Development of a Nutrient Film Technique Culture System for Arbuscular Mycorrhizal Plants

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Abstract. A nutrient film technique (NFT) culture system was developed to allow nursery production of arbuscular mycorrhizal horticultural crops. This would benefit horticultural production and allow for uncomplicated production of mycorrhizal hyphae. Roots of lettuce (Lactuca sativa var. capitata) plants were highly colonized by the arbuscular mycorrhizal fungus, Glomus mosseae (BEG 107) after 4 weeks in the NFT system, following an initial phase of five weeks in inoculated in Perlite substrate. In the NFT system, a thin layer of glass beads was used to provide solid support for plant and fungus growth and nutrient solution was supplied intermittently (15 min, six times per day). A modified nutrient solution (80 µM P) was used and was replaced with fresh solution every 3 days. A significantly higher dry weight was found for the mycorrhizal versus the nonmycorrhizal lettuce plants in Perlite during the precolonization period. The root colonization rate was also high at rates up to 80 µM P supply. On the NFT system, growth differences between mycorrhizal and nonmycorrhizal plants were less than in Perlite. However, root colonization rate was not reduced during the NFT culture period. In this system, high amounts of fungal biomass were produced. This would allow the determination of metal and other nutrient concentrations in fungal hyphae. Furthermore, we found large amounts of external fungal hyphae surrounding the root surface. As much as 130 mg fungal biomass were collected per culture plate (three plants). Therefore, we suggest that this modified NFT culture system would be suitable for fungal biomass production on a large scale with an additional aeration by intermittent nutrient supply, optimum P supply, and a use of glass beads as support materials. Furthermore, bulk inoculum composition with a mixture of spores, colonized roots, and hyphae grown in soilless media by the modified NFT system might be a useful way to mass-produce mycorrhizal crops and inoculum for commercial horticultural purposes.

Materials and Methods

Two experiments were conducted. The first experiment was a trial to determine in Perlite substrate the optimum P concentration of the nutrient solution to avoid root growth while maintaining an adequate root colonization rate. The second experiment tested whether the modified NFT could be used for the commercial production of mycorrhizal crop plants under greenhouse conditions.

Materials and Methods

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hyphae in supernatant were collected using sieve (30 µm pore size). After rinsing the fungal hyphae on the sieve carefully with double distilled water, fungal hyphae were collected into Eppendorf tube and dried at 50 °C in an oven for 2 d to determine dry weights. Shoot and roots were harvested for dry weight determination after sampling the fresh root (0.5 g fresh weight per plant) for the determination of mycorrhizal colonization. Shoot and roots sampled were dried at 70 °C in an oven for three days and thereafter determined dry weights. The experiments were carried out in a growth chamber with a PAR of 400 to 500 µmol·m–2·s–1 (mercury halide lamps, Osram Powerstar HQI-T-2000 W/D), a day/night temperature of 25/20 °C and a relative humidity of about 60%.

Nutrient media. The plants in Perlite culture were supplied with 1/10 strength nutrient solution for the first 4 d in all experiments and thereafter with a full strength of nutrient solution for three days and thereafter determined dry weights. The nutrient solutions was circulated and pH of 6.0 was maintained by 0.15 mM MES-H2O (0.0752), K2HPO4 2H2O (0.0048), K2SO4 (0.7), KCl (0.1), MgSO4 7H2O (0.5) and micronutrients (µM): H3BO3 (10), MnSO4 H2O (3), ZnSO4 7H2O (0.5), CuSO4 5H2O (0.2), (NH4)2MoO4 24H2O (0.01), FeEDTA (10). A pH of 6.0 was maintained by 0.15 mM MES-KOH. The nutrient solutions was circulated and replaced every 3 d. The nutrient solution was supplied by water pump, which connected to a time switch so as to supply nutrient solution for 15 min once every 4 h, i.e., six times per day, so that each plate received 2 L per day in total.

Mycorrhizal root colonization. Percentage of root length colonized by mycorrhizal fungi was determined on roots stained in trypan blue (Koske and Gemma, 1989) using the gridline-intersect method (Giovannetti and Mosse, 1980).

Plant P analysis. The P concentrations of the dried, pulverized shoot and roots material were determined using the molybdo-P-blue method (Murphy and Riley, 1962).

Statistics. Four replications per treatment were used in both experiments. Pots and NFT plates were placed randomly in the growth chamber and the position of the plates was varied each time after replacing the nutrient solution. The Student’s t test and one-way analysis of variance were applied to determine differences due to P concentration in the first experiment and mycorrhizal colonization in the second experiment.

Results

Roots colonization of mycorrhizal plants in Perlite culture was colonized to approximately 60% when 10 µM P was supplied (Table 1). When more P was supplied, root colonization rates further increased. However, there was no significant difference in root colonization rate between 40 and 80 µM P supply (Table 1).

After 5 weeks of colonization in Perlite, the shoot fresh weight of lettuce had increased as P supply increased in the nutrient solution. However, there was no significant difference between 40 and 80 µM P supply (Table 1).

After colonization in Perlite for 5 weeks in the second experiment, lettuce was transplanted into NFT culture plates. After four weeks in NFT culture, the total root colonization percent-

| P supply (µM) | 10      | 40      | 80      |
|--------------|---------|---------|---------|
| Colonization rate (%) | 58.7 ± 2.1 a | 86.3 ± 4.2 b | 77.5 ± 3.9 b |
| Shoot fresh weight (g) | 13.94 ± 0.61 a | 19.00 ± 2.12 b | 23.95 ± 1.80 b |

The results indicate that it would be possible to produce arbuscular mycorrhizal plants with root colonization in a NFT culture system. Therefore, NFT would be one way to mass-produce inoculum and commercially produce mycorrhizal horticultural crop under greenhouse conditions. Growth or Puptake of mycorrhizal plants on NFT culture system was not clearly superior to that of nonmycorrhizal plants (Tables 2 and 3). This result was expected because nutrients are delivered to the root surface by the solution flow in NFT culture, so that the additional absorbing surface of the extraradical hyphae is of no benefit to the plant (George, 2000). Nevertheless, the NFT system can be used to raise mycorrhizal plants that may be superior in outplanting success, nutritional composition, or selling price.

In the modified NFT system, the AM fungus remained high (about 85.3%) compared to the initial percentage root colonized in Perlite. The fungal biomass per culture plate (three plants) was as much as 130 mg of dry weight. Furthermore, extraradical hyphae of AM were well developed in this system. After 4 weeks in this system, we found large amounts of external hyphae surrounding the root (Fig. 1). Better developed root mats were formed in mycorrhizal plants compared to nonmycorrhizal plants due to aggregation with fungal hyphae (Fig. 1). Noninoculated plants showed no colonization in this system.

The shoot dry weights of mycorrhizal and nonmycorrhizal plants grown in the NFT system were significantly different 4 weeks after transplanting into the NFT system (Table 2). Dry weight of mycorrhizal lettuce shoot and root were higher than those of nonmycorrhizal lettuce.

The P concentrations in roots were also higher in mycorrhizal plants compared to nonmycorrhizal plants (Table 3). However, no significant difference in shoot P concentration between mycorrhizal and nonmycorrhizal plants was observed.

Table 1. Total root colonization and shoot fresh weights of mycorrhizal lettuce plant after 5 weeks of precolonization in Perlite. Different letters indicate statistical difference due to P supply (P < 0.05, one-way ANOVA). Data are means of four replications ± SE.

| P supply (µM) | 10      | 40      | 80      |
|--------------|---------|---------|---------|
| Colonization rate (%) | 58.7 ± 2.1 a | 86.3 ± 4.2 b | 77.5 ± 3.9 b |
| Shoot fresh weight (g) | 13.94 ± 0.61 a | 19.00 ± 2.12 b | 23.95 ± 1.80 b |

Table 2. Dry weights of nonmycorrhizal (NAM) and mycorrhizal (AM) lettuce plants grown in the NFT system after 5 weeks of precolonization in Perlite. Different letters indicate statistical difference due to P concentration within one treatment (P < 0.05, Student’s t test). Data are means of four plates with three plants per plate ± SE.

| Treatment | NAM | AM |
|-----------|-----|----|
| Shoot     | 3.09 ± 0.49a | 10.79 ± 0.15b |
| Root      | 1.84 ± 0.16a | 3.16 ± 0.27b |

Table 3. P concentrations of nonmycorrhizal (NAM) and mycorrhizal (AM) lettuce plants grown in the NFT system after 5 weeks of precolonization in Perlite. Different letters indicate statistical difference between NAM and AM within one treatment (P < 0.05, Student’s t test). Data are means of four plates with three plants per plate ± SE.

| Treatment | NAM | AM |
|-----------|-----|----|
| Shoot     | 1.23 ± 0.13a | 1.16 ± 0.05a |
| Root      | 1.48 ± 0.11a | 2.22 ± 0.15b |
Using a thin layer glass beads as substrate in this system might be helpful to support plant and fungal growth and additionally to avoid complete drying of the root during the nonwatering period. However, it was somewhat difficult to collect most of the external hyphae from the root and glass beads because the mycorrhizal root had developed well and aggregated tightly to the glass beads together with the external hyphae. So it may be preferable in future experiments to use a membrane bag (30 µm in diameter) to separate root and hyphae for the better collection of hyphae.

A NFT culture system may have some advantages in comparison to other culture system. In aeroponics, wilting of plants is a possibility when the water supply is briefly interrupted by some technical problem. In hydroponics, fungal growth may be inhibited by limited aeration. Therefore, it is suggested that NFT is a better production system compared to aeroponics and hydroponics due to not only for inoculum and mycorrhizal crop production but also for investigating activity of external and elemental composition of fungal hyphae to nutrient uptake.

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