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Arbuscular mycorrhiza facilitates growth of micropropagated plants and seedlings of black plum, *Syzygium cumini*

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ABSTRACT

One of the major impediments to the success of micropropagation is the poor establishment and survival of in vitro developed plants on transfer to soil. To ameliorate this problem, effects of two arbuscular mycorrhiza fungi (AMF), *Rhizophagus fasciculatus* and *R. macrocarpus*, either alone or in combination, were studied on the growth and nutrient status of micropropagated plants and seedlings of *Syzygium cumini*. Symbiotic association with AMF was beneficial for the overall growth of both types of plants. However, the degree of positive effects on growth varied with the plant type as well as the species of AM fungus used. *R. macrocarpus* induced profuse lateral branching of the main root, while *R. fasciculatus* induced elongation of the main roots. The combination of the two proved to be the best for the overall growth of seedlings as well as the micropropagated plants. In seedlings, colonization of AM occurred in the lateral roots arising from the maturation zone of the taproot, indicating recognition and establishment of AM during the early stages of development. This was further confirmed by the observed higher mycorrhizal dependency (MD) during the initial growth period. With time, MD declined slightly as the plants became partially independent of AM. Except for Mn^{2+}, the levels of cations *viz.,* K^{+}, Mg^{2+}, Fe^{2+}, Cu^{2+} and Zn^{2+}, and two anions *viz.,* PO_4^{2-} and NO_3^{2-} were higher in AM treated plants than the respective controls. The present study demonstrates the potential of AMF in the alleviation of transplantation stress and better growth of micropropagated plants of *S. cumini*.

Keywords: Micropropagation, Mycorrhiza, *Myrtaceae, Rhizophagus,* Tissue culture, Transplantation

INTRODUCTION

The technique of micropropagation has been widely used for the large-scale propagation of several tree species in a limited time and space. However, the success of this technique is delimited by various factors. The most important being a low survival and poor establishment of plants, developed in vitro, to the soils (Wang *et al.,* 1993; Sharmila *et al.,* 2000). This is mainly attributed to the poor cuticle of roots and the development of weak root systems, which do not enable the plants to sustain the harsh and altered environmental conditions. Arbuscular mycorrhiza (AM), a mutualistic symbiotic association occurring between plant roots and fungi belonging to phylum *Glomeromycota* offers an important option to overcome this hindrance. Symbiotic association of mycorrhizal species with roots of micropropagated plants at the weaning stage helps to develop a strong root system, thereby improving their nutrient and water acquisition capacity (Kapoor *et al.,* 2008; Costa *et al.,* 2021). In addition, this association makes the micro-propagated plants more resistant to transplantation shock and attacks of root pathogens (Agustini *et al.,* 2020). The application of AM fungi also provides effective protection against fungal pathogens as the freshly transplanted plants are highly prone to fungal attack due to poor cuticular and root development (Krishna *et al.,* 2006; Berruti *et al.,* 2016). In addition, the association of AM fungi helps the roots of host plants in the uptake of various macro-and micro-nutrients from the soil. The key role of AM fungi in the uptake of phosphorus (P) has been well documented (Smith and Smith, 2011). Besides P, the association of AM also improves the availability and acquisition of other nutrients, such as nitrogen (N), potassium (K), calcium (Ca), magnesium (Mg), and copper (Cu) from the soil (Wang *et al.,* 2017; Srivastava and Singh 2019; Huang *et al.,* 2020). Further, the association of AM plays an indispensable role in the assimilation of various micronutrient ions by either (i) increased uptake of these relatively immobile cations from the soil and (ii) by acting as a sink for these cations by storing them and thus preventing their concentration to reach toxic levels in plants (Marschner and Dell, 1994).

Syzygium cumini (L.) Skeels is a multipurpose fruit tree of the family *Myrtaceae*. It is often used as a windbreak in various plantations and is a common roadside tree due to its dense canopy. Its timber is widely used for the construction of boats, buildings, and plywood. The leaves are used as cattle fodder as well as for rearing 'tussar silkworms'. The decoction of fruit and bark is widely used in the Ayurvedic system of medicine (Anonymous, 1969).

The large-scale propagation of this taxon is hampered by the poor viability of the seeds. Besides this, the frost during the winter accounts for the high mortality rate of the seedlings developed from the freshly fallen seeds (Ramasubbu *et al.,* 2016). The perusal of literature revealed that the association of AM fungi is very effective for promoting the growth of seedlings/in vitro raised plants under unfavourable conditions (Kapoor *et al.,* 2008). The present study was undertaken to assess the impact of the symbiotic association between AM fungi and roots of micropropagated plants as well as seedlings of *S. cumini* on their establishment, growth, and nutrient status. Earlier, the positive influence of AM fungi, *Funneliformis mosseae*, *Rhizophagus fasciculatus* and *R. irregularis*, on the seed germination and seedling growth of *S. cumini* has been reported (Bukhari and Rodrigues, 2008; Devachandra *et al.,* 2008). Likewise, Thoke *et al.* (2011) reported the beneficial effect of *R. fasciculatus* on seed germination, graft takes and graft survival of *S. cumini*.

MATERIALS AND METHODS

Two species of AM fungi *viz., Rhizophagus macrocarpus* and *R. fasciculatus* were utilized in the present study. The inocula of both species were procured from the Applied Mycology Laboratory, Department of Botany, University of Delhi, Delhi. The spores of *R. macrocarpus* and *R. fasciculatus* were

isolated from the soil of Delhi ridge, employing Wet sieving and decantation method (Gerdemann and Nicolson, 1963). This was followed by their multiplication separately in the pot cultures of *Trigonella* for four months and later maintained in the pot cultures of *Sorghum* for additional four months before using as a source of inoculum. The inoculum consisted of roots of *Sorghum* colonized by soil-based AM fungal inoculum consisting of extramatrical hyphae, and chlamydospores.

For studying the effect of AM fungal association, plants were raised/transferred to either garden pots or paper cups, each containing autoclaved (15 lbs at 121^{0}C for 15 min) garden soil with or without fungal inoculum. The soil used in the experiment was loamy with pH 7.3, electrical conductivity 1.3 mmol cm^{-1} at 25^{0C}, and had high nutrient content with organic carbon 1.21%, available P (3.51 kg ha^{-1}), and average N (817 kg ha^{-1}). For each treatment, sterilized soil was mixed with inoculum (2:1) of either *R. macrocarpus* or *R. fasciculatus* or a combination (1:1) of both the species. Control plants were grown in non-inoculated soil. For each treatment in paper cups, 200 g of sterilized soil was mixed with 100 g of inoculum, while for experiments in earthen pots 3.0 kg soil was used.

Seedlings were raised from seeds sown directly in the different treatments of soil as mentioned above. However, in vitro raised plants with an average shoot length of 2.8 cm and root length of 3.0 cm were used as experimental material. These plants were obtained following the pre-standardized protocol for the micropropagation of *S. cumini* (Jain and Babbar, 2000). The plants were irrigated with tap water daily. The pots with seedlings were kept outdoors in the shade, while the paper cups with the micropropagated plants were maintained in the culture room (temperature 25± 2^{0}C, light intensity 60 µmol m^{-2}s^{-1}, provided by 40W cool daylight Fluorescent tubes).

For analyzing the effect of AM on the growth, micropropagated plants and seedlings were randomly uprooted from each treatment at monthly intervals for four months. The uprooted plants were washed thoroughly with tap water. Subsequently, lengths of root and shoot, dry weights of shoots and roots, and the number of leaves of individual plants were measured. The experiment was replicated twice using the same number of plants, and the data were subjected to Students t-test (P≤0.05).

Mycorrhizal status in the roots was ascertained by clearing the roots in 10% KOH followed by staining with trypan blue (Philips and Hayman, 1970). The percent colonization was calculated as

% colonization = (No. of colonized segments x 100)/ Total number of root segments screened

Mycorrhizal dependency was estimated as the Relative Mycorrhizal Dependency Index (RMDI) (Plenchette *et al.,* 1983).

RMDI = 100 x (d.m. M$^+$ - d.m. M$^-$/ d.m. M$^+$)

Where, d.m. M$^+$ - dry matter of mycorrhizal plant; d.m. M$^-$ - dry matter of non-mycorrhizal plant.

Fe, Cu, Mg, Mn, Zn, and K contents of roots and shoots of plants subjected to various treatments, were estimated following Allen *et al.* (1974), using Atomic Absorption Spectrophotometer (Shimadzu AA-640-130), calibrated with 1 ppm and 2 ppm standard solutions.

The oven-dried samples (0.2 g) were digested in Kjeldahl's flasks (30 ml capacity) in a combination of 60% (v/v) perchloric acid (1 mL), nitric acid (5 mL) and sulphuric acid (0.5 mL) using micro Kjeldahl's digestion unit. The colourless digest was filtered through Whatman filter paper number 44 and diluted to 50 mL with distilled water. The digest was aspirated at 324.8 nm (Cu), 248.3 nm (Fe), 285.2 nm (Mg), 279.5 nm (Mg), 213.8 nm (Zn), and 766.5 nm (K) using air-acetylene combinations as fuel. The content of different cations was calculated as

Cation (µg/g) = [C (ppm) x solution volume (mL)] / sample weight (mg)

Nitrogen and P contents were estimated employing Indole-phenol blue and Molybdenum blue (Allen *et al.,* 1974) methods, respectively. Using calibration curves, mg-P and mg-NH_3 in the samples were estimated and % contents were calculated as:

P/N (%) =C (mg) x solution volume (mL)/ 10 x aliquot volume (mL) x sample weight (g)

The significance of differences among results of treatments for each experiment were tested employing Student's t-test (P≤0.05).

RESULTS

Root colonization by AM fungi was initiated following germination of the chlamydospores (Fig. 1A). The external hyphae developed appressoria on the root surface (Fig. 1B), through which the fungal hyphae entered the roots. The fungal hyphae initially remained confined to the epidermis, but gradually spread in the cortex (Fig. 1C). The roots of control plants did not exhibit any such structures (Fig. 1D).

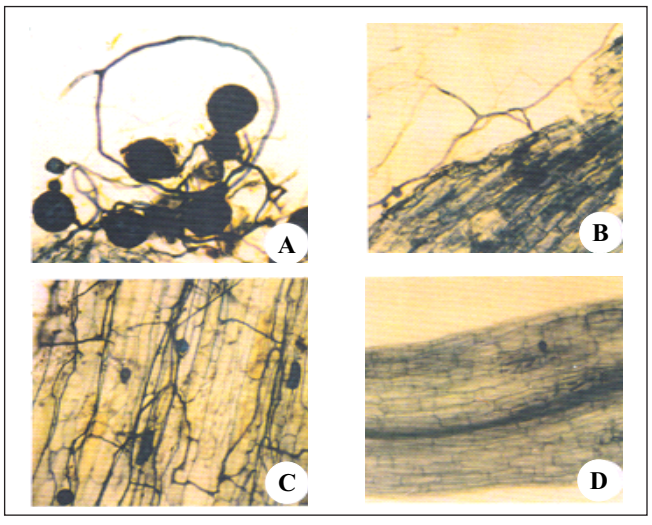

Fig. 1: Colonization of AM in the roots of *S. cumini*. A: Chlamydospores. B: A portion of the root segment with an appressorium through which the fungal hyphae enter the root. C: The fungal hyphae in the root cortex with vesicles. D: A portion of the root of control plants with no colonization.

Once in the cortex, the AM fungi developed all the characteristic structures like arbuscules (Fig. 2A, B), hyphal coils (Fig. 2C), and vesicles (Fig. 2D).

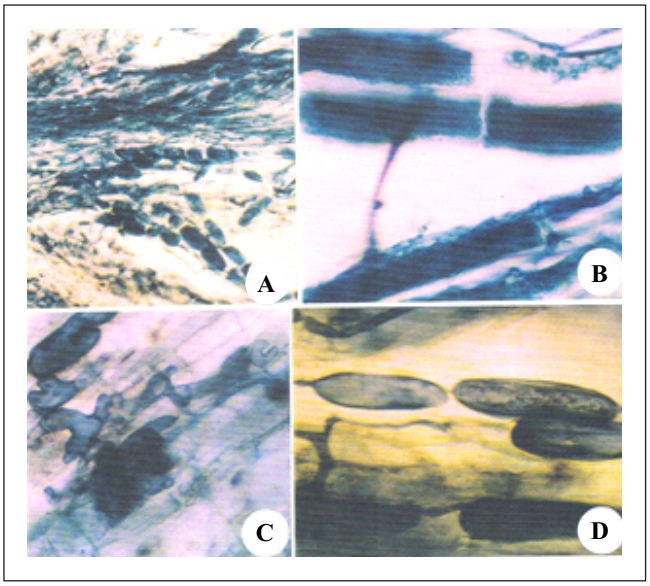

Fig. 2: Structural characteristics of AM fungus in the root cortex. A. A portion of the root segment fully colonized with mycorrhizal hyphae with well-developed arbuscules. B. Close up view of arbuscules. C. Hyphal coils. D. Vesicles.

The percent root colonization was maximum after four months in both seedlings and micropropagated plants. Out of the four treatments used, both types of plants grown in the soil having both *R. macrocarpus* and *R. fasciculatus* exhibited maximum colonization (Fig. 3). Moreover, within a single root of a seedling at any time, the lateral roots near the maturation zone (a-zone) had maximum colonization, with the least being in the region adjacent to the root tip (c-zone; Fig. 4).

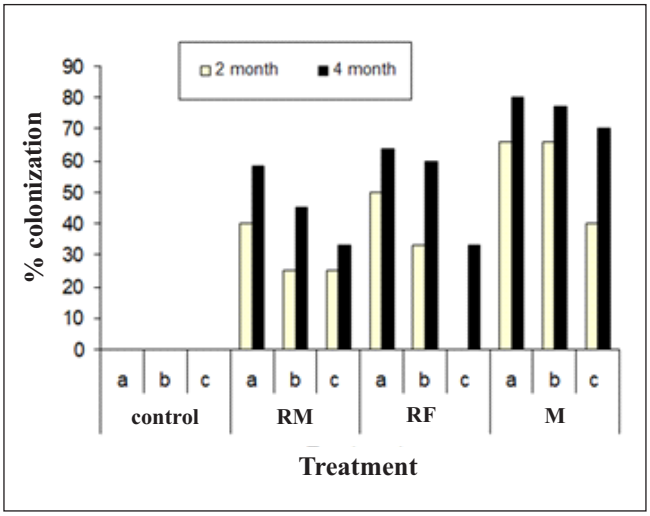

Fig. 3: Root colonization in micropropagated plants of *S. cumini* by two AM fungi. (RM: *R. macrocarpus*, RF: *R. fasciculatus*, M: Combination of two AM fungi).

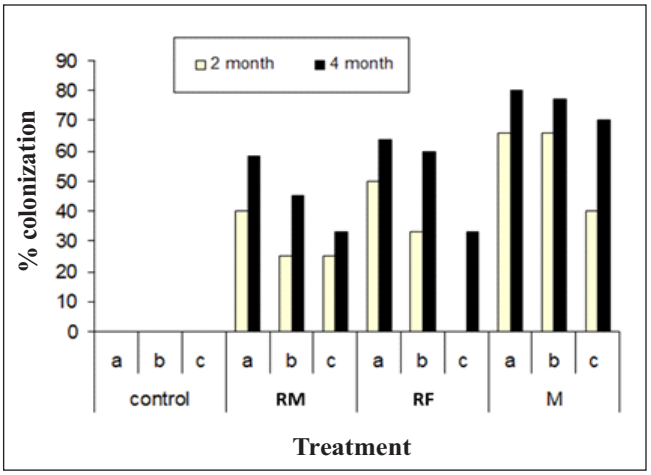

Fig. 4: Percent root colonization in seedlings of *S. cumini* by two AM fungi. (RM: *R. macrocarpus,* RF: *R. fasciculatus,* M: Combination of two AM fungi, a: zone of maturation, b: zone of elongation, c: zone of division).

To compare the growth of the plants, both micropropagated and seedlings, among controls and three treatments, various parameters such as the number of leaves, average length of root and shoot, fresh and dry weights of the shoots and roots were recorded (Table 1). No statistically significant differences were discernible in the average shoot lengths of the treated and control plants after two months. However, by the end of the fourth month, the average shoot length of the plants grown on soil treated either with only *R. macrocarpus* or a combination of *R. macrocarpus* and *R. fasciculatus* was significantly higher than the control. Throughout the four months of observation, there was no significant difference between the number of leaves of the plants grown on untreated or variously treated soils (Table 1).

Table 1: Effect of *Rhizophagus macrocarpus* (RM) and *R. fasciculatus* (RF) alone or in combination (M) on growth parameters of the micropropagated plants and seedlings of *S. cumini.*

Growth parameters	Seedlings		Micropropagated plants	
	2 month	4 month	2 month	4 month
Average shoot length (cm)				
Control	8.90 ± 5.09*a	10.17 ± 3.50b	6.25 ± 2.25a	6.91 ± 1.47b
RM	11.71 ± 2.26a	15.52 ± 4.10a	6.91 ± 1.95a	8.50 ± 1.33ab
RF	11.74 ± 2.05a	13.56 ± 4.60ab	7.20 ± 1.05a	9.50 ± 1.00ab
M	10.93 ± 1.93a	17.54 ±2.71a	6.95 ± 1.00a	10.13 ± 1.31a
Average root length (cm)				
Control	6.95 ± 2.19b	10.99 ± 3.71b	6.80 ±2.12b	7.46 ± 2.61b
RM	8.45 ± 3.45ab	12.96 ± 2.45ab	7.80 ± 3.07ab	9.27 ± 1.32ab
RF	11.45 ± 4.57a	15.26 ± 2.80a	8.34 ± 2.23a	9.87 ± 2.47ab
M	10.44 ± 3.92ab	14.48 ± 3.19ab	9.14 ± 2.10a	11.71 ± 2.31a
Number of leaves				
Control	7.23 ± 2.14a	12.01 ± 2.80a	6.40 ± 2.30 a	6.51 ± 1.24b
GM	9.14 ± 4.81a	12.10 ± 3.81a	6.33 ± 1.33a	7.25 ± 2.52ab
GF	8.36 ± 3.20a	10.42 ± 4.27a	6.75 ± 2.13a	10.81 ± 3.91a
M	8.04 ± 2.48a	12.50 ± 2.83a	7.63 ± 3.13a	9.76 ± 3.29a
Dry weight of shoots (g)				
Control	0.07 ± 0.03c	0.15 ± 0.07d	0.02 ± 0.01c	0.04 ±0.03b
RM	0.14 ± 0.11a	0.21 ± 0.13b	0.05 ± 0.03b	0.12 ± 0.10a
RF	0.12 ± 0.05b	0.18 ± 0.14c	0.03 ± 0.01c	0.10 ± 0.02a
M	0.14 ± 0.06a	0.26 ± 0.15a	0.07 ± 0.03a	0.40 ± 0.12a
Dry weight of roots (g)				
Control	0.02 ± 0.03c	0.07 ± 0.02b	0.02 ± 0.03b	0.04 ± 0.03c
RM	0.04 ± 0.13b	0.08 ± 0.03ab	0.04 ± 0.12a	0.06 ± 0.06b
RF	0.06 ± 0.03a	0.09 ± 0.05a	0.05 ± 0.01a	0.07 ± 0.02ab
M	0.06 ± 0.03a	0.09 ± 0.04a	0.05 ± 0.02a	0.08 ± 0.03a

*mean standard deviation. Values followed by the same letter within a column are not statistically different. (t-test, P=0.05)

The beneficial effect of AM treatment on root length was evident even after two months, as the average root length of the plants grown on soil treated with *R. fasciculatus* was significantly higher than the control. In other treatments also, there was a numerical increase in average root length, however, these differences were not statistically significant. Unlike the shoot length, the positive effect of the treatments on dry weights of roots became evident even after two months and was maintained up to the fourth month. The treatment with a combination of both the AM species appeared to be the best when dry weight was taken into consideration. Among the three treatments, those involving *R. fasciculatus* alone and the combination had a better and comparable positive effect on these parameters than those involving only *R. macrocarpus* (Table 1).

After four months of growth, among the micropropagated plants, only those grown on soil inoculated with both the AM species revealed a clear positive effect on the average shoot length. The beneficial influence of treatments involving only *R. fasciculatus* and the combination on root length was evident even after two months. However, by the end of the fourth month, the average root length of only the plants grown on soil treated with combination was significantly higher than the control. Like average shoot length, the positive influence of AM treatments on the number of leaves became evident only after the fourth month, with the plants grown on soil treated with either *R. fasciculatus* alone or with the combination of AM fungi showing a significantly higher number of leaves. The beneficial effect of mycorrhizal treatments became more evident on dry weights of shoots. Among the treatments, those involving *R. macrocarpus* and the combination had a better effect than with *R. fasciculatus*. However, if only dry weight after four months was taken into consideration, all three treatments had similar beneficial effects. The observations on dry weights of roots indicate that all three treatments have a clear-cut beneficial effect. However, among the treatments, those involving *R. fasciculatus* and the combination appeared to be better than that involving only *R. macrocarpus* (Table 1).

In the case of seedlings, the Relative Mycorrhizal Dependency Index (RMDI) increased gradually during the first three months in all three treatments. However, during the fourth month, it decreased in all the treatments. For micropropagated plants, initial RMDI values were considerably higher than the corresponding values recorded for seedlings in all the treatments taken after two months. However, even with micropropagated plants, this value decreased during the fourth month (Table 2).

The endogenous levels of six mineral cations *viz.,* K^+, Mg^{2+}, Mn^{2+}, Zn^{2+}, Fe^{2+}, Cu^{2+} and two anions *viz.,* PO_4^{2-} and NO_3^{2-} were determined in roots and shoots after an interval of two months for micropropagated plants as well as seedlings for up to four months. The level of P increased gradually in shoots and roots of both the seedlings and micropropagated plants with time in all three treatments as well as in the control. There were large differences in the endogenous levels of PO_4^{2-} in the shoots of the seedlings as well as in micropropagated plants after two months of growth on the treated soil. Among the three, the treatment involving both *R. fasciculatus* and *R. macrocarpus* resulted in the highest levels of P in the shoots followed by the one with *R. macrocarpus*. With time the levels of PO_4^{2-} in the shoots of both seedlings and micropropagated plants were higher than the control (Table 3).

Table 2: Relative Mycorrhizal Dependency Index (RMDI) of the micropropagated plants and seedlings of *S. cumini*, treated with *R. macrocarpus* (RM) and *R. fasciculatus* (RF) or in combination (M).

Treatments	Seedlings				Micropropagated plants	
Time period (months)	1	2	3	4	2	4
RM	0.18	0.35	0.38	0.26	0.73	0.65
RF	0.18	0.35	0.35	0.08	0.60	0.56
M	0.35	0.42	0.51	0.32	0.76	0.68

The endogenous level of nitrate was higher in roots as well as shoots of the treated seedlings than those of the control after two months. However, after four months the levels were almost equal in the roots and shoots of the treated as well as untreated plants. Though the differences were minute, the levels of nitrate were consistently higher in plants grown on the soils treated with both the AM fungi than the controls as well as those raised on soils inoculated with either of two AM fungi. Unlike the seedlings, both roots and shoots of the micropropagated plants had higher levels of N than those found in control at two- and four- month intervals (Table 3).

The levels of K^+ in both roots and shoots of the seedlings as well as the in vitro regenerated plants treated with AM were always higher than those recorded for the control. Among the treatments, the combined AM treatment proved to be the best followed by *R. macrocarpus* alone. Almost similar trends were observed for two cations *viz.,* Mg^{2+} and Fe^{2+}. Although, the levels of Zn^{2+} in shoots of the treated seedlings as well as the micropropagated plants were higher than the controls, the differences were minute at least in the case of seedlings. On the other hand, roots of the treated plants of both categories had consistently higher levels of Zn^{2+} than those of the controls with the maximum increment being in the plants receiving combined treatment of both AM fungi, followed by those inoculated with only *R. macrocarpus*. The differences between the levels of Mn^{2+} in the various treatments were minute in both shoots and roots at all the times. The notable higher levels of Cu^{2+} were recorded at different time intervals in shoots as well as roots inoculated with combined AM treatments than the corresponding controls. In the other treatments, although differences were observed, no clear trends were evident (Table 3).

DISCUSSION

The effect of AM on the establishment of micropropagated plants have earlier been documented in grapevine (Krishna *et al.,* 2006), citrus (Wu *et al.,* 2011), eucalyptus (Agustini *et al.,* 2020), argon tree (Ouallal *et al.,* 2018) and apple (Costa *et al.,*

Table 3: Effect of *Rhizophagus macrocarpus* (RM) and *R. fasciculatus* (RF) or combination (M) on endogenous levels of nutrient ions in the shoots and roots of the micropropagated plants and seedlings of *S. cumini*.

Treatments	Seedlings				*In vitro* regenerated plants			
	2 month		4 month		2 month		4 month	
	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots
Phosphate (PO_4^{2-}, %)								
C	0.89a	0.61b	1.42c	1.06b	0.66c	0.73b	1.08b	1.06b
RM	0.92a	0.69b	1.59b	1.02b	0.83b	0.85ab	1.20ab	1.05b
RF	0.97a	0.64b	1.51bc	1.01b	0.85ab	0.76b	1.16b	1.02b
M	1.02a	0.82a	1.69a	1.23a	0.92a	0.92a	1.42a	1.29a
Nitrate (NO_3^{2-}, %)								
C	0.18b	0.17b	0.20b	0.20a	0.13c	0.08c	0.19c	0.13c
RM	0.20ab	0.20a	0.21b	0.21a	0.22a	0.13b	0.29b	0.17b
RF	0.19ab	0.19ab	0.19b	0.20a	0.21ab	0.18ab	0.23b	0.19ab
M	0.22a	0.21a	0.28a	0.23a	0.23a	0.20a	0.33a	0.21a
K^+ (%)								
C	2.05b	1.62c	2.25c	1.94c	1.61c	2.25c	3.62c	2.18b
RM	2.18b	1.86b	2.52b	2.25b	2.07b	2.15b	4.32b	2.21b
RF	2.05a	1.77bc	2.46b	2.08b	2.12b	2.05b	4.22b	2.02b
M	2.51a	2.02a	3.06a	2.62a	3.12a	2.25a	5.91a	2.71a
Mg^{2+} (%)								
C	2.10b	2.00b	5.10c	3.10c	6.40d	4.90c	7.10c	6.40c
RM	3.20a	2.70b	7.30b	4.20b	8.00b	6.40b	8.70b	7.30b
RF	2.80b	2.30b	6.80b	3.90c	7.60c	6.30b	8.40b	7.10b
M	3.70a	4.00a	9.80a	5.20a	9.50a	7.60a	10.20a	8.40a
Fe^{2+}(µg/g)								
C	31.30c	33.50c	52.33c	40.90d	22.50c	16.50c	30.50d	22.50d
RM	38.10b	36.31a	56.50b	56.50b	24.37b	24.50a	36.60b	36.67a
RF	30.80d	30.58d	42.75d	47.50c	23.60bc	22.50b	31.20c	30.30c
M	40.08a	35.30b	87.25a	58.60a	36.50a	23.60ab	41.80a	32.37b
Zn^{2+}(µg/g)								
C	12.08d	2.10c	14.20c	4.70c	3.75c	3.75c	6.25d	5.00c
RM	12.58b	3.50b	15.70b	5.20b	5.00b	6.50a	8.50b	7.50b
RF	12.25c	2.70c	14.80c	4.80c	4.75b	5.20b	7.50c	7.20b
M	13.30a	4.10a	20.30a	6.25a	6.25a	6.50a	12.50a	10.00a
Mn^{2+}(µg/g)								
C	12.30a	10.00c	24.20a	16.16d	11.25c	10.00c	12.50b	11.25d
RM	12.60b	11.30b	22.30b	18.60b	12.50b	11.25b	12.90ab	12.50c
RF	12.80b	10.80c	20.70c	17.90c	12.20b	12.50b	12.70b	13.70b
M	13.60a	12.80a	24.10a	19.30a	13.10a	13.20a	13.25a	15.10a
Cu^{2+}(µg/g)								
C	208.0b	166.0c	250.0c	233.0c	150.0d	130.0d	169.0b	210.0c
RM	191.0c	175.0b	297.0b	251.0b	182.0b	150.0c	175.0b	220.0b
RF	189.0c	166.0c	250.0c	225.0d	162.0c	175.0b	170.0b	212.0c
M	258.0a	197.0a	330.0a	287.0a	212.0a	182.0a	260.0a	247.0a

Values followed by the same letter within a column are not statistically different. (t-test, P=0.05)

2021). AM fungi have been shown to have stimulatory (Costa *et al.,* 2021) or inhibitory (Hršelová *et al.,* 1989) or no effect (Kiernan *et al.,* 1983), on the micropropagated plants. In the present study, a symbiotic association between AM fungi and micropropagated plants and seedlings of *S. cumini* was found to be beneficial as observed in earlier reports on *Gloriosa superba* (Yadav *et al.,* 2013) *Comanthera mucugensis* (Pereira *et al.,* 2017), and *Malus prunifolia* (Costa *et al.,* 2021). The degree of beneficial effects on growth and nutrient uptake depends on the plant species as well as the strains of AM fungus used (Marin *et al.,* 2003; Novais *et al.,* 2014). Similar variations were observed for plants of *S. cumini* using two species of *Rhizophagus viz., R. macrocarpus* and *R. fasciculatus*. *Rhizophagus. macrocarpus* induced profuse lateral branching of the main root, while *R. fasciculatus* induced elongation of the main roots. Combined inoculation with the two AM species proved to be the best for the growth of seedlings as well as micropropagated plants.

AM colonized plants of *S. cumini* exhibited an increase in dry matter content with the increase in the time period, while the root/shoot ratio in terms of growth declined. Similar observations have been reported earlier for other tree taxa *viz., M. prunifolia* (Gastol *et al.,* 2016), and *Argania spinosa* (Ganoudi *et al.,* 2021). The increase in dry weight happens due to the high efficiency of the mycorrhizal root system for nutrient uptake. The latter observed effect could be due to the extra-radical hyphae which increases the overall absorptive surface area of the root, thereby improving the nutrient and water uptake (Smith and Read, 2008). In seedlings of *S. cumini*, colonization of AM was encountered with lateral roots arising from the maturation zone of the taproot, indicating that recognition and establishment took place in the early stages of development. This was further confirmed by high RMDI values during the initial growth period. With time, RMDI declined slightly as the plants became partially independent of AM. Similar observations have been recorded for *A. spinosa* (Ouallal *et al.,* 2018), *Ziziphus* spp (Thioye *et al.,* 2018), and *Acmella oleacea* (Vieira *et al.,* 2021).

Out of six cations *viz.,* K^+, Mg^{2+}, Mn^{2+}, Fe^{2+}, Cu^{2+} and Zn^{2+}, and two anions *viz.,* PO_4^{2-} and NO_3^{2-}, endogenous levels of which were studied in shoots and roots of seedlings and *in vitro* regenerated plants of *S. cumini,* except for Mn^{2+}, the levels of all other ions were found to be higher in AM-treated plants than in the controls. The beneficial effect of AM fungi in the uptake of macronutrients, especially P has been documented for many plant species, such as *Citrus tangerina* (Wu *et al.,* 2011), *Juglans regia* (Huang *et al.,* 2020), and *Malus prunifolia* (Costa *et al.,* 2021). It happens mainly due to the absorption and translocation of P from distant areas, which are otherwise inaccessible to plant roots (Smith and Read, 2008). In addition to P, AM fungi also enhance the acquisition of relatively immobile cations, particularly Zn and Cu, due to strong absorption by soil colloids (Wang *et al.,* 2017). This could be due to (i) alteration in the root morphology, (ii) root physiology, or (iii) due to higher mobilization of micronutrient cations in the rhizosphere through AM exudations (Govindarajulu *et al.,* 2005; Smith and Read, 2008). The increased growth due to the enhanced uptake of PO_4^{2-}, NO_3^{2-}, K^+ and various micronutrient elements (Zn, Fe, Cu, Mg, Mn) has been directly correlated with the rate of colonization as has been reported in peach (Rapparini *et al.,* 1994) and walnut (Dolcet-Sanjuan *et al.,* 1996). The comparison of nutrient analysis of mycorrhizal and non-mycorrhizal plants reflects that the numerical values of all the cations were higher in the plants colonized by mycorrhiza. This could be due to the ability of AM hyphae to explore a much larger soil volume than the non-mycorrhizal plants, thereby increasing the overall absorption of ions from the rhizosphere.

The results of the present study revealed the potential of AM fungi in the alleviation of transplantation stress in micropropagated plants and the establishment of seedlings of *S. cumini*. Further, among the three AM treatments, the one involving the combination of both AM species had the best effects. To conclude, the present study demonstrates unequivocally the tremendous potential of exploitation of AM technology for mass propagation of *S. cumini*.

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