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Growth of *Glomus intraradices* and its effect on linseed (*Linum usitatissimum* L.) in hydroponic culture

Abstract This paper presents a hydroponic system for culturing and maintaining the VAM fungus *Glomus intraradices* in symbiosis with linseed (*Linum usitatissimum* L.) under greenhouse conditions in pure nutrient solution. It was possible to obtain large quantities of mycorrhizal host plant roots as well as extramatrical mycelium and chlamydospores free of impeding residues of solid substrate components. Starting from linseed donor plants inoculated in sand and transferred to the nutrient solution, new infections arose within the fast growing root system, hyphae spread out into the liquid and infected mycorrhiza-free receptor plants. Data for infection rates and plant growth parameters are presented. In comparsion to other culture systems for VAM fungi, the advantages of this hydroponic system are discussed and potential uses suggested.

Key words VA mycorrhiza · *Glomus intraradices* *Linum usitatissimum* · Hydroponic culture Extramatrical hyphae

Introduction

Glomus spp. develop an obligate, biotrophic VA mycorrhiza with a large number of plant families from which host plants benefit in several respects: improved acquisition of nutrients and water enables plant growth under unfavourable conditions; effects on the phytohormone balance can stimulate flowering behaviour and fruit development; there is generally increased resistance towards soil-borne pathogens. Such pronounced effects have recently been reviewed by Schönbeck et al. (1994).

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In other reports, the interest lay in biochemical and molecular aspects of VA mycorrhiza that govern the compatibility between the symbiont and its host plant (Balestrini et al. 1992; Palma et al. 1993). To study these interactions, it is desirable to have culture systems for the production of VA mycorrhiza with an adequate amount of extramatrical mycelium and chlamydospores free of solid substrate components.

Axenic culture of VA mycorrhiza has been reported (Janardhanan et al. 1990). However, no artificial medium has yet been developed that permits the culture and maintenance of these obligate symbionts with sufficient yield of mycelium and spores without loss of viability and infectivity. Since spore germination and mycelial growth of VAM fungi are apparently stimulated by the presence of host root factors (Lei et al. 1991), soil-less methods for host plant culture seem to be best for culturing VA mycorrhiza for certain objectives. Mosse and Thompson (1984) have reported such a culture method with *Phaseolus vulgaris* in nutrient flow culture, which unfortunately is difficult to manage. The Beltsville method for soil-less VA mycorrhiza production (Millner and Kitt 1992) is based on culture in sand, demanding separation procedures during the harvest of fungal structures, which may disturb in particular the harvesting of mycelium (Vilariño et al. 1993).

Although Peuss (1958) reported vigorous growth of extramatrical hyphae of *Glomus mosseae* on tobacco plants in Knop's nutrient solution, her observations have been used neither in the development of a production system for VAM fungi nor as a subject of mycorrhizal research. Furthermore, there is little information about the infection, spreading and establishment of these fungi in pure nutrient solution. This present paper describes a hydroponic culture system in pure nutrient solution adapted for two isolates of *Glomus intraradices* on linseed plants, which permits production of large quantities of extramatrical fungal structures free of soil residues.

Materials and methods

Rearing of host plants in sand

Linseed (*Linum usitatissimum* cv. Atalante) was grown in plastic pots (10 plants/pot) in pure sand under greenhouse conditions (24/20° C, 75% relative humidity and 16-h daylength) with supplementary light of 15 W/m^2. The plants were irrigated sufficiently and fertilized weekly with a 0.3% complete nutrient solution (Wuxal Normal, Schering, Germany) containing N:P:K as 12:4:6, 50 ml/pot.

Inoculum used

Two isolates of *G. intraradices* from the collection of the Institut für Pflanzenkrankheiten und Pflanzenschutz der Universität Hannover (isolate 49 and 510, originating from Skandinavia and Tunesia, respectively) were used as VA mycorrhiza inocula. These were produced on carrier material (expanded clay, 2–4 mm in diameter, Leca) on *Tagetes erecta* L. as described by Dehne and Backhaus (1986). Inoculation was performed at sowing by mixing inoculum carrier material with culture substrate to 5% volume.

Hydroponic culture

The hydroponic culture was performed in a pure nutrient solution based on Knop's and Hoagland's nutrient solution (Urbach et al. 1983) with some modifications. First, the stock solutions were prepared from a 20-fold concentrated macronutrient solution, an 18-fold concentrated micronutrient solution and an iron chelate solution. The stock solution of iron chelate was prepared by dissolving 12.05 g of Titriplex II (EDTA, Merck) in 134 ml 1 N NaOH to which 13.45 g $FeSO_4.7H_2O$ was added. After making the solution up to 500 ml with distilled water, it was aerated overnight with a pump to facilitate the formation of iron chelate. The final nutrient solution was prepared by diluting the macronutrient stock solution 1:40, and the micronutrient stock and iron chelate stock solutions 1:1000 with tap water; the pH of this solution was 7–7.2.

Fifteen days after sowing, one set of plants was separated carefully from the culture substrate, washed with tap water and transferred into nutrient solution. The roots of VA mycorrhiza-inoculated linseed plants at this time had 30% and 25% infection by isolates 49 and 510, respectively. In the case of infection experiments in the nutrient solution, one mycorrhizal plant and one non-mycorrhizal plant were grown in a test tube filled with 90 ml of the solution. The test tubes were made up daily to 90 ml with nutrient solution and the solution changed completely every 5 days. In addition, 3-l plastic pots were tested for large scale culture of VAM fungi, in which case six mycorrhizal linseed plants were transferred into 2.5 l nutrient solution. The solution was aerated daily for about 30 min with a pump and changed completely every 7 days.

Assessment

Root samples were collected at intervals from hydroponic culture as well as from sand culture for microscopic analysis of VAM fungal growth. The colonization by VAM fungi is presented as percentage infection of roots. Plant growth parameters such as stem and root dry weights, number of capsules per plant and number of seeds per capsule were determined at the end of 12 weeks of culture in plastic pots in pure nutrient solution.

Results

Fungal growth

From the initial infections of 30% and 25% for isolates 49 and 510, respectively, the symbiosis developed rapidly. Within 2 days it was possible to observe vigorous extramatrical mycelium in the nutrient solution with the naked eye. Spreading of the VAM fungus from older infected root parts continued (a) internally with typical mycelium and arbuscule development in the old roots, (b) with robust hyphae on the root surface. Furthermore, intensively produced extramatrical hyphae infected nonmycorrhizal adjacent roots. The establishment of such new infections in pure nutrient solution was examined in detail with nonmycorrhizal roots of receptor plants grown together with preinoculated mycorrhizal donor plants. Both isolates of *G. intraradices* were able to infect the nonmycorrhizal root systems within 15 days of hydroponic culture . The infection of the receptor plant proceeded in known infection steps: the attachment of robust hyphae to the root surface, development of appressorium, penetration, development of arbuscules, and finally internal and external growth of mycelium and sporulation after a further 7–10 days.

Both isolates of *G. intraradices* showed identical growth in the nutrient solution; therefore the percentage infection of linseed roots is presented for isolate 510 only (Fig. 1). At the beginning of hydroponic culture, the VAM fungus spread more slowly than in sand culture. However, the percentage infection increased strongly during the 5th week and reached its maximum, identical to the percentage infection in sand culture, after 8 weeks.

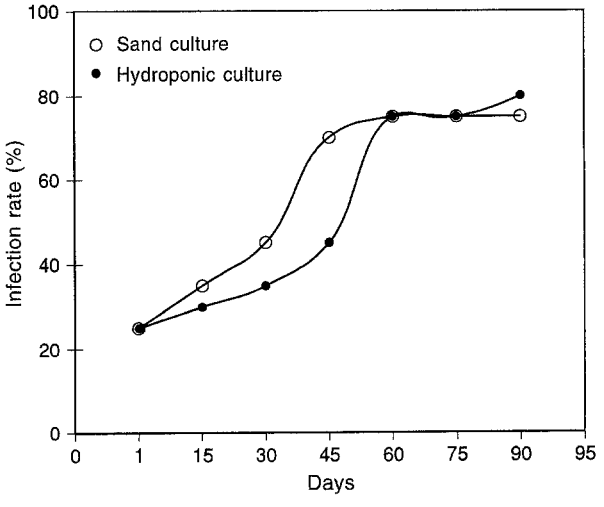

Fig. 1 Infection of linseed plants with *Glomus intraradices* isolate 510 in sand and hydroponic culture. At day 1, one set of plants was transferred to hydroponic culture

Table 1 Effect of *Glomus intraradices* isolates on dry weight and number of capsules of linseed plants after 12 weeks growth in nutrient solution

Treatment	Dry weight/plant (g)		
	Stem	Root	Capsules/plant
Control	3.40±0.4	0.85±0.02	52.94±13.3
Isolate 49	3.85±0.3	0.78±0.02	55.94±11.7
Isolate 510	3.30±0.2	0.65±0.02	47.11±10.2

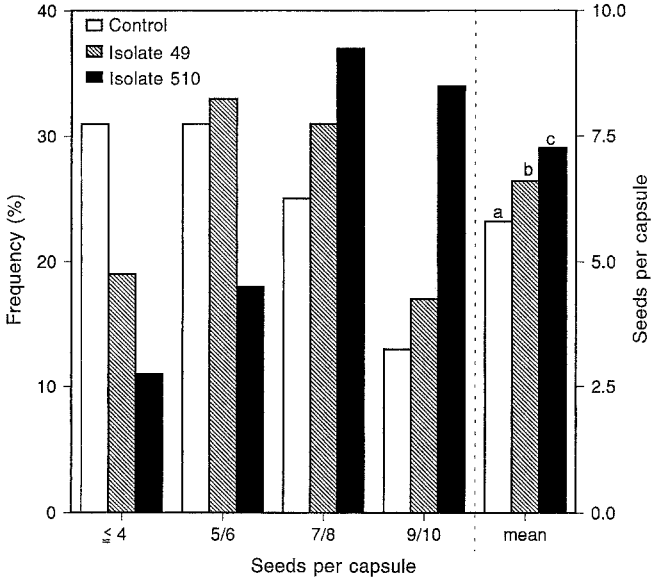

Fig. 2 Effects of *G.intraradices* isolates on number of seeds per capsule of linseed plants in hydroponic culture ($n=300$) *Left* Frequency distribution of seeds per capsule, *right* mean number of seeds per capsule. Means followed by different letters are significantly different according to Tukey's multiple range test ($P=0.01$)

Host growth in nutrient solution

Linseed plants grew further without any visible deleterious effects after their transfer from sand culture into pure nutrient solution. An increase in the growth rate of main and of first- and second-order lateral roots was observed, but lateral roots of higher orders developed only after 10–15 days culture. Mycorrhizal and nonmycorrhizal linseed plants showed no differences in the growth process or in the habit of the shoot. Stem dry weights and numbers of capsules per plant were also not significantly different (Table 1). In contrast, mycorrhizal root systems had lower root dry weights than nonmycorrhizal roots, the effect being stronger with isolate 510 than with isolate 49. The mean number of seeds per capsule was in part markedly increased in the mycorrhizal treatments (Fig. 2).

Discussion

The growth and development of VAM fungi during contact and interaction with their host plants are especially dependent on the growth conditions for both partners provided by the culture system. This is apparently optimal in the case of the hydroponic culture system described. Linseed plants can be grown easily and very quickly in different solid substrates but also in pure nutrient solution. They formed mycorrhiza with different isolates of *Glomus* spp. within a short time.

The degree of infection in the nutrient solution was comparable with that in solid culture substrates. This seems to contradict a number of observations on the low colonization of aquatic plants by *Glomus* spp. (Stenlund and Charvat 1994), and their rare occurrence at moist-wet locations or in occasionally flooded root systems of marshy and aquatic trees (Khan 1993), which appear to be unfavourable conditions for infection and growth of VAM fungi. The lower redox potential in the rhizosphere is cited as a particular limiting factor. Probably because of such observations over many years the results of Peuss (1958) did not lead to a detailed study of VAM fungi in pure nutrient solution.

A negative correlation between abundance of root hairs and colonization of VAM fungi was recently reported by Michelsen (1993), and thus the observed lower infection by *G. intraradices* at the beginning of hydroponic culture, in comparison to that in sand culture, is probably related to rapid growth of the linseed root system in nutrient solution. At this time, nutrients and compounds rich in energy absorbed through arbuscules from the host plant, are apparently preferentially used for intensive development of arbuscules and mycelium in older roots or vigorous growth of extramatrical hyphae rater than for the colonization of rapidly growing young root parts. The hypothesis of Peuss (1958) that *G. mosseae* can spread to newly grown roots of tobacco plants via extramatrical hyphae in the nutrient solution is supported by our infection results under comparable conditions. The culture of *G. intraradices* in nutrient solution with linseed plants seems to solve the problem of producing large quantities of mycelium and spores free of solid residues. Such a hydroponic culture, which has not been previously reported, was carried out with two isolates of *G. intraradices* that are not different in their growth, development or spreading in the nutrient solution. It would also be interesting to try with other *Glomus* species that can develop a symbiosis with linseed.

In comparison to other systems, such as the hydroponic sand culture described by Ojala and Jarrell (1980), the Beltsville method of sand culture (Millner and Kitt 1992), nutrient flow culture (Elmes and Mosse 1984) and aeroponic culture (Sylvia and Hubbell 1986), the present system permits rapid culture of VAM fungi as well as the inoculation of nonmycorrhizal root systems at low cost. The particular advantage of this sys-

tem is the easy separation of extramatrical fungal structures from roots, both of them being free of solid substrate residues. Due to the large number of chylamydospores produced, application of this system can be extended, e.g. to the splitting of populations, the development of single-spore cultures, the quantification of the reproductive potential of a specific isolate, and the production of mycorrhizal roots, mycelium and spores for biochemical and biomolecular analysis. The quantity of fungal structures obtained can be optimized by varying container size and duration of culture.

In contrast to the dramatic stimulation of growth and the generative phase of linseed plants in sand and compost culture (Dugassa unpublished work), neither isolate of *G. intraradices* in nutrient solution had any influence on shoot growth or number of capsules. Since the shoot growth of mycorrhizal and nonmycorrhizal linseed plants exceeded that of sand cultures by about 40%, and the number of capsules was twice that in sand culture, the conditions in the hydroponic culture with its permanent nutrient and water availability are clearly superior. Under such conditions, VAM fungi obviously cannot further promote plant growth; however, VAM fungi do alter the physiology of host plants, as is manifested by the isolate-specific reduction of root dry weight and increase in number of seeds per capsules.

The simple hydroponic system presented here seems to be suitable not only for the production of VAM fungi but also for investigations of their interactions with host plants.

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