Development of the On-farm Production of Indigenous AM Fungi inoculum and Its Potential for Sustainable Growth and Nutrition of Native Plants in Kuwait's Desert

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Abstract:

Arbuscular mycorrhizal (AM) fungi are the most important soil microorganisms found in the desert soil that form symbiotic association with the roots of most desert plants. AM fungi are known to influence the uptake of mineral nutrients, water relations, soil structure, growth and development, and disease resistance of plants. One way of ensuring these benefits to seedling production and consequently, the successful revegetation of desert lands is to achieve mycorrhizal associations by inoculation with efficient mycorrhizal fungi. The on-farm production of AM fungi inoculum using indigenous desert fungal strains could be beneficial in terms of taxonomically diverse and ecologically adapted characteristics of the produced inoculum. Mycorrhizal spores, pieces of colonized plant roots, and viable mycorrhizal hyphae can function as active propagules of AM fungi and can be used as inoculums to form symbiotic association with target plants. Effective AM fungi inoculum can be produced by various methods. On-farm inoculum production system is cost-effective, simple, and readily adaptable for seedling producers and large-scale revegetation purposes. Our aim is to develop an on-farm inoculum production system, which is exceptionally simple and inexpensive, using fungal strains adapted to Kuwait's desert climate and expected to produce an effective and species diverse inoculum. The potential benefits and utilization of on-farm produced AM fungi inoculum for the improvement of native desert plant seedling production and revegetation success are discussed.

Keywords: Mycorrhizal fungi; inoculum production; inoculation; biofertilizer; seedling production; revegetation.

Introduction

Arbuscular mycorrhizal (AM) fungi are essential components of nearly all terrestrial ecosystems, developing beneficial symbiotic associations with the roots of around 95% of vascular plants with diverse communities of mycorrhizal fungi. Particularly, in arid environments, AM fungi are recognized as a critical component of plant-soil systems of deserts (Requena, et al. 1996), and plants can survive in harsh and low-nutrients environments because of their role in stress alleviation and enhanced nutrient acquisition capacity (Smith and Read, 2008; Sylvia and Williams, 1992). The mycelial network of AM fungi extends into the soil volume and greatly increases the surface area for the uptake of immobile nutrients and water relations. Many studies have indicated that AM symbioses improve plants' tolerance to drought (Jayne and Quigley, 2014; Smith and Read, 2008), enhance plants' tolerance of or resistance to root pathogens, and perform a role in the soil aggregation process (Smith and Read, 2008; Rillig and Mummey, 2006; Dalpé, 2005). In recent times, the utilization of mycorrhizal symbiosis is becoming an important biological tool in environmental land reclamation programs, agriculture, forest seedling production, revegetation, and horticultural sectors, to improve plant growth and survival and to reduce the use of extensive fertilizers and pesticides (IJdo, et al. 2011; Gianinazzi, et al. 2002). Considering this importance, the optimal exploitation of the AM fungi is essential for farmers, seedling producers, and reclamation industry. There are two scenarios for the ideal utilization of mycorrhizal fungi. One is to effectively manage the mycorrhizal populations if already present in the systems or to inoculate plants in nursery, planting fields with indigenous and site adapted isolates of mycorrhizal fungi. The current paper will consider only the on-farm production and use of AM fungal inocula.

The use of indigenous and site adapted AM fungal inoculum produced on-farm may demonstrate an appropriate substitute to commercial AM fungal inocula and may offer economically and ecologically important advantages in many areas of agriculture, horticulture, and forestry. AM fungal inocula, presently, are being produced using various techniques and that are commercially available. Some of the available techniques that are currently used, for example, soil/sand/vermiculite/potting mix substrate-based carrier materials that are mixed with the starter inocula of AM fungi; substrate free cultivation systems, namely, -hydroponics and aeroponics; and the excised roots organ culture systems (IJdo et al. 2011). The last two techniques involve high production cost, are not suitable for large-scale production, and are mainly limited to research purposes. Nevertheless, all of the developed techniques and products have their own advantages and disadvantages, field of application, as well as differences in the cost involved in production. Substrate-based on-farm production of AM fungal inocula have been developed and described previously by many researchers in which a starter inoculum of AM fungi was mixed into beds of fumigated soil, raised bed enclosure containing mixtures of compost and vermiculite, and pot culture in greenhouses or field-based methods in which one or a series of suitable host plants are grown (Sieverding, 1991; Gaur, 1997; Douds, et al. 2000; Gaur, et al. 2000; Gaur and Adholeya, 2002; Gianinazzi and Vosatka, 2004; Douds, et al. 2005, 2006).

In this report, we intend to describe a simple and inexpensive modified technique for the on-farm production of indigenous AM fungal inoculum, mixing desert sand with nursery potting soil as a carrier-substrate without sterilization, fumigation, or use of chemicals for the application in Kuwait's desert climates. This simple method of on-farm crude inoculum production is

appropriate for nursery growers, other farmers, and seedling producers, where growers can easily mix the locally adapted inocula into growing media to produce inoculated seedlings.

Materials, Methods, Results, and Discussion

Materials Required for On-farm AM Fungus Inoculum Production

The on-farm production system was established in a greenhouse located at the Shuwaikh Campus of the Kuwait Institute for Scientific Research. The following materials were required to space an on-farm production of AM inoculum: desert sand, nursery potting mix, black plastic grow bags, starter inoculum soil, root fragments collected from the rhizosphere of desert plants, and seeds of host plants.

Host Plant

Corn plants (*Zea mays* L.) were selected as the host plant (trap plant) for AM fungus inoculum production because it has been exhibited, in many greenhouse experiments, as a suitable host for most of the AM fungi examined. Several other trap host plants are commonly used for the large-scale production of AM fungi, such as *Allium* spp., *Zea mays* L, *Paspalum notatum* Flugge, and *Sorghum sudanese* (Piper) stapf. All of these host plants have suitable characteristics with shorter life cycle, profuse root system development, colonization capacity by a wider range of AM fungi, and tolerance to low levels of phosphorus.

Methods of the On-farm Production of AM Fungus Inoculum

The current modified method of indigenous AM fungus inoculum production was developed based on the procedures previously descried by many authours (Douds, et al. 2010; Douds, et al. 2005; Gaur, 1997). The on-farm inoculum production system began with the collection of indigenous starter inoculum from the rhizospheric zone of naturally growing plants in Kuwait's desert. The mixed starter inoculum usually consists of rhizospheric soil, native populations of AM spores and fungal hyphae, and chopped root pieces obtained from the rhizosphere regions (top 10 to 15 cm) of different native plants growing in Kuwait's desert and pooled for the starter inoculum. Besides fungal spores, colonized root fragments and hyphae are also prospective infective propagules of AM fungi (Klironomos and Hart, 2002). In this on-farm production system, starter inoculum species are indigenous to the Kuwait desert sites and not identified to the species level.

To propagate indigenous AM fungal strains present in the starter inoculum, the starter inoculum was multiplied by growing *Zea mays* L. as a trap host plant in a culture mix for a full growing season, for about four months, in black plastic bags (grow bags) filled with 15 L of 1:4:1 (v/v) mixture of potting soil (Plantaflor® SAB potting soil) (Table 1), desert sand, and starter inoculum, respectively. Corn (*Zea mays* L) seeds were collected locally and surface sterilized with Clorox (2%) for 15 min and rinsed 3 times with sterilized water before putting onto a germination plate with filter paper under the room temperature. Ten germinated corn seeds were directly transferred to each culture bag, and the bags with germinated seeds (10 seedlings/bag) were allowed to grow in greenhouse conditions for the entire growing season (Fig. 1). In this way, AM fungi propagules present in the starter soil proliferate throughout the carrier substrate in the bag and colonize the roots as the host plant grows over the growing season. The culture bags were watered throughout the growing season as needed, and the

watering was restricted during the last two weeks before harvesting. No additional fertilizer was added. At the end of the growing season, the shoots of the plant were allowed to die and subsequently removed from the bags. The carrier substrate in the bag was allowed to dry in a greenhouse for one week. The roots were harvested and chopped and mixed with the substrate. The inoculum substrates from several bags were mixed together and then further allowed to be air dried and mixed thoroughly to obtain a homogenous inoculum mixture. This inoculum mix contained spores, living hyphae, and colonized root fragments. A step-by-step schematic representation of the procedure for the production of indigenous on-farm inoculum is shown in Figure 2.

Assessment and Estimation of AM Root Colonization and Spore Population

After the completion of inoculum production, fine root samples were collected and randomly selected (i.e., three replicate batches of root samples) for detecting the AM root colonization status. The root samples were washed with tap water and cut into small pieces of about 1 cm in size. The root samples were then cleared and stained with Chlorozol Black E as per the procedures described by Brundrett, et al. (1984), and the colonization rate of the host plant roots was observed and estimated under compound a microscope (Fig. 3). The host root samples exhibited profuse AM colonization structure with arbuscules, vesicles, and hyphae, and the colonization rate by AM fungi was estimated to be 98-100% when 100g of inoculum was used in the bioassy experiment. AM fungal spores in the produced inoculum substrate were detected and counted in triplicate samples by following the modified sucrose centrifugation method (Smith and Skipper, 1979). The AM spore population was observed and estimated to be between 95 and 134 per 50 g of inoculum. Then, the produced inoculum was stored at 4°C and ready to use for subsequent inoculation purposes by the researchers, farmers, and nursery owners.

Table 1. Chemical characteristics of the potting soil mix used for on-farm indigenous inoculum production

pHs	**Ece (mS/cm)**	**Ca^{+2}**	**Mg^{+2}**	**K^{+1}**	**N**	**P mg/kg**	**Organic matter %**
		mg/kg					**%**
7.13 ± 0.1	1.29 ± 0.07	1540 ± 213.1	133 ± 14.18	250 ± 10	1400 ± 0.01	2.36 ± 0.92	8.62 ± 0.94

Figure 1. On-farm inoculum production bags showing corn plants growing as the trap host plant for inoculum production under greenhouse conditions for four months.

Colonization Rates of the Produced On-farm Inoculum

The number of AM infectious propagules (IP) in the produced inoculum was assessed by trapping bioassay (Gaur, et al. 1998), which confirmed the establishment of mycorrhizal association. The values of the IP were calculated as follows:

1. The rate of infection of the initial inoculum was evaluated by inoculating 25, 50, and 100 g of the produced inoculum into a sterile bioassay setup in which corn seedling used as trap plant (autoclaved desert sand, 30 min, twice at 121^{o}C) (Fig. 4). Similarly, 100 g of inoculum was sterilized twice in an autoclave at 121^{o}C for 30 minutes. The sterile inoculum was used as control treatment. The inocula and sterilized desert sand was mixed properly and placed in a 500 ml plastic container. The host seedlings (corn seedlings) were then grown in all combinations of inoculum under the greenhouse conditions for four weeks before the evaluation of the percentage rate of colonization by AM fungi (Fig. 4). The experiment was space in triplicate.
2. The seedling roots were harvested and the colonization rate by AM fungi was measured by counting the number of vesicles and arbuscules in 100 cm of corn root after clearing and staining with Chlorozol Black E as described by Brundrett, et al. (1984). (Fig. 5, Table 2). The % colonization rate was estimated as the percentage of root length that was colonized by the formation of arbuscules, vesicles, and hyphae. The percent colonization rate was calculated using the formula:

$$\frac{\text{No. of root bits colonized by AM fungal structures}}{\text{Total length of root bits studied.}} * 100$$

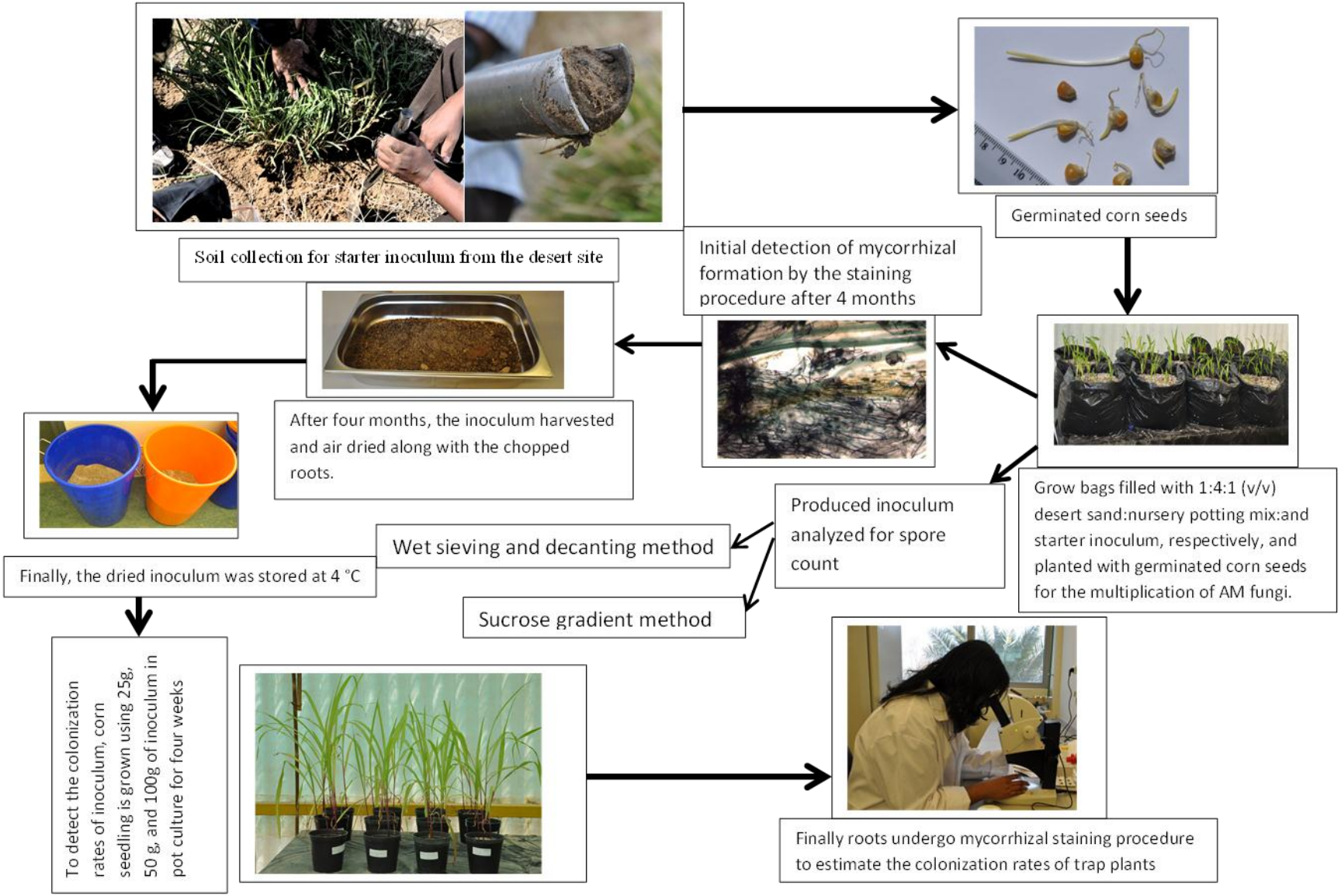

Figure 2. Flow chart showing the step-by-step protocol for indigenous on-farm inoculum production.

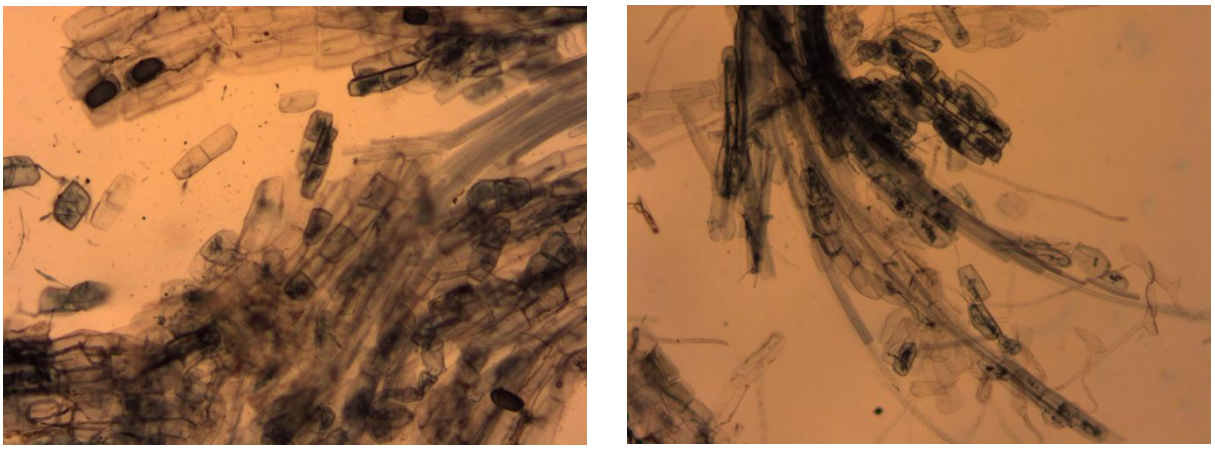

Figure 3. Abundant arbuscules and vesicles observed in root samples from corn plants used for the indigenous inoculum production.

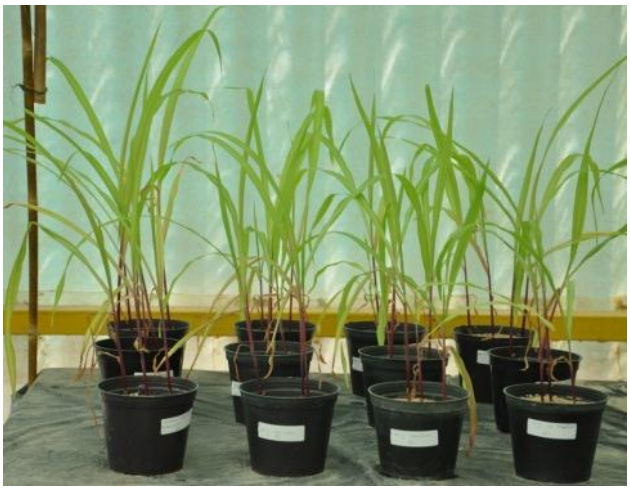

Figure 4. Photo showing trapping bioassay setup using corn seedlings inoculated with 25, 50, and 100 g of inoculum and a 100 g sterile inoculum as control in triplicates.

Table 2. Percentage of AM colonization on 100 cm of corn root when grown on different concentrations of the produced inoculum in a bioassay study

Sample ID	**% colonization by vesicles**	**% colonization by arbuscule**
Sterile inoculum 100 g	0 ± 0	0 ± 0
25 g	89.33 ± 2.5	21.33 ± 6.8
50 g	91.33 ± 15	87.17 ± 22.2
100 g	100 ± 0	98.17 ± 3.2

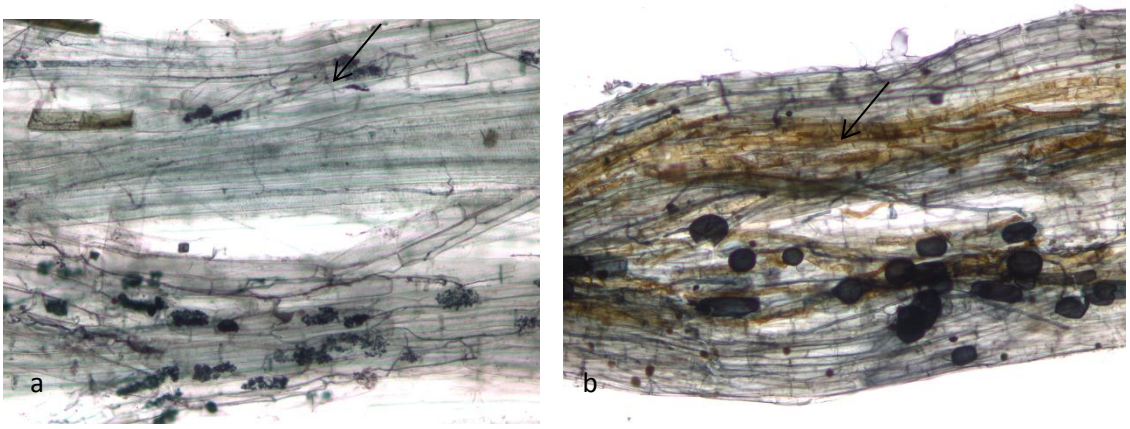

Figure 5. Corn roots from the produced inoculum stained with Chlorozol Black E showing well developed arbuscules (a) vesicles (b), and hyphae demonstrating the successful development of AM fungal colonization in the bioassay study.

On-farm AM Inoculum Production System and Utilization in Kuwait

Substrate-based production of AM fungal inoculum is the most commonly accepted techniques for AM fungal inoculum production, particularly in on-farm inoculum production because of it being a very simple method, the low maintenance required, and the low production cost. As a whole, on-farm production is considered as the most convenient system for large-scale production of indigenous AM fungi inoculum. On-farm produced approach of indigenous AM fungi inoculum potentially extends several advantages over commercially available inocula. The details about the advantages and disadvantages of on-farm inoculum were discussed earlier by several authors (IJdo, et al. 2011; Gaur, et al. 2000; Douds, et al. 2005, 2006). However, our current modified method of indigenous AM fungal inoculum production successfully produced inoculum using a mixture of desert sand and nursery potting media, and corn as a trap plant. The aim was to use desert sand and potting mix as a carrier substrate in which seedlings are commonly grown in nursery, as well as to adopt a low-cost simpler technique, which is easy to integrate into any nursery and farming system.

There are different kinds of IP of AM fungi present in the on-farm inoculum such as spores, fragments of colonized roots containing AM structures of arbuscules, vesicles, and viable hyphae. Our results demonstrated an average of 109.67 ± 17.32 spores produced per 50 g of inoculum mixture. Apparently, the number of spores produced in this inoculum production system seems lower in range. Spore production may vary depending on various reasons, particularly depending upon the species in the starter indigenous AM fungal community. However, results from 100 g of the produced on-farm inoculum, using bioassay, clearly showed (Table 2) the presence of abundant colonization structures in the roots of bioassay trap plants with 98-100% colonization rate. The result suggests that the infectious propagule numbers are likely to be greater in our current on-farm produced AM inoculum as it may contain more arbuscules, vesicles, and active hyphae than spore numbers per gram of inoculum. Spore production and root colonization rates may not be directly proportional in many instances (Hetrick and Bloom, 1986). Therefore, it is indicated that most of the propagules present in the inoculum may be in the form of colonized root fragments and infective extraradical hyphae than spores.

The method developed here is reasonable and adapted to Kuwait's desert conditions compared to other described methods; furthermore, this technique requires no fumigation or sterilization, does not involve pre-colonization steps, and is inexpensive. Nevertheless, the method of on-farm production and utilization of indigenous AM fungal inoculum is more realistic for several reasons. Published reports show that indigenous AM fungal community may be more effective for the growth and development of plants in their native soil than the introduced commercial inoculum (Oliveira, et al. 2005). Since the starter inoculum was collected from diverse areas and associated host plant in desert, the possibility is to have a taxonomically diverse inoculum already suitable for desert sites. However, the use of different host plant species in separate grow bags for diverse AM fungi propagation is suggested for further taxonomically diverse high-quality on-farm inocula (Douds, et al. 2010).

Conclusion

Commonly, plants can be inoculated either in the field at planting or seedlings inoculated during the production of nursery seedlings for later outplanting in the field. Our produced on-farm inoculum could be useful to seedling producers for revegetation purposes or greenhouse vegetable growers as this inoculum can be easily and economically produced and mixed into potting media. The on-farm production of indigenous AM fungi inoculum is probably a functional approach in Kuwait. The inoculum could be mixed into commonly practiced potting media in nurseries for the production of pre-colonized seedlings prior to their transplant to field sites. In Kuwait, it is the beginning of the utilization of mycorrhizal products in seedling production and is yet in the research stage. The continued development of high-quality, adapted to particular needs and inexpensive inoculum production system may be appealing to farmers and seedling growers in the near future. Further research is needed to optimize the maximum number of spore production in the on-farm production system.

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