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Short communication

Arbuscular mycorrhizal inoculum production for commercial use

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ABSTRACT

Introduction of arbuscular mycorrhiza (Am) to soils in which they are lacking, present in low numbers or to soils having inefficient species is limited due to the non-availability of a commercially viable proper inoculum. In this paper we report a simple and low cost method of Am inoculum production using an organic medium that can be used in commercial scale inoculum production using *Pueraria phaseoloides* as the host plant. Preinoculated (with *Acaulospora spinosa*) *Pueraria* seedlings were grown in large wooden boxes filled with a mixture of fine coir dust and finely ground rice husk (1:1) as the medium. These were grown for 3-4 months to multiply the Am fungus in the media before it was tested as a suitable inoculum.

Key words: *Acaulospora spinosa*, Arbuscular mycorrhiza, inoculum, media

Arbuscular mycorrhiza (Am) has been described as a "Universal Symbiosis" in plant kingdom by Nicolson (1967). The beneficial effects of these intimate associations are many and are well documented.

Arbuscular mycorhizae (Am) are symbiotic associations formed between zygomycetes (order glomales) and the roots of most terrestrial higher plants. These associations create an intimate link between plant roots and the soil in many types of natural ecosystems throughout the world. Am associations play an important role in the acquisition of mineral nutrients, specially the slowly mobile ions such as phosphorus (Abbott and Robson 1984). In addition to growth promoting effects mainly due to efficient acquisition of mineral nutrients, it had been shown that Am plants are more resistant to biotic and abiotic stresses in nature. (Nelson and Safir 1982; Sylvia *et al.* 1993; Al-Karaki and Al-Radded 1997).

Artificial introduction of more efficient species of Am fungi into soils, where they are lacking or present in low numbers or inefficient species, can improve the growth of many plants (Govinda Rao *et al.* 1983; Islam *et al.* 1980; Khan 1972; Ibijbijen *et al.* 1996). However, since these fungi associated with arbuscular mycorrhiza cannot be grown on artificial synthetic media, the production of inoculum in large quantities has so far not been very successful. Therefore Am fungal collections are maintained in living host plants under controlled conditions.

Large scale multiplication of efficient fungi has been achieved by inoculating appropriate host plants such as clover, raygrass, sudan grass or maize that are grown in sterilized soil or any other rooting media such as clay, bark, pumice, sawdust, peat mixtures and glass beads (Feldmann and Idezak 1992; Vestberg and Uosukainen 1992; Declerek *et al.* 1996; Redecker *et al.* 1995). Spores, hyphae, infected root pieces, and infested soil or rooting medium obtained from these cultures can be used either separately or in a mixture as a source of crude inoculum. However application of this pot culture methodology for commercial inoculum production has been minimal due to several practical limitations. Sterilized coir dust has been tried as the main substrate by Jayaratne and Wettasinghe (1987). They found that no spores were produced by the fungus *Gigaspora margarita* although the host plants had fairly high levels of infection. However, in-contrast to this, Van Holm (1998) reported coir dust to be a good medium for the VAM inoculum production. Here we report a very simple and economical medium to multiply the Am inoculum and inoculate nursery or field growing plants.

Wooden boxes (90 x 30 x 14 cm) lined with polythene films were filled with a mixture of fine coir dust (by passing through a 2 cm mesh) and finely ground rice husk 1:1. Potting medium was used without sterilization as the preliminary work proved that this mixture does not carry any Am inocula to infect the plants. The trays were planted with 15 *Pueraria* seedlings preinoculated with *Acaulospora spinosa*. The seedlings were evenly planted to cover the entire tray. Plants were provided with 50 ml/tray of 1/5th Rorison's nutrient medium minus phosphorus (Rorison 1960) once in two weeks.

Pot cultures maintained in the glass house were checked for the purity once in 3 months by examining the spore types. They were reisolated and the cultures were initiated on sterilized soil/sand 1:1 mixtures once in 6 months to maintain the purity.

The pot cultures that were used to prepare pre-inoculated *Pueraria* seedlings were established in the following manner:

A Spores of *Acaulospora spinosa* were isolated from green house pot cultures maintained at the Rubber Research Institute of Sri Lanka using wet seiving method (Gerdemann and Nicolson 1963).

B Spores were given a chill treatment by storing them at 5°C for 4-5 days prior to sterilization to facilitate germination. These spores were surface sterilized for 20 minutes using 2% chloramine T with 0.02% streptomycin. After washing well with sterile distilled water, the spores were germinated on 1% DIFCO BACTO Agar.

C Germinated spores were placed on to the seedlings of *Pueraria* that were growing in small plastic vials (3.5 cm diameter x 5 cm) filled with sterile sand. After 4-5 weeks of growth, the plants were transferred to large cement pots filled with sterile soil:sand (1:1) mixture (autoclaved for 1 hr. at 15 lbs) to initiate the pot cultures. Plants were allowed to grow inside a glass house for 3 months before these pot cultures were used to produce preinoculated plants.

Preinoculated plants of *Pueraria* were produced by sowing surface sterilized seeds on to these pot cultures. These seedlings were allowed to grow for 4-5 weeks at which time the root infections were checked by staining with Trypan blue after cleaning in 10% KOH (Phillips and Hayman 1970).

Plants were allowed to grow in the wooden boxes with organic medium for 3-4 months with regular pruning of the shoots and watering inside a glass house. The infection percentages were determined after staining the roots with Trypan blue after cleaning in 10% KOH. (Phillips and Hayman 1970). Spore numbers in the media were determined using wet-sieving decanting techniques (Gerdemann and Nicolson 1963).

The shoots were cut-off at the collar region and the contents of the box were mixed thoroughly and the roots were chopped into small fragments with the help of a mechanical mincer. The contents were mixed up well again in a large polythene bag and allowed to air dry over-night under a slow fan to attain a moisture content of about 40%. The final inoculum was packed into 500 g packets in polythene bags and were stored in a refrigerator (8-10°C).

One of these samples were used to check the viability of the produced inoculum at zero time. This was carried out by incorporating 10 g of the inoculum into 500 g of sterilized soil sand (1: 1) mixture in small plastic pots and sown with germinating *Pueraria* seeds. Pots with dead inoculum (autoclaved inoculum) served as the controls. Ten replicates were used with 5 plants per pot. These seedlings were allowed to grow for 2 months and the infection percentages were determined after staining with Trypan blue (Phillips and Hayman 1970) using grid line intersect method (Giovanetti and Mosse 1980).

Viability of the stored samples was ascertained by the same procedure at every 3rd month up to the 18th month.

Rice husk coir dust media had a pH of 4.4, 1.5% N, 0.09% P, 0.02% K, 0.47% Ca and 0.05% Mg. Preinoculated plants developed from the pot cultures had high percentage of root infections (between 40-50%) at the time of transferring them into large wooden trays. The root samples obtained from trays harvested after 4 months had more than 90% of the root length infected with arbuscular mycorrhizal fungi. The counts revealed the presence of 20 spores of *Acaulospora spinosa* per 1 g of wet medium.

Viability of the harvested inoculum is quite high even after 18 months of storage (Table 1). Pots with dead inoculum had zero infection in all pots. Percentage root lengths infected in *Pueraria* plants used to test the viability of inoculum are given in Table 1.

Table 1. Percentage root length infection of test plants used to determine the viability of stored inoculum. Each value represents mean of 10 replicates of 5 plants each.

	Duration of storage, months						
Time (Months)	0	3	4	6	10	15	18
% Root infection ±SD	85±3	80±6	82±8	94±4	98±2	90±3	96±3

Although the use of arbuscular mycorrhizal fungi in the improved plant production has been tested and described by many authors, specially under tropical conditions (Sieverding and Saif 1984; Hayman 1987), its large scale use in plant production systems have not been commercialized yet. This is mainly due to two practical difficulties.

A Difficulties involved in the selection of more efficient fungi that could be introduced into plant production systems.

B Difficulties in production of a suitable low cost inoculum that can be stored and transported to the users.

High infection levels shown in the plants used in the bioassay to determine the viability of stored inoculum in the present study clearly indicated that,

even after 18 months of storage at low temperatures. inoculum does not loose its viability. Therefore this medium could be used to produce *Acaulospora spinosa* inoculum in a large scale at a very low cost. Moreover, this inoculum can be compressed and made into briquettes giving the advantage of storage facilities and transportation. However, the only disadvantage is the fairly long time (3-4 months) taken to produce this inoculum.

The transplanted perennial crops such as rubber and tea can be inoculated at nursery stage by incorporating the inoculum into nursery beds or polythene bags. The annual crops such as vegetables that are grown on specially prepared seed beds can be inoculated with this fungus by directly incorporating the inocula into soil at the time of preparation of seed beds.

Further, the fact that coir dust medium has been proved to be a suitable medium to produce *Rhizobial* inoculum (Van Holm 1998) will be of an added advantage in inoculation procedures at field level.

The quantification of the inoculum potential of the produced inoculum is of prime importance before the application in the field and in nurseries. It can reduce inoculation costs as it will allow the use of the minimum amount of inoculum needed to guarantee the colonization of the host plants. A possible method to determine the infectivity level (inoculum potential) is to carry out the most probable number method (MNP) described by Porter 1979.

Results of the present study indicate that the medium described here is suitable to multiply the fungus *Acaulospora spinosa* to be used in large scale commercial application. This medium may be useful in inoculum production of other species of arbuscular mycorrhiza as well. This aspect has to be investigated separately for other species.

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