

Original Research Article

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Effect of Arbuscular Mycorrhiza Fungi (AMF) on Early Seedling Growth of Some Multipurpose Tree Species

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ABSTRACT

In present study, three important multipurpose tree species, namely *Aegle marmelos*, *Leucaena leucocephala* and *Parkia roxburghii* were inoculated with better performing native arbuscular mycorrhiza fungi (AMF) species of the region. The AMF species viz., *Acaulospora scrobiculata* and *Rhizophagus irregularis* were inoculated individually and in combination under nursery conditions. Study consisted of four treatments i.e. three mycorrhizal [individual inoculation (*A. scrobiculata*, *R. irregularis*) and combined inoculation (*A. scrobiculata* + *R. irregularis*)] and a control (un-inoculated). All mycorrhizal treatments (individual and combined) significantly increased plant height, collar diameter, shoot, root and total dry weights of seedlings over control, barring few exceptions. This can be due to more volume of soil exploration for available nutrients and water by mycorrhizal plants than non-mycorrhizal plants, which improves plant biomass. The mycorrhizal dependency (MD) for AMF inoculants ranged from 42.6 to 44.3% (mean MD: 43.3%) in *A. marmelos*, -30.3 to 25.2% (Mean MD: 2.5%) in *L. leucocephala* and 51.2 to 58.8% (mean MD: 55.5%) in *P. roxburghii*. The seedling quality index, computed on the basis of growth parameters, also, showed that mycorrhizal treatments significantly improved plant health. Thus, based on the results obtained from this study, we may expect a better establishment, survival and growth of the mycorrhizal inoculated seedlings in the fields.

Keywords

Aegle marmelos,
Arbuscular Mycorrhiza
Fungi, *Leucaena
leucocephala*, *Parkia
roxburghii*, Seedling
quality index.

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Introduction

Arbuscular mycorrhiza fungi (AMF) are obligate symbionts that colonize the roots of most cultivated plant species (Kumar *et al.*, 2007; Meddad-Hamza *et al.*, 2010). Mycorrhizal symbiosis can be found in nearly all types of ecological situations and most plant species are able to form this symbiosis naturally (Jaizme-Vega *et al.*, 2006; Shukla *et al.*, 2012). These associations occur naturally in the fields, favoring plant development by increasing nutrient uptake, growth rates and

hormonal activities (Shukla *et al.*, 2010; Hashmi *et al.*, 2010; Jha *et al.*, 2012, 2014, 2015). The positive effects of mycorrhizae on seedlings survival and growth under nursery conditions are well documented (Smith *et al.*, 2009; Guissou *et al.*, 2016).

To develop any agroforestry/forestry model, healthy seedlings of woody perennials are pre-requisite (Jha *et al.*, 2015). When the nursery raised seedlings are transplanted, they

may face transplantation shocks in the fields and consequently plants become weaker and poorly established (Hartmann and Kester, 1986). Navarro-Garcia *et al.*, (2011) postulated that preconditioning of young seedlings with efficient AMF not only makes plant stronger but also helps in their establishment in fields. Early inoculation of seedlings with AMF under nursery conditions can be beneficial in two ways i.e. superior and stronger growth of the seedlings, and better performance in the fields. But before going for AMF inoculation in nurseries, better understanding of the mycorrhizal system for tree species, nursery and out-planting site is required (Turjaman *et al.*, 2008). Caravaca *et al.*, (2003) suggested that native AMF species can produce more vigorous seedlings than non-native species. Moreover, several researchers have suggested that consortium of AMF species give better results in terms of growth as compared to individual AMF species (Jansa *et al.*, 2008; Wehner *et al.*, 2010; Shukla *et al.*, 2014). However, Jansa *et al.*, (2008) advocated that consortium of mycorrhizae, having multiple AMF species varies in competitive ability. Therefore, in present study, better performing native AMF species of the region (*Acaulospora scrobiculata* Trappe and *Rhizophagus irregularis* (Blaszk., Wubet, Renker & Buscot) C. Walker & A. Schüßler) were tested individually and in combination for their growth promotional activities under nursery conditions in important multipurpose tree species, namely *Aegle marmelos* (L.) Correa (Family: Rutaceae; Bael), *Leucaena leucocephala* (Lam.) de Wit (Family: Fabaceae; Subabul) and *Parkia roxburghii* G. Don (Fabaceae; Tree bean).

A. marmelos and *L. leucocephala* are being evaluated for their suitability in various agroforestry models at ICAR-Central Agroforestry Research Institute, Jhansi. *P. roxburghii* was also included in the study, as

it is one of the important tree legumes (Firake *et al.*, 2013). According to Rathi *et al.*, (2012), *P. roxburghii* is known for its economic value as vegetable, medicinal, industrial and fire wood. It is a fast growing, easier to grow and hardy in nature. It is also being tested in agroforestry systems (Teklehaimanot, 2004).

Materials and Methods

The study was conducted at ICAR-Central Agroforestry Research Institute (CAFRI), Jhansi (25° 27' N latitude, 78° 35' E longitude and at 271 m above msl) during 2016–2017. On agro-ecological zone map of India, Jhansi lies in the hot semi-arid region. The area receives annual rainfall between 700 and 1150 mm mostly during South West monsoon period (Mid June–September) with an average of 52 rainy days per year. Mean maximum temperature ranges from 47.4°C (June) to 23.5°C (January) and mean minimum temperature from 27.2°C (June) to 4.1°C (December). Diurnal variation in temperature is quite high. May and June are the hottest months. The maximum recorded temperature on a particular day often touches 47–48 °C during summer.

Separate net house experiments were conducted for *A. marmelos*, *L. leucocephala* and *P. roxburghii*, to study the response of their seedlings to mycorrhizae fungi. Two AMF species, namely *A. scrobiculata* and *R. irregularis* were used individually and in combination, in the present study. Both species were selected on the basis of their relative abundance in our region and their ability to improve plant health (Hashmi *et al.*, 2010; Jha *et al.*, 2012; Shukla *et al.*, 2016). A total of four treatments viz., *A. scrobiculata* (individual); *R. irregularis* (individual); *A. scrobiculata* + *R. irregularis* (combined) and control (un-inoculated seedlings) were employed in the study. Each treatment was

replicated eight times in completely randomized design (CRD). Experiment was carried out in red soil (alfisol; pH: 6.29 (1:2.5 H₂O), EC: 134 μS cm⁻¹, organic carbon: 0.27%, Olsen P: 2.5 ppm), collected from experimental farm of the institute. Mixture of red soil: sand: FYM (2:1:1) was used as potting substrate. Each polythene bag containing 3 kg substrate represented a unit. Treatments were imposed in the substrate by mixing mycorrhizal inocula with substrate @ 10 kg inoculum in 1000 kg substrate. For individual inoculations (either *A. scrobiculata* or *R. irregularis*), 10 kg inocula were mixed in 1000 kg substrate but for combined inoculation (*A. scrobiculata* + *R. irregularis*), 5 kg of each inoculum were mixed in 1000 kg substrate. This mycorrhiza-substrate mixture was filled in respective polythene bags. Surface sterilized seeds of *A. marmelos*, *L. leucocephala* and *P. roxburghii* were sown in the bags, and bags were transferred to net-house and watered as and when required.

After germination, one healthy plant was maintained in each bag. After four month of sowing, the effect of mycorrhizae inoculations were evaluated based on five morphological variables viz. plant height (cm), measured with a ruler; collar diameter (mm), measured by using a digital caliper; shoot dry weight (g), root dry weight (g) and total dry weight (g plant⁻¹). Dry weights were determined, after drying the material in a hot air oven at 70 °C for 72 hrs. Mycorrhizal dependency (MD) was calculated in terms of plant growth as [(M-NM)/M] × 100, using dry weights of individual mycorrhizal plants (M) and mean dry weight of corresponding non-mycorrhizal (NM) plants (Plenchette *et al.*, 1983). The data recorded on above mentioned morphological features of the seedlings were used to compute seedling quality parameters. The seedling quality index was, also, calculated by using the formula given by Dickson (1960):

$$\text{Seedling Quality Index} = \frac{\text{Total dry weight of plant (g)}}{\frac{\text{Plant height (cm)}}{\text{Collar diameter (mm)}} + \frac{\text{Shoot dry weight (g)}}{\text{Root dry weight (g)}}}$$

Statistical analysis

Data were subjected to analysis of variance using the ANOVA procedure of the Web Agri Stat Package developed by ICAR Research Complex Goa, India. Statistical significance was determined at the 5% probability level. Means were compared by applying Duncan's Multiple Range Test (DMRT).

Results and Discussion

In *A. marmelos*, all mycorrhizal treatments significantly increased plant height, collar diameter, shoot, root and total dry weights over control. Maximum plant height (16.4 cm) was recorded in *R. irregularis*, followed

by *A. scrobiculata* (15.3 cm) and *A. scrobiculata* + *R. irregularis* (14.6 cm). However, differences among mycorrhizal treatments for plant height, shoot, root and total dry weight were statistically non-significant. Maximum collar diameter (6.00 mm) was recorded in *A. scrobiculata* which was comparable with *R. irregularis* and significantly superior to dual inoculation (*A. scrobiculata* + *R. irregularis*). MD was recorded maximum (44.3%) in *R. irregularis* which was at par with other mycorrhizal treatments (Table 1). In *L. leucocephala*, maximum plant height (74.9 cm) was recorded in *R. irregularis* which was at par with *A. scrobiculata* and significantly higher than combined inoculation. More or less

similar results were obtained for shoot, root and total dry weight. Difference in various mycorrhizal treatments for collar diameter was statistically non-significant. MD was recorded maximum (25.2%) in *R. irregularis* which was at par with *A. scrobiculata* (Table 2). In *P. roxburghii*, all the treatments significantly increased the studied parameters over control. Maximum plant height (59.7 cm) was recorded in *R. irregularis*, followed by *A. scrobiculata* and *A. scrobiculata* + *R. irregularis*. Higher values of collar diameter (8.6 mm), shoot (14.88 g), root (4.56 g) and total dry weight (19.45 g) were recorded in *A. scrobiculata*-inoculated plants which were at par with *R. irregularis*. Maximum dependency for dry matter production in *P. roxburghii* (58.8%) was recorded in *A. scrobiculata* which was at par with *R. irregularis* (Table 3). Thus, the results of

present study showed that all mycorrhizal treatments (either individual or combined inoculation) increased growth of the test plant species when compared with un-inoculated. This could be due to more volume of soil exploration for available nutrients and water by mycorrhizal plants than non-mycorrhizal plants. Better nutrients, especially phosphorus and water uptake by AM-inoculated plants generally leads to secondary indirect effect such as improved plant biomass (Jha *et al.*, 2012; Shukla *et al.*, 2012). Beneficial effects of AM inoculation on growth and health of various plant species, including *A. marmelos*, *L. leucocephala* and *Parkia* species have been reported by several researchers (Habte and Fox 1989; Manjunath *et al.*, 1989; Awotoye *et al.*, 1992; Osundina 1995; Guissou *et al.*, 1998; Schneider *et al.*, 2013; Verma *et al.*, 2015).

Table.1 Effect of arbuscular mycorrhizal fungi (AMF) inoculation on growth parameters of *Aegle marmelos*

Treatment	Plant height (cm)	Collar diameter (mm)	Dry weight (g)			MD (%)	SQI
			Shoot	Root	Plant		
<i>A. scrobiculata</i>	15.3 ^a	6.0 ^a	3.15 ^a	4.12 ^a	7.27 ^a	42.6 ^a	2.177 ^a
<i>R. irregularis</i>	16.4 ^a	5.5 ^a	2.87 ^a	4.39 ^a	7.26 ^a	44.3 ^a	1.987 ^a
<i>A. scrobiculata</i> + <i>R. irregularis</i>	14.6 ^a	4.6 ^b	2.78 ^a	4.42 ^a	7.20 ^a	42.9 ^a	1.891 ^a
Un-inoculated	9.8 ^b	3.3 ^c	1.64 ^b	2.32 ^b	3.96 ^b	--	1.093 ^b

Different letters within the column indicate significant differences

Table.2 Effect of arbuscular mycorrhizal fungi (AMF) inoculation on growth parameters of *Leucaena leucocephala*

Treatment	Plant height (cm)	Collar diameter (mm)	Dry weight (g)			MD (%)	SQI
			Shoot	Root	Plant		
<i>A. scrobiculata</i>	74.8 ^a	7.5 ^a	8.41 ^{ab}	3.58 ^{ab}	11.99 ^{ab}	12.6 ^a	0.951 ^a
<i>R. irregularis</i>	74.9 ^a	8.0 ^a	9.70 ^a	4.30 ^a	13.99 ^a	25.2 ^a	1.208 ^a
<i>A. scrobiculata</i> + <i>R. irregularis</i>	57.0 ^b	6.9 ^a	5.49 ^b	2.38 ^b	7.87 ^b	-30.3 ^b	0.746 ^a
Un-inoculated	58.8 ^b	7.0 ^a	6.28 ^b	3.07 ^{ab}	9.35 ^b	--	0.894 ^a

Different letters within the column indicate significant differences

Table.3 Effect of arbuscular mycorrhizal fungi (AMF) inoculation on growth parameters of *Parkia roxburghii*

Treatment	Plant height (cm)	Collar diameter (mm)	Dry weight (g)			MD (%)	SQI
			Shoot	Root	Plant		
<i>A. scrobiculata</i>	57.3 ^a	8.6 ^a	14.88 ^a	4.56 ^a	19.45 ^a	58.8 ^a	1.936 ^a
<i>R. irregularis</i>	59.7 ^a	8.3 ^{ab}	14.36 ^{ab}	3.53 ^{ab}	17.89 ^{ab}	56.5 ^a	1.587 ^b
<i>A. scrobiculata</i> + <i>R. irregularis</i>	54.4 ^a	7.8 ^b	12.73 ^b	3.34 ^b	16.07 ^b	51.2 ^b	1.484 ^b
Un-inoculated	29.9 ^b	6.9 ^c	5.70 ^c	2.00 ^c	7.70 ^c	--	1.034 ^c

Different letters within the column indicate significant differences

The results, also, showed that the dependency of test plant species for dry matter production on the inoculated fungi i.e. MD varied from 42.6 to 44.3% (mean MD: 43.3%) in *A. marmelos*, -30.3 to 25.2% (Mean MD: 2.5%) in *L. leucocephala* and 51.2 to 58.8% (mean MD: 55.5%) in *P. roxburghii*. Further, results showed that plants inoculated with individual inoculants (either *A. scrobiculata* or *R. irregularis*) showed higher MD value than combined inoculation (*A. scrobiculata* + *R. irregularis*) in all studied plant species.

This could be due to functional compatibility between AMF and plant species (Genre and Bonfante, 2005). According to de Novais *et al.*, (2014), different AMF species (even different strains of AMF) have different strategy for colonization of plant roots, which may be related to taxonomic differences at the family level. AMF species, having different mode of actions were included in the present study. *A. scrobiculata* forms vesicles inside root, spores outside the roots, does not produce sporocarps, etc. while *R. irregularis* produces vesicles, spores inside the roots and sporocarps (Shukla *et al.*, 2010; Jha *et al.*, 2014, 2015). This could explain the reason that why tested AMF species did not work synergistically with each other. It was postulated that there may be a preference for a particular AMF species by studied plant species. Seedling quality index computed on

the basis of growth parameters suggested that in *A. marmelos*, all treatments significantly improved its quality, wherein maximum seedling quality index (2.177) was recorded in *A. scrobiculata*-inoculated plants, followed by *R. irregularis* and combined inoculation (*A. scrobiculata* + *R. irregularis*). In *L. leucocephala*, it was recorded maximum (1.208) in *R. irregularis*, followed by *A. scrobiculata*.

In *P. roxburghii*, all the treatments significantly improved the seedling quality, wherein maximum seedling quality index (1.936) was recorded in *A. scrobiculata*-inoculated plants, followed by *R. irregularis*.

Thus, from present study it is concluded that the seedling quality parameters of all the tested plant species have been improved, when they were inoculated with AMF under nursery conditions. Thus, we may expect a better establishment, survival and growth of these AM-inoculated seedlings in the fields after out planting.

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Conflict of interest

Authors declared that there is no conflict of interest.

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