RESEARCH PAPER

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Influence of surface sterilization and cold treatment on germination of AM spores

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Vesicular arbuscular mycorrhizal(VAM) fungi are a unique group of ubiquitous soil microorganism known to form symbiotic association with roots of economically important crop plants. AM propagules such as isolated spores, sheared mycorrhizal roots are virtually able to initiate AM symbiosis and establish the pre-symbiotic phase with the transformed root. The spores were sterilized with 96 % ethyl alcohol and treated for 30, 60 and 90 seconds. Surface sterilization of AM spore *viz.*, *Glomus mosseae, G. intrardices* and *G. caledonium* with 96 per cent ethyl alcohol for 60 sec exhibited higher germination per cent without affecting the viability of spores. Further cold treatment of spores at 4^0C for a period of 30 days improved the spore germination to 90 % and 56.5 % in *Glomus intraradices* and *Glomus caledonium,* respectively, whereas *Glomus mosseae,* recorded maximum with 20 days of cold treatment. Though germination was observed higher with 30 days and cold treatment, stratification at 4^0C for a perod of 16-20 days resulted better germination of spores.

Key words : AM fungi, Sterilization, Spore germination, *Glomus*

INTRODUCTION

The first culture of hairy roots colonized by the AM fungus was achieved by Mugnier and Mosse (1987). AM propagules such as isolated spores, vesicles and sheared mycorrhizal roots are used to initiate AM symbiosis and establish the pre-symbiotic phase with the transformed root. The first *in vitro* sporulation of AM fungus was obtained by Becard and Fortin (1988) using carrot hairy roots colonized by *Glomus intraradices*

AM propagules such as isolated spores, vesicles and sheared mycorrhizal roots are virtually able to initiate AM symbiosis and establish the pre-symbiotic phase with the transformed root. Chlamydospores of *Glomus* sp (Mosse and Hepper, 1975; Mugnier and Mosse, 1987) and non-sporocarpic azygospore of *Gigaspora margarita* (Becard and Fortin, 1988; Diop *et al*., 1992) are also preferred as starter inoculum. Recently, sporocarps of *Glomus mosseae* have also been used in an attempt to establish *in vitro* cultures (Budi *et al*., 1999).

Spores are usually collected from the field, or from pot cultures, by wet sieving (Gerdemann and Nicolson, 1963). Mycorrhizal roots used to initiate monoxenic cultures come from plants grown in pot cultures, with field collected soil or AM propagules. Leek (*Allium porrum* L.) plants are widely used because of their susceptibility to colonization.

Proper selection and efficiency of sterilization process are keys for the success of axenic or monoxenic AM fungal cultures. Variations in the sterilization time, the composition and concentration of the sterilizing agent may have significant impact on the process of sterilization.

Viable, surface sterilized spores are a prerequisite not only for *in vitro* experiments but also for producing starter inoculum, free of contaminants, for commercial purposes. Germination of AM spores may also get affected by the use of surface sterilants. Hence, inorder to study the effect of sterilization and cold treatment on germination of AM fungal spores following studies were conducted.

Spore dormancy is a common phenomenon in the fungal kingdom and the term "dormancy" has been used to describe a large range of physiologically inactive stages. Dormancy can be broken by critical activation step, which results in spore germination (Tommerup, 1985). Studies showed that incubation at low temperature (5^0C) for several weeks often induced spore germination (Hepper and Smith, 1976). Hence, to study the influence of surface sterilants as well as cold treatment on germination of AM spores lab experiments were conducted under controlled condition.

MATERIALS AND METHODS

AM cultures used :

The arbuscular mycorrhizal cultures *viz.*, *Glomus mosseae, Glomus intraradices* and *Glomus caledonium* were used in this study. The cultures were obtained from the culture collection centre at TERI, New Delhi. The cultures were maintained in 1:1 sterile sand: soil mixture either with maize or onion as a host.