TECHNIQUES FOR EXTRACTION OF ARBUSCULAR MYCORRHIZAL FUNGI SPORES

S. Shamini\textsuperscript{1} and Dr. K. Amutha\textsuperscript{2}\textsuperscript{*}

\textsuperscript{1}Research Scholar, Department of Biotechnology, Vels University, Chennai-17
\textsuperscript{2}Associate Professor, Department of Biotechnology, Vels University, Chennai-17

ABSTRACT

Arbuscular mycorrhizal fungi (AMF) spores are normally isolated by wet sieving and decanting method. In my present study AMF spores were isolated by capillary rise method. The rise in the capillary is due to the result of surface tension of liquid. Gerdemann and Nicolson, 1963 were developed wet sieving and decanting method to isolate different size of spores. Sucrose density gradient centrifugation (Daniel and Skipper, 1982), New plate counting method (Mosse and Bowen, 1968) and Adhesion floatation technique (Sutton and Barron, 1972) are also useful for extraction of spores and our research article will help the researchers to pick out the suitable method to extract AMF spores from soil.

Keywords: Mycorrhiza, Plant roots, Rhizosphere, Capillary rise method.

Corresponding author: Dr.K. Amutha Email i.d: amutharavi40@gmail.com

INTRODUCTION

Mycorrhiza is the keystone organism that forms an interface between soils and plant roots (Power and Mills 1995). Mutualistic associations of AMF with host plants have been observed in various natural and agricultural ecosystems (Sylvia and Williams, 1992). Spores are adequate in number during the summer and the growth rate of roots will be maximum during this time period (Hayman, 1982). The arbuscular mycorrhizal fungi density is based on seasonal changes and host metabolic pathway (Lugo M, 2002). Based on morphological character like spore wall, size, colour and hyphal attachment the AMF spore was identified (Schenck and Perez 1990). In some species the hyphae is viewed under maximum magnification because of the presence of lipid droplets (Brundrett et al., 1996). The beneficial effects of arbuscular mycorrhiza are used as bio-fertilizers for plant growth, especially in soils of low fertility. The morphology of arbuscular fungi were mainly analysed based on
spores isolated from collected soil samples. Vilarino and Arines, 1990, An et al., 1990 and Pacioni, 1994 explained wet sieving and decanting method in the research work. Walket et al., 1982 used sugar solution for centrifugation to extract the AMF spores.

MATERIALS AND METHODS

Plant roots and rhizosphere soil samples were collected from various locations of different ecological zones of Chennai. For the isolation of AMF spores various techniques were carried out.


The wet sieving and decanting is one of the popular technique when compare to other techniques. This technique is used for sieving the coarse particles of the soil and retaining AMF spores and organic particles on sieves of different sizes. 10 g of soil was mixed with 100 ml of water in the 500 ml conical flask. The soil mixture was agitated vigorously to free the AMF spores from soil and allowed to settle for 15-45 minutes and the supernatant was decanted through standard sieves. By using a dissecting microscope, spores were picked by means of pipette or needle.

SUCROSE CENTRIFUGATION TECHNIQUE-Daniel and Skipper, 1982

Spores were purified by re-suspending the sieving in the 40% sucrose solution and centrifugation was carried out. Centrifugation was carried out at 1750 rpm for 5 minutes. The supernatant was removed and poured into the sieves. The spores that hold on the sieves are carefully rinsed with tap water. The spores were collected by using dissecting microscope.

NEW PLATE METHOD-Mosse and Bowen, 1968
The plate count method was first used by Mosse and Bowen (1968). 10 gram of soil was added to 90 ml of distilled water and 10 ml was promptly transferred onto a filter paper by using a pipette. The filter paper was viewed under dissecting microscope.

**ADHESION-FLOTATION TECHNIQUE-Sutton and Barron, 1972**

This method was developed based on their properties of flotation in aqueous solutions and adhesion to glass surfaces. 10 gram of soil sample was mixed with 1 litre and shook vigorously. The suspension was allowed to settle for 2 minutes and then decanted into 150 ml separatory funnel. The materials adhering to the inner surface of the separatory funnel was washed down onto filter paper and viewed under dissecting microscope.

**EXTRACTION OF AMF SPORES BY USING CAPILLARY RISE METHOD**

Extraction of AMF spores by using the capillary tube. Capillarity denotes that the rise and fall of the liquid due the surface tension. The soil was collected from the rhizosphere region. And 10 grams of the soil was added to the beaker containing 100 ml water then mixed well using rod. And the jar was kept undisturbed on the table and leave it settle down. Two same size funnel were taken and connected with ½ inch diameter plastic tube. Then the plastic tube was connected with the check valve tap. The water above the settled soil was gently poured in the funnel. By adjusting the tube the water level was raised. Hence the spore floating on top of the water was settled onto a filter paper while transferring to the funnel and using the dissecting microscope the spores was viewed.

**RESULTS**

The recovery of AMF spores for quantitative studies was done by various techniques
Table No. 1: Isolation of AMF using various techniques

<table>
<thead>
<tr>
<th>S.NO</th>
<th>TECHNIQUES</th>
<th>NO. OF SPORES/ kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>wet sieving and decanting technique (WS)</td>
<td>124</td>
</tr>
<tr>
<td>2.</td>
<td>sucrose density gradient centrifugation (SD)</td>
<td>97</td>
</tr>
<tr>
<td>3.</td>
<td>New plate counting method (PC)</td>
<td>110</td>
</tr>
<tr>
<td>4.</td>
<td>Adhesion floatation technique (AF)</td>
<td>133</td>
</tr>
<tr>
<td>5.</td>
<td>Extraction of AMF spore using capillary rise method (CR)</td>
<td>121</td>
</tr>
</tbody>
</table>

Figure No. 1: Isolation of AMF using various techniques

DISCUSSION

It may be concluded from this studies that the spore extraction using capillary rise method also have advantage when compared with other technique during extraction this can
prevent the hyphal breakage, more convenient, economic method, time consuming method and easy to extract similar size spores. The comparative studies for the extraction of AMF spores were carried out by five methods. When applying water force it may lead to the breakage of hyphae in the wet sieving and Decanting method and sometimes the debris will also get attached with the spore during extraction process. In the density gradient technique there is some disadvantage during the extraction process like other than spore with the same density materials will also be extracted from the sample and spore losses also seen due to centrifugation process. The plate counting method is mainly depends on number of spores present in the soil and in many field samples the spore count will be very less so it will take more times for extracting spores by using this technique.

References

1. An ZQ, Hendrix ZQ, Hershman DE, Hendrix JW and Henson GT. Evaluation of the “Most Probable Number” (MPN) and wet-sieving methods for determining soil borne populations of endogonaceoussmycorrhizal fungi. Mycologia; (1990), 82, p. 516-581.


