observing and interpret the chlorophyll fluorescence. Students need to write a scientific report about the experiment which is corrected and marked.
Characterization and identification of symbionts using molecular techniques	University of Basel,	Winter Semester 2006-2007	Dr. Dirk Redecker	9 full days of laboratory work for the fourth semester undergraduate students. Consists of 3 groups, every group spends 3 full days in learning the following skills: DNA extraction from root samples and spores, nested PCRs, pouring and running an agarose gel, restriction digest, sequence editing, using BLAST, phylogenetic analysis with the PAUP*4 program, evaluating the obtained phylogenetic trees.
Plant Biology Practical course – photosynthesis	University of Basel,	Summer semester 2007	Dr. Dirk Redecker	Same as summer semester 2006

International Courses

- Techniques in Arbuscular Mycorrhiza Research, including: Molecular Approaches to AM Research. University of York, York, UK. 18-22 March 2002

Awards

- Sultan Qaboos University award for academic outstanding performance 1997

Memberships and representations:

- Oman coordinator on the European scientific program COST (COST838 action). The program lasted until 2004
-

Employment History:

- 20 February – 30 June 1998: Administrative work. Ibri College of Education, Oman.
- 12 August 1998 - 4 September 1999: Agricultural Extension Engineer. General Directorate of Agriculture, Sohar, Oman.
- 5 September 1999- 30 October 2003: Soil Microbiologist, Plant Protection Research Center, Ministry of Agriculture and Fisheries. Sultanate of Oman.
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