Influence of Biofertilizers on *In-Vitro* Raised Plantlets of *Santalum album*(Linn.) for Better Growth and Survival under Nursery Conditions

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Abstract:

A novel approach was undertaken to assess the growth performance and survival of *in vitro* raised *Santalum album* plantlets under nursery conditions in response to various biofertilizer applications. Due to parasitic nature, *S. album* finds it difficult to absorb a sufficient amount of moisture and essential nutrients directly from the soil on its own. Furthermore, plantlets raised through *in vitro* conditions often face a hard time to survive in the initial stages of hardening in the nursery. To overcome this problem, experiments were conducted to stimulate the uptake of water and nutrients by adding biofertilizers directly into the media (sand: soil: compost at 3:3:1 ratio) during secondary hardening. In the present study it was observed that among all treatments, a combination of *Azotobacter* and *Pseudomonas* (T7) reflected maximum height, collar diameter and the number of leaves per plant with a maximum percentage of survival. Therefore, the application of biofertilizers revealed a positive effect on the growth and survival of the overall plantlets of *S. album*.

Keywords: *Biofertilizers, Santalum album, micropropagation, nursery conditions*

INTRODUCTION

Santalum album (Linn.) commonly known as white sandalwood belongs to family Santalaceae. Indian sandalwood is highly prized for its fragrant heartwood and oil. Sandalwood is a hemi-root parasite tree species. For moisture and simple nutrients, it has to be partially dependent on the host plant even though they are able to manufacture their own complex compounds (Wanntorp & De Craene, 2009). Bio-fertilizers contain living microorganisms that play a significant role in regulating the dynamics of organic matter decomposition, promote the growth of the plant by increasing the supply or availability of primary nutrients such as N, P and S to the host plant (Jen-Hshuan Chen, 2006).

Tissue culture raised plants are often structurally and physiologically abnormal. The abnormal leaf morphology and anatomy, poor photosynthetic efficiency (Donelly and Vidaver, 1984) malfunctioning of stomata and marked decrease in epicuticular waxes are some of the limitations which are faced during the initial hardening.

It is proven through various studies that *S. album* do require the help of other microorganisms to absorb nutrients. *S. album* root zone contains more nitrogen-fixing bacteria and VAM fungi compared to the pigeon pea (Subbarao et. al., 1990). Studies of the association between arbuscular mycorrhizal fungi (AMF) and growth of seedlings show that spores extracted from the rhizosphere of sandal are predominantly of *Glomus* and *Gigaspora* species. (Subbarao et. al., 1990, Thappar et. al., 1992). *S. album* seedlings inoculated with *Glomus sp*. performed better than uninoculated seedlings in terms of water relations and nutrient content (Nagaveni et al. 1998). Ananthpadmanabha et. al., 1988 observed a positive correlation in good host species to the shoot growth and root system development with high production of haustoria in *S. album*. The best growth of *S. album* seedlings was obtained with a host of *Alternanthera sessilis*, followed by *Mimosa pudica* enhancing significant growth and nutrient status (NPK) (Annapurna et. al., 2006).

No report has been recorded on the effect of biofertilizers on tissue culture raised planting material of sandalwood for survival and growth studies.

In view of the above, an investigation was carried out to observe the role of different biofertilizers inoculation on *in vitro* raised plantlets of *S. album* for better growth and survival under nursery conditions.

MATERIAL AND METHODOLOGY

Preparation orInoculation of biofertilizers in the soil:

Six different types of biofertilizers [Azotobacter, Phosphate solubilizing bacteria (PSB), Pseudomonas, Rhizobium, Trichoderma and Vesicular-Arbuscular Mycorrhiza (VAM)] was procured from the

University of Agricultural Sciences, Bangalore. For each treatment,1g of inoculum per plant is added directly to the soil where they have to compete with microbes already living in the soil that are already adapted to local conditions.

Tissue culture raised primary hardened healthy *S. album*(clone T1) plantlets of uniform size were used to test the effect of bio-fertilizers and the following experiments were carried out. The micro propagated *S. album* of similar height were planted in root trainers (250cc) containing the potting mixture comprising sand: soil: compost in the ratio of 3:3:1. The compost was pre-sterilized in an autoclave at 121^{o}C (20 min) to ensure the elimination of microbes in the organic compost. A total of nine plantlets per treatment were planted with and without a primary host. Initially, primary hardened plantlets were maintained in the mist chambers for 3-4 weeks and acclimatized to the nursery conditions. To improve the quality of plants and enhance survival percentage different biofertilizers were tested alone and combinations along with control.

Treatment no.	Biofertilizer/s
T1	Azotobacter
T2	Pseudomonas
T3	Rhizobium
T4	PSB
T5	VAM
T6	Trichoderma viride
T7	Azotobacter+Pseudomonas
T8	Azotobacter+Rhizobium
T9	Azotobacter+PSB
T10	Azotobacter+VAM
T11	Pseudomonas+Rhizobium
T12	Pseudomonas+PSB
T13	Pseudomonas+VAM
T14	Rhizobium+PSB
T15	Rhizobium+VAM
T16	PSB+VAM
T17	Azotobacter+Pseudomonas+Rhizobium
T18	Pseudomonas+Rhizobium+PSB
T19	Rhizobium+PSB+VAM
T20	Azotobacter+Pseudomonas+Rhizobium+VAM+PSB
T21	Control

Experiment 1: Effect of biofertilizers with hostAlternanthera brasiliana

An experiment was conducted, with the primary host of *Alternanthera brasiliana* to check the growth and survival percentage of *S. album*. After the application of biofertilizers, the plantlets were kept in the polytunnel for six months and irrigated regularly with sterile water. The host plants were pruned at regular intervals to check the overgrowth. The data was recorded periodically till the sixth month from the date of application.

Experiment 2: Effect of biofertilizers without host

The above experiment was repeated but this time without the host to see whether the same results are reflecting irrespective of the role of host in the growth and survival percentage of *S. album* plantlets. The plantlets were kept in the same polytunnel along and provided with the same environment and maintenance provided to the experiment with the host. Till sixth-month data was recorded for the growth and performance of the plantlets.

Experiment 3: Effectiveness of the biofertilizers over time

The best four treatments which gave considerably higher results over the other treatments were selected along with the control to further investigate the effectiveness of the biofertilizers over time. Observations recorded for the parameters like shoot length, collar diameter and survival percentage of the plantlets over time. The data was recorded periodically after every two months of interval till the sixth month from the date of application.

Sr. No.	Treatment
T1	Azotobacter+Pseudomonas
T2	Azotobacter+Pseudomonas+Rhizobium+VAM+PSB
T3	Azotobacter+VAM
T4	Azotobacter+PSB
T5	Control

Parameters Measured

The growth parameters taken into consideration comprise of plant height, collar diameter, the number of leaves and percentage of survival were recorded for each treatment at an interval of 2 months. All the parameters were accessed after secondary hardening. Plantlet height was measured from the base of the stem to the angle made between the youngest at the tip. The girth of the stem was measured 1 cm above from the base of the pseudostem using Vernier Caliper. Three replicates for each treatment and each replicate has nine plantlets.

RESULT AND DISCUSSION

The outcomes of the study revealed that the application of biofertilizers on the micropropagated plantlets at the nursery stage significantly enhanced the growth parameters (plantlet height, collar diameter and the number of leaves per plant) as well as the overall quality of *S. album* plantlets as compared to the control.

Exp. 1: Effect of Biofertilizers with primary host:

In the First experiment, among all the treatment tested,application of biofertilizers in T7 (Azotobacter + Pseudomonas) has resulted in higher shoot length (16.50cm), collar diameter (3.58mm) and number of leaves per plant (27.33) followed by T20(24.11), T10(21.77) and T9(16.55). The performance of the treated plants was better than that of the uninoculated ones and the degree of infection was higher in the root trainer (250cc) than with a 'polybag' container. The root trainer being narrow at the root zone increases the greater chances of infection as all the inoculum will contact the feeder roots of plants (Nagaveni *et. al.*, 2014). Similar findings were reported by Balasubramanian and Srinivasan (1995) that inoculation with four AM fungi significantly increased the total biomass, leaf area and total chlorophyll content in leaves of *Ailanthus excelsa*, *Tectona grandis* and *Dalbergia sissoo* seedlings in the nursery.

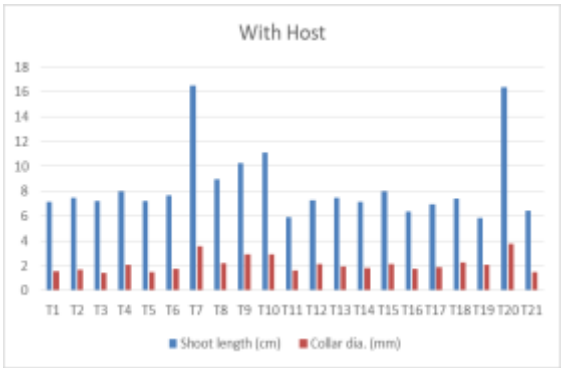

Fig. 1: Effect of Biofertilizers (alone and in combination) on growth of tissue culture raised Sandalwood plantlets with primary host (*Alternanthera brasiliana*) in nursery condition after three months from application.

The interaction between the host plant and biofertilizer was also found significantly affecting the number of leaves per plant at the nursery stage. The maximum number of leaves per plant (27.33) was observed in T7 followed by T20 (24.11), T10 (21.77) and the lowest number of leaves per plant was recorded in T6 (5.55). The overall plant growth and survival percentage were enhanced when the biofertilizers were used in combination. The results were supported with the findings of positive and dynamic interactions among bacterial and cyanobacterial strains and their promise in integrated nutrient management of wheat crop by Nain, L. *et. al.* 2010. Kumar N. *et. al.* 2018, concluded that a combination of AMF+Rhi/Azo+PSB may be used to enhance the yield of *A. hypogaea* and *S. indicum* in Bundelkhand region of central India.

Exp. 2: Effect of Biofertilizers without primary host:

A second experiment was conducted without a host plant in view of the better performance and establishment of micropropagated *S. album* plantlets under nursery conditions. It was conducted to see whether sandal plants can perform better without the help of the host plant in the presence of biofertilizers. It was observed that the *S. album* plantlets did not survive well without a host plant even though the plants were fortified with fertilizers inoculum (Fig. 2). This shows that *S. album* can only do well with a host plant because the major nutrients were taken through the host roots by haustorial connection (Sreenivasa Rao 1933; Patthasarathi *et. al.*, 1974; Ananthapadmanabha *et. al.*, 1988). Nagaveni *et. al.*, (1998) observed that the root region of Sandalwood had more N_2 fixing bacteria and VAM than that of the host.

The use of biofertilizers not only brings down the cost over the expensive chemical fertilizers but also increases soil fertility for a longer duration. The increment in the shoot length and collar diameter in treatment T7 was mainly due to the use of living bacteria Azotobacter, which is known for its ability to fix atmospheric nitrogen and also its antifungal compounds to fight against many plant pathogens as the *S. album* is more prone to fungal diseases in early stage of growth especially under nursery conditions. Whereas the Pseudomonas helps to control the fungal diseases as well.

Muthukumar and Udaiyan (2010) found a positive response in all growth parameters and nutrient uptake in *Casuarina equisetifolia* seedlings inoculated with biofertilizers under tropical nursery conditions. Rashmi and Bhavana (2015) stated that the inoculation of VAM in seedlings of woody species at the nursery stage can be aided in the development of agroforestry models and other floristic vegetation in the degraded land. Binu *et. al.*, (2015) reported the response of sandalwood seedlings to AMF inoculation, increased the seedling height, number of leaves, leaf area and shoot weight. Verma *et. al.*, (2012) screened out different plant growth-promoting micro-organisms for sandalwood in the nursery for better survival and growth of the seedlings.

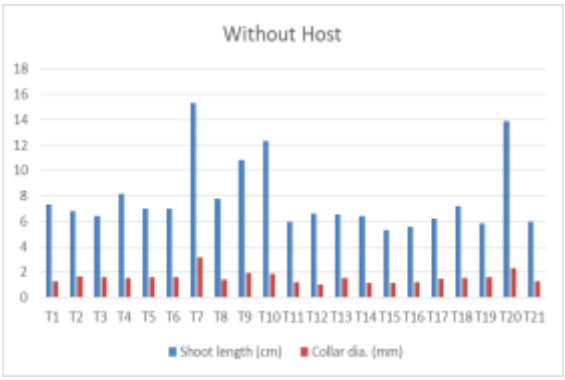

Fig. 2: Effect of Biofertilizers (alone and in combination) on growth of tissue culture raised Sandalwood plantlets without primary host in nursery condition after three months from application.

Exp. 3: Effectiveness of the biofertilizers over time:

Among all the treatment tested, the best-performed treatments were selected for further studies on the biofertilizer effect on the plantlets over time. The plantlets were kept in the same polytunnel and gradually exposed to the 50% shade net for the next six months and observations were recorded periodically.

Plantlets in the media inoculated with different combinations of biofertilizer showed more than 80% survival compared with the control 55% (Fig.1). As the seedlings treated with combinations of biofertilizers performed better than individuals, further work was carried out for different aged Sandal plantlets. Haustoria formed on sandalwood seedlings in the absence of a host, but were much more abundant in their presence.

Table 1: Selected best four treatments for further experiments along with control

Sr. No.	Age of plantlets	Treatment	Shoot length (cm)	Collar dia. (mm)	Survival %
T1	After 2 months	Azotobacter+Pseudomonas	12.6	2.69	100
T2		Azotobacter+Pseudomonas+Rhizobium+VAM+PSB	11.7	1.93	100
T3		Azotobacter+VAM	11.5	2.08	95
T4		Azotobacter+PSB	8	1.83	100
T5		Control	7.2	1.59	80
T1	After 4 months	Azotobacter+Pseudomonas	18.6	3.01	100
T2		Azotobacter+Pseudomonas+Rhizobium+VAM+PSB	17.9	2.24	100
T3		Azotobacter+VAM	13.3	2.20	95
T4		Azotobacter+PSB	10.3	2.11	85
T5		Control	8.9	1.68	60
T1	After 6 months	Azotobacter+Pseudomonas	22.6	3.78	100
T2		Azotobacter+Pseudomonas+Rhizobium+VAM+PSB	21.3	2.62	100
T3		Azotobacter+VAM	17.7	2.57	90
T4		Azotobacter+PSB	13.2	2.35	85
T5		Control	9.6	2.09	35

Plate 1: *a) S. album*plantlets with host *Alternanthera brasiliana*. b) *S. album* plantlets without host. c& d) *S. album* plantletsshowing haustorial connections and growth after four months of biofertilizer application.

CONCLUSION

The use of biofertilizers and their different combinations to enhance the survival percentage of sandalwood plantlets is a current need of the day for the promotion of eco-friendly planting material. The present study throws light on the utility of proper biofertilizers and their suitable combinations of different bio-inoculants with positive synergistic interactions, under nursery conditions. Thus, biofertilizers during the initial stage of growth (nursery stage) increase plants vigour and enhance growth by improving root activity and robustness of plantlets for proper field establishment.

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