ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 5, Issue, 07, pp.1730-1734, July, 2013

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

CONCENTRATION MEDIATED EFFECT OF ARBUSCULAR MYCORRHIZAL FUNGI (AMF) ON GROWTH AND NUTRITION OF AIR-LAYERED LITCHI PLANTS

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ARTICLE INFO

Article History:
Received 18th April, 2013
Received in revised form
14th May, 2013
Accepted 05th June, 2013
Published online 18th July, 2013

Key words:
Thick roots, Brittle roots,
Nursery, Arbuscular Mycorrhizal fungi,
Infective Propagules,
Growth, Development

ABSTRACT

For centuries, Litchi (*Litchi chinensis* Sonn.) has been commercially propagated by air-layering. But still, the results are not optimum due to the production of thick and brittle roots by the air-layers, which are sometimes difficult to establish in the nursery and field as well. It is established fact that Litchi quite readily forms natural symbiotic associations with Arbuscular Mycorrhizal fungi (AMF). Despite, the effect of AM fungi on the growth and development of the fruit crops have been studied extensively, still the substantial results are lacking towards the mycorrhizal inoculum optimizations for best desirable effects. So, optimum concentrations of the AMF were worked out in terms of Infective Propagules (IP). In most of the characters leaving apart some characters, which were at par with the other treatments, most of the growth characters and nutrient levels were found to be significantly higher in the plants inoculated with higher concentrations of the AMF which in our case was of 800 IP. All the mycorrhizal treated plants showed good survival with only one mortality. AMF has proved its pivotal role in the development of healthy and vigorous planting material at the nursery level. The results have initiated the first step towards standardizing the optimum dose of AMF which will prove to be quite beneficial to the farmers and a strong impetus for the fruit growing industry.

INTRODUCTION

Litchi (*Litchi chinensis* Sonn.) is an evergreen sub-tropical tree which is commercially important for its fruits, having translucent and scented aril. A Sapindaceae member, it is amongst the leading income generating fruit, particularly in the *Tarai* region of Uttarakhand state of India. Litchi has been historically propagated by marcottage, and this is the most common method of propagation employed by commercial nurseries. Other methods of propagation like seeds, cutting, budding and grafting are not expedient, as they may lead to either long juvenile period or improper establishment of the litchi seedlings (Pandey and Sharma, 1989). Marcottage (air-layering, branch-layering, Chinese layering, air-grafting, gootee, guti or marcotting) has been practiced by the Chinese for over 3000 years (Li and Li, 1949) for propagating litchi. Marcots come into bearing early, although they have a shallow root system and thus lead to obtaining profitable returns quite early. The arbuscular mycorrhizae are quite inseparable to the Litchi crop, as the investigations have indicated that diverse species of AMF form the symbiosis with Litchi roots. This is the reason why in India, the practice of acquiring the soil from the old litchi orchards and then employing it for the planting of new litchi saplings, is a necessary followed step prior to the establishment of the litchi orchards (Sharma *et al*., 2009). This method has several demerits too; primarily the association of litchi roots with AMF cannot be established. Eventhough lucrative benefits are witnessed in litchi orchards there is a research gap to facilitate the harnessing of full potential of AMI from fruit crop. Nursery is the backbone of the fruit production and healthy planting material is the prerequisite for establishment of the orchard. Hence, the poor establishment of the air-layers in the nursery is the major hindrance in obtaining optimum returns. This may be due to several factors namely, root thickness, genetic difference, insect and pathogen attack, unfavorable climatic conditions, low phosphorus uptake and other essential nutrients. Considerable work has been done regarding the effect of various species of AMF, but till date no satisfactory work has been done with respect to the effect of varying concentrations of AMF. The optimization of the concentration of the mycorrhizae is an important aspect in the fruit production, as the correct quantity of the inoculum will prove to be beneficial in obtaining optimum growth in the nursery grown litchi plants. This area has been neglected till now, nevertheless, the role of AMF has been well pronounced since ancient times. From the findings of the previous workers, no doubt arises in quoting that AMF has positive effect in enhancing the growth and nutrition of the litchi plants. But, how the air-layered plants respond to the different concentrations of the AMF in the pots was the main aim behind the whole experiment.

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MATERIALS AND METHODS

Preparation of air-layers

For the experiment, one to two year old shoots and 2.5 to 4 cm in diameter, were selected and tagged during end of July, 2009. The shoots having almost same vigour were selected and a ring of bark, about 2-3 cm wide just below a bud was removed. Subsequently, girdled area was covered with a layer of moistened sphagnum moss grass and then wrapped with a piece of 200 gauge polyethylene film of 15×15 cm^2 size to prevent desiccation and it was firmly tied with the help of a jute string. On October 1, the air-layers were then severed from the mother plant and selective pruning of shoots and leaves were performed to avoid desiccation of the plants. The use of rooting hormone was avoided during the preparation of the layers.

Preparation of media

The layers were transplanted into 2 kg pots. The pots were first washed properly and disinfected by the bleach (5% NaOCl). The potting media was created by mixing 1 part soil, 2 parts sand and 1 part well rotten Farmyard manure. The pH was maintained at 7.3. The alkaline $KMNO_4$ hydrolyzed Nitrogen (kg ha^{-1}) was 1792, while the neutral normal Ammonium acetate extractable Potassium (kg ha^{-1}) was 313.6 and Olsen's Phosphorus (mg kg^{-1}) was 6.3. The media was then sterilized in an autoclave at 121°C for 1-1.5 hr subsequently for 2 days.

AMF inoculum production

Soil samples were collected for isolation of AMF at the depth of 0-20 cm from an undisturbed site in GBPUA&T, Pantnagar, U.S. Nagar, Uttarakhand, India, situated at 29° N latitude, 79° 3' E longitudes and at an altitude of 243 m above MSL in tarai belt in Shivalik range of Kumaun Himalayas. The soils were sieved, air-dried and processed by wet sieving and decanting method for collection of AM fungal spores. These were picked and identified after sucrose gradient density centrifugation (Tommerup and Kidby, 1979). The inoculum was produced in steam sterilized soil: sand (1:1) mixture placed in 2 kg pots. Four holes were made in the soil and 50 spores were placed in each hole. One maize seed (*Zea mays* L., variety "Naveen") was sown in each hole and grown for two cycles each of 60 days of plant growth to ensure sufficient production of fresh spores. The plants were watered as required. After two cycles of AMF multiplication, the most probable number (MPN) of infective AMF propagules were assessed using a dilution technique (Porter, 1979). After two cycles of AMF culturing, the soil-based inoculum had 100 infective propagules (IP) g^{-1}. The dominant AMF genera were *Gigaspora albida*, *Glomus intraradices* and *Acaulospora scrobiculata*. For various treatments the varying concentrations was given as 2g inoculum/ air-layer/ pot for 200 IP, 4g for 400 IP, 6g for 600 IP and 8g for 800 IP respectively. The pots were filled by the media and a central hole was dug in the pot wherein the inoculum was added prior to the transplanting of the layers so as to facilitate the symbiosis between the roots of the litchi and the arbuscular mycorrhizae.

Physico-chemical analysis

The litchi air layers were harvested 120 days after planting and all the observations were recorded thereafter. The various observations comprised of the number, length and thickness of the lateral roots (cm), number of leaves and leaflets, height and stem diameter (cm), total leaf area, total biomass, mycorrhizal dependency and leaf nutrient analysis. leaf area was calculated using leaf area meter (Li-COR Portable leaf area meter LI-3000). Mycorrhizal dependency (%) was calculated by the formula: (Average Biomass of mycorrhizal plants – Average biomass of non-mycorrhizal plants) X 100/biomass of mycorrhizal plants. The roots, leaves and stem were collected separately and oven dried at 58±2° C till constant weight (approx. 48 hrs). A representative sub-sample of the fine roots and leaves were kept aside for the further analysis of the percent root colonization and leaf nutrient analysis. Quantification of Nitrogen was done by micro-kjeldahl method (Cliffes, 1958). The Phosphorus was estimated by vanadomolybdophosphoric yellow color method (Jackson, 1973) and K with the help of flame photometer. Available micronutrients namely, Fe, and Zn estimated using 0.005 M DTPA extractions (Lindsay and Norvell, 1978) on Atomic Absorption Spectrophotometer (GBC, Model No. 902, Australia).

Assessment of root colonization

Fine feeder roots were extracted from the root samples, washed to remove soil and debris in running tap water and cut into 1 cm pieces. The roots were then dipped in Hydrogen Peroxide solution and were kept overnight in a dark place. After removing the roots from the Hydrogen Peroxide solution, they were then subsequently treated with 10% KOH solution and were subjected to boiling in a water bath at 90°C for 45 min. Thereafter, the root-pieces were washed 3–5 times with sterilized distilled water and treated with 1% HCl for 3–4 min (Sharma *et al*., 1988). The root-pieces were finally stained with 0.05% trypan blue according to Phillips and Hayman (1970). The assessment of per cent root colonization was done by the slide method proposed by Giovannetti and Mosse (1980).

Statistical analysis

The experiment was conducted on a Completely Randomized Block Design (CRD) method according to Gomez and Gomez (1984). It comprised of 6 treatments with concentration of 100 IP, 200 IP, 400 IP, 600 IP, 800 IP of the AMF and control. Each treatment was replicated eight times. The data thus obtained was subjected to statistical analysis to evaluate the comparative efficacy among different treatments. Data were analyzed using SPSS software (Version 7.5, SPSS Inc., Illinois, USA).The significance of variation among the treatments was observed by applying 'F' test and critical difference was calculated at 5% level of probability.

RESULTS AND DISCUSSION

Root characteristics

The Fig.1 and data in Table 1 reveals that the mycorrhizal concentration of 800 IP significantly had higher root number (21) and root length (14.8cm) than the other treatments.

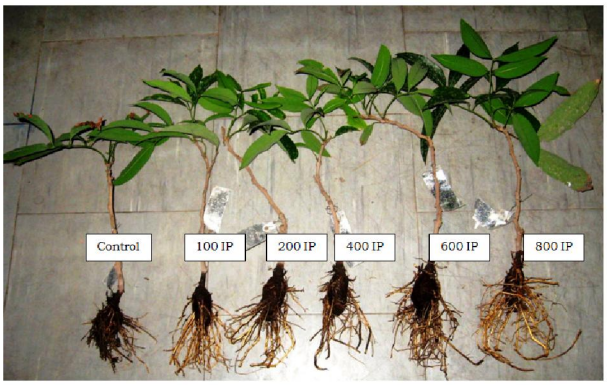

Fig. 1. Effect of different concentrations of AM fungi on root architecture. IP = Infective Propagules

Table 1. Effect of different concentrations of AM fungi on root characteristics in Air-layered Litchi plants. IP = Infective Propagules

	Treatment	Number of lateral roots	Thickness of roots (mm)	Length of laterals (cm)
T_1	Control	9.37	1.81	5.43
T_2	100 IP	12.37	1.77	7.46
T_3	200 IP	13.62	1.32	8.33
T_4	400 IP	15.75	1.27	10.51
T_5	600 IP	17.12	1.31	11.35
T_6	800 IP	21.00	1.02	14.80
S. Em. ±		0.95	0.17	0.89
CD at 5%		2.71	0.51	2.55

The minimum root thickness (1.02 mm) was also observed from the plants having concentration of 800 IP. As compared to the control plants, the mycorrhizal inoculated plants showed a better root architecture. Sharma *et al*. (2009) observed that *Glomus fasciculatum* significantly increased the total root length of the Litchi plants. Wu *et al*. (2010) found that mycorrhizal citrus seedlings had greater root length, root projected area and root surface area than the non-mycorrhizal control seedlings. Modification in the root geometry and morphology might be morphogenetic effects mediated by IAA and Gibberellins (Allen *et al*., 1980). The relationship between root system architecture and phytohormones has been well established (Perez-Perez, 2007), because phytohormones are key signaling factors conferring the effects of environmental cues on the root system

architecture (Jiang *et al*., 2007). In the present experiment, it can also be suggested that the modified auxin level by AM inoculation was responsible for the changes in root morphology. The differential degree of stimulation of root parameters may be further related to differential production of these compounds by AMF.

Shoot characteristics

The litchi plants responded positively to the application of varying concentrations of the AMF (Table 2 and Fig. 2). The mycorrhizal association significantly affected the plant height, stem thickness, number of leaves and leaflets and total leaf area. All the mycorrhizal inoculated plants showed higher shoot characters than uninoculated control plants. The mycorrhizal concentration of 800 IP comparatively increased the plant height (44.21cm), stem diameter (3.58mm), number of leaves (13), number of leaflets (28.12) and total leaf area (271.68cm^2). Gera *et al*. (2001) postulated that the increase in height and stem diameter might be due to greater transpiration which may have possibly increased the flow of water and dissolved N to the roots of mycorrhizal treated plants. Also Patil *et al*. (2004) showed that AM inoculation significantly increased the stem diameter and height in the Papaya. David *et al*. (2001) noticed shoot growth enhancement in air-layered litchi trees inoculated with indigenous AM fungal species The increased leaf area can be explained as there is increase in number of leaves, so even though the area of the individual leaf of mycorrhizal plants is approximately same as that of the control plants, but due to the increased number of leaves, the total leaf area of the mycorrhizal plants is more as compared to the control plants. There are reports of increased growth in *Dalbergia sissoo, Alianthes excelsa, Tectona grandis and Terminalia alata* using VAM inoculum (Agarwal and Chauhan, 1995; Khan and Uniyal, 1999).

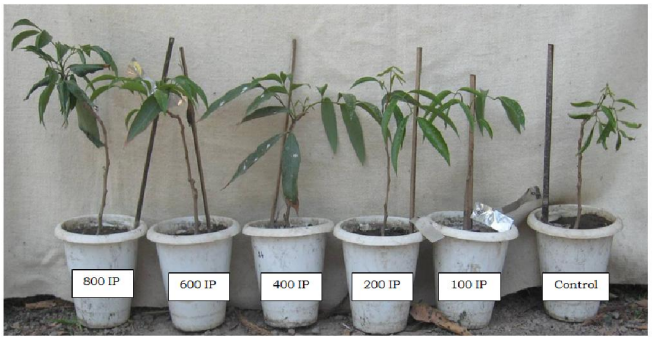

Fig. 2. Effect on Plant Height and Shoot Growth, as influenced by different concentrations of AM fungi. IP = Infective Propagules

Table 2. Effect of different concentrations of AM fungi on shoot characteristics in Air-layered Litchi plants. IP = Infective Propagules

	Treatment	Plant Height (cm)	Stem Diameter (mm)	Number of leaves	Number of Leaflets	Total Leaf Area (cm^2)
T_1	Control	35.73	3.13	5.37	12.50	133.25
T_2	100 IP	36.57	3.17	5.87	14.12	144.49
T_3	200 IP	38.68	3.27	7.12	16.75	169.17
T_4	400 IP	42.32	3.30	8.75	20.12	198.83
T_5	600 IP	43.73	3.37	11.00	24.50	237.16
T_6	800 IP	44.21	3.58	13.00	28.12	271.68
S.Em.±		0.98	0.08	0.58	1.03	10.05
CD at 5%		2.81	0.25	1.65	2.96	28.85

Studies with *Arabidopsis* indicated that cytokinin activated cell division was responsible for leaf formation (Smalle *et al*., 2002). Therefore, it can also be suggested that AM inoculation probably influenced the leaf number and leaf area by modifying the endogenous cytokinin levels in litchi air-layers.

Biomass

There was a considerable increase in the fresh weight and dry weight of both the roots as well as the shoots, with the rise in the concentration of the AM inoculum from nil to 800 IP (Fig. 3).

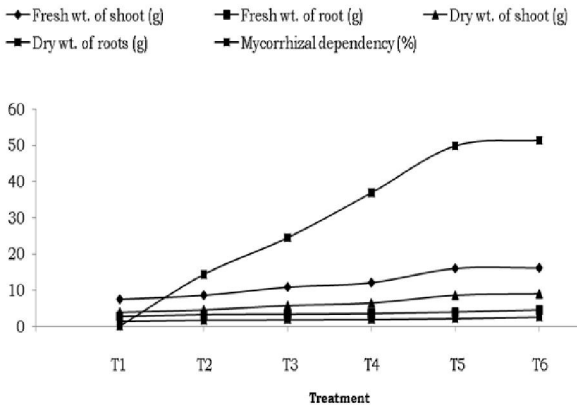

Fig. 3. Effect of AM inoculation on fresh wt. and dry wt. of both root and shoot, and Mycorrhizal dependency. T1= Control, T2= 100 IP, T3= 200 IP, T4=400 IP, T5=600 IP, T6= 800 IP. IP= Infective propagules, wt. = weight

The plants provided with 800 IP of the mycorrhizal inoculum exhibited preeminent results with highest fresh weight and dry weight of roots (4.49g and 2.50g) and shoots (16.05g and 8.88g). The result may be attributed to the increased number of leaves and lateral roots as affected by the AMF. The increased number of leaves resulted in more photosynthesis, higher whole plant carbon assimilation and higher plant dry weight accumulation. The increased rooting enhanced the water absorption and nutrient availability, thus increasing the biomass. Janos *et al*. (2001) observed similar results, where they found that the litchi air-layers inoculated with the field-collected AMF had 39% higher shoot dry weight than the control plants. The findings in this experiment also corroborate with the findings of Nemac and Vu (1990) for increase in the biomass of the AM inoculated plants. Against the increased fresh and dry weight of roots and shoots, the root:shoot ratio on both fresh weight as well as dry weight basis showed a negative trend with the maximum observed in the control plants (0.37) and the minimum in the 800 IP inoculated plants (0.24 and 0.25). The result can be explained by the fact that the mycorrhizal infection induces change in the root architecture and reduces the root: shoot ratio, making the plants less capable of independent nutrient absorption and more dependent on endophyte association (Hetrick *et al*., 1988). Such results related to the root: shoot ratio has also been obtained from studies related to some forest species and crop plants, inoculated with different species of VAM (Smith, 1980; Sugavanam *et al*., 1998; Singh and Jha, 2005).

Percent root colonization, survival percentage and mycorrhizal dependency

The mycorrhizal infection rate in terms of percent root colonization varied from 12.3-26.7% with a significant difference among the treatments. The roots of the control plants showed no mycorrhizal infection. Mycorrhizal treated plants exhibited good survival percentage over non mycorrhizal. In total 3 plants showed mortality: 2 from uninoculated plants and 1 from inoculated ones. It was evident from the data that the plants provided with the AM fungal inoculum showed greater mycorrhizal dependency. It ranged from 14.2-51.2% (Fig.3). Such results can be explained by the fact that different concentrations of the AMF have different interactions with the root morphology and architecture hence different values of mycorrhizal dependency are obtained. Such variations in mycorrhizal dependency was obtained by Yao *et al*. (2005) in their studies on litchi seedlings, inoculated with AMF.

Leaf nutrient analysis

There occurred a significant variation amongst the various macro and micro nutrient levels (Table 3). The effect of AMF could be well pronounced in escalating the leaf N, P, K which varied from 1.3-1.6%, 0.16-0.32%, 1.2-1.4%, respectively. The Zn and Fe level also

Table 3. Effect of different concentrations of AM fungi on nutrient levels in Air-layered Litchi plants. IP = Infective Propagules

	Treatment	N (%)	P (%)	K (%)	Zn (mg kg^{-1})	Fe (mg kg^{-1})
T_1	Control	1.32	0.16	1.20	15.22	46.55
T_2	100 IP	1.46	0.18	1.29	22.74	65.79
T_3	200 IP	1.51	0.20	1.34	23.49	70.58
T_4	400 IP	1.57	0.25	1.37	24.96	76.43
T_5	600 IP	1.61	0.28	1.41	25.86	81.91
T_6	800 IP	1.66	0.32	1.48	26.43	87.25
S.Em.±		0.08	0.06	0.05	0.56	0.57
CD at 5%		0.23	0.18	0.16	1.63	1.65

showed steady rise in their levels in the leaves (15.2-26.4 and 46.5-86.4 $mg^{-1}kg$). The plants inoculated with 800 IP of AMF showed significantly higher levels of N, P and Zn, while the levels of K and Fe was also higher but they were at par with the other treatments. The increased root geometry, nutrient assess and supply mediated by AMF results in the development of extramatricular hyphae that might have further contributed to improved growth resulting in improved nutrient uptake. Hooker *et al*. (1992) was the first to demonstrate both direct nutrient uptake and indirect growth effects of fungal inoculation on the plants. AMF had beneficial effects on mango, citrus, papaya and jamun seedlings and the mycorrhizae readily increase the uptake of the nutrients which have low mobility otherwise. The result is in accordance with the findings of Rhodes and Gerdemann (1975), which have shown that mycorrhizal plants are able to extract more nutrients from the soil through their extramatricular mycelium and thus facilitated easier absorption in lower levels of fertility. Sanders and Tinker (1973) hypothesized that the uptake of any element would be enhanced by mycorrhizae if it is slowly available. The improved nutrition can also be attributed to increased AM fungal association in terms of percent root colonization and spore count leading to increased surface area for absorption and uptake of nutrients. Besides this, AMF are also known to release growth substances, growth regulators, hormones and enzymes (acid phosphatase) in the rhizosphere, which help in the conversion of insoluble nutrients to soluble form and increase their availability to the plants resulting in increased contents of major nutrients like N, P and K and micronutrients like Fe, Mg, Mn, Mo and Co (Adivappar *et al*., 2004). Normally, acquisition of those nutrients with low mobility in the soil, such as P, Zn and Cu, may be enhanced in the plants by AM inoculation (Turk *et al*., 1996). In plants, particularly those with weak root system, hyphal connections act as a bridge between roots and nutrient sites in soil and facilitate efficient uptake of immobile nutrients by host plant (Azcon-Aguilar and Barea, 1996). Later, findings of many workers have confirmed that AMF has a major effect in increasing the uptake of the nutrients (Janos *et al*., 2001; Yao *et al*., 2005; Plenchette *et al*., 1981and Granger *et al*., 1983).

Table 4. Regression Analysis of the macro and micro nutrients in leaf in VAM inoculated litchi layers with the growth (plant height) co-efficient

Model	Unstandardized co-efficients		Standardized co-efficients	t
	B	Std. Error	B	
1 (Constant)	26.754	1.603		16.693
P	58.07	6.720	0.974	8.641
Model Summary				
Model	R	R 2	Adjusted R2	Durbin Watson residual
1	0.974	0.949	0.936	0.250

Regression Analysis of the leaf macro and micro nutrients associated with plant height

Analysis of relationship of plant height with the different macro and micro nutrients using stepwise regression resulted in identification of one parameter phosphorous (Table 4) showing positively highly significant regression with the growth (plant height). This confirms that the VAM IP's play a significant role in phosphorous uptake which in-turn enhances the plant height in litchi layers. This confirms the existence of correlation between two qualitative parameters.

The co-efficient of determination i.e, the adjusted R2 value of phosphorous alone was 0.936 (93.6%).

Conclusion

Thus the present work suggests that 800 IP of AMF is quite effective in influencing the growth characters and nutrient status of Litchi substantially. With the increasing concentrations of AMF, the responses were found to be significantly higher leaving only some characters that were at par with others. While dealing with different concentrations of AMF, the problem of low survival of the air-layers and poor establishment in the nursery has been resolved to a much larger extent. AMF has lead to the development of healthy planting material in the nursery which could reduce the time taken by the young plants to reach the maturity; hence early fruiting can be observed bringing monetary benefits to the farmers. Thus, optimization of the standard dose of AMF would definitely create a landmark in the field of fruit growing and more research work is to be needed regarding the same.

Acknowledgement

We are extremely thankful to Dr. A.K. Sharma, Assoc. Director, Department of Biological Science, College of Basic Sciences and Humanities, GBPUA&T, Pantnagar and Mrs. Shruti Chaturvedi (SRF), for providing us with the AMF inoculums and their necessary guidance during the course of completing the research work successfully.

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