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Status of Arbuscular mycorrhiza (AM) in nurseries of willow, poplar and pine seedlings in Himachal Pradesh

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ABSTRACT

Poplar and willow are economically-important, fast-growing tree species with the ability to colonize nutrient-poor environments. Willow (*Salix* sp.) offers a great potential as a source of renewable energy and for bioremediation and polluted environments. To initiate a study on the possible contribution of arbuscular mycorrhiza to this ability, we isolated mycorrhial fungi from in and around the rhizosphere of native poplar (*Populus* sp.), willow (*Salix* sp.) and pine (*Pinus* sp.) seedlings grown in research nurseries at Dr. Y.S. Parmar University of Horticulture and Forestry, Solan (H.P.). Several species of mycorrhizal fungi grew well in the rhizosphere of these trees, were characterized based on morphological studies. The number of spores per 50 g of rhizosphere soil from pinus, willow and poplar were found to be 1380, 1290, 1300 and 540, 490, 530 spores at 106 µ and 250 µ mesh sieves, respectively. The presence of these AM fungi may help explain the ability of these pioneering tree species to grow under nitrogen limitation. Their presence will be helpful in mitigating the losses due to soil borne diseases as well as enhancing the plant vigor.

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INTRODUCTION

Plant-microbe interactions have been utilized to improve plant growth for the production of food, fibre, biofuels and key metabolites. The mutualistic interaction can be beneficial in directly providing nutrients to the plant (biofertilizer) or increasing the availability of compounds such as iron or phosphate. Mycorrhizal fungi are an important integral component of the plant-soil system, forming symbiotic associations with most land plants and mediating a range of crucial ecosystem processes. In addition, other non-nutritional benefits, such as soil aggregation and stability, increased drought tolerance, and protection against pathogens (Azcon-Aguilar *et al*., 2002 and Gosling *et al*., 2006) can be conferred upon the associated host. The mycorrhizas have been found associated with several biomass-producing plant species, such as *Populus* and *Salix* species (Baum *et al.*, 2002 and Trowbridge and Jumpponen, 2004). AM are commonly found on the roots of forest trees like pine, oak, beech and eucalyptus. AM are the main organs for nutrient uptake in many woody plants, often connect seedlings to mature trees, and influence biogeochemical cycling and the composition of plant communities in a forest ecosystem. The abundant mycorrhizae found in the upper slope enhanced water uptake by the pines, mitigated drought stress, and thereby decreased the mortality from pine wilt disease (Akema and Futai, 2005). Mycorrhizal fungi are also able to utilize soil organic nitrogen and phosphorus pools. However, mycorrhiza also impart a considerable cost to the host plant, as mycorrhizal roots may consume a 2.5-fold greater amount of host carbon compared with non-mycorrhizal roots (Jones *et al.*, 1991). Pine seedling when inoculated with VAM showed a significant increase in shoot height and a doubling of shoot dry weight. The content of N, P and K in plants with VAM was higher than for uninfected control plants (Heslin and Douglas, 1986).The presence of mycorrhizae makes possible the coexistence of symbiotic organisms in hostile environment or in places where the competition is very strong. Beneficial effects on growth and biomass production of *Salix x dasyclados* inoculated with an ectomycorrhizal fungus were observed in a phytoextraction study conducted by Baum *et al.* (2006).

In addition to their protective role, mycorrhizae may contribute to the resistance of plant–microbial associations through enhanced degradation of organic pollutants in the mycorrhizosphere (Meharg and Cairney, 2000), thus lowering the bioavailable concentration of the pollutant in soil. VAM colonization of *Salix reinii* seedlings strongly depended on nearby established *S. reinii* shrubs. Growth and nitrogen content of seedlings increased significantly with number of associated VAM fungal species and VAM root tips (Nara and Hogetsu, 2004 and Nara, 2006). Sell *et al.* (2005) showed that VAM fungi like *Paxillus involutus* significantly enhanced total Cd extraction by *Salix viminalis* and *Populus canadensis*. Therefore VAM fungi became important tool for bioremediation of heavy metals. The beneficial effect of mycorrhizal associations is the enhanced uptake of mineral nutrients, namely phosphorus and increased photosynthetic activity by assimilating P and N in willow ectomycorrhizas (Reid *et al.*, 1983; *Jones et al.*, 1990; Tam and Griffiths, 1993; Smith and Read, 1997; Bougher *et al.*, 1990; Dosskey *et al.*, 1990; Rousseau and Reid, 1990 and Martins *et al.*, 1997). The importance of ectomycorrhiza in forest plantations has received much attention when it was observed that trees often fail to establish at new sites if the ectomycorrhizal symbiont is absent. This effect has been observed in exotic pine transplantation in different parts of the world. In Western Australia, *Pinus radiata* and *P. pinaster* failed to establish in nursery beds in the absence of mycorrhizal fungi (Lakhanpal, 2000). It was revealed that a strain of the ectomycorrhizal fungus *Paxillus involutus*, which synthesized significantly higher amounts of acid phosphatases than other tested strain of this species, also promoted the mycorrhiza formation and biomass production of willows (*Salix* spp.) more successfully (Hrynkiewicz *et al.*, 2010). Heinemeyer *et al.* (2007) described contradicting results in their *in situ* study of the ability of Lodge pole pine associated mycorrhiza to store soil C over a period of 1-year, where the fungus was thought to return plant surplus C directly back to the atmosphere. Labrecque *et al.* (1995) showed that fast growing willows (*Salix viminalis* and *discolor*) accumulated high contents of trace metals such as mercury, copper, lead, nickel, and zinc in their roots and stems from soils treated with wastewater sludge.The present studies were, therefore, carried out to know the status and role of AM fungi in rhizosphere of willow, pine and poplar seedling grown in the nursery.

MATERIAL AND METHODS

Seeds:

Willow, pine and poplar seeds were collected from the Department of Tree improvement and Genetic Resources, and sown in the nursery in RBD design at Experimental Farm of Tree improvement and Genetic Resources.

Isolation and identification:

For isolation of mycorrhizal fungi from rhizosphere of different economic trees soil samples were collected from 3 to 4 regions, 5-8 inches deep from the plant bases, mixed thoroughly and was made into a composite sample and brought to the laboratory for isolation . The technique used for isolation of fungal spores was estimated by wet sieving and decanting method (Gerdemann and Nicolson, 1963). The spores were picked from the counting plate through syringe under stereoscopic binocular microscope and examined for the counting of the number as well as descriptive characters for their identification. The distinct morphological characteristic used for identification and characterization was based on size and shape, colour, subtending hyphae and wall layer characteristics.

Spore population:

The spore population of AM fungi in soil was estimated by wet sieving and decanting method (Gerdemann and Nicolson, 1963). The soil sample drawn from each treatment was air dried in laboratory of which 100 gm sample was suspended in 350 ml water contained in 500 ml flask. This was stirred vigorously. Heavier particle were allowed to settle down for few seconds and the suspension was thereafter decanted through series of sieves (300, 250, 106 and 45µ) arranged one over another in descending order. The residue left in each sieve was thoroughly washed under tap water and after washing whatever residue left in the sieve was collected in a beaker and final volume was made upto 50 ml. Suspension was then transferred in to counting plate and examined under stereoscopic binocular microscope and the number of spore per 100 gm dry soil basis were thus calculated.

Characterization of AM fungi:

Mycorrhizal fungi obtained as spore of sporocarps or chlamydospores in soil through wet sieving and decanting method of Gerdemann and Nicolson (1963) were picked up with syringe and were mounted in lactophenol (Omar *et al.*, 1979). VAM fungal spores were characterized on the basis of their morphological characters like shape, size, structure of spore and attachment of subtending hypahe as per by synoptic key (Benny *et al.*, 2001; Morton and Redecker, 2001; Mukerji, 1996; Trappe, 1982 and Hall and Fish, 1979). Genus as well as species of mycorrhizal fungi was characterized by morphology of their resting spores and attachment of subtending hypha.

RESULTS AND DISCUSSION

The findings of the present study as well as relevant discussion have been presented under the following heads:

Isolation and identification:

Rhizospheric soil samples from different locations were subjected to the recovery of AM fungal spores. The soil samples of different locations showed different types of spores. All the recovered spores represent five genera namely; *Acaulospora*, *Gigaspora*, *Scutellospora*, *Glomus* and *Entrophospora*. The entire collected rhizosphere sample exhibited the presence of varied range of spore population in the soil profile. Various genera of AM fungi present in the rhizosphere of willow, pine and poplar were characterized based on morphological characteristics and presented in Table 3.

Spore population:

Number of mycorrhizal spores (per 50 g of soil) present in rhizosphere of willow, pine and poplar was observed with highest spore number (1380 and 540 in 106 µ and 250 µ sieve sizes, respectively) in the rhizosphere soil of *Pinus* sp. followed by *Populus tremula* sp. collected form Nursery raised in the Experimental Farm of the Department of Tree improvement and genetic resources, Dr.Y.S.Parmar University of Horticulture and Forestry, Nauni Solan. H.P. However, lowest spore number per 50 g in the rhizosphere was noticed in rhizosperic soil of *Salix babylonica* (1290 and 490 spores in 106 µ and 250 µ sieve size).

Table 1 : Population of AM spore in rhizosphere soils of willow, pines and poplar

Number of spore /50 g in the rhizosphere soil enumerated by two different sized sieves (µ)					
Willow (*Salix babylonica*)		Pine (*Pinus roxburghii*)		Poplar (*Populus tremula*)	
106 µ	250 µ	106 µ	250 µ	106 µ	250 µ
1290	490	1380	540	1300	530

Table 2 : Enumeration of AM spores in willow, pines and poplar under 10X microscopic field

Av. No. of spores in Rhizosphere soils enumerated by two different sized sieves (µ)					
Willow (*Salix babylonica*)		Pines (*Pinus roxburghii*)		Poplar (*Populus tremula*)	
106 µ	250 µ	106 µ	250 µ	106 µ	250 µ
21.08	9.44	34.14	25.88	27	12.55

Table 3 : Descriptive identification of different mycorrhizal fungi of willow, pine and poplar plants

Isolated Genera	Tree source	Characteristic features of VAM fungi
Glomus magnicaulis	Willow	Spores formed singly in soil; light brown with varying amount of adhering debris, globose to subglobose measuring 132-179 µm in size.
G. macrocarpus	Willow	Sporocarps are fragmentary, Spores are usually slightly longer than wide, subglobose or globose to irregular,110-120 µm.
Gigaspora gigentea	Willow	Spore formed terminally or laterally on a bulbous sporogenous cell; greenish yellow, globose to subglobose, 245-264 x 262-366 µm.
Acaulospora bireticulata	Willow	Azygospores with grayish brown, globose to subglobose vesicle, surface ornamented with a polygonal reticulum, 274-412 µm size.
Entrophosphora spp.	Willow	Azygospores smaller in diameter, multiple layered walls, walls are thinner and hyaline measuring 48-60 µm in diameter.
Scutellospora pellucida	Willow	Spores singly in soil formed terminally on a bulbous subtending hyphae; hyaline to yolk yellow, globose to subglobose, sometimes ovoid size ranges between 130-155 x 160-235 µm.
Glomus fasciculatum	Willow	Pale yellow to bright brown, 72-123 µm size, Globose to sub-globose, Spores produced directly with one or more subtending hyphae, 3 layered walled.
Acaulospora scrobiculata	Willow	Spores with light brown, surface evenly pitted with depressions/profuse minute pores, consisting of wall layers when ruptured
Acaulospora spinosa	Willow	Cream to pale orange brown, 138-218 µm size, Spores formed from sporiferous saccule. Double walled, Globose to sub-globose
Glomus clarum	Willow	Shape of spore is globose to subglobose, yellowing brown measuring 70-276 µm.
Acaulospora scrobiculata	Popular	Spores with light brown, surface evenly pitted with depressions/profuse minute pores, consisting of wall layers when ruptured
Aculospora bireticulata	Popular	Azygospores with grayish brown, globose to subglobose vesicle, surface ornamented with a polygonal reticulum, 274-412 µm size.
Glomus mosseae	Popular	Brown to orange-brown, 194-200 µm size, Globose to sub-globose, Hyphae double layered, 3 walled
Acaulospora gerdemannii	Popular	Spores red to brown, round to spherical, 100-200 µm in diameter, surface of outermost wall layer ornamented with cerebriform folds upto 12 µm in breadth
Acaulospora rehmii	Popular	Spores reddish brown in color, >100 µm in diameter, almost sessile, round and spherical, spore wall not much distinct, ornamented with labyrinthine channels or network on the outer surface
Glomus mossae	Pinus	Chlamydospores yellow to brown, globose to subglobose sometimes ellipsoid to irregular, spore size ranges between 122-127 µm.
G. fasciculatum	Pinus	Pale yellow to bright brown, 72-123 µm size, Globose to sub-globose, Spores produced directly with one or more subtending hyphae, 3 layered walled
Glomus taiwanensis	Pinus	Chlamydospores yellowing brown measuring 42- 86 µm. Double walled layer.
G. gigentea	Pinus	Spore formed terminally or laterally on a bulbous sporogenous cell; greenish yellow, globose to subglobose, 245-264 x 262-366 µm.
Aculospora scrobiculata	Pinus	Spore borne singly in the soil; produced laterally on the neck of a sporiferous saccule; yellowish white to pale yellow; globose to subglobose; 120- 142 µm diam.
Aculospora alpine	Pinus	Spores single in soil, sporiferous saccule; pale yellow to deep yellow to orange-brown; globose to subglobose; rarely ovoid to irregular, 60-81 µm.
A. tuberculata	Pinus	Spore red orange to dark red brown, Globose to subglobose in shape,125-176 µm.

Number of AM fungal spores per microscopic field:

Average number of AM fungal spores per microscopic field (10X) was significantly maximum in 106 µ (34.14 spores) from pine rhizosphere soil than in 250 µ (25.88 spores) followed by poplar whereas lowest number of AM fungal spores were observed in *Salix babylonica* with 21.08 in 106 µ and 9.44 in 250 µ sieve size. This shows the prevalence of more AM population with pines and poplar and then the willows.

In the present study, the population dynamics of AM fungi from willow, popular and pine was determined by collecting the resting spores from different soils. As AM fungi are widespread, they occurred in almost all soils, but with a variation both in number and type of spores and sporocarps. The AM fungal species belonging to the genera of *Glomus*, *Acaulospora*, *Gigaspora*, *Scutellospora* and *Entrophospora* were isolated**.** It is well known fact that nutritionally deficient soils, phosphorus soils in particular, harbor more AMF. The present research findings also support this. In the present study, *Glomus* dominated the soils supporting pine and willow seedlings followed by *Acaulospora* sp., which was similar with earlier reports of AM association on other forest trees (Baum *et al.*, 2006; Akema and Futai, 2005; Lakhanpal 2000 and Hrynkiewicz *et al.*, 2010). However, the *Acaulospora* spp. was found more in frequency in poplar than *Glomus* sp. Two different species such as *Entrophosphora* sp. and *Scutellospora pellucida* were also observed in case of willows which were identified based on description of the characteristics of sporophores, shape, size, colour, wall layers of isolated AM fungi from the rhizosphere.

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