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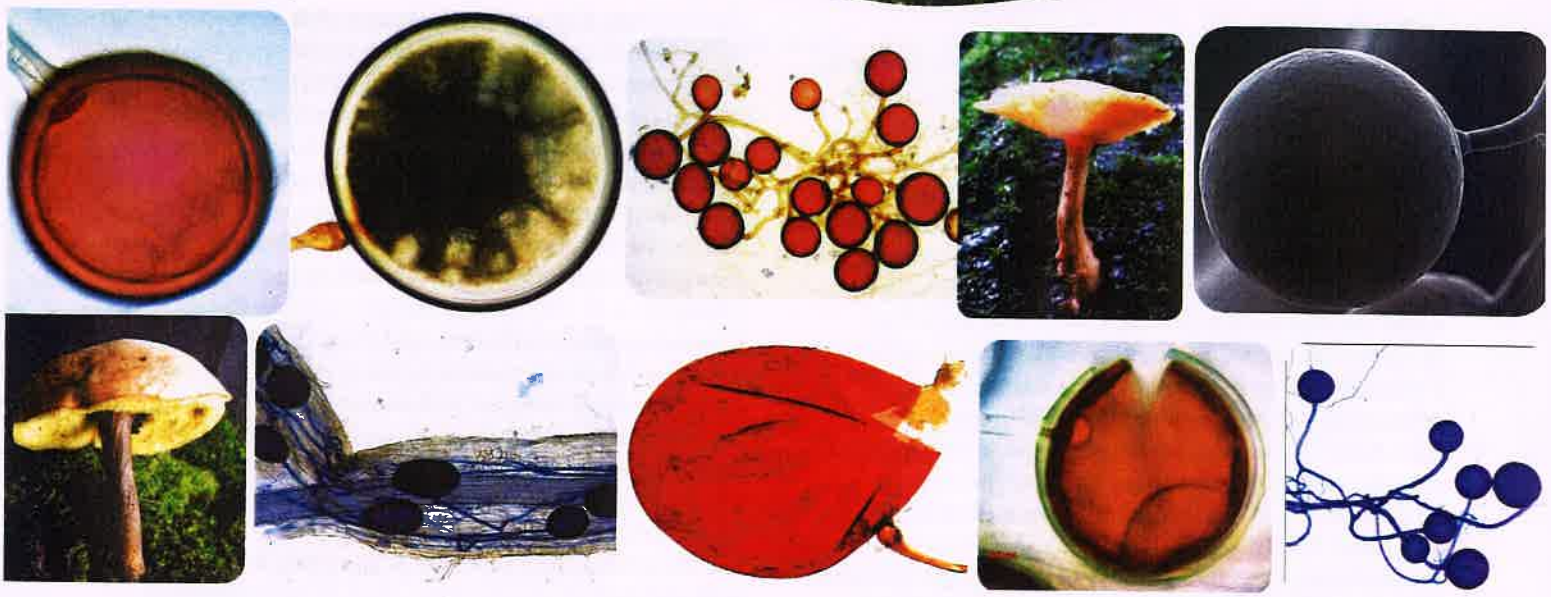

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TERI's Mycorrhiza Network is primarily responsible for establishing the Mycorrhiza Information Centre (MIC), the Centre for Mycorrhiza Culture Collection (CMCC), and publishing *Mycorrhiza News*. The Network helps scientists carry out research in mycorrhiza and promotes communication among mycorrhiza scientists.

Mycorrhiza News

The *Mycorrhiza News* provides a forum for the dissemination of scientific information on mycorrhiza research and activities; publishes state-of-the-art papers from eminent scientists; notes on important breakthroughs; brief accounts of new approaches and techniques; publishes papers complied from its RIZA database; provides information on forthcoming events on mycorrhiza and related subjects; lists important research references published during the quarter; and highlights the activities of the CMCC.

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RESEARCH FINDING PAPERS

Impact of Abiotic Stresses on Arbuscular Mycorrhiza Fungus: A Review

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Introduction

Abiotic stresses, such as salinity, extreme temperature, drought, acidity, and pollution are considered as the primary cause of crop yield loss worldwide as they result in the deterioration of the soil and present serious threats to agriculture (Wang *et al.* 2003). Fortunately, there are some species of bacteria and fungi that have the ability to overcome the detrimental effects and to ameliorate plant performance under stress conditions (Levy *et al.* 1983). The majority of terrestrial plants live in close collaboration with a diversity of soil microorganisms among which arbuscular mycorrhiza fungus (AMF) plays a major role. AMF that belongs to the *Glomeromycota* phylum contributes to plant growth and plant protection against various environmental stresses (Smith and Read 2008). The resistance mechanisms of mycorrhizal plants to abiotic stresses, such as drought, salinity, and pollution are well documented (Abdel Latef and Miransari 2014; Cicatelli *et al.* 2014; Miransari 2011; Porcel *et al.* 2012; Seguel *et al.* 2013; Wu *et al.* 2013a, b), little is known today concerning the mechanisms implemented by AMF themselves to tolerate the deleterious effects induced by the stresses. This review provides an overview of the impact of various abiotic stresses (drought, salinity, pollution, extreme temperature, CO_2, calcareous, and acidity) on biodiversity, abundance, and development of AMF.

Impact of Abiotic Stress on AMF Abundance and Biodiversity

It is evident that the overall AMF biodiversity is underestimated (Wang and Li 2013) despite its widespread distribution in several ecosystems. Application of new techniques and development of methodology will broaden our understanding of AMF species diversity. The AMF diversity is generally high in non-disturbed lands (grassland, forest) and various factors, such as host plant, soil, environmental conditions, and agricultural practices greatly influence their distribution (Hayman 1982; Wang and Li 2013). So far, upto 52 AMF taxa in forests and 43 taxa per habitat have been recorded in grasslands (Zangaro *et al.* 2013). *Claroideoglomus claroideum*, *Claroideoglomus etunicatum*, and *Funneliformis mosseae* were the dominant species in many studies that were found through different techniques of identification (molecular or morphological, such as restriction fragment-length polymorphism, denaturing gradient gel electrophoresis, cloning and sequencing, and pyrosequencing).

However, AMF diversity is generally lower in soils exposed to abiotic stress than in non-disturbed soils with a predominance of *Glomeraceae*. They have evolved their characteristics in such a way that are advantageous in adverse environments (Sy′ korová *et al.* 2007) and invest their energy mainly in the production of many offsprings due to their opportunistic behaviour. *Rhizophagus irregularis* is an example of rapid colonizer of plant roots that generates a high number of spores in a short amount of time.

The production of spores in *Glomeraceae* occurred in parallel to plant growth suggesting a direct investment of carbon in spore formation (Declerck *et al.* 2001). To survive in different environmental conditions, AMF species must also have the genetic and physiological characteristics (Picone 2000; Silva *et al.* 2010). Even though AMF are sensitive to the

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environment, there are exceptions when it comes to certain individual species or isolates that can tolerate different environmental conditions (Stahl and Christensen 1991). Long-term adaptations to soils with extreme properties result in indigenous AMF ecotypes (Sylvia and Williams 1992). Under high-salinity conditions, *Glomus* species are able to grow as they are typical of semi-arid Mediterranean ecosystems (Requena *et al.* 1996; Ferrol *et al.* 2004; Juniper and Abbott 2006; Sánchez-Castro *et al.* 2012a, b). In several salt marshes, *Funneliformis geosporum* was found to be the predominant species (Carvalho *et al.* 2003; Hildebrandt *et al.* 2007; Sonjak *et al.* 2009; Wilde *et al.* 2009). *F. mosseae* has a global distribution and tolerates different environmental conditions (Stahl and Christensen 1991; Öpik *et al.* 2006). This species is the typical early-stage colonizer and appears to be adapted to frequent soil disturbance (Sy´ korová *et al.* 2007), including hydrocarbon (Huang *et al.* 2007a,b), fungicide (Ipsilantis *et al.* 2012) and trace metal pollutants (Abdel-Azeem *et al.* 2007; Hassan *et al.* 2011; Ortega-Larrocea *et al.* 2010; Zarei *et al.* 2010), salinity (Krishnamoorthy *et al.* 2014), drought (Mohammad *et al.* 2003; Panwar and Tarafdar 2006; Tian *et al.* 2009; Verma *et al.* 2008), or cold (Gai *et al.* 2009).

Impact of Abiotic Stress on Development and Morphological Adaptations

Several studies have shown that the main stages of AMF development cycle (germination, colonization, sporulation, and extraradical hyphal elongation) could be hampered by the presence of different abiotic stresses (Enkhtuya *et al.* 2002). Various studies have revealed that in the presence of fungicides (Calonne *et al.* 2010; Twanabasu *et al.* 2013; Venedikian *et al.* 1999; Zocco *et al.* 2008), salinity (Juniper and Abbott 2006), trace metals (Pawlowska and Charvat 2004), drought (Estaun 1990) or polycyclic aromatic compounds (PAH) (Alarcón *et al.* 2006; Franco-Ramírez *et al.* 2007; Verdin *et al.* 2006), spore germination, and germinative hyphae elongation get inhibited. Most of the abiotic stresses can induce a reduction of the total root colonization.

This is either due to a disruption at later stages in the colonization process after host contact or due to an inability of spores to germinate and to detect a suitable host root (Juniper and Abbott 2006; Bolgiano *et al.* 1983; Estaun 1990; Debiane *et al.* 2009; Jacobson 1997). Moreover, this negative impact could be described either by an indirect effect due to an inhibition of root development or by a direct effect on the AMF itself.

Abiotic stresses (trace metals, salinity, drought, cold, PAH, fungicides, and acidity) also affect the post-symbiotic steps which consist of the post-colonization formation of new spores and extraradical mycelium. This could be as a result from a direct impact of stress on spores and extraradical mycelium development or from an indirect impact of stress on spores and extraradical mycelium. The number of branched absorbing structures (BAS) gets reduced in the presence of NaCl, fungicide and PAH (Zocco *et al.* 2008; Juniper and Abbott 2006; Aranda *et al.* 2013; Estrada *et al.* 2012; Jahromi *et al.* 2008). BAS are nutrient uptake structures formed by the extraradical mycelium of *R. irregularis* (Bago *et al.* 1998).

To avoid local, metal-polluted microenvironments, many fungi exposed to toxic compounds increase hyphal elongation (Fomina *et al.* 2003). AMF are able to survive in trace metal-contaminated soils by using a metal avoidance strategy (Cardenas-Flores *et al.* 2011).

Increase of root colonization in the presence of drought (Klironomos *et al.* 2001), trace metals (Bona *et al.* 2011; Todeschini *et al.* 2007), and waterlogging (Sah 2006) have also been reported. Increased temperature can increase the acquisition of phosphorus, enhance carbon allocation to AMF, and increase mycorrhizal root-colonization rates (Kytöviita and Ruotsalainen 2007). Several studies have shown that the warming of soils induced an increase of the length of extraradical hyphae (Rillig and Allen 1999; Gavito *et al.* 2003; Heinemeyer and Fitter 2004; Bunn *et al.* 2009) without any change of plant biomass, thereby suggesting that hyphal elongation may be a direct fungal response to temperature (Heinemeyer and Fitter 2004).

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Scope and Limitations of AMF Biofertilizer Production

*Sankrita S Gaonkar and B F Rodrigues**

Introduction

Arbuscular mycorrhizal (AM) fungi are soil-borne microbes belonging to phylum Glomeromycota that form a symbiotic association with roots of higher plants. Hyphae colonize their host roots and form a mycelial network in the rhizosphere to facilitate nutrient uptake, especially P (Rodrigues and Rodrigues 2014), and in turn acquire photosynthates from the host plant. Around 90% of vascular plants form AM association (Smith and Read 2008). Plant genes and signal molecules enable hyphal entry and development of the fungus in the plant (Parniske 2008). The extra-radical mycelium extends several centimetres beyond the depletion zone absorbing nutrients that are transported to host roots (Khan *et al.* 2000). These fungi play an important role in agriculture, forestry, and horticulture by increasing crop yield, health, and resistance to stress by reducing the cost of agrochemicals (Johansson *et al.* 2004). Occurrence of AM symbiosis is dated back to >460 million years ago (Read *et al.* 2000). Based on the spore morphology, approximately 240 AM fungal taxa belonging to order Glomales have been described (Schubler and Walker 2010; Kruger *et al.* 2012), although molecular analysis data shows that the actual number of AM fungal taxa can be much higher (Vandenkoornhuyse *et al.* 2002).

Culture Techniques for AM Fungal Inoculum

Various cultivation techniques of AM fungal inoculum production have been attempted in the last few decades. Sand/soil- and substrate-based production techniques, substrate-free culture techniques (hydroponics and aeroponics), and *in vitro* cultivation methods have been attempted in the large-scale production of AM fungi. Several parameters must be taken into consideration for the culture of AM fungi, such as controlled or semi-controlled conditions in greenhouses, AM fungal species, the host plant, substrate, and amendments.

Substrate-Based Production System

Conventional production of AM fungi is commonly achieved by the cultivation of host plants and their symbionts in a soil- or sand-based substrate (substrate-based production system). The inoculum to initiate production consists of dried root fragments or colonized root fragments, AM spores, sporocarps, and fragments of hyphae. When spores are extracted from the soil and used as inoculum directly they tend to have very low viability or may even be dead or parasitized. To overcome this, initially, the rhizosphere soil is used to prepare a 'trap culture' using a suitable host plant. This increases the number of viable spore propagules for further isolation, multiplication, and production of monospecific cultures. The pure culture inoculum thus produced consists of spores, colonized root fragments, and AM hyphae of a single species.

Selection of host plant is based on numerous criteria, such as plants exhibiting a short life cycle, rapid growth, adaptation to the prevailing growing conditions, and ready colonization by a range of AM fungal species. A large quantity of roots should also be produced in a relatively short period, and resistance to pest and diseases common to the inocula production environment.

A range of plant species, such as *Zea mays* (corn), *Allium cepa* (onion), *Arachis hypogaea* (peanut), *Paspalum notatum* (bahia grass), *Pueraria phaseoloides* (kudzu), coleus (*Plectranthus scutellarioides*), ragi (*Eleusine coracana*), etc., have been used as hosts with encouraging results.

Various substrates, such as soil, sand, peat, vermiculite, perlite, calcinated clay, and compost have been used to propagate AM fungi (Ijdo *et al.* 2011). Addition of different organic amendments also influences AM fungal colonization. Chitin and humic substances increase colonization levels (Gryndler *et al.* 2003; Gryndler *et al.* 2005). Manipulation of nutrient content has a further impact on AM fungal propagule production (Douds and Schenck 1990). The substrate-based culture technique is the most widely used method for AM fungal production as it requires a relatively little less technical support, is cheap, is the least artificial, and a large set of AM fungal species can be cultured (Ijdo *et al.* 2011). Conversely, the sand/soil-based systems have certain disadvantages such as the presence of unwanted contaminants, even with good phytosanitary care, fewer viable spores than *in vitro* system, and parasitized spores.

Substrate-Free Production System

Substrate-free cultivation systems, such as hydroponic and aeroponic have also been used for the

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multiplication of AM fungi wherein a continuous flow or mist of nutrient solution is provided for the plant and the symbionts. Although this system offers the advantage of providing inoculum which is free from attached substrate particles, a disadvantage has been that the nutrient solution is prone to microbial contamination and algal growth (Elmes and Mosse 1984).

Monoxenic Culture System

The first attempt to culture AM fungi monoxenically dates back to the late 1950s (Mosse 1959). Thereafter, tremendous progress has been made for the mass production of AM fungi using Ri T-DNA transformed roots (Mugnier and Mosse 1987). Different *in vitro* culture techniques have been derived such as the bicompartment system wherein AM fungal mycelia and spore are produced free from roots (St-Arnaud *et al.* 1996), and manipulation of culture medium to induce sporulation (Becard and Piche 1992). These developments have enabled studies in spore ontogeny (Pawlowska *et al.* 1999), sporulation dynamics (Declerck *et al.* 2001), response of AM fungi to cell wall-associated phenolics (Douds *et al.* 1996) and flavonoids (Morandi *et al.* 1992), lipid metabolism (Bago *et al.* 2002), transport of mineral nutrients to roots (Dupre de Boulois *et al.* 2005) and isolation of contaminant-free spores for molecular analysis (Pawlowska and Taylor 2004). A wide number of AM fungal species belonging to Glomeraceae and a few Gigasporaceae have been successfully cultured in the root organ culture (ROC) system.

Species, such as *Acaulospora rehmii* (Dalpe and Declerck 2002), *Gigaspora rosea* (Bago *et al.*1998c), *Gi. margarita* (Miller-Wideman and Watrud 1984; Diop *et al.* 1992; Gadkar and Adholeya 2000), *Gi. gigantea* (Gadkar *et al.* 1997), *Gi. decipiens* (Fernandez Bidondo *et al.* 2012), *Glomus etunicatum* (Schreiner and Koide 1993), *G. versiforme* (Diop *et al.* 1994; Declerck *et al.* 1996), *G. deserticola* (Mathur and Vyas 1995), *G. fistulosum* (Nuutila *et al.* 1995; Gryndler *et al.* 1998), *G. clarum* (De-Souza and Berbara 1999; Rodrigues and Rodrigues 2012), *Funneliformis caledonius* (Hepper 1981; Karandashov *et al.* 2000), *F. geosporus* (Declerck *et al.* 1998), *F. mosseae* (Douds 1997; Rodrigues and Rodrigues 2015), *Rhizophagus irregularis* (Chabot *et al.* 1992; St-Arnaud *et al.* 1996), *R. fasciculatus* (Declerck *et al.* 1998), *R. proliferus* (Declerck *et al.* 2000) and *Sclerocystis sinuosa* (Bi *et al.*2004) have been successfully cultured *in vitro*.

Culture media such as minimal (M) medium (Becard and Fortin 1988) and modified Strullu Romand (MSR) medium (Strullu and Romand 1986, modified by Declerck *et al.* 1998) are often used to culture AM fungi. The growth of germ tube is inhibited by the presence of sucrose in MSR medium. Healthy germination of AM fungal spores in MSR medium without sucrose was achieved by D'Souza *et al.* (2013). During the pre-symbiotic phase, AM spore use the reserve materials from propagules for the germination and growth of germ tubes (Clark 1997). Root Organ Culture (ROC) was first developed by White (1943), followed by development of further Ri T-DNA transformed roots of different plant species, *viz.*, clover (*Trifolium*) (Mosse and Hepper 1975), bindweed (*Convolvulus sepium*) (Tepfer and Tempe 1981), onion (*Allium cepa*), tomato (*Solanum lycopersicum*) (Strullu and Romand 1986, 1987), carrot (*Daucus carota*) (Mugnier and Mosse 1987), strawberry (*Fragaria x ananassa*), chicory (*Cichorium intybus*) (Boisson-Dernier *et al.* 2001), barrel medic (*Medicago truncatula*) (Fontaine *et al.* 2004) and linum (*Linum usitatissimum*) (Rodrigues and Rodrigues 2015).

Fungal inocula such as isolated spores or propagules from intra-radical phase (colonized root fragments and isolated vesicles) of AM fungi can be used to initiate monoxenic cultures (Rodrigues and Rodrigues 2015). The culture established needs to be maintained by continuous sub-culturing, transferring the mycorrhizal roots onto fresh medium (St-Arnaud *et al.* 1996). Under aseptic conditions, AM symbiosis with the transformed roots takes place by development of extra-radical mycelium which is often accompanied by formation of arbuscule-like structures (ALS) (Bago *et al.* 1998a) or branched absorbing structures (BAS) (Bago *et al.*1998b). These structures are probably nutrient-exchange sites between the fungus and its host (Diop 2003). Sporulation in AM fungi differs between species as well as between isolates of the single species and is related to spore size (Declerck *et al.* 2001).

The most important advantage offered by *in vitro* cultivation system is the absence of undesirable organisms. Contamination by other undesirable microorganisms can occur, however, during the establishment of culture process or during the later stages of culture maintenance. This type of system can be used for the large-scale production of AM fungi consisting of high-quality inoculum with minimum space. Also, the factors influencing optimum production can be easily detected and controlled, and harvesting time can be determined. The maintenance of a successfully established culture is easily achieved by sub-culture and maintaining the plates in dark condition. As a disadvantage, the *in vitro*-grown AM fungal diversity is lower than that under-pot culture system (Rodrigues and Rodrigues 2013). Furthermore, the *in vitro* production is expensive, requiring skilled technicians and sophisticated

laboratory equipment to carry out the whole process in sterile and controlled conditions (Ijdo *et al. 2011*). Further studies are in progress to identify and eliminate contaminants in established cultures.

AM Fungi as Biofertilizers

It has been observed that AM fungal inoculation provides beneficial results in plant growth both in controlled and open-field conditions. AM fungi have been confirmed to show better performance in terms of plant growth and yield characteristics. This would make the AM fungal technology more suitable to sustainable cropping systems (Berruti *et al.* 2016). Khan *et al.* (2008) reported that the inoculation of a single or dual AM fungi increased the growth and nutrient uptake of *Medicago sativa* which resulted in the increased dry weight of shoot and root. Bhat *et al.* (2010) studied the effect of AM fungi and *Rhizobium* on green gram (*Vigna radiata*) and reported a significant effect on nodulation, yield, crude protein content, and NPK content in grain. Various further studies have proved that AM fungi are an effective resource when used as biofertilizers with no adverse environmental effect.

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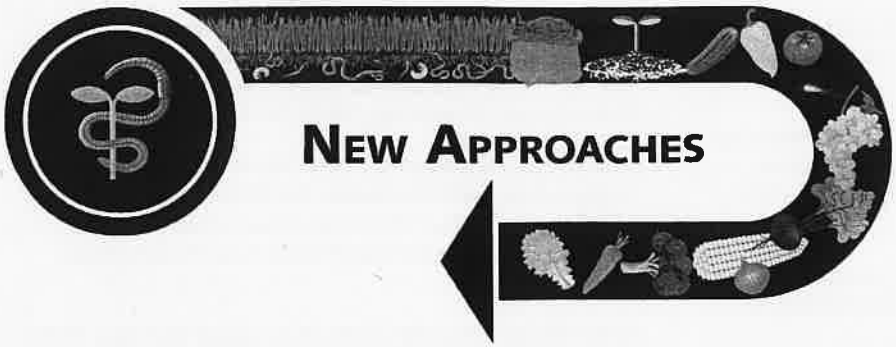

Promising Farming Strategy that Involves Inoculation of AMF with Plastic Mulching in Rain-Fed Wheat

Zhu Y, Lv G C, Chen Y L, Gong X G, Peng Y N, Wang Z Y, Ren A T, and Xiong Y C (2017) conducted two relatively independent but closely related field trials to evaluate the inoculation effects of AMF species on grain yield, biomass accumulation, water use efficiency (WUE), soil organic carbon, and economic profitability in field-grown wheat under plastic film mulching (PFM) in a typical semiarid site of northwest China (*Field Crops Research* 207: 229–241). Trial 1 included two treatments: traditional flat planting (CK-1) and PFM; while Trial 2 consisted of PFM treatment inoculated with three AMF species (*Acaulospora laevis, Glomus monosporum*, and *G. intraradices*) alone or in combination, or without inoculation as the control (CK-2). PFM resulted in significant increases in grain yield and WUE due to improved hydro-thermal balance in both growing seasons. Importantly, across all the species under PFM, the AMF inoculation increased grain yield and above-ground biomass by 46.6% and 56.5%, respectively, in wet year 2014, and 16.6% and 27.4%, respectively, in warm dry year 2015, comparing with the non-inoculation treatment. AMF symbiosis also significantly enhanced harvest index and population fitness, and higher inoculation rates generally led to greater increases in yield, biomass, and WUE.

Modification and Future Perspective of Phytoremediation Strategies for Soils Contaminated with Heavy Metals

In this review, *Sarwar N, Imran M, Shaheen M R, Ishaque W, Kamran M A, Matloob A, Rehim A, and Hussain S* (2017) have discussed various sources and harmful effects of some important heavy metals and metalloids, traditional phytoremediation strategies, mechanisms involved in phytoremediation of these metals, limitations, and some recent advances in phytoremediation approaches (*Chemosphere* 171: 710–721). Since traditional phytoremediation approach poses some limitations regarding their applications at large scale, there is a dire need to modify this strategy using modern chemical, biological, and genetic engineering tools. In view of above, the present manuscript brings both traditional and advanced phytoremediation techniques together in order to compare, understand, and apply these strategies effectively to exclude heavy metals from soil, keeping in view the economics and effectiveness of phytoremediation strategies.

The Need for Phosphorus Fertilizer Application to Corn is Reduced by White Clover Living Mulch

White clover living mulch (LM) increases uptake of phosphorus (P) and yield of the main crop by promoting colonization of arbuscular mycorrhiza (AM). However, the extent to which the P fertilizer application rate can be reduced by using LM is not yet known. This study by *Deguchi S, Uozomi S, Tuono E, Uchino H, Kaneko M, and Tawaraya K* aimed to address this question. Two field experiments were conducted from 2008 to 2009 (Experiment 1) and from 2009 to 2010 (Experiment 2) at the fields where the available P of soil fluctuated near the lower limit of the optimum P level (43.6 mg/kg: Truog method) (*European Journal of Agronomy* 86: 87–92, 2017). Experiment 1 had a randomized block design, and Experiment 2 had a split-plot design with a factorial arrangement of two cropping systems (LM and no LM) with three P application treatments (0 kg/ha, 43.6 kg/ha, and 87.3 kg/ha). LM increased P concentrations in the early stages of growth and the yield of corn. This can be attributed to increased AM colonization rate in the early stages of growth. The yield and total digestible nutrient yield of corn in LM with no P application was comparable to the maximum yield in no LM with or without P application. Therefore, LM could make unnecessary P fertilization in soils, where P fertilization is required for silage corn.

Morpho-taxonomy of Acaulospora mellea (Accession CMCC/AM-2403)

*Aditi Pandit and Alok Adholeya**

A microorganism is described by its morphological characters, which are done at different taxonomic levels. Arbuscular mycorrhiza fungi (AMF) shows different morphological characters across different families and genera; so this approach has been very well explored in case of AMF taxonomy, which has helped mycorrhizologists in classification and feature identification. Morphological features, such as spore structure, size, number of wall layers, hyphal attachments, hyphae size, wall ornamentations, reaction with reagents, etc., are of great significance for nomenclature of the AMF (Souza 2015). With reference to our previously published articles, this recent issue will take up morphological study of arbuscular mycorrhizal fungus that was collected from Centre for Mycorrhizal Culture Collection Bank, TERI, with the accession number CMCC/AM-2403. This article gives a detailed morphological description of this accession, which was critically observed to reach a level of morphotaxonomic identification.

Monosporal Establishment

The culture of accession CMCC/AM-2403 was raised from the soil of Germany. For the propagation of trap cultures of this culture, it was raised in pot conditions with *Sorghum* plant as a host for a period of three months under greenhouse conditions. After three months of growth cycle, the soil samples were analysed for AMF spore density, types, spores of varying sizes, colour, and diversity by wet sieving and decanting method (Gerdemann and Nicolson 1963). Firstly, suspending the soil samples in water and allowing them to pass through a series of sieves of 60, 100, and 300 British Standard Size, respectively.

The sieving from each fraction was critically observed under a stereo-zoom microscope. Next, morphologically similar looking (size, structure, and colour) and healthy spores were collected and categorized and were used to obtain pure single species of AMF. Next, voucher specimens were prepared to investigate the morphotaxonomic characterization. Voucher slides were prepared with two mounting agents, polyvinyl lacto glycerol (PVLG) and polyvinyl lacto glycerol: Melzer's reagents (1:1). Voucher specimen were analysed for spore and its wall layers, hyphal attachment, etc., under a compound microscope (under 10×, 40×, and 100× magnifications). Healthy single AMF spore was used to raise monospecific cultures that were inoculated to pre-germinated seed of a suitable host (*Sorghum*). After 3 to 6 months, depending upon successful growth period, the host roots were screened for root colonization with the AMF and cultures for the spores. Cultures showing spores and colonized roots were considered as successful cultures and pure only when the spores isolated were morpho-taxonomically similar to the voucher specimen prepared from the mother cultures, which were used during the initiation of the monosporals (Figure 1).

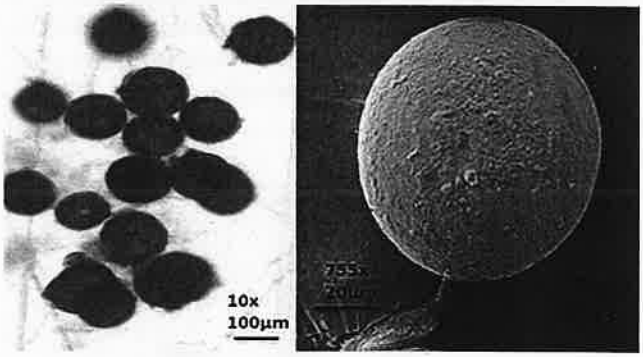

Figure 1 Compound microscopic images (10×) and scanning electron micrographs (SEM) of CMCC/AM-2403 showing spore with small subtending hyphae

Spore Morphology and Shape

Spores that were isolated from the monosporals were borne singly in the soil developed laterally on the neck of a sporiferous saccule and each of these were suspended with a single prominent subtending hyphae.

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Most of the spores were pale yellow to pale orange-brown to orange in colour. The shape of the spores varied from globose to sub-globose and occasionally irregular. Scanning electron micrograph (SEM) of the spores showed that the outer surface of the spores is slightly rough. The outer wall of the spores is mucilaginous and appears rough with organic debris sticking to it (Figure 2). The average diameters of the spores were found to be in the range of 90–120 µm (Figure 3).

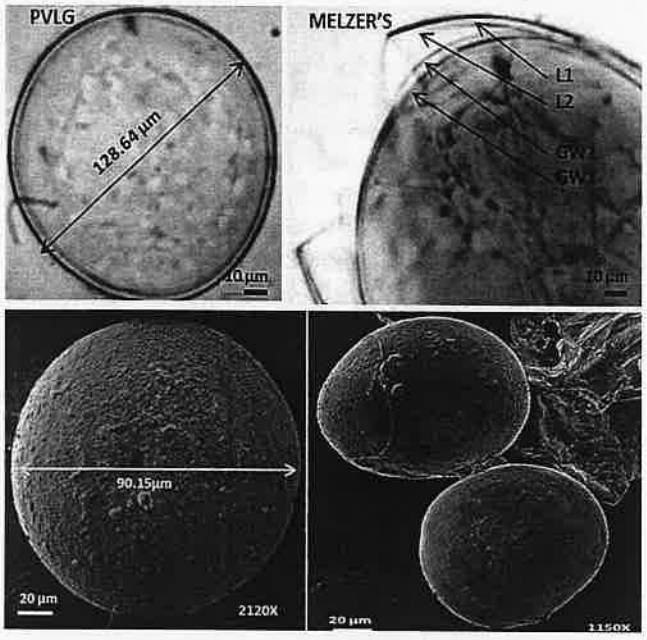

Figure 2 Compound microscopic images (40×) and SEM of mature spores of accession CMCC/AM-2403 showing globose- to sub-globose-shaped spores with single subtending hyphae. SEM images reveal slightly rough outer layer of the spore

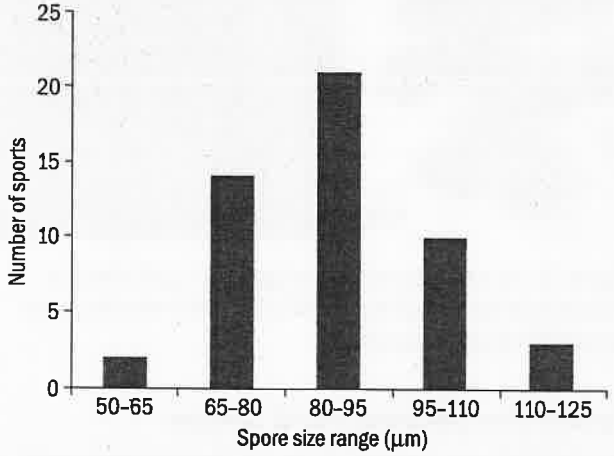

Figure 3 Analysis of spore diameter of 50 healthy and cleaned spores obtained from 1-year-old monosporal culture of accession CMCC/AM-2403

Subcellular Structure of the Spore

Spores of this accession show no or very less reaction with PVLG but show reaction with polyvinyl lacto glycerol: Melzer's reagents. However, mature spores are composed of the following subcellular structures and wall layers (Figure 4):

Spore Wall Layer 1 (L1)

The first and the outermost hyaline layer of the spore is designated as L1. The average thickness of this outer wall layer is <0.5 µm. This layer is flexible and often produces wrinkles in reaction with PVLG. This layer is continuous with the wall of a Sporiferous saccule, usually completely sloughed in mature spores. When this layer does not slough, it produces numerous folds on the spore surface and appears 'rugose'.

Spore Wall Layer 2 (L2)

This layer consists of fine and adherent sub-layers. L2 is smooth, and at maturity, the pore between spore and saccule neck is closed by continuous sub-layers of this layer encloses the spore contents in an 'endospore'. The thickness of this layer is 2.5–4.0 µm thick.

Spore Wall Layer 3 (L3)

This layer is flexible and is separated from L2. It consists of sub-layers that are separated from each other. It is pale yellow-brown and its thickness is around 1–4 µm.

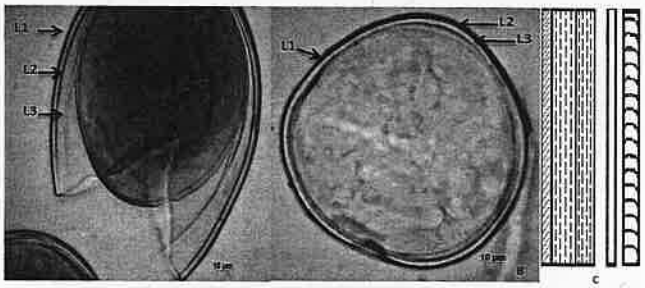

Figure 4 Compound microscopic images of spore wall layers of CMCC/AM-2403 after mounting in PVLG: Melzer's and PVLG reagent (A and B). Murograph (Walker 1983) of the mature spore showing three distinct wall layers (C)

Germinal walls: Two flexible inner walls (GW1 and GW2) are present that readily separate from each other and from the spore wall.

GW1: This layer can be seen easily as it is separated from the spore wall. Layers separate slightly in only few spores, where these can be measured. This germinal wall layer is rigid because it breaks with the spore wall layer and is described as a 'semi-rigid unit wall'.

GW2: This layer stains red-purple to dark red-purple with Melzer's reagent. It is composed of two adherent layers.

Sporiferous Saccule

They are mostly hyaline in colour and mostly globose to sub-globose in shape. They consist of one layer, which is smooth.

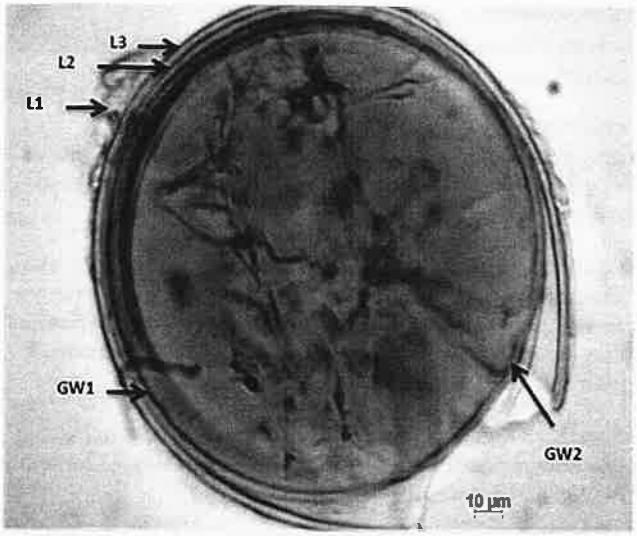

Figure 5 Compound microscopic images of spore germinal wall layers of CMCC/AM-2403 after mounting in PVLG: Melzer's

Mycorrhiza

Mycorrhiza structures, such as intra and extra-radical hyphae, arbuscules, and vesicles, were observed in *Sorghum bicolor* roots when stained in ink vinegar. Root colonization assays showed presence of both extra-radical and intra-radical hyphae and spores. Arbuscules are abundant and are dispersed evenly through the root cortex. Intra-radical vesicles were present. Extra-radical hyphae were abundant and were usually seen bearing spores bearing or in a group of two to three (Cannon and Kirk 2007) (Figure 6).

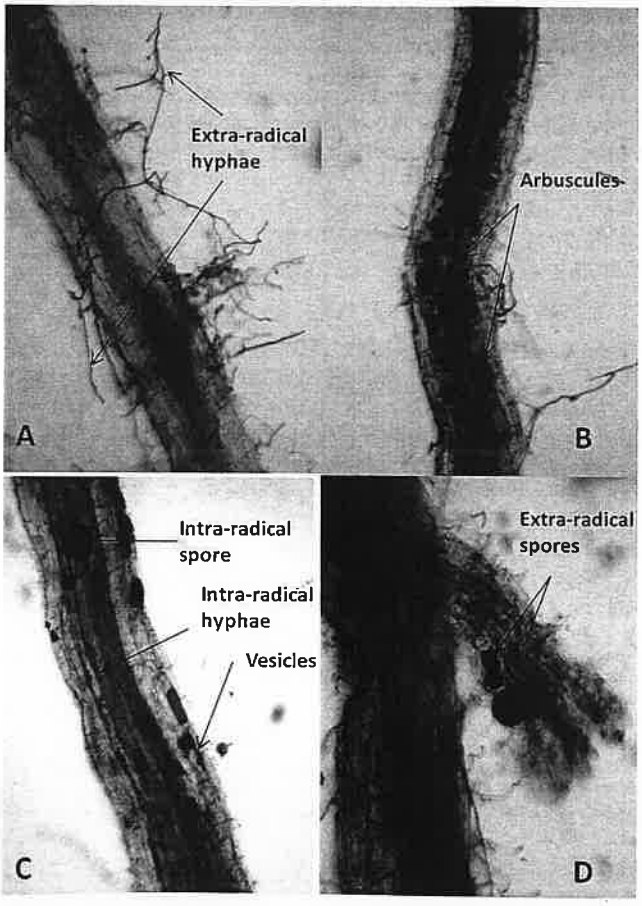

Figure 6 Compound microscopic images (10x) of roots of *Sorghum bicolor* stained in ink vinegar and observed for root colonization by CMCC/AM-2403 showing extra radical hypha (A), arbuscules (B), and abundant vesicles in the cortical cells (C). Spores in the extra-radical hyphae are also seen in small clusters (D).

Conclusion and Classification Level

On the basis of above morpho-taxonomic analysis of the accession CMCC/AM-2403, many distinguishing features regarding the family, genera, and the species could be derived (Morton and Benny 1990). The following features were taken into consideration for characterization and identification:

- Globose, asexual spores produced singly with three-layered spore walls.
- Spore wall layer is composed of outer hyaline layer followed by one inner fine sub-layer followed by a third layer that is flexible.
- Spores are of varying shapes and sizes ranging from globose to sub-sub-globose.
- Formation of both intra-radical and extra-radical hyphae and abundant vesicles and intracellular arbuscules.

All these features suggest that the culture CMCC/AM-2403 belongs to the family *Acaulospora mellea* (Schenck 1984).

Following are some of the unique morpho-taxonomic features of the accession:

- From pale yellow to pale orange-brown to orange in colour, globose to sub-globose, asexual spores produced singly with layered spore walls; spores are of varying shapes and sizes ranging from globose to sub-globose. Size ranges 50–(86)–120 µm.
- Spore wall layer is composed of outer hyaline layer, inner fine layer, a third flexible innermost layer that is continuous with the subtending germinal wall.
- Formation of both intra-radical and extra-radical hyphae and abundant vesicles and intracellular arbuscules.

The taxonomic feature of the accession CMCC/AM-2403 matches the characters of *Acaulospora mellea* (Schenck 1984).

Systematic Classification

Glomeromycota
Glomeromycetes
Diversisporales
Acaulosporaceae
Acaulospora
A. mellea

Most of the work on AMF focusses on phylogeny, ecology, genetics, taxonomy, and functional properties. Identification of morphological features of an organism has been used as a conventional method for identifying an organism and comparing it with existing reference or type species. Morphological features about AMF spore morphology, wall layers, hyphal attachment, reaction with reagents, and so on help to reveal information about genera and species to which the AMF belongs. But sometimes this approach can create a problem in identification of the organism at species level. Therefore, using alternative methods have more significant advantages over traditional taxonomic approaches. Characterization techniques, such as biochemical characterization through fatty acid methyl ester profiles and sequencing of ITS region of 18s rDNA (molecular characterisation), have become more popular. Analyses of rDNA regions have often confirmed the morphologically defined species (Schüßler and Walker 2010).

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