Improved aeroponic culture technique for production of inocula of arbuscular mycorrhizal fungi

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Abstract We compared the conventional atomizing disc aeroponic technology with the latest ultrasonic nebulizer technology for the production of *Glomus intraradices* inocula. The piezo ceramic element technology used in the ultrasonic nebulizer employed high frequency sound that blasted the nutrient solution and nibulized it into micro-droplets size of 1μm in diameter. Growth of the pre-colonized AMF-roots of Sudan grass was successfully achieved in both the chambers used in this study but root growth and mycorrhization was statistically faster and higher in the ultrasonic nebulizer aeroponic system than in the atomizing disc aeroponic system. The shearing of the AMF infected roots from both the systems did not result in the loss of inoculum viability as evident from the MPN data. However, MPN assay showed that sheared roots produced using the ultra-sonic nebulizer system had a significantly higher number of infective propagules than those produced by the atomizing disc system. Our findings indicate that the latest ultra-sonic nebulizer aeroponic technology is superior, and can be an alternative to conventional atomizing disc or spray nozzle systems for a high quality concentration of AMF isolates which can be used in small doses to produce large response, a pre-requisite for commercialization of AMF technology.

Key words arbuscular mycorrhiza; aeroponic culture; ultrasonic nebulizer; inoculum; MPN

Introduction

Mycorrhizal fungi, especially those that are arbuscular (AM), are ubiquitous soil inhabitants forming symbioses with most naturally growing terrestrial (Jeffries 1987) and most aquatic (Khan and Belik 1995) plants. Their potential to enhance plant growth is well documented and recognized but not fully exploited. They are rarely found in nurseries due to the use of composted soil-less mixes, high levels of fertilizer and regular application of fungicidal drenches. The potential advantages of inoculation of nursery plants with AM fungi (AMF) in horticulture, agriculture, and forestry is not perceived by these industries as a significant issue. This is partially due to inadequate methods for large-scale inoculum production. Thus, the exploitation of AM fungi still presents a great challenge. Pot culture in pasteurized soils has been the most widely used method for producing AMF inocula but it is time consuming, bulky, and often not pathogen free. To overcome these problems, various soil-free methods, namely soil-less growth media (for references see Sylvia and Jarstfer 1994), aeroponic system (Hung *et al*. 1991; Jarsfter and Sylvia 1995), hydroponic (Elmes and Mosse 1984; Mosse and Thompson 1984), and axenic culture of AM fungi with transformed or non transformed living roots of various hosts (Chabot *et al*. 1992; Diop *et al*. 1994) have been used successfully to produce AMF-colonized root inocula. Colonized roots produced without substrate can be sheared

(Sylvia and Jarstfer 1992). Jarstfer and Sylvia (1995) tested three types of aeroponic systems and chambers, i.e. an atomizing disc; pressurized spray through micro-irrigation nozzle; or ultrasonically generated fog of nutrient solution with a droplet size 3 to 10 μm in diameter, and concluded that the pump and spray nozzle systems were the most versatile and reliable for aeroponic production of AM fungi. The authors found that the ultrasonic fog system producing relatively large size (>3 μm in diameter) droplets failed to provide enough free moisture on the surface of the roots resulting in poor root growth and failure of AM fungal infection to spread and less spore production.

Our objective was to conduct a comparative assessment (based on MPN bioassay and percentage colonization of roots) of the atomizing disc and the latest ultrasonic nebulizer technology for the production of sheared roots of AMF-colonized Sudan grass as inocula. The conventional spray nozzle and ultrasonic fog to produce fine mist of nutrient solution results in rapid loss of nutrient solution through evaporation and diminishing the possibility of rapid absorption of nutrients by the aeroponically cultured roots (Carruthers 1992). Another disadvantage of spraying roots with a nutrient solution with larger droplets is stifled root growth (Carruthers 1992). These problems limit rapid growth and AMF colonization of roots in the system. To overcome these limitations we used a piezo ceramic elements technology which employs high frequency sound that blasts the nutrient solution and nebulizes it into micro-droplets size of 1μm in diameter (Carruthers 1992).

Materials and methods

Three parts of coarse sand and one part vermiculite, pasteurized twice (1 h steaming separated by a 24 h cooling period), was used as growth medium for the initiation of AM infection in Sudan grass (*Sorghum sudanese* Staph.)seedlings. The pH of the growth medium ranged between 5.9 - 6.7. Approximately 1.2 kg of the growth medium was placed in 14 x 13 cm polyethylene lined, free-draining plastic pots . The pots were watered with distilled water and stored at room temperature before use. Twenty pre-germinated Sudan grass seedlings, raised from surface sterilized seeds, were planted in each of the pots. Before inserting the seed into the medium, 10 g of a sand culture of *Glomus intraradices* Schenk & Smith (INVAM isolate WV 994A-1) was layered 1cm below the surface as AM fungal inoculum for the roots. The pots were placed in a shaded greenhouse (temperature range 25 – 29 C^0; 16 h photoperiod; photosynthetic photon flux density of 500 μ mol m^{-2} s^{-1} using a 500 watt metal-halide lamp (Osram HQ1-T 250 W/D) 1.5 m above the pots. The plants were initially watered and fertilized with approximately 100 ml of the low- P (0.408 mg l^{-1} of KH_2PO_4) Hoagland nutrient solution (Sylvia and Hubbell 1986) every 2 days. After 4 wk of growth in pots, the roots from each pot were examined for AM fungal infection with an inverted microscope using fluorescence (Ames *et al.* 1982). Healthy and infected 4 wk old Sudan grass seedlings were secured in holes of the lids of the ultrasonic nebuliser aeroponic nutrient chamber (Carruthers 1992) as well as the atomizer disc aeroponic chamber. Schematics diagrams of the two systems can be found elsewhere (see Carruthers 1992). Both chambers contained modified Hoagland nutrient solution (Sylvia and Hubbell 1986). The chambers were maintained in the same green house under the conditions as above The solution in the ultra-sonic nebuliser system was supplied as a fog-like mist whereas, in the atomiser disk chamber, it is sprayed onto the plant roots. The fast growing roots in both the chambers were trimmed above the solution at 4, 8, and 12 weeks, assessed for AM fungal colonization by the auto fluorescence method (Ames *et al.* 1982). The harvested root samples were mixed with de-ionized water in a 1:10 ratio (w/v) and sheared with a blender (Black and Decker Shortcut) for about 80 seconds to produce a slurry of the AM infected roots as per procedure by Jarsfter and Sylvia (1992). The slurry containing root fragments, inter-radical vesicles, mycelia and spores, were passed through a 450 μm sieve and air dried at room

temperature for 72 h. The number of viable AM fungal propagules per 100 g of the sheared root inoculum so produced were determined by the most probable number (MPN) technique (Porter 1979, as modified by Woomer 1994), using pre-germinated Sudan grass seedlings as the test plant. Ten-fold dilutions of the sheared root inoculum was prepared by mixing one g dried sheared-root inoculum with 500 g of pasteurized sand to make the first dilution. A series of 10-fold dilutions were then prepared using pasteurized sand as diluent. The diluted samples (approximately 70 g per tube) were then transferred into 50 ml modified corning modified centrifuge tubes (Aldrich Chemical Company Inc.) with a narrow opening at the base for drainage. Two pre-germinated Sudan grass seedlings were then planted to each of the vials. Five replicates per dilution were randomized in trays in the glass house with growth conditions as above. The seedlings were maintained at field capacity by frequent watering to a constant weight. After 6 weeks the seedlings were harvested, their roots were stained with aniline blue and assessed for AM fungal infection. The MPN of AM fungal propagules for each inoculum were calculated by using the table provided by Cochran (1950). The experiment was repeated 4 times and the data was subjected to one way ANOVAR.

Results and discussion

Growth of the pre-colonized AMF-roots of Sudan grass was successfully achieved in both the aeroponic chambers used in this study. This supports similar observations by Sylvia and his co-workers (Sylvia and Jarsfter 1992). However, in the present study, root growth and mycorrhization was much faster and higher in the ultrasonic nebulizer aeroponic system than in the atomizing disc aeroponic system (Table 1). Within 8 to 12 weeks of growth in the ultra-sonic nebulizer system each seedling produced numerous adventitious roots, microscopic examination of which revealed 80% of root segments to contain numerous inter-radical vesicles, hyphae and terminal spores on the extramatrical mycelium (Table 1). On the other hand, the percentage of AM fungal infection in the roots produced by the atomising disc system ranged from 61 to 65%. Extramatrical hyphal growth around root surfaces was comparatively denser in the ultrasonic chamber than that observed with the atomising disc system. No attempt was made to quantify the extramatrical hyphae. The percentage colonization of the roots growing in the ultra-sonic chamber was significantly ($p < 0.5$) greater than that in the disk system used (Table 1). Root sub sampling every 4 weeks, however, showed an upward trend in mycorrhization of the roots in both the systems employed to produce AM fungal inoculum.

The shearing of the AMF infected roots did not result in the loss of inoculum viability as evident from the MPN data. MPN assay also showed that sheared roots of Sudan grass produced using the ultra sonic nebulizer system had a significantly higher number of infective propagules than those produced by the atomising disc system.

Some of the data differ with a previous study where root growth and mycorrhization using an ultrasonic chamber was inferior to that achieved with spray nozzle or atomising disc systems (Jarsfter and Sylvia 1995). The authors reported that the ultrasonic system failed to provide enough free moisture on the root surface. This failure may have been due to excessive evaporation of nutrient solution caused by the fan used to distribute the nutrient micro-droplets inside the chamber.

Our findings indicate that the ultra sonic nebulizer aeroponic technology can be an alternative to conventional atomising disc or spray nozzle systems for production of AM fungi in aeroponic culture. Our results support Carruthers (1992) who reported that plant growth using the ultra-sonic nebulizer system was far superior to those grown using conventional spray nozzle or atomising disc systems. The author also reported that the growth of marigold seedlings transplanted into the ultra-sonic nebulizer system was much faster than those growing in the conventional systems. The superiority of nebulizer systems is most likely related to the

production of finer mists. These allow improved humidity inside the chamber which, in turn, helps the rapid absorption of nutrients from the cloud by the roots and improves aeration of both the plant and the fungus (Carruthers 1992). It has been reported that improved aeration enhances mycorrhization of roots (Saif 1981; Bagyaraj 1991). With the atomising disk and spray nozzle system used here, we experienced the blocking of the siphon tube and entanglement and destruction of fine roots by the drive shaft and/or impeller. With the nebulizer, mechanical breakdowns are virtually eliminated as it is based on solid state technology, power consumption is low, and installation costs are low because irrigation delivery lines and nozzles are no longer required to deliver nutrients to the plant root (Carruthers 1992).

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Table 1: Percentage AMF infection in the Sudan grass roots produced by the atomising disc and ultra-sonic aeroponic culture systems and their inoculum potentials (MPN values).

Method used	Weeks after planting	% root length infected* (each value is the mean of 4 replications)	Number of viable AM propagules g^{-1} inoculum (MPN values)
Ultra-sonic nebulizer system	4	52.4^{a}	
	8	61.8^{b}	
	12	80.0^{c}	$175{,}000^{a}$
Atomising disc system	4	30.6^{a}	
	8	50.7^{b}	
	12	65.0^{c}	$140{,}000^{b}$

*Values suffixed by different alphabets are statistically different at $P<0.05$.