Spore production and mycorrhizal development in various tropical crop hosts infected with *Glomus clarum*

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Abstract

Plant growth, mycorrhizal development and vesicular arbuscular spore production were examined in five tropical crop host species inoculated with *Glomus clarum* and grown in a glasshouse. In one of the two experiments, sequential harvests of maize, sorghum and chickpea were made in order to study spore production in relation to plant growth and mycorrhizal development. Spore numbers in each of these hosts increased at a fairly constant rate until maximum plant dry weight, when spore production ceased. Sorghum and maize produced considerably more spores than chickpea, with spore numbers being closely correlated with mycorrhizal root length. In the second experiment, *Glomus clarum* was cultured on each of maize, millet, sorghum, groundnut and chickpea for three consecutive generations before cross-inoculation of the spores from each host onto all five hosts. Sporulation with respect to host size was generally greatest when the inoculum used to infect a host had been produced on that host. The growth-promoting effects of the fungus were not influenced by the source of the inoculum. More spores were produced on the cereals than the legumes. Differences in spore numbers amongst hosts and plant generations were apparently influenced mainly by infected root length and by the growth period.

Introduction

Vesicular-arbuscular mycorrhizal (VAM) fungi form symbiotic associations with most economically important crop plants. These fungi can improve plant growth under low-fertility conditions and have attracted considerable research interest into their potential agricultural use. However, as the fungi are considered obligate symbionts, unable to be grown in pure culture (Hepper 1984), their exploitation is dependent on either producing commercially viable inoculum on plant hosts or manipulating agricultural systems to develop and exploit indigenous VAM populations. Several authors have tested different host-fungus combinations for spore production (Hetrick and Bloom, 1986, Struble and Skipper, 1988), and also the influence of environmental conditions on sporulation (Ferguson and Menge, 1982; Schenke and Smith, 1982). Suggested hosts for inoculum production have included cassava (Potty, 1985), bahiagrass (Struble and Skipper, 1988) and Rhodes grass (Sreenivasa and Bagyaraj, 1988), with the choice of host apparently more important for some VAM fungi than others (Hetrick and Bloom, 1986). Little research, however, has been done into what plant characteristics enhance spore production. This study examined spore production of *Glomus clarum* in relation to host type, stage of plant growth and mycorrhizal development, and also investigated whether there were differences in effectiveness of the VAM inocula produced on the different hosts.
Materials and methods

Hosts

The hosts used were *Arachis hypogaea* L (groundnut cv P1259747), *Cicer arietinum* (chickpea cvs Rabat and L-550), *Sorghum bicolor* L (sorghum cv CSH5), *Pennisetum americanum* L (millet cv BJ104) and *Zea mays* (maize cvs Earlibelle and Drocan P01). In Experiment 1, both chickpea and both maize varieties were used, along with the sorghum. In Experiment 2, groundnut, sorghum and millet were used, and also maize cv Drocan P01 and chickpea cv Rabat. All seeds, except for Earlibelle, were obtained from ICRISAT, Hyderabad.

Inoculation

Two hundred spores of *Glomus clarum* (Nicolson and Schenck), obtained by wet-sieving from pot cultures of millet, were spread across a layer half-way down each pot, and two seeds per pot of the appropriate plant host sown just above this layer. This inoculation regime was used for Experiment 1 and in the first stage of Experiment 2. At maturity of stage 1 in Experiment 2, spores were extracted and a new set of plants of each host inoculated with 200 spores of *Glomus clarum* obtained from the same host. This was repeated for a third generation of hosts. In the fourth stage of the experiment, 350 spores from each host were re-inoculated onto the same host and also inoculated onto the other 4 hosts (see below).

<table>
<thead>
<tr>
<th>Generation</th>
<th>1 chickpea</th>
<th>2 chickpea</th>
<th>3 chickpea</th>
<th>4 chickpea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source of inoculum</td>
<td>groundnut</td>
<td>groundnut</td>
<td>groundnut</td>
<td>groundnut</td>
</tr>
<tr>
<td>ex millet</td>
<td>maize</td>
<td>maize</td>
<td>maize</td>
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<tr>
<td>sorghum</td>
<td>sorghum</td>
<td>sorghum</td>
<td>sorghum</td>
<td>sorghum</td>
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<tr>
<td>5 hosts</td>
<td>5 hosts</td>
<td>5 hosts</td>
<td>5 hosts</td>
<td>5 hosts</td>
</tr>
</tbody>
</table>

Cultural conditions

After emergence the plants were thinned to give 1 seedling per pot. Temperature in the glasshouse was maintained at a minimum of 25°C, with a daylength in the winter months of 13 h; during the spring and summer seasons the plants were exposed to the natural photoperiods. Except for the cereals in the final stage of Experiment 2, the plants were grown in 12.5-cm plastic pots containing 1 kg of washed and sterilised seashore sand (pH 7.0). In stage 4 of Experiment 2, the cereals were grown in 18-cm pots containing 3 kg of sand. This was to give any growth differences more chance to develop; previous work with these hosts had shown that pot size did not affect such parameters as % infection or spores produced per unit root length, but that a larger pot-size allowed more growth response to mycorrhizal inoculation. In each experiment, granular Kodjari rock phosphate (8.1% P) was mixed with the sand at a rate of 2.25 g 10 kg⁻¹ sand. The plants were fed twice weekly with 30 mL of Hoaglands nutrient solution minus the phosphate ion and watered as required.

Plants given the various treatments were arranged in blocks, with each host being kept separate within a block to limit shading. The hosts were frequently rotated to minimise positional effects.

Harvesting

In Experiment 1, the first harvest was carried out seven weeks after sowing, with plants then being harvested at approximately two weekly intervals until senescence for each host. Four plants of each host were taken for each harvest. For chickpea, harvesting ceased after 13.5 weeks from sowing, whereas for the cereals they continued until 21.5 weeks. Subsequent inspection of additional plants confirmed no further increase in plant weight or mycorrhizal spore production after these times. In Experiment 2, each host was harvested at maturity. The time in weeks for this varied between the different stages of the experiment, depending on photoperiod and temperature. Replication differed with experimental stage: in stage 4 there were six replications for each host and inoculation treatment.

Assays

Shoots were oven-dried at 85°C for 48 h and then