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A review of taxonomic studies of Arbuscular Mycorrhizal Fungi in Iran

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Abstract: Arbuscular Mycorrhizal Fungi (AMF) are one of the most essential beneficial soil microorganisms that can form a mutualistic symbiotic relationship with plants. AMF receive carbon from the host plant to complete their life cycle. In return, these fungi have copious roles for the host plants, including plant protection against pathogens, increasing abiotic stresses tolerance (drought and salinity), and enhancing water and mineral nutrient acquisition. Formerly, AMF was identified and classified merely based on morphological features of fungal spores such as mode of formation, wall structure, and subtending hyphal characteristics. Later on, molecular procedures were incorporated for AMF identification. PCR-based techniques led to the direct identification of AMF species that existed in plant roots or the rhizosphere. Several primers have been developed to increase the accuracy of AMF identification. Nowadays, classification systems of AMF are based on both morphological and molecular techniques. This paper aims to review the researches on the identification of AMF in Iran. Identification of AMF has received increasing interest over the past few decades. Authors have used a combination of morphological characteristics and DNA-based techniques to the identification of AMF. So far, more than 115 AMF species belonging to 22 genera have been identified and reported from different regions and plant communities such as croplands, forests, grasslands, etc. Understanding the community composition and diversity of AMF is vital for using them as biofertilizer in agriculture to reduce chemical inputs and increase sustainable crop production.

KEYWORDS: Community composition, Diversity, *Glomus*, Spore morphology, Symbiosis.

INTRODUCTION

Mycorrhiza is a symbiotic association between the plant root systems and soilborne fungi (Willis et al., 2013). Among the different types of mycorrhizae, arbuscular mycorrhizal fungi (AMF) are the most common symbiotic relationship with plants. AMF are obligate biotrophs of over 80 % of vascular plant species, which play a crucial role in nutrient cycling processes and maintaining ecosystem stability (Smith & Smith 2011). These fungi have an enormous ecological and economic impacts. The extra-radical hyphae of AMF, which are much thinner than fine roots, penetrate unreachable small spaces of soil and enhance the root surface area (Zou et al., 2021). Subsequently, they improve the growth of host plants and alleviate the effects of biotic and abiotic stresses (Chen et al., 2018, Zhang et al., 2020). AMF symbiosis enhances the growth and nutrition of host plants and reduces the need to fertilizers (Balestrini & Lumini 2018).

AMF species belonging to various genera have been reported from diverse habitats of the world, e.g. meadows, frigid regions, tropical regions, croplands, grasslands, tropical to temperate forests, alpine, dunes, deserts, etc. (Lovelock et al., 2003, Al-Yahya'ei et al., 2011, Varga et al., 2015). It should be noted that a small number of AMF species are limited to specific environmental conditions (Rosendahl 2008). The composition, abundance, and diversity of AMF are affected by many factors, like seasonal variations, edaphic characteristics, plant species, etc. (Bencherif et al., 2016, Turrini et al., 2017, Wang et al., 2021).

Morphological characteristics of spores are a basis for the identification of AMF species. The identification and classification of AMF based on morphological differences of the spores is complicated and frequently misleading or impossible and require a lot of information and experience (Kruger et al., 2012). Most of the recent molecular works have focused on using PCR-based techniques to analyze target sequences within the ribosomal DNA (rDNA) genes. This region is very suitable for designing general and specific primers due to having coded/conserved genes (SSU, 5S, and LSU) and non-coded / variable genes (ITS and IGS) (Symanczik 2016). A part of the large subunit (LSU)

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or small subunit (SSU) rDNA gene or combination of both with internal transcribed spacer (ITS) have been chosen as a target for the Polymerase Chain Reaction (PCR) in many studies. These regions contain enough variation to distinguish and differentiate species of AMF (Binet et al., 2011, Johnson et al., 2016, Nielsen et al., 2016). Each of these regions alone is not enough for species resolution of AMF.

DNA is extracted from a certain amount of host plant roots or one spore or multispore or soil sample and is amplified using the AMF-specific primers (Ferrol & Lanfranco 2020). Until now, many researchers designed specific primers that would amplify all known AMF species. Molecular identification methods have the potential to study AMF ecology because they allow to identification and evaluation of the AMF taxa in root samples without the need for fungal spores. They also have the potential to identify AMF hyphae in the soil (Sanders 2004). So, the choice of the DNA region used for amplification is critically essential (Gorzelak et al., 2012).

The inability to accurately and adequately identify AMF species is caused by the nature of their obligate symbiont and the failure to independently culture any of them (Berruti et al., 2016). Identification and classification of the AMF are made based on morphological characteristics of spores and molecular tools applied separately or combined (Kruger et al., 2012, Wetzel et al., 2014). By far, more than 340 AMF species have been identified and described worldwide, and the taxonomy of this fungal group is constantly being developed. The molecular techniques and morphological observations are shown that number of AMF species is greater than the present estimations and is expected up to 1600 (Tedersoo et al., 2018).

Isolation and identification of AMF species in each region are crucial steps prior to producing of fungal inoculum and employment in plantations and afforestation sites. In this regard, it is essential to know which types of AMF have symbiotic relationships with native plant species (Smith & Smith 2011). This review provides an overview of the morphological and molecular identification of AMF species in Iran over the past few decades.

Arbuscular Mycorrhiza Fungi

Mycorrhizal fungi are the most widespread and abundant fungi in nature and form a symbiosis relationship with many plants. The fungi belong to Ascomycota, Glomeromycota, Basidiomycota, and Mucoromycota (Smith & Read 2008). Mycorrhizal associations based on the type of fungus, host plant lineages, and structures produced by the root-fungus combination are classified into seven groups: endomycorrhiza (AM), ectomycorrhiza (EC), ectendomycorrhiza, ericoid, arbutoid, orchid and monotropoid (Tao et al., 2016) (Fig. 1). The movement of carbon from plant to fungus is common in all types of mycorrhizal association (Allen et al., 2003). Most often, mycorrhizal fungi are beneficial for host plants by improving the nutritional status of the plant (Chen et al., 2018).

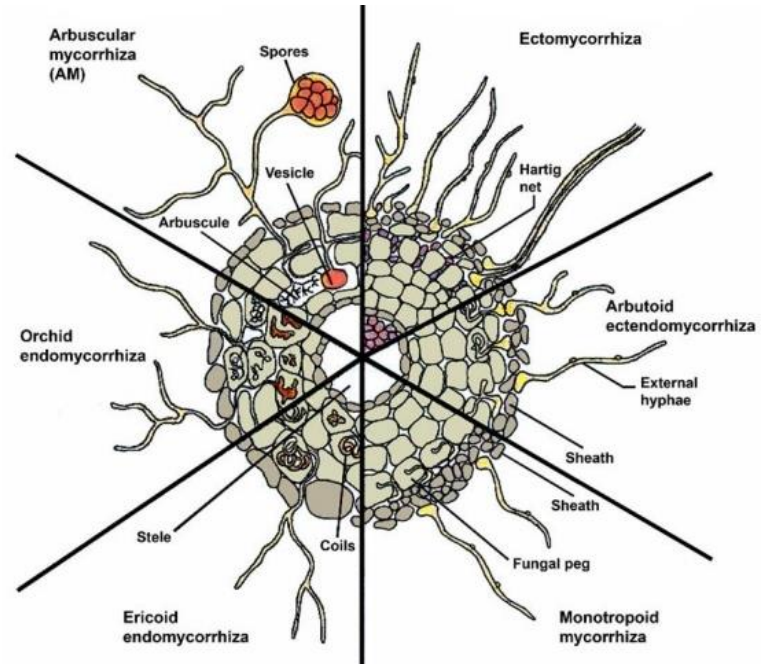

Fig. 1. Schematic representation of various types of mycorrhizal fungi (Amballa & Bhumi 2017).

The arbuscular mycorrhizal symbiosis is probably the most widely beneficial interaction between fungi belonging to the Glomeromycota phylum and the host plant (Giovannini et al., 2020). AMF are obligate symbionts that able to establish a mutualistic symbiotic association with the majority (upwards of 80%) of vascular plants, including Bryophytes, Hepatics, Pteridophytes, Gymnosperms and Angiosperms, in terrestrial ecosystems (Gao et al., 2019, Zhang et al., 2020). AMF receives the carbohydrates and lipids necessary for the survival of the photosynthetically produced carbohydrates by the host plant; Such that 10 to 40 % of the carbohydrates can be absorbed by fungi (Begum et al., 2019, Genre et al., 2020). In return, AMF improve the acquisition of essential mineral nutrients (particularly phosphorus and nitrogen) and water supply. This improvement is done using development a branching mycelial network in the soil that can be expanded the host root surface area by 40 times (Hayashi et al., 2018, Zhang et al., 2019). Fundamentally, these fungi provide other benefits such as carbon recycling, improvement soil aggregation, production of plant growth hormones, enhance the photosynthetic efficiency of the host, and helping the plant to tolerance abiotic (such as drought, salinity, and heavy metals contamination) and biotic stresses (pathogens and insects) through the implementation of various mechanisms (Ferrol et al. 2019, Lakmali et al., 2019, Li et al., 2020, Wu et al., 2021). As obligate biotrophs, AMF cannot complete their life stages without a compatible and living host plant (Smith & Read 2008). Under optimal conditions, an AMF spore (s) germinates, and after receiving signals released by the symbiotic partner, the hyphal germ tube navigates toward the host plant root (Tahat & Sijam 2012). Once in contact with the surface of the host's root, fungal hyphae form attachment structures, called "hyphopodia or appressoria" (Bonfante & Genre 2015). Then, hyphae penetrate toward the inner cortex cell and produce intraradical tree-like branched hyphae structures called "arbuscules". Arbuscules are the inorganic minerals (especially phosphorus and carbon compounds) and water exchange sites among the symbionts (Ivanov et al., 2019). Several AMF species, including Glomeraceae and Paraglomeraceae, produce vesicles the outer to middle cortical layers of root. Vesicles are storage structures contain lipids and glycogen and have a thin to thick wall layer (Smith & Read 2008). AMF structures in host plant roots are usually not observed without appropriate staining (Fig. 2).

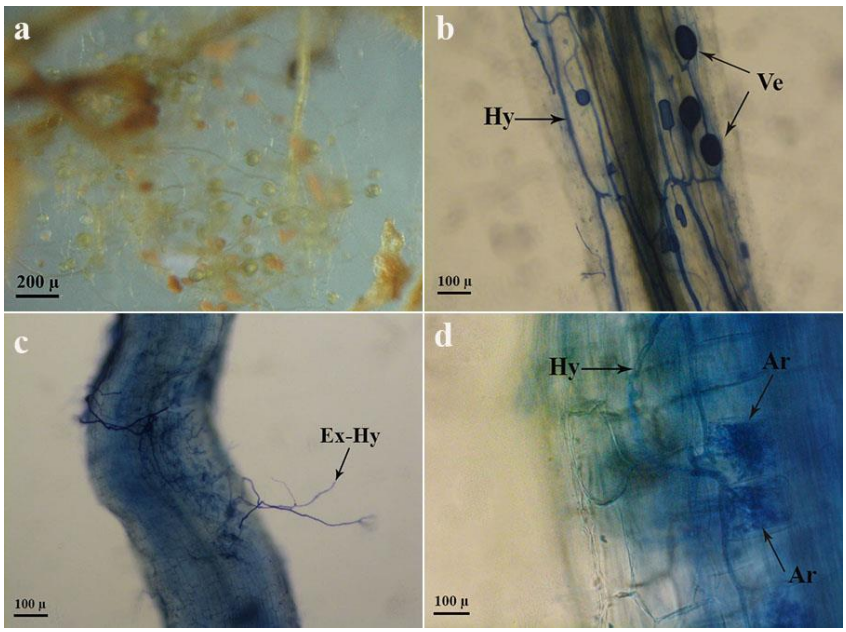

Fig. 2. Structures of arbuscular mycorrhizal fungi inside and outside of host plant roots. a: Spores; b: Intra-radical hyphae (Hy) and Vesicle (Ve); c: Extra-radical hyphae (Ex-Hy); d: Hyphae (Hy) and Arbuscule (Ar) (Picture by the researcher)

Intra-radical and extra-radical hyphae of AMF are composed of aseptate multinuclear mycelia (Redecker et al., 2013). AMF spores are produced singly, in loose clusters, tight clusters, or sporocarps in the soil or within host plant roots (Schüßler & Walker 2010). Molecular studies have suggested that spores AMF are multinucleate, and a single spore may contain multiple sequences of the same gene. Moreover, genetic variation of AMF is high not only between species but also nuclei in a single spore, and DNA polymorphism within fungal isolates was identified (Fitter 2005). To date, sexual reproduction has not been observed in AMF, although hidden sexual events were recently

suggested to happen (Corradi & Brachmann 2017). It was hypothesized the exchange of genetic material is carried out through anastomosis between AMF mycelia (Chagnon 2014, Novais et al., 2017).

History of the taxonomy of AMF

Fossil records and molecular evidence suggest that AMF symbiotic relationship with host plants formed 400-480 million years ago (between the Ordovician and the Devonian) and would have had essential roles in the establishment of terrestrial plants on land (Redecker et al., 2000). The symbiosis name of arbuscular mycorrhiza has changed over the years. First, the symbiosis was called "phycomycetous endomycorrhiza" to distinguish it from the endomycorrhizal symbioses, that formed between members of the Orchidaceae or Ericaceae and a fungus of endotrophic mycorrhiza. After discovering fungal structures, vesicles and arbuscules, the name "vesicular-arbuscular mycorrhiza" was established and persisted until recently. With regard to not all fungi forming vesicles, this symbiosis was renamed "arbuscular mycorrhiza" (Koide & Mosse 2004).

In recent years, the taxonomy and systematics of the AMF have experienced radical transformations. Kamienski (1881) discovered the symbiotic relationship between fungi and the roots of *Monotropa hypopitys*. Frank (1885) later used the term "mycorrhiza" for this relationship which means 'fungus root.' The Tulasne brothers (Tulasne & Tulasne 1845) were the first to describe the genus *Glomus* (*G. microcarpus* and *G. macrocarpus*) that considered this genus phylogenetically close to *Endogone*, a genus established by Link in 1809. Thaxter (1922) revised the Endogonaceae family, that included the genera *Endogone*, *Glaziella*, *Sclerocystis*, and *Sphaerocreas*. Peyronel (1923) was the first to suggest that the term "vesicular–arbuscular mycorrhizae" for members of the genus *Endogone*. Mosse (1953) reported the first study of adding the sporocarps of *Endogone* species to sterile soils with strawberry seedlings that resulted in mycorrhizal colonization.

Mosse (1953) reported the first study of inoculation the sporocarps of *Endogone* species to strawberry seedlings in sterile soil, which led to mycorrhizal colonization. This fungus was later named *Endogone mosseae* (= *Glomus mosseae* = *Funneliformis mosseae*) in her honor. After the device of the widely used wet sieving method by Gerdemann & Nicolson (1963) to extract fungal spores and sporocarps from soils, the interest in taxonomy of AMF increased dramatically. In the early 1970s, Gerdemann & Trappe split *Endogone* into seven nonmycorrhizal (*Endogone*, *Modicella*, *Glaziella*) and mycorrhizal (*Glomus*, *Sclerocystis*, *Gigaspora*, *Acaulospora*) genera, that were all placed in the family Endogonaceae, order Mucorales and phylum Zygomycota. As *Glaziella* and *Modicella* were not forming arbuscular mycorrhizal symbiosis, Trappe (1982) transferred *Modicella* to the family Mortierellaceae, and Gibson et al. (1986) placed *Glaziella* in the Ascomycota. This classification provided a sound basis for a taxonomy of AMF for up to several years.

Ames & Schneider (1979) recognized and introduced a new genus, *Entrophospora*. Walker & Sanders (1986) separated a new genus called *Scutellospora* from the *Gigaspora*. For the ease of identification of AMF, Schenck and Perez (1988) have published "Manual for the Identification of VA Mycorrhizal Fungi", which included all 126 AMF species descriptions. At the same time, different keys for the identification of AMF species developed. These manuals and keys are still being used in some laboratories to identify AMF species. During this time, AMF were identified based on morphological features and subcellular structures of spores. Morton & Benny (1990) placed the arbuscule-forming fungi into three families (Glomaceae, Acaulosporaceae, and Gigasporaceae) in a new order, the Glomales (now Glomerales). Cavalier-Smith (1998) proposed AMF could be placed in a new class, the Glomeromycetes within a new phylum Archeomycota. Analysis of available species of AMF and the examination of fossil records led to the suggestion of new taxa and the transfer of species to other genera. Taylor et al. (1995) proposed the genus *Glomites*. They described *Glomites rhyniensis* from aerial stems and rhizomes of the 400 million-year-old fossil plant *Aglaophyton major* in the Devonian period based on extra-radical and intrar-adical hyphae, chlamydospore-like spores, and arbuscule-like structures in the fossil plant.

Before molecular techniques, the only way to identify AMF was by microscopic examination of fungal spores. As early as 2001, Morton & Redecker erected two new families, Archaeosporaceae and Paraglomaceae in the order Glomales, by the data from molecular, morphological, and biochemical. Schüßler et al. (2001), using molecular data (SSU rDNA gene) and morphological characteristics, separated AMF from Zygomycota and grouped them in a monophyletic group, the phylum Glomeromycota, that is closely related to the Basidiomycota and the Ascomycota. Further, they proposed four new orders, i.e. Archaeosporales, Paraglomerales, Glomerales, and Diversisporales. Oehl & Sieverding (2004), identified four new species and after re-described two species of *Glomus*, classified them as a new genus *Pacispora* in the family Glomeraceae. In 2006, Sieverding & Oehl emended five species of *Entrophospora* and created the genera *Kuklospora* and *Intraspora*. Walker et al. (2007), based on morphological characteristics and rDNA data (SSU and ITS regions) proposed and described the genus *Ambispora* and the family Ambisporaceae. Oehl et al. (2008) introduced three new families in AMF

(Dentiscutataceae, Racocetraceae and, Scutellosporaceae).

Schüβler & Walker (2010) proposed a classification of AMF based on almost the entire length of the SSU rDNA gene. They separated *Glomus* into the genera *Funneliformis*, *Rhizophagus*, and *Sclerocystis* in the family Glomeraceae. They also erected the genus *Redeckera* in the family Diversisporaceae and the remaining species of *Glomus* along with *Claroideoglomus* in the family Claroideoglomeraceae. Oehl et al. (2011a) proposed a classification based on combined genetic (partial sequences of β-tubulin, and SSU and LSU rRNA) and phenotypic (traits associated with subtending hypha, e.g., color, shape and thickness, pore closure) characters. They divided the phylum Glomeromycota into three classes (Glomeromycetes, Archaeosporomycetes, and Paraglomeromycetes) and rearranged species of *Sclerocystis* and *Rhizophagus* and transferred back to the genus *Glomus*. In the same year, Oehl et al. (2011b) have been proposed new classes and orders. Goto et al. (2012), based on combined molecular and morphological studies, proposed a new classification and erected a new family called Intraornatosporaceae with the genera *Intraornatospora* and *Paradentiscutata*. Then, Kruger et al. (2012), based on morphological and molecular analyses of rDNA sequences (SSU, LSU, and ITS), divided this phylum into four orders: Paraglomales, Archaeosporales, Glomerales, and Diversisporales and ten genera. According to the classification made by Redecker et al. (2013), more than 250 species of AMF were reported, which were divided into one class (Glomeromycetes), four orders (Diversisporales, Glomerales, Archaeosporales, and Paraglomerales), 11 families and 25 genera. They rejected some genera like *Viscospora*, *Simiglomus*, and *Orbispora* and accepted *Dentiscutata*, *Sacculospora*, and *Corymbiglomus* but required verification. Blaszkowski et al. (2015) described two new genera, *Dominikia* and *Kamienskia*. Based on the phylogenetic analysis performed by Sieverding et al. (2015), *Rhizoglomus* genus forms a separate clade within the family Glomeraceae.

Taxonomy and classification of this fungal group have been rapidly updated over the last years, and several reports have been made. Walker et al. (2018) classified the phylum Glomeromycota into a single class (Glomeromycetes) which includes four orders (Diversisporales, Glomerales, Archaeosporales, and Paraglomerales), 12 families (Acaulosporaceae, Ambisporaceae, Archaeosporaceae, Claroideoglomeraceae, Diversisporaceae, Geosiphonaecae, Gigasporaceae, Glomeraceae, Pacisporaceae, Paraglomeraceae, Pervetustaceae, and Sacculosporaceae), 34 genera and approximately 316 species. In the same year, the phylum Glomeromycota was divided by Tedersoo et al. into classes Glomeromycetes (Glomerale, Gigasporales, and Diversisporales), Paraglomeromycetes (Paraglomeromerales) and Archaeosporomycetes (Archaeosporales). Then, Wijayawardeneet et al. (2018) proposed another classification for Glomeromycota, which included two classes (Glomeromycetes, Archaeosporomycetes, and Paraglomeromycetes), four orders (Archaeosporales, Diversisporales, Glomerales, and Paraglomerales), 12 families and 38 genera and Gigasporales was not accepted. According to the latest unofficial classification presented on the website amf-phylogeny.com, AMF include four orders Diversisporales, Glomerales, Archaeosporales, and Paraglomerales, 12 families, 41 genera, and 342 species. Considering that new genera and species of this fungal group are being identified, such as *Microkamienskia* and *Microkamienskia peruviana* (Corazon-Guivin et al. 2019), the classification of Glomeromycota is debatable. Nevertheless, the classification performed by Wijayawardeneet al. (2018) is used as a reference.

Morphological identification of AMF

Traditionally, AMF species have been identified by the morphological and anatomical characteristics of their spores (Traditionally, identification of AMF species has been made by spore extraction from soil and investigation of the morphological and anatomical characteristics of their spores) (Blaszkowski 2012). The morphological method is commonly used because it is fast, easy, cost-effective, and economical and can identify at the genus level. In the absence of spores, the intraradical and extra-radical structures of AMF (arbuscules, vesicles, hyphae, auxiliary cells) allow taxon identification at the family level (Merryweather & Fitter 1998, Sanders 2004).

There are several disadvantages to the morphological method: So far, an exact and comprehensive identification key has not been provided for AMF. AMF spores have simple structures, and morphological characteristics of the spores are scarce. For example, *Paraglomus* and *Glomus* cannot be distinguished from each other using spore morphology (Redecker et al., 2003). On the other hand, some species produce two types of spores or dimorphic spores (e.g., *Archaeospora leptoticha* and *G. dimorphicum*). Beside this, spore morphology may be high even within an AMF species (Merryweather & Fitter 1998). Also, spores collected from field soil may be degraded or parasitized and unidentifiable (Oehl et al., 2015, 2016). Another limitation of morphological identification is the spore production in this fungal group is strongly affected by environmental conditions and some species of AMF might sporulate rarely or only in a seasonal manner, such as *Acaulospora* spp. (Oehl et al., 2011b, 2012).

Aside from that, several AMFs may perform vegetative reproduction without the production of spores (Marleau et al., 2012). Thereby, the morphological diversity of AMF spores is insufficient for their variety in ecosystems. It is still common to the identification of AMF species by morphological features, notwithstanding the problems that are known in this method.

The first step in morphological identification of AMF species is the isolation of fungal. For this purpose, AMF spores were extracted from air-dried soil samples by wet sieving (Gerdemann & Nicolson 1963) and sucrose centrifugation (Jenkins 1964) methods. Intact and healthy spores are mounted on slides with PVLG + Melzer's reagent (1:1, v/v) (Hall 1984) and structural features are recorded, including spore color, shape, size, surface ornamentations, wall structure, hyphal attachments, and the mode of spore germination. Eventually, fungal spores are identified according to comprehensive identification manuals (Schenck & Perez 1988, Schüßler & Walker 2010, Oehl et al. 2011a, Wang & Liu 2017) and reference websites (http://www.invam.wvu.edu, http://www.zor.zut.edu.pl).

Molecular identification of AMF

In recent years, the development of new molecular methods has made it possible to identify symbiotic AMF species with different plant roots in a shorter period (Souza 2015). Molecular analysis is faster and provides more accurate and precise results. Molecular methods have revolutionized our understanding of phylogenetic relationships between fungi and fundamentally altered the morphological classification system (Hempel et al., 2007).

DNA extraction involves roots colonization, multiple spores, or single spore (Ferrol & Lanfranco 2020). The amount of AMF DNA extracted from different kinds of templates could lead to significant differences in the amplification of the target region (Shi et al., 2012). Field collected mycorrhizal roots contain various amounts of AMF tissue, and PCR inhibitors such as polysaccharides, proteins and phenolic compounds (Shafiqua & Stephan 2013). Also, the amount of fungal DNA compared to DNA plant is very low. So, selection of the suitable DNA extraction method and AMF-specific primers are necessary because otherwise, numerous saprophytic and pathogenic fungi will be identified simultaneously (Redecker et al. 2003).

Spores isolated from a soil sample are divided based on spore morphology using a stereo-microscope. The healthy spores that show numerous lipid globules inside, were selected and used for DNA extraction (Redecker 2020). Afterward, each spore type is grouped into three groups for molecular identification, morphological identification and purification. Cleaning spores by ultrasonic treatment and surface sterilization are helpful to remove debris and DNA from contaminating organisms (Sasvari et al., 2012). DNA of spore (s) is extracted by crushing the spore using a micropipette. The most crucial reason for DNA extraction from a single spore, is reduced contaminations of other microbial species embedded in the spore wall. Removal of contamination attached to the single spore surface is more straightforward in comparison with multiple spores and mycorrhizal roots (Schwarzott et al., 2001).

Almost all molecular identification approaches for AMF are based on ribosomal DNA and the methods of Polymerase Chain Reaction (PCR) with specific primers (Kohout et al., 2014). Nested PCR using AMF-specific primers is a susceptible method that allows investigating of the mycorrhizal community structure and detection of fungal species present in roots and soil (Jacquot et al., 2000). Molecules other than rDNA, such as the mitochondrial genome, fatty acids, isozymes, and proteins (actin and ß-tubulin), have been analyzed for AMF molecular identification (Raab 2007, Formey et al. 2012, Aminiannasab et al. 2021). Still, they are not widely used.

The ITS (internal transcribed spacer) region has been used for AMF molecular studies and sequencing (Opik et al., 2014, Tedersoo et al., 2014). But this region has a high degree of variation within AMF species, and it is difficult to find distinct features of closely related fungal species (Victorino et al., 2020). Therefore, ITS is of limited utility for AMF. Most data concerning molecular identification of AMF have been obtained using the SSU rDNA gene due to has a highly conserved gene (less variable than ITS) (Victorino et al. 2020) and provides intraspecific variation in this fungal group (Thiery et al. 2013, Lekberg et al. 2018, Perez-Lamarque et al. 2020). However, the slow evolution of this region is its most crucial disadvantage (Kruger et al., 2009, Bruns & Taylor 2016). Considering the LSU gene contains enough variation to distinguish species of AMF, this region has been chosen as a target for molecular identification and classification of AMF in some studies (House et al., 2016, Schutte et al., 2019). The use of the ITS variable region along with LSU and SSU regions, due to the high polymorphism, allows the identification of AMF to the species level and the differentiation of closely related species (Kruger et al. 2009). However, the SSU region is still the most frequent segment used in AMF molecular studies.

The selection of AMF-specific primers is essential as general primers will co-amplify the DNA of other organisms (Kruger et al., 2009). The first SSU sequences from rDNA of AMF were determined by Simon et al. (1992), who designed a specific primer for these fungi termed VANS1. The VANS1 primer was used in some studies (Clapp et al. 1995, Redecker et al. 2000), but it became clear that this primer is not well-conserved in all species of the AMF. Later, different specific primers were

designed to identify other species of AMF. Currently, the primer sets SSUmAf/ LSUmAr and SSUmCf/LSUmBr designed by Kruger et al. (2009) are more specific and amplifies a partial on rDNA with a length of 1.5 kb. This 1.5 kb barcode consists of a part of the SSU, the complete ITS region (ITS1, 5.8S, ITS2 gene), and 800 bp of the LSU and avoids nonspecific amplification (Kruger et al., 2009). These primers should allow the distinction and separation of closely related AMF species such as *F. mosseae* and *F. coronatus* (Rosendahl & Matzen 2008), which the SSU or LSU regions alone cannot do (Schüßler 2013).

To evaluate the success of PCR amplification, PCR products are separated by electrophoresis through agarose gel in a TBE buffer. Much information about the sequences of rDNA of AMF species are accumulated in the GenBank database NCBI (http://www.ncbi.nlm.nih.gov). PCR-based techniques require continuous improvement in primer design as more sequences are introduced in the database. Each DNA sequence is compared with the nucleotide sequences in GenBank database NCBI using suitable software (Thompson et al., 1994). Sometimes the sequences obtained using PCR, may be unknown or uncultured Glomeromycota. This is because the rDNA sequences of some AMF species are not available in the GenBank database.

Today, critical progress has been made in the identification of AMF using morphological characteristics of AMF spores and molecular analysis targeting specific rDNA genes, which can easily be deduced from the numbers of AMF taxa detected (Njeru et al., 2015, Pontes et al., 2017). Molecular methods, due to their high identification accuracy can be complementary to morphological methods and lead to more confidence in identification and more stability in AMF taxonomy (Crossay et al., 2017).

Arbuscular Mycorrhizal Fungi in Iran

In Iran, many researchers have surveyed AMF communities and its diversity among different plant species. Since about 30 years ago, identification and description of AMF were made in Iran. For the first time, Mehravaran and Minasian (1984) identified 10 species of AMF, including *Rhizophagus fasciculatus* and *Gigaspora margarita*, from citrus orchards in the north and south of Iran. With the increase of information and the development of the methods used, several AMF species from different regions of Iran, combining molecular and morphological techniques, have been reported (e.g., Sedaghati et al., 2005; Rezaee Danesh et al., 2006; Sabet Jahromi et al., 2013; Yazdanpanah et al., 2017; Zangeneh 2021). However, most studies on AMF communities used morphological approaches and spores characteristics. Most AMF molecular identification systems used were based on rDNA genome and the Nested-PCR method. Among the primers used can be mentioned to SSU-Glom1/LSU & ITS4/ITS5 (Salehi Jouzani et al. 2012; Sabet Jahromi et al. 2013), NS4/AML1 & AML1/AML2 (Yazdanpanah et al. 2017) and SSUmAf-LSUmAr/LSUmAr & SSUmCf/LSUmBr (Bazgir et al. 2020). It should be noted in the study conducted by Aminian et al. (2021) the partial sequences of the ß-tubulin gene were amplified by three Nested-PCR using C2F/FBtub4R, GiH4R/IB36F and, Fsp/GiH3R primers.

Obviously, in Iran similar to other regions of the world, the composition and diversity of AMF species are affected by plant communities, soil and, climatic characteristics. These plants include food crops, vegetables, fruitful and non-fruitful trees, ornamental plants, medical plants, wild weeds, etc. In Table 1, some of the studies on identifying AMF species using morphological, molecular, and combination of both of these methods in Iran are listed. According to published articles, more than 115 AMF species have been identified at the species level (Table 2). Based on the results, the Glomeraceae species were dominant under different conditions, suggesting the higher and better adaptation of members of this family than other species. The predominance of Glomeraceae is consistent with the results of some studies in different countries (Ndoye et al., 2012, Bouazza et al., 2015, Mosbah et al., 2018). Members of some families, such as Acaulosporaceae, have occurred almost in the natural and intact areas. Moreover, it is probably the majority of AMF species that are still to be discovered and described.

CONCLUSION

The physiological and ecological benefits of mycorrhizal symbiosis for their host plants have long been acknowledged. The obligate biotrophic nature of AM fungi is one of the significant constraints of studying their taxonomy (Johnson et al., 2016). Detection and identification of AMFs based on spore morphology along with molecular methods provides a more comprehensive estimate of the composition, abundance, and diversity of the fungal community (Mosbah et al., 2018). Most AMF molecular identification studies have used primers that amplify ribosomal DNA, because the genes in this region have highly conserved coding sequences (Bati et al., 2015).

In Iran, many mycorrhizasts have done considerable amount of works on the identification of AMF species associated with plants located in different geographical regions of the country. They have used molecular and morphological methods to investigate the diversity and community composition of AMF symbiosis with varying plants in different areas. According to studies, the genera belonging to the family Glomeraceae, particular, *Glomus* and *Funneliformis*, are present in most areas of Iran that indicating greater adaptability of these genera to

varied soil conditions. In the future, AMF identification and classification must develop, and more applied research should be carried out to make AMF commercially available in the agricultural sectors of Iran.

Table 1: AMF species/genera reported from different plants and regions in Iran

Plant/Region	Identification method	Result	Reference
Citrus	Morphological	Ten species	Mehravaran & Minassian 1984
Saffron	Morphological	15 species	Kianmehr et al., 1999
wheat, barley, maize, and sorghum	Morphological	21 species	Sadravi et al., 1999
Crops/Khorasan	Morphological	Six species	Balali et al., 2001
Grapes/Khorasan and Qazvin	Morphological	13 species	Sedaghati 2002
Different plants	Morphological	Five species	Sadravi 2002
Citrus	Morphological	Five species	Zangeneh et al., 2004
Kharturan	Morphological	12 species	Karimi et al., 2005
Sugarcane/Khuzestan	Morphological	17 species and two genera	Kariman et al., 2005
Pistachio/Rafsanjan	Morphological	Ten species and seven genera	Sedaghati et al., 2005
Citrus	Morphological	23 species	Zangeneh et al., 2005
Soybean	Morphological	21 species and five genera	Rezaee Danesh et al., 2006
Sugarcane	Morphological	14 species and four genera	Rokni et al., 2006
Wheat/Golestan	Morphological	19 species	Sadravi 2007
Sugarcane	Morphological	10 species and four genera	Rokni et al., 2010
Sugarcane	Morphological	16 species and four genera	Rokni & Goltapeh 2011
Grapevine/West Azerbaijan	Morphological	12 species and two genera	Mahdavi et al., 2012
Alfalfa/Kohgiluyeh & Boierahmad	Morphological	23 species and nine genera	Sadravi 2012
Wheat, barley and weeds	Morphological and Molecular	Seven species	Salehi Jouzani et al., 2012
Barley/Damghan	Morphological	16 species and four genera	Rezaee Danesh 2013
Wheat and barley	Morphological and Molecular	Nine species	Sabet Jahromi et al., 2013
***Crataegus pontica*/ Ilam**	Morphological	13 species and five genera	Mirzaei et al., 2014
Some Trees/Kiasar	Morphological	Ten species and three genera	Modarresi Chahardehi et al., 2014
Potato/Ardabil	Morphological	Four species and three genera	Rostami Hir et al., 2014
Thymus daenensis	Morphological	Nine species and two genera	Ahmadi et al., 2016
Amygdalus scoparia	Morphological	13 species and eight genera	Mirzaei & Moradi 2017
Pistachio/Kerman	Morphological and Molecular	Three species and three genera	Yazdanpanah et al., 2017
Manesht and Ghalarang protected area	Morphological	35 species and ten genera	Mirzaei et al., 2018
Pistachio/Rafsanjan	Morphological	Seven species and four genera	Aminizadeh et al., 2018
Saffron	Morphological and Molecular	Three species and three genera	Pooryousef et al., 2018
Medicinal plants/Kerman	Morphological and Molecular	Five species and four genera	Bazgir et al., 2020
Different plants/Rafsanjan	Morphological and Molecular	Five species and four genera	Aminiannasab et al., 2021
Different plants	Morphological	Five species and one genus	Zangeneh 2021

Table 2. List of number of the AMF species described in Iran

Families	Genus	Species
Glomeraceae	*Glomus*	*G. ambisporum, G. gibbosum, G. macrocarpum, G. pansihalos, G. sinuosum, G. microcarpum, G. badium, G. aureum, G. corymbiform, G. liquidambaris, G. botryoides, G. fulvum, G. gerdemanni, G. trimurales, G. albidum, G. rubiforme, G. fuegianum, G. dimorphicum, G. reticulatum, G. boreale, G. manihotis, G. tortuosum, G. invermaium, G. magnicaulis, G. pubescens, G. multisubstensum,*
	Septoglomus	*Se. constrictum, Se. africanum, Se. deserticola, Se. turnauae, Se. xanthium, Se. jasnowskae, Se. viscosum, Se. nakheelum*
	Sclerocystis	*Sc. coremioides*
	Funneliformis	*F. mosseae, F. caesaris, F. geosporus, F. caledonius, F. multiforus, F. coronatus, F. verruculosum, F. fragilistratum*
	Rhizophagus	*Rh. aggregatus, Rh. fasciculatus, Rh. intraradices, Rh. irregularis, Rh. diaphanus, Rh. clarus, Rh. microaggregatum, Rh. iranicus, Rh. arabicus*
	Simiglomus	*Si. hoi*
	Dominikia	Do. *disticha, Do. minuta*
	Microkamienskia	M. *perpusilla*
Claroideoglomeraceae	*Claroideoglomus*	*C. etunicatum, C. luteum, C. claroideum, C. walkeri, C. drummondii, C. lamellosum*
	Viscospora	*V. viscosa*
Diversisporaceae	*Diversispora*	*D. spurca, D. aurantia, D. eburnea, D. arenaria, D. varaderana, D. celata, D. pustulata, D. epigaea, D. versiformis, D. aurntium, D. globifera*
	Desertispora	*Des. omaniana*
Paraglomeraceae	*Paraglomus*	*Pa. laccatum, Pa. occultum, Pa. franciscana, Pa. scintillans*
Archeosporaceae	*Archaeospora*	*Ar. trappei, Ar. schenckii*
Acaulosporaceae	*Acaulospora*	*A. cavernata, A. mellea, A. dilatata, A. bireticulata, A. alpina, A. gedanensis, A. herrerae, A. punctata, A. scrobiculata, A. thomii, A. paulinae, A. capsicula, A. delicate, A. rugosa, A. colombiana*
Ambisporaceae	*Ambispora*	*Am. fennica*
Pacisporaceae	*Pacispora*	*P. boliviana, P. scintillans, P. franciscana*
Entrophosporaraceae	*Entrophospora*	*E. infrequens*
Pervetustaceae	*Pervetustus*	*Pe. simplex*
Gigasporaceae	*Gigaspora*	*Gi. gigantea, Gi. rosea, Gi. decipiens, Gi. candida, Gi. albida, Gi. margarita*
	Scutellospora	*S. calospora, S. armeniaca, S. pellucida S. coralloidea, S. fulgida, S. erythropa, S. dipurpurascens*
Dentiscutataceae	*Dentiscutata*	*De. reticulate, De. biornata*

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مروری بر مطالعات تاکسونومیکی قارچ‌های میکوریز آربوسکولار در ایران

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چکیده: قارچ‌های میکوریز آربوسکولار (AMF) یکی از مهم‌ترین ریزجانداران مفید خاک هستند که قادر به برقراری رابطه همزیستی همیاری با طیف وسیعی از گیاهان می‌باشند. AMF کربن مورد نیاز برای تکمیل چرخه زندگی خود را از گیاه میزبان دریافت می‌کنند. در مقابل، این گروه قارچی فواید متعددی برای گیاهان میزبان دارند، از جمله محافظت از گیاه در برابر بیمارگرها، افزایش تحمل تنش‌های غیرزیستی (خشکی و شوری) و افزایش جذب آب و مواد مغذی. در گذشته، شناسایی و طبقه‌بندی AMF عمدتا براساس ویژگی‌های ریخت‌شناسی اسپورها مانند نحوه تشکیل، ساختار دیواره و ویژگی‌های هیف اتصال انجام می‌گردید. سپس، روش‌های مولکولی برای شناسایی این قارچ‌ها مورد استفاده قرار گرفتند. روش‌های مبتنی بر PCR منجر به شناسایی مستقیم گونه‌های AMF موجود در ریشه‌های گیاه یا ناحیه فاریشه می‌شوند. تاکنون، پرایمرهای متعددی برای افزایش دقت شناسایی AMF طراحی شده است. امروزه، طبقه‌بندی این گروه قارچی مبتنی بر روش‌های ریخت‌شناسی و مولکولی می‌باشد. در این مطالعه، مروری بر پژوهش‌های انجام شده در زمینه شناسایی AMF در ایران ارائه می‌گردد. طی چند دهه اخیر، شناسایی AMF در ایران مورد توجه فزاینده‌ای قرار گرفته است. بدین‌منظور، پژوهش‌گران از ویژگی‌های ریخت‌شناسی و روش‌های مبتنی بر DNA برای شناسایی این گروه قارچی استفاده کرده‌اند. تاکنون بیش از ۱۱۵ گونه AMF متعلق به ۲۲ جنس از مناطق و جوامع گیاهی مختلف مانند زمین‌های زراعی، جنگل‌ها، مراتع و غیره، شناسایی و گزارش شده است. شناخت ترکیب و تنوع AMF برای استفاده از آن‌ها به عنوان کودهای زیستی در کشاورزی جهت کاهش نهاده‌های شیمیایی و افزایش تولید محصول حائز اهمیت می‌باشد.

کلمات کلیدی: ترکیب جامعه، تنوع، ریخت‌شناسی اسپور، همزیستی، *Glomus*.

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