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SHORT NOTE

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Improved aeroponic culture of inocula of arbuscular mycorrhizal fungi

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Abstract We compared conventional atomizing disc aeroponic technology with the latest ultrasonic nebulizer technology for production of *Glomus intraradices* inocula. The piezo ceramic element technology used in the ultrasonic nebulizer employs high-frequency sound to nebulize nutrient solution into microdroplets 1 μm in diameter. Growth of pre-colonized arbuscular mycorrhizal (AM) roots of Sudan grass was achieved in both chambers used but both root growth and mycorrhization were significantly faster and more extensive in the ultrasonic nebulizer system than in the atomizing disc system. Shearing of the AM fungi (AMF) infected roots in both the systems did not reduce inoculum viability, as evident from the MPN data. However, sheared roots from the ultrasonic nebulizer system had significantly more infective propagules than those produced in the atomizing disc system. Thus, the latest ultra-sonic nebulizer aeroponic technology appears to be superior and an alternative to conventional atomizing disc or spray nozzle systems for the production of high-quality AMF inocula. These can be used in small doses to produce a large response, which is a prerequisite for commercialization of AMF technology.

Key words Arbuscular mycorrhiza · Aeroponic culture · Ultrasonic nebulizer · Inoculum · MPN

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Introduction

Mycorrhizal fungi, especially those that are arbuscular (AM), are ubiquitous soil inhabitants forming symbioses with most naturally growing terrestrial (Jeffries 1987) and 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 the inoculation of nursery plants with AM fungi (AMF) in horticulture, agriculture, and forestry are not perceived by these industries as significant. This is partially due to inadequate methods for large-scale inoculum production. 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, soil-free methods such as soil-less growth media (for references see Sylvia and Jarstfer 1994) and the aeroponic (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: an atomizing disc, pressurized spray through a micro-irrigation nozzle and an ultrasonically generated fog of nutrient solution with droplets 3–10 μm diameter. They concluded that the pump and spray nozzle systems were the most versatile and reliable for aeroponic production of AM fungi. The ultrasonic fog system which produces relatively large droplets provided insufficient free moisture on the surface of the roots. This resulted in poor root growth, failure of AM fungal spread and lower spore production.

Our objective was to compare by MPN bioassay and percentage colonization of roots, the atomizing disc and the latest ultrasonic nebulizer technology for the production of sheared roots of AMF-colonized Sudan grass. Conventional spray nozzles and ultrasonic fogging used to produce a fine mist of nutrient solution suffer from rapid loss of the solution through evaporation and a consequent reduction in the absorption of nutrients by aeroponically cultured roots (Carruthers 1992). Another consequence of spraying roots with a nutrient solution in large droplets is stifled root growth (Carruthers 1992). To overcome these factors limiting growth and AMF colonization of roots, we used a piezo ceramic elements technology employing high-frequency sound to nebulize the nutrient solution into micro droplets 1 μm 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 a growth medium for the initiation of AM infection in Sudan grass (*Sorghum sudanese* Staph.) seedlings. The pH of the growth medium was 5.9–6.7. Approximately 1.2 kg of the growth medium was placed in 14 × 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 cm below the surface as an AM fungal inoculum. The pots were placed in a shaded greenhouse (25–29 °C; 16-h photoperiod; photosynthetic photon flux density of 500 μ mol m^{-2} s^{-1} using a 500-W 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 KH_2PO_4) Hoagland nutrient solution (Sylvia and Hubbell 1986) every 2 days. After 4 weeks growth in pots, the roots from each pot were examined for AM fungal infection with an inverted microscope using fluorescence illumination (Ames et al. 1982). Healthy and infected 4-week-old seedlings were secured in holes in the lids of an ultrasonic nebulizer aeroponic nutrient chamber and an atomizer disc aeroponic chamber. Diagrams of the two systems can be found elsewhere (Carruthers 1992). The two chambers, containing modified Hoagland nutrient solution (Sylvia and Hubbell 1986), were maintained in the greenhouse under the conditions described. In the ultrasonic nebulizer system, the solution was supplied as a mist whereas, in the atomizer disk chamber, it was sprayed onto the plant roots. The fast-growing roots in both chambers were trimmed above the solution at 4, 8, and 12 weeks and assessed for AM fungal colonization by the autofluorescence method (Ames et al. 1982). The harvested root samples were mixed 1 : 10 ratio (w/v) with de-ionized water and sheared with a blender (Black and Decker Shortcut) for about 80 s to produce a slurry of AM infected roots (Jarsfter and Sylvia 1992). The slurry, containing root fragments, inter-radical vesicles, mycelia and spores, was passed through a 450-μm sieve and air dried at room temperature for 72 h. The number of viable AM fungal propagules per g of inoculum was 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. Tenfold dilutions of the sheared root inoculum with pasteurized sand were prepared after first mixing 1 g of dried sheared-root inoculum with 500 g of pasteurized sand to make the first dilution. The diluted samples (approximately 70 g per tube) were then transferred into 50-ml Corning centrifuge tubes (Aldrich Chemical Company Inc.) modified with a narrow opening at the base for drainage. Two pre-germinated Sudan grass seedlings were then planted in each of the vials. Five replicates per dilution were randomized in trays in the greenhouse 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 and their roots stained with aniline blue for assessment of AM fungal infection. The MPN of AM fungal propagules for each inoculum was calculated using the table provided by Cochran (1950). The experiment was repeated four times and the data subjected to one-way ANOVA.

Results and discussion

Pre-colonized AMF-roots of Sudan grass grew in both aeroponic chambers used in this study, as also reported by Sylvia and Jarsfter (1992). However, in the present study, root growth and mycorrhization were much faster and more extensive in the ultrasonic nebulizer than in the atomizing disc aeroponic system (Table 1). After 8–12 weeks of growth in the ultrasonic nebulizer system, each seedling produced numerous adventitious roots. Microscopic examination revealed that 80% of root segments contained numerous interradical vesicles, hyphae and terminal spores on the extramatrical mycelium (Table 1). On the other hand, AM fungal infection in the roots produced by the atomizing disc system ranged from 61 to 65%. Extramatrical hyphal growth around root surfaces was denser in the ultrasonic chamber than in the atomizing disc system. No attempt was made to quantify the extramatrical hyphae. The percentage colonization of roots was significantly ($P < 0.05$) greater in the ultrasonic chamber than in the disk system (Table 1). Root subsampling every 4 weeks, however, showed a trend towards increased mycorrhization in both systems.

Shearing of AMF-infected roots did not reduce inoculum viability, as evident from the MPN data. The

Table 1 Percentage colonization of Sudan grass roots by arbuscular mycorrhizal fungi in atomizing disc and ultrasonic aeroponic culture systems and their inoculum potentials (MPN). Values marked by different letters are significantly different at $P < 0.05$

Culture system	Weeks after planting	% root length colonized ($n = 4$)	Number of viable AM propagules g^{-1} inoculum (MPN)
Ultrasonic nebulizer	4	52.4[a]	
	8	61.8[b]	
	12	80.0[c]	175000[a]
Atomizing disc	4	30.6[a]	
	8	50.7[b]	
	12	65.0[c]	140000[b]

MPN assay also showed that sheared roots of Sudan grass produced in the ultrasonic nebulizer system had a significantly higher number of infective propagules than those produced in the atomizing disc system (Table 1).

To some extent, the data here differ from a previous study where root growth and mycorrhization in an ultrasonic chamber was inferior to that achieved with spray nozzle or atomizing disc systems (Jarsfter and Sylvia 1995). The authors reported that the ultrasonic system provided insufficient free moisture on the root surface. This may have been due to excessive evaporation of nutrient solution caused by the fan distributing the nutrient microdroplets inside the chamber. Furthermore, host plant, light intensity and temperature differences may influence system efficiency.

Our findings indicate that ultrasonic nebulizer technology is an alternative to conventional atomizing disc or spray nozzle systems for production of AM fungi in aeroponic culture. Our results support those of Carruthers (1992), who reported that plant growth was far superior using the ultrasonic nebulizer system than conventional spray nozzle or atomizing disc systems. This author also reported that the growth of marigold seedlings was much faster in the ultrasonic nebulizer system than in the conventional systems. The superiority of nebulizer systems is most likely related to the production of a finer mist. This improves humidity inside the chamber, which in turn promotes 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 atomizing disk and spray nozzle system used here, we experienced 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. Furthermore, power consumption is lower, and installation is cheaper because irrigation delivery lines and nozzles are no longer required to deliver nutrients to the plant root (Carruthers 1992).

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