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New Hosts for Large Scale Inoculum Production of Arbuscular Mycorrhizal Fungi from Saudi Soils

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ABSTRACT

Arbuscular mycorrhizal Fungi (AMF) are available in most of the soils. They can form symbiotic association with many terrestrial plants. This association plays an important role in plant growth, plant protection, stress tolerance, soil quality etc. Methods of commercial high quality inoculum production are discussed in present paper. Many different cultivation techniques are available for AM fungi. For large scale inoculum production, substrate-based including soil based production techniques and substrate-free culture techniques (hydroponics and aeroponics) are being practiced. But suitable indicator plants for inoculum production of AMF from Saudi soils are not available. In the present research program, we have developed suitable methods of large scale inoculum production techniques, from Saudi soils, with indigenous and exotic plant species under Saudi conditions. Our results indicated that either single plant or 2-3 plants in one pot was suitable for good infection. In our study, *Sesbania* sp. and *Cassia tora* were new hosts for inoculum production along with *Z. mays*, *Allium* sp. and *Sorghum bicolor*. To our knowledge not many researchers have used *Sesbania* sp. as host for inoculum production. Main advantage of *Sesbania* sp. plants as host or indicator plants for inoculum production was that this plant can produce nodulation for N fixation along with mycorrhizal inoculum production.
KEY WORDS: AM fungi, *Sesbania* sp., Saudi Soil, Inoculum Production

INTRODUCTION

In most of the soils of Saudi Arabia are calcareous, sandy in nature and low fertility level. It has low water holding capacities, high infiltration rates and high evaporation rates. Saudi has very low organic matter content. In many tropical soils including Saudi Arabia, Phosphorus availability is limited due to P fixation. These limitation makes the agriculture in Saudi Arabia little difficult. Arbuscular Mycorrhizal Fungi (AMF) may, partly, overcome this problem. Arbuscular mycorrhizal (AM) fungi play a significant role in increasing plant productivity under natural and semi-natural conditions [1]. Now a day, the AM fungi are widely used in agriculture, horticulture, and forestry systems. They are also used for environmental reclamation and to limited application of agrochemicals [2]. The AMF can improve plant growth by up taking P and to help to absorb N, K, Ca, S, Cu, and Zn [3]. They can improve water absorption [4, 5] during plant production systems. Inoculation with AMF can help in reducing the transplantation shocks and ultimately minimize the plant losses during field growth [6]. The mycorrhizal fungi play a regulatory role under high temperature [7, 8]. Mycorrhizal colonization also improved plant growth under salt stress conditions [9]. During AMF activities in plant growth it produce glomalin which act as soil binding agent [10]. AMF can decrease disease incidence [5] and nematodes causing disease [11]. Because of obligatory nature of AMF, it is not so easy to produce and use the AMF in plant production systems. Usually AMF inoculum were developed through pot-culture [12].

In a review [13], authors are mentioned many different types of hosts and methodology. The advantage and disadvantage of the hosts plants and methods are mentioned elaborately [14]. Out of the many different hosts, Leek (*Allium porrum* L.), Sudan grass (*Sorghum bicolor* L. Moench), corn (*Z. mays* L.), and bahia grass (*Paspalum notatum* Flüggé) are widely used plant species [15]. Menge [16] and Bagyaraj and Manjunath [17] reported that soil-based pot culture is a common method for production of AMF inoculum.

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MATERIAL AND METHODS

The proposed research program was performed under green-house conditions in collaboration with the Department of Soil Science, King Saud University, Saudi Arabia. Starter inoculum composed of sufficient number of AMF spores, fragments of mycelium and 80-90 percent infected root segments was used for inoculation. The inoculum was developed under green-house condition with *S. bicolor* as a trap crop (hosts).

Few exotic and indigenous plant species were used for the present study as trap crops or indicator plants species. They were *Cassia tora*, *Allium* sp., *Sesbania* sp., *Z. mays* and *S. bicolor*. The seeds before planting were surface sterilized with house hold bleach (containing 5% sodium hypochlorite) having 1:9 (bleach: water) ration for approximately 5-10 minutes. A mixture of 2:1:1 volume of Sandy soils, peat moss and perlite were used as base materials. The medium were sterilized under direct sunlight (40-50°C) for 3 days. The medium was placed in 1 kg pots keeping appx. 2 cm vacant to hold water properly. The infectivity was very high in the starter inoculum. Half of the rhizosphere soils and root segments were used for inoculation of one set of pots and other half was sterilized in the autoclave at 121°C at 15 PSI for 45 minutes three times and it was used as alternative of mycorrhizal inoculum in another set of pots to minimize or maintain the same level of nutrient available in the soil and root inoculum. The starter inoculum was thoroughly mixed at a ratio of 1:10 (inoculum: mixture). Five germinated seeds were placed in each pot at a depth of 1-2 cm. The inoculated pots were placed in a greenhouse. The pots were watered daily until water begins to drip from the bottom of the pot. The data were taken after 60 days of planting the seeds. The roots were stained and assessed by following the methods mentioned below. Preserved root segments were stained by following the methods of Phillips & Hayman [18]. The stained root segments were mounted in lacto glycerol solution on glass slides for observation of different AM structures (mycelium, vesicles, arbuscules, and hyphal coils). Percentage of mycorrhizal colonization, intensity of AM structural colonization (mycelium, vesicles and arbuscular colonization) were estimated in the stained roots for total infection.

Mycelial colonization was regarded as total root colonization. The percentage of AM structural colonization was calculated as the equation [19] below.

$$\%\ \text{Colonization} = \frac{\text{Total number of AM positive segments}}{\text{Total number of segments studied}} \times 100$$

Statistical analysis: The data were statistically analyzed using analysis of variance (ANOVA) for a completely randomized design using the program SPSS and the differences in means were determined by the least significant differences (LSD) (a=0.05) test.

DISCUSSION AND CONCLUSION

Propagation of inocula arbuscular mycorrhizal fungi (AMF) for large-scale inoculation is a major problem in popularizing the AMF for use in cultivation systems. Different types of host plants which are commonly used for large scale inoculum production of AM fungi are mentioned in a review [13]. Gaur and Adholeya [20], reported that host plants played a major role in inoculum production as was found in our study. Burrows and Pfleger [21] also demonstrated that the production of AMF spores size dependent on the hosts. The population of plants species and individuals plant species their age and health may significantly influence the inoculum production.

Several tests were performed under greenhouse conditions to screen or find out suitable plants for large scale inoculum productions in Saudi conditions. We use basic inoculum comprises of different species of *Glomus* grown with *Z. mays* plants for inoculation study. Plants were grown in single or in combination of different plants in same pot. It was observed from the table 1 that when single (*C. tora*, *Allium* sp., *Sesbania* sp., *Z. mays* and *S. bicolor* plants were grown, the infectivity differed independently and widely. Out of five plants, highest arbuscular infectivity was recorded with *S. bicolor* and which was followed by *Z. mays*, *Sesbania* sp. and *Allium sp.* and the lowest infection was recorded with *C. tora*. In case of infection with vesicles, highest infection was found with *Allium* sp. which was followed by *S. bicolor*, *Z. mays* and *Sesbania* sp. and lowest infection was found with *C. tora* again. In case of mycelial infection, the lowest infection was found again with *C. tora* and the highest infection was recorded with *S. bicolor*. The overall good infection was found with *S. bicolor*, *Allium* sp. and *Z. mays*, but lowest infection was recorded with *C. tora*. Although less infection with all the parameter was found with *C. tora* but it was recorded as a very good mycotrophic plants under Bangladesh conditions (M.A.U. Mridha, personal communication). When more than one selected plants were grown in single pot, the infectivity differed, but not too much. In case of four plants grown in single pot, the infectivity reduced significantly (Table 1), this may be attributed to density of the plants. Our results indicated that either single plant or 2-3 plants in one pot was suitable for good infection. In our study, *Sesbania* sp. and *C. tora* were new hosts for inoculum production along with *Z. mays*, *Allium* sp. and *S. bicolor*. To our knowledge not many researchers have used *Sesbania* sp. as host for inoculum production. Main advantage of *Sesbania* sp. plants as host or indicator plants for inoculum production was that this plant can produce nodulation for N fixation along with mycorrhizal inoculum production.

Table 1: Intensity of infection of arbuscular mycorrhizal fungi in different hosts.

Culture combination	Plant	Arbuscular %		Vesicles%		Mycelium %	
		Mean	SD	Mean	SD	Mean	SD
Single culture	*C. tora*	26.3	5.5	42.3	2.5	53.7	3.2
	Allium sp.	63.0	3.6	86.3	5.5	76.3	5.5
	Sesbania sp.	63.0	2.6	53.0	3.0	47.3	2.5
	Z. mays	68.7	1.5	78.0	2.0	50.7	6.1
	S. bicolor	78.7	5.7	81.3	3.1	79.3	4.7
	P-value	<0.0005		<0.0005		<0.0005	
***S. bicolor* + *Sesbania* sp.**	*Sesbania* sp.	81.0	3.6	92.0	4.4	91.3	3.2
	S. bicolor	76.3	6.5	80.0	5.0	80.3	4.5
	P-value	0.880		0.714		0.520	
Allium* sp. + *Z. mays	*Allium* sp.	67.3	2.5	78.0	7.5	90.3	5.5
	Z. mays	83.0	3.6	92.3	4.9	76.3	5.5
	P-value	0.004		0.051		0.036	
Sesbania* sp. + *Z. mays	*Sesbania* sp.	70.7	4.0	85.7	6.7	94.0	4.6
	Z. mays	28.0	2.6	67.3	2.5	50.7	5.0
	P-value	<0.0005		0.0		<0.0005	
Allium* sp. + *Sesbania* sp. + *Z. mays	*Allium* sp.	94.7	4.2	92.3	2.5	69.3	4.0
	Sesbania sp.	52.7	3.1	42.3	2.5	27.0	3.6
	Z. mays	74.3	3.8	86.3	5.5	32.0	2.0
	P-value	<0.0005		<0.0005		<0.0005	
Allium* sp. + *Sesbania* sp. + *Z. mays* + *S. bicolor	*Allium* sp.	42.7	2.5	52.7	2.5	64.0	3.6
	Sesbania sp.	53.3	3.1	51.0	4.6	37.3	3.1
	Z. mays	35.0	4.4	33.3	4.2	32.3	10.8
	S. bicolor	33.7	3.2	42.7	2.5	38.7	4.2
	P-value	<0.0005		0.001		0.001	

The results obtained in the present investigations are expected, because most of the plants are mycotrophic. Under Saudi conditions, these plants may be used for inoculum production of arbuscular mycorrhizal fungi. Some of the selected plants (*Allium* sp.) although highly mycotrophic but their growth was slow under cold condition and infectivity was also low. Most of the selected plants are highly mycotrophic under Saudi conditions with basic inoculum from Saudi soils. The inoculum production technology may be used here in Saudi Arabia and may be utilized for growth improvement of Saudi plants under green house and field conditions.

The outcome of the present research program will be highly useful for introduction of AMF in Saudi agricultural system for inoculation to improve the growth of the plantation as well as other type of plants. In most of the cases the Saudi soils are devoid of AM fungi and or diminish because of high temperature and drought. In this situation the AMF inoculum will be very useful for reintroductions of these beneficial fungi. The soil based AMF inoculum which will be developed under the proposed program will be utilized in the nursery as well as plantations crops in the field. The inoculum either will be incorporated with the soil in the nursery or placed in the planting hole to have greater contact with the root systems of the plantation crops.

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