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Naresh Kumar

ICAR-Central Agroforestry Research Institute, Jhansi, Uttar Pradesh, India

Anil Kumar

ICAR-Central Agroforestry Research Institute, Jhansi, Uttar Pradesh, India

Ashok Shukla ICAR-Central Agroforestry Research Institute, Jhansi, Uttar Pradesh, India

AR Uthappa

ICAR-Central Agroforestry Research Institute, Jhansi, Uttar Pradesh, India

Sudhir Kumar

ICAR-Central Agroforestry Research Institute, Jhansi, Uttar Pradesh, India

Correspondence Anil Kumar ICAR-Central Agroforestry Research Institute, Jhansi, Uttar Pradesh, India

Effect of arbuscular mycorrhizal inoculations on growth and seedling quality of four *Leucaena* species

Naresh Kumar, Anil Kumar, Ashok Shukla, AR Uthappa and Sudhir Kumar

Abstract

An experiment was conducted to study the suitability of arbuscular mycorrhizal (AM) fungi for inoculation of four *Leucaena* species, namely *L. collinsii*, *L. shannoni*, *L. diversifolia* and *L. leucocephala* for assessing their effect on growth and seedling quality index (SQI) at nursery stage. Study consisted of three mycorrhizal treatments (*Acaulospora scrobiculata*, *Rhizophagus irregularis* and *A. scrobiculata* + *R. irregularis*) and a control. Inoculation of *A. scrobiculata* significantly increased only root length, while inoculation of *R. irregularis* increased almost all studied parameters. Among tested *Leucaena* species, *L. collinsii* and *L. leucocephala* showed better response towards inoculated fungi. Plants inoculated with *R. irregularis* showed higher mycorrhizal dependency (20.75%) than other inoculants. *L. leucocephala* recorded maximum mycorrhizal dependency (MD) followed by *L. collinsii*. *R. irregularis* increased SQI significantly, irrespective of tested *Leucaena* species. Thus, the results suggested that the seeds of two *Leucaena* species viz., *L. leucocephala* and *L. collinsii* may be inoculated with *R. irregularis* to obtain more vigorous seedlings.

Keywords: arbuscular mycorrhizal inoculation, L. collinsii, L. shannoni, L. diversifolia and L. leucocephala

Introduction

Arbuscular mycorrhizal (AM) fungi are obligate symbiont, occurring in nearly all natural and agricultural soils and colonize roots of most of the plant species (Smith and Read, 1997; Oseni *et al.*, 2010) ^[30, 23]. The primary effect of AM fungi on the host plant is an increase in nutrients uptake and plant growth (Kumar *et al.*, 2017) ^[17]. Plants with mycorrhiza are potentially more effective in nutrient and water uptake (Shukla *et al.*, 2010) ^[29] and less susceptible to diseases (Shukla *et al.*, 2014) ^[27]. They can increase uptake of nutrients especially which are relatively immobile in the soil such as phosphorus (P) and consequently increase the plant biomass and growth (Ryan and Angus, 2003; Jha *et al.*, 2014) ^[26, 24].

Genus Leucaena, member of family Leguminosae, consists of 22 identified species (Brewbaker, 2016)^[3]. Botanical literature claims a sum of 55 species. The only valid ones appear to be L. collinsii, L. diversifolia, L. esculenta, L. lanceolata, L. leucocephala, L. macrophylla, L. pulverulenta, L. retusa, L. shannoni and L. trichodes. All these species have value for the tropics but only L. leucocephala (Lam.) de Wit has been exploited extensively so far (National Research Council, 1984)^[21]. At ICAR-Central Agroforestry Research Institute (CAFRI), Jhansi, 25 germplasms of Leucaena species (L. collinsii, L. diversifolia, L. *leucocephala* and *L. shannoni*) have been maintained (Uthappa *et al.*, 2015)^[33]. Positive effect of AM inoculation on growth of L. leucocephala has been reported by various researchers under different conditions (Manjunath et al., 1989; Dixon et al., 1993; Habte et al., 2011; Zhan et al., 2016)^[19, 9, 10, 34]. Ramadhani et al. (2015)^[25] suggested that the effectiveness of AM inoculations can be different among species or even among varieties (cultivars) of a species. According to Chakravarty et al. (2018)^[5], different species of a genus can exhibit variable response in terms of mycorrhizal dependency (MD) i.e. the dependency of plant species on AM inoculation for dry matter production. Differences in the MD values of species of any genus could be due to the ability of plant roots to acquire P from soil (Tawaraya, 2003) [31]. Thus, selection of optimal relationship between AM inoculants and host plants could result in better growth of the plant. Hence, a study was carried out to test the response of four different Leucaena species to AM inoculation.

Materials and Methods

Separate experiments on effect of AM inoculations on growth and seedling quality of four species of Leucaena, namely L. collinsii, L. shannoni, L. diversifolia and L. leucocephala were conducted at ICAR-CAFRI, Jhansi (78°17'E, 24°11'N) under net-house conditions. The study consisted of four treatments viz., inoculations with Acaulospora scrobiculata, *Rhizophagus irregularis, A. scrobiculata* + *R. irregularis* and a control. Seven replicates were maintained for each treatment in completely randomized design. Red soil (alfisol; pH- 6.5, EC- 134 µScm⁻¹, OC- 0.27%, Olsen P- 2.5 ppm) was used as substrate, which was filled in plastic pots (4-5 kg capacity) and AM inoculum (50 g) was applied 4-5 cm below the seed as per treatments. The pots were transferred to net-house and watered as per need. Thinning of the plants was carried out 15 days after sowing, leaving one plant per pot. The seedlings were harvested after three months and observations on plant height (cm), root length (cm), collar diameter (mm), dry shoot biomass (g) plant⁻¹ and dry root biomass (g) plant⁻¹ were recorded. MD and SQI were calculated by using the formulae given by Plenchette et al. (1983)^[34] and Dickson (1960)^[7], respectively.

Mycorrhizal dependency (%) =
$$\frac{M - NM}{M} \times 100$$



The data were analyzed statistically by using a general linear model for analysis of variance in a completely randomized design. Least significant difference (LSD_{0.05}) was used to compare treatment differences.

Results and Discussion

Effect of AM inoculations on growth

Among treatments, maximum plant height was recorded in *R. irregularis*, which was significantly higher than other treatments. Remaining two treatments (*A. scrobiculata* and *A. scrobiculata* + *R. irregularis*) were at par with control. Among tested *Leucaena* species, maximum plant height was recorded in *L. collinsii*, followed by *L. shannoni*, *L. diversifolia* and *L. leucocephala*. Two-way interaction between the treatments and tested species was found significant. *R. irregularis* and *A. scrobiculata* + *R. irregularis* in *L. collinsii*, and all the treatments in *L. leucocephala* significantly increased the plant height (Table 1).

Among different treatments, single inoculations of A. scrobiculata and R. irregularis significantly increased root length. Remaining treatment (A. scrobiculata + R. irregularis) was found at par with control. On the other hand, maximum root length was recorded in L. shannoni, followed by L. collinsii, L. leucocephala and L. diversifolia. Single inoculations of A. scrobiculata and R. irregularis significantly increased root length of L. leucocephala (Table 1).

Differences in collar diameter in different AM treatments were found non-significant. However, results showed that AM inoculations increased the diameter when compared with control. Among tested species, maximum collar diameter was recorded in *L. shannoni*, followed by *L. collinsii*, *L. diversifolia* and *L. leucocephala*. Two-way interaction was found non-significant; hence comparison could not be made (Table 1).

Effect of AM inoculations on dry biomass

Maximum shoot dry biomass was recorded in R. irregularis, which was significantly higher than other treatments. Remaining treatments (A. scrobiculata + R. irregularis and A. scrobiculata) were found statistically at par with control. Among tested species, maximum shoot dry biomass was recorded in L. diversifolia which was at par with L. shannoni and L. collinsii. In L. collinsii, two treatments (R. irregularis and A. scrobiculata + R. irregularis) and in L. leucocephala, all treatments significantly increased the dry biomass (Table 2). More or less similar result was recorded in case of root dry biomass. Single inoculation of R. irregularis significantly increased root biomass. Maximum root biomass was recorded in L. shannoni, followed by L. collinsii and L. diversifolia. Two-way interaction was statistically non-significant (Table 2). Total dry biomass was significantly increased by R. irregularis. Among tested species, maximum biomass was recorded in L. shannoni, followed by L. collinsii, L. diversifolia and L. leucocephala. In L. shannoni, R. irregularis; in L. collinsii, R. irregularis and A. scrobiculata + R. irregularis; and in L. leucocephala, all AM treatments significantly increased total biomass (Table 2).

Mycorrhizal dependency (MD) of various AM treatments ranged from 4.65 to 20.75%. Among AM fungi, maximum MD value was recorded for *R. irregularis* (20.75%), which was significantly higher than *A. scrobiculata* + *R. irregularis* (8.11%) and *A. scrobiculata* (4.65%). Among tested species, *L. leucocephala* showed maximum dependency on AM inoculation for dry matter production followed by *L. collinsii* (Table 3).

Thus, the results showed that various AM treatments significantly increased the studied parameters. Plant height was increased by 4.4 to 17.1%, root length by -6.3 to 12.0%, collar diameter by 0.8 to 7.0%, shoot biomass by 4.4 to 27.8%, root biomass by 9.1 to 28.2% and total biomass by 6.9 to 28.1%, over control. The increase in above mentioned parameters can be attributed to the increase in soil volume explored for nutrients/ water uptake by mycorrhizal plants as compared to non-mycorrhizal ones (Shukla et al., 2012)^[28]. Results also showed that different tested AM fungi showed variable effects. Single inoculation of A. scrobiculata significantly increased only one parameter (root length) while single inoculation of R. irregularis increased most of the studied parameters (plant height, root length, shoot and root dry biomass). Such variable effects of the fungi could be due to differences in the uptake of P and other nutrients in AM inoculated plants (Shukla et al., 2012)^[28]. These differences might be attributed to (1) differences among AM fungi for hyphal spread and density away from roots (Bürkert and Robson, 1994)^[4], (2) ability of AM fungi to increase nutrient availability in soil through enhanced phosphatase/phytase activity (Dinkelaker and Marschner, 1992; Khalil et al., 1994) ^[8, 15] and/or excretion of solubilizing materials such as ethylene (Ishii et al., 1996)^[13], flavonoides (Ishii et al., 1997) ^[12] and growth regulating compounds (Danneberg et al., 1992; Thiagarajan and Ahmad, 1994)^[6, 32], and (3) ability of AM fungi to change rhizosphere soil pH (Li et al., 1991)^[18]. Beneficial effects of AM inoculations on growth of L. leucocephala have been reported by various workers (Bhagyaraj et al., 1989; Manjunath et al., 1989; Dixon et al., 1993; Habte et al., 2011; Zhan et al., 2016) [2, 19, 9, 10, 34]. The seedling quality index, computed on the basis of the recorded

parameters also showed that all AM treatments significantly improved the quality of the seedlings (Table 3). Maximum index value was recorded in *R. irregularis*-inoculated plants, which was found statistically at par with *A. scrobiculata* + *R. irreguaris*.

Results also showed that among tested *Leucaena* species, *L. collinsii* and *L. leucocephala* gave better response towards inoculated fungi than other two species. This could be due to different genetic make-up of tested *Leucaena* species. Variation in terms of response of cultivars and genotypes towards inoculated AM fungi has been reported in many plants (Nemec, 1978; Ibijbijen *et al.*, 1996; Khalil *et al.*, 1999) ^[22, 11, 16]. Similar results in tree species are meager.

Monzon and Azcon (2001)^[20] reported variation in response of three *Alnus* species (*A. cordata*, *A. glutinosa* and *A. incana*) towards AM inoculation. The MD value of *A. glutinosa* was ten times as large as that of the other two species. Adjoud *et al.* (1996)^[1] also reported variation in MD values of four *Eucalyptus* species (*E. bosistoana*, *E. delegantensis*, *E. dumosa* and *E. macarthurii*). The MD values in these four species varied from 14-27%.

Thus, based on the results obtained in present study it can be concluded that the seeds of two *Leucaena* species viz., *L. leucocephala* and *L. collinsii* may be inoculated with *R. irregularis* to obtain more vigorous seedlings.

Treatments	L. diversifolia	L. shannoni	L. collinsii	L. leucocephala	Mean				
Plant height (cm)									
A. scrobiculata (As)	67.0	77.2	77.0	58.0	69.8				
R. irregularis (Ri)	80.3	83.3	84.0	59.2	76.7				
As+Ri	61.7	73.7	83.0	55.2	68.4				
Control	72.0	80.5	71.0	38.7	65.5				
Mean	70.3	78.7	78.8	52.8					
Root length (cm)									
A. scrobiculata	33.8	42.0	32.3	33.8	35.5				
R. irregularis	28.3	41.2	37.2	33.7	35.1				
As+Ri	25.3	30.7	34.2	28.5	29.7				
Control	29.3	36.7	35.3	25.5	31.7				
Mean	29.2	37.6	34.8	30.4					
Collar diameter (mm)									
A. scrobiculata	6.65	8.93	7.31	7.42	7.58				
R. irregularis	7.91	8.59	8.23	7.28	8.00				
As+Ri	7.18	8.04	8.00	6.94	7.54				
Control	7.76	8.23	7.51	6.41	7.48				
Mean	7.37	8.45	7.76	7.01					
		LSD (0.05)							
	Shoot length	Root length	Col	Collar diameter					
Treatment	5.5	2.7	NS						
Species	5.5	2.7	0.54						
Interaction	11.0	5.5	NS						

Table 1: Effect of arbuscular mycorrhizal inoculations on growth of *Leucaena* species

Table 2: Effect of arbuscular mycorrhizal inoculations on dry biomass of Leucaena species

Treatments	L. diversifolia	L. shannoni	L. collinsii	L. leucocephala	Mean			
Shoot dry biomass (g)								
A. scrobiculata (As)	9.95	9.69	8.64	8.01	9.07			
R. irregularis (Ri)	11.89	12.43	12.40	7.73	11.11			
As+Ri	9.76	9.52	11.67	7.49	9.61			
Control	10.80	10.33	8.90	4.73	8.69			
Mean	10.60	10.49	10.40	6.99				
Root dry biomass (g)								
A. scrobiculata	5.23	5.96	4.84	4.65	5.17			
R. irregularis	5.66	6.58	6.34	5.21	5.95			
As+Ri	4.55	5.49	5.51	4.69	5.06			
Control	5.34	5.35	4.95	2.90	4.64			
Mean	5.20	5.85	5.41	4.36				
Total dry biomass (g)/plant								
A. scrobiculata	15.18	15.65	13.47	12.66	14.24			
R. irregularis	17.55	19.01	18.73	12.94	17.06			
As+Ri	14.31	15.01	17.18	12.18	14.67			
Control	16.14	15.68	13.85	7.63	13.32			
Mean	15.79	16.34	15.81	11.35				
	Shoot dry biomass	Root dry biomass	Total dry biomass					
Treatment	1.14	0.61	1.45					
Species	1.14	0.61	1.45					
Interaction	2.28	NS	2.89					

Table 3: Effect of arbuscular mycorrhizal inoculations on seedling quality index (SQI) and mycorrhizal dependency (MD) of Leucaena species

Treatments	L. diversifolia	L. shannoni	L. collinsii	L. leucocephala	Mean			
SQI								
A. scrobiculata (As)	1.279	1.516	1.093	1.346	1.309			
R. irregularis (Ri)	1.405	1.642	1.536	1.357	1.485			
As+Ri	1.331	1.384	1.385	1.251	1.338			
Control	1.417	1.371	1.225	1.015	1.257			
Mean	1.358	1.478	1.310	1.242				
MD (%)								
A. scrobiculata	-11.98	-2.70	-5.24	38.51	4.65			
R. irregularis	1.69	16.18	25.25	39.87	20.75			
As+Ri	-14.63	-7.93	18.49	36.50	8.11			
Mean	-8.31	1.85	12.84	38.29				
	LSD (0.05)							
	SQI	MD						
Treatment	0.145	10.01						
Species	0.145	10.01						
Interaction	NS	NS						

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