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The root of *Michelia champaca* L. was studied under green house condition for arbuscular mycorrhizal fungal morphology. Both *Paris* and *Arum* type of arbuscular mycorrhizal fungal morphology were found in the plant species. The co-occurrence of both the types of arbuscular mycorrhizal fungal morphology is reported to be first in this plant species.

Key words: *Michelia champaca,* arbuscular mycorrhizal fungi, arbuscular mycorrhizal fungal morphology, AM fungal colonization

INTRODUCTION

Gallaud (1905) distinguishes the arbuscular mycorrhizal (AM) fungal morphology into *Arum*-type and *Paris*-type. Most cultivated plants produce *Arum*-type mycorrhizal colonization consisting of intercellular hyphae and arbuscules, whereas many trees and forest herbs form *Paris*-type intracellular hyphae, coils and arbusculate coils (Dickson, 2004). Morphological types of AM fungi have been examined in many plant taxa (Yamato and Iwasaki, 2002; Kubota and Hyakumachi, 2004; Yamato, 2004; Dickson 2004, Matsuda *et al*., 2005; Ahulu *et al*. 2005; Kubota *et al*.; 2005; Muthukumar *et al*. 2006; Muthukumar and Prakash, 2009).

Michelia champaca L. (Magnoliaceae) is famous for its striking appearance with large, very aromatic yellow blossoms, smooth trunk, and large ovate, glossy leaves. The species has high economic value for perfume and timber industries in India. Flower buds of *M. champaca* are used in most of the herbal preparations for several diseases and possess active constituents (Jarald *et al*. 2008).

Paris type of AM fungi morphology is reported earlier in *M. champaca* (Muthukumar *et al*. 2006; Das and Kayang, 2010). The above study is conducted from the roots collected from plants growing in natural ecosystem and plantations. However, our present study is conducted to evaluate the morphology of AM fungal morphology from the plants grown in greenhouse conditions inoculated with AM fungi.

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MATERIALS AND METHODS

Plant materials

The seeds of *M. champaca* were disinfected with 1% sodium hypochlorite. Seeds were then soaked in distilled water for two days. The arils of seed were removed and 8-10 seeds were placed on double sterilized river bed sand: pine forest soil (1:1w/w) in 200 ml disposable plastic containers. The germination set up were kept in B.O.D. incubator at 25°C under white fluorescent tubes (photoperiod 12 h) and watered whenever it was required to keep the soil mixture moist. After one month, about 90 % of the seeds germinated.

Fungal material

For bulk production of inoculum, seeds of *Paspalum notatum* Flügge procured from Indian Grassland and Fodder Research Institute, Palampur, India were placed on double sterilized river bed sand:

pine forest soil (1:1) on 3 kg garden pots which were inoculated by spreading 2-3 cm below the seeds with 20 g of supplied mycorrhizal inoculant (*Glomus intraradices* Schenck & Smith AM WG19) collected from TERI (The Energy and Resource Institute, India).

Inoculation

The germinated seedlings of *M. champaca* were transferred in sterilized 2 kg garden bags containing same sand and soil mixture. On November, 2009 inoculation was done with *G. intraradices* by adding 10 g of dried crushed inoculum raised with *P. notatum* all around the root of *M. champaca*, dug with a glass rod of about 12 cm deep and seedling was placed in it. The set up was maintained in green house and watered whenever it was required every week.

Sampling

For AM fungal morphology and colonization studies, three mycorrhizal inoculated seedlings were harvested from greenhouse after 180 days of inoculation. The roots were cut and made into 1 cm approximately and all the three subsamples were made into composite samples.

AM fungal morphology and colonization

The washed root samples were processed and stained with black Faber Castell stamp pad ink (Das and Kayang, 2008). Stained root segments of approximately 1 cm long were mounted on slides in lactoglycerol and examined for AM fungal morphology under light microscope (Olympus 41209 & LEICA DM 1000). The colonization in the root segments from the plant were estimated using the magnified intersection method of McGonigle *et al*. (1990).

Data analysis

Means and standard errors were calculated for the mycorrhizal colonization. Means were separated using *post-hoc* test (Fischer's LSD). All the statistical analysis was done using Statistica software 9.0.

RESULTS

In the plants grown in greenhouse inoculated with AM fungi, root hairs along with extraradical hyphae and both types of AM fungal morphology were observed. The mean mycorrhizal structural colonization of arbuscules, vesicles and hyphae were observed to be 29.67 ± 2.72, 9.70 ±1.49 and 65.07 (±5.17), respectively. There were significant differences in between mycorrhizal structural colonization (p<0.001). *Paris*-type AM fungal morphology was observed in the roots with intracellular hyphal coils (Fig. 1 a & b) and *Arum*- type was detected in a few segments with penetrating arbuscules into the cells (Fig. 1 c & d).

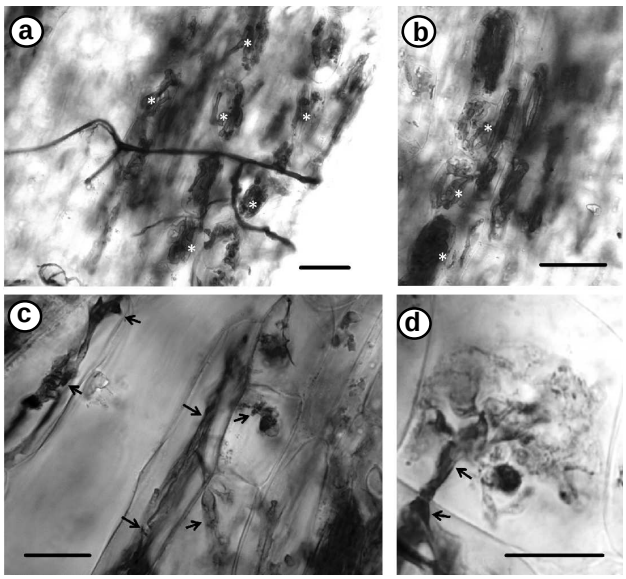

Fig.1. Light microscopy images of mycorrhizal colonization of root of *Michelia champaca* plants from greenhouse (a-d). (a&b) Root portion showing intracellular hyphal coils [asterisk] exhibiting *Paris*-type of AMF morphology. Scale bar=200 ìm & 100 ìm (c) Root segment showing intercellular hyphae with penetrating hyphae into the cells. Scale bar=100 ìm. (d) Arbuscules showing *Arum*-type of

DISCUSSION

The co-occurrence of both the types of arbuscular mycorrhizal fungal morphology is reported to be first in this plant species. The intracellular hyphal coils and intracellular arbusculate coils indicate *Paris* type and intercellular hyphae with penetrating arbuscules into the cortical cells specify *Arum* type AM fungal morphology in this species. However, *Paris* type morphology was reported earlier (Muthukumar *et al*., 2006; Das and Kayang, 2010). The coincidence of different AM fungal morphology within the same root is consistent with the previous report (Kubota *et al*, 2005; Kubota and Hyakumachi, 2004). Bonfante-Fasolo and Fontana (1985) reported co-occurrence of *Arum* and *Paris* type morphologies in the same root system, but

Paris type morphology was less. This is in contrast to the study of Kobato *et al*. (2005) where in tomato and cucumber both morphological types were well represented. *Lycopersicon esculentum* also formed both *Arum* and *Paris* type but with high occurrence of *Paris* type AM fungal morphology (Kubota and Hyakumachi, 2004) which is consistent with our study.

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