Development of the VAM fungus, *Glomus mosseae* in groundnut in static solution culture

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Abstract. The establishment of a mycorrhizal fungus, *Glomus mosseae* in groundnut was studied in a static nutrient solution having 0.25 mM of phosphorus and inoculated with powdered infected groundnut roots or resting spores. Initiation of the mycelial growth in roots was observed after 8 days of contact with the fungal inoculum.

In a second experiment initial concentrations of P in the range of 0.25 to 1 mM resulted in maximum colonization by the fungus and increased production of the plant biomass. Plant growth and VAM development was slightly less in a pot culture without the addition of P to the soil. It is suggested that the static solution culture method can successfully be adopted to determine the requirement of initial levels of essential elements in culture solutions for investigating similar mycorrhizal associations of crop plants.

Keywords. *Glomus mosseae*; groundnut; phosphorus; static solution culture; vesicular arbuscular mycorrhiza.

1. Introduction

The importance of vesicular-arbuscular mycorrhiza (VAM) in the uptake of phosphorus (P) in many plants, especially under low nutrient conditions, has been fully realized in recent years (Gerdemann 1975; Khan 1975; Mosse 1973). Most of the reports of VAM in crop plants are restricted to cool-temperate climates. They seem to indicate a rapid colonization of the plants by the VAM fungi. However, an initial lag in the establishment of mycelium and production of vesicles or spores of *Glomus mosseae* in the roots of groundnut was reported in this laboratory (Rao and Parvathi 1982). In the present investigation, an attempt was made to determine the pattern of colonization of groundnut roots by the fungus and also the influence of phosphorus level in the medium on the establishment of the fungus in a static solution culture.

2. Materials and methods

Clean seeds of groundnut cv TMV-2 were sown in pots containing sterilized sand and watered regularly with sterile water. After 10 days of sowing, the seedlings were carefully removed without damaging the roots and the adhering sand particles washed off with sterile distilled water. A porcelain tray (43.3 x 36.5 x 10 cm) was filled with a mineral salts solution, except for the top 2 cm and covered with a bamboo grid, with gaps of about 1 cm² to hold the plants in position. The culture solution employed was essentially the same as that used for rice culture by Ishizuka (1933). Micronutrients (Mn, Zn, Ca, B and Mo) were added according to the formulation of Johnson *et al* (1957). The solution had a P content of 0.25 mM in the form of NaH₂PO₄ · 2H₂O and
the pH was adjusted to 4.8-5. About 101 of medium was provided for 64 plants in the tray. The inoculum of *Glomus mosseae*, obtained by powdering air-dried, infected groundnut roots and passing the powder through a 2 mm sieve or by collecting the resting spores from field soil infested with the fungus, by a wet-sieving method (Gerdemann and Nicolson 1963), was added to this solution. The inoculum consisted of either 1 g of root powder or 50 spores/1 l medium. The seedlings were placed on the grid so that the roots were completely immersed in the solution. They were firmly supported by placing large pebbles on the grid. The tray was left in open shade in the laboratory (room temperature 27°C ± 5°C). The culture solution was aerated 3 to 4 times daily by bubbling air through it.

Plant samples in triplicate were withdrawn at desired intervals. At the end of the 20th day, the entire inoculated medium was drained off from the tray and substituted with an equal quantity of fresh uninoculated medium. A total of 25 one cm segments of root collected from three plants at random were sampled each time and observed for the presence or absence and the nature of mycorrhizal structures, after staining as described earlier (Rao and Parvathi 1982). The average number of vesicles and/or spores per one cm root length and the number of segments showing the fungus were observed to determine the per cent infection.

An experiment was also set up in pots using sterilized lateritic soil (pH 7-8; organic matter, 0.26%; total nitrogen 0.0616%; total phosphorus, 0.0045%) inoculated with powdered roots from infected plants. No external source of P was added to the soil. About 10 seeds were sown in each of the 20 pots prepared this way and the pots were incubated as mentioned above and mycorrhizal development was assessed.

In another experiment, groundnut seedlings raised in sterilized sand were transferred to the bamboo grid placed over a glass tank (16.2 × 6.5 × 18 cm) containing 2 l of the culture solution with different initial concentrations of phosphorus (0, 0.1, 1 and 10 mM). Uninoculated solution with 0.25 mM of P was maintained as the control. About 20 plants were raised in two tanks for each treatment. The sides of the tanks were covered with black paper to avoid algal growth in the medium and incubated in the laboratory.

After 20 days the entire solution was removed and the tanks were refilled with fresh uninoculated nutrient solution with the same concentrations of phosphorus. Subsequently, three plants were taken out at intervals of 5 days and the roots examined for the nature and extent of mycorrhizal formation. At the time of the last sampling, five plants were removed for each treatment, oven-dried to constant weight at 70°C and the weights recorded. The phosphorus content of shoots and roots was determined by the method of Fogg and Wilkinson (1958).

3. Results

The data on colonization of groundnut roots by *Glomus mosseae* under static conditions of nutrient culture are presented in figure 1. The data of the corresponding experiment with sterilized soil are presented in figure 2. In the culture solution, the mycorrhizal fungus appeared in host roots as sparse hyphae only after 8 days of contact with the inoculum. The vegetative growth of the fungus increased progressively up to 18 or 19 days and a sudden development of tiny vesicles was noticed on the 20th day in the medium. In the plants raised in inoculated sterilized soil, vesicles started to develop
VA micorrhiza in groundnut

Figure 1. Development of G. mosseae in groundnut roots in static solution culture. Ten-day old seedlings raised in sterilized sand transferred to the culture solution. a. Per cent mycorrhizal infection and b. average number of vesicles and/or spores per cm root. Solid line (----), root powder inoculum and broken line (-----), spore inoculum.

Figure 2. Development of G. mosseae in groundnut roots raised in sterilized soil in the presence of root powder inoculum. Solid line (----), per cent mycorrhizal infection and broken line (-----), average number of vesicles and/or spores per cm root.

after 28 days. Arbuscules were noticed prior to the appearance of vesicles in the culture solution experiment with spore inoculum and in sterilized soil with powdered root inoculum. With the appearance of vesicles, the arbuscules started to disappear. In all the cases, root infection as well as vesicle formation increased progressively. After 46 days of plant growth, some of the vesicles started developing into thick-walled spores. During the 40-day incubation period in culture solution, about 44% of the root segments examined were colonized and the vesicle and/or spore formation reached a maximum of 8-9/cm root. In sterilized soil, 46% of root segments were colonized but
Table 1. Effect of phosphorus level in culture solution on the establishment of *Glomus mosseae* in groundnut roots.

<table>
<thead>
<tr>
<th>P level in culture solution (mM)</th>
<th>Incubation (days)*</th>
<th>Root length (cm)</th>
<th>Total dry wt. (g/plan0</th>
<th>P content (mg/g material)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20</td>
<td>25</td>
<td>30</td>
<td>35</td>
</tr>
<tr>
<td>Inoculated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>12</td>
<td>12</td>
<td>14(0-2)</td>
<td>14(0-4)</td>
</tr>
<tr>
<td>0.1</td>
<td>16(0-2)</td>
<td>20(1-9)</td>
<td>22(2-0)</td>
<td>24(2-6)</td>
</tr>
<tr>
<td>1.0</td>
<td>24(0-6)</td>
<td>28(2-2)</td>
<td>34(3-9)</td>
<td>38(4-5)</td>
</tr>
<tr>
<td>10.0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Uninoculated</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Ten-day old seedlings grown in sterilized sand were transferred into the culture solution containing inoculum (0.1% infected root powder, w/v) and incubated further; *Per-cent root length infected; *Figures given in parantheses represent the average number of vesicles or spores/cm root length.

In the study involving different initial levels of phosphorus in the nutrient solution, root infections were observed even in the plants exposed to inoculum in the medium receiving no phosphorus. However, the development of vesicles was delayed up to 30 days of incubation (table 1). The roots of plants receiving 0.1 and 1 mM of initial P concentrations exhibited comparatively better hyphal growth and the vesicles started developing at the end of 20 days. The plants showed maximum root length and total dry weight at 1 mM of initial P content. There was virtually no mycorrhizal infection at a higher level (10 mM) and this concentration also appeared to be toxic to the plant as seen in reduced dry weight, root length and general appearance. The plants, however, had 1% P in tops and 0.8% in roots. The growth of plants raised in uninoculated solution containing 0.25 mM P was less than in the solution without P but inoculated with the fungus.

4. Discussion

In the present investigation, an attempt was made to ascertain the applicability of static conditions of nutrient solution culture for the effective establishment of the VAM fungus, *G. mosseae* in groundnut roots. In the plants raised in nutrient solution as well as in sterilized soil, the fungus appeared only after 8 days of transfer to the solution or after 18 days of sowing, which was confirmed in repeat experiments. The occurrence of only the hyphal phase of the fungus in the roots up to 28 days also clearly shows that the development of the mycorrhizal endophyte in the culture solution with an initial concentration of 0.25 mM of P proceeds in a manner similar to that obtained in soil.

The arbuscules, implicated to be the structures involved in active uptake of P by plants (Mosse 1973) were observed at 28 days of plant growth, prior to the initiation of vesicles. However, the arbuscules were found only when the laboratory temperature
was relatively low (27°C) both in solution culture and in sterilized soil. In a repeat experiment conducted with the solution culture at a higher temperature (32°C), arbuscules did not appear. The development of vesicles after 28 days, however, was uniform in all cases. This finding extends our earlier report that, in the semi-arid soil conditions as exist in this locality, a lag of 3 to 4 weeks occurs before the vegetative mycelium of *G. mosseae* in groundnut roots is transformed into vesicles (Rao and Parvathi 1982). On the other hand, in some Australian soils, maximum number of entry points on the roots of *Medicago truncatula* and the development of arbuscules occurred on 8th day at 12–16°C (Smith and Bowen 1979). However, they reported that, for *Trifolium subterraneum* there was a delay of 8–10 days for the entry of the fungus even at these temperatures. In another study, Schenck and Schroder (1974) found that development of arbuscules and per cent infection of soybean roots declined above 30°C with no infection at 41°C. The total absence of arbuscules at 32°C and a relatively long lag phase of 28 days in the development of vesicles in the present study are perhaps due to the higher temperatures prevailing under our experimental conditions.

In soil-grown plants, P uptake is commonly limited to a narrow zone of soil adjacent to the root and to eliminate this limitation a flowing culture technique with very low P concentration was developed by Howeler et al (1982) to establish an effective endomycorrhizal association on cassava. The present investigation indicates that a much simpler method of static solution culture providing higher level of P can be successfully used for mycorrhizal development. The limitation of available phosphorus that occurs in soil would not be a limiting factor in a liquid medium as the concentration would adjust itself through diffusion.

To ascertain the influence of initial P concentration on the growth of the plants and for the development of the fungus in the solution culture, three different levels (0.1, 1 and 10 mM) of P were employed. The ten-day old seedlings transferred to the culture solution containing the inoculum continued to grow, although there was no added P in the medium. They had 1.1 mg P/g plant material at the time of transfer which got reduced to 0.16 mg P/g shoot material by 35 days. Apparently, the P already present in the seedlings was sufficient for further growth. Under these conditions the mycorrhizal fungus showed poor vegetative growth and a delay in the development of vesicles. But the degree of infection and the development of vesicles and/or spores was more or less the same in 1 mM level of P in culture medium as at 0.25 mM of P in the previous experiments. Hence, a range of 0.25 mM to 1 mM of initial P in the culture medium may be considered adequate for the growth of the plant and for mycorrhizal development.

The flowing culture techniques developed by Howeler et al (1981) and Macdonald (1981) are more elaborate and require large quantities of nutrient solution. The present technique, which is simpler than the flowing culture technique, needs only daily aeration and a change in the nutrient solution after 20 days, while indicating the response of the plant and the fungus clearly.

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