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Role of external nutrient supply in establishing host-sandalwood interactions

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ABSTRACT

Sandalwood is semi root parasite plants that required host plant for water and nutrients. So, for successful sandalwood plantation, it is important to study the host parasite interactions and role of external supply of water and nutrient, both theoretically and practically. To find out long-term host species for sandalwood, a randomized block design experiment was conducted to identify the suitability of host and effect of externally supplied nutrient medium on sandalwood planted with five selected host (Alternanthera sp., Azadirachata indica, Dalbergia sissoo, Melia dubia, and Aquilaria malaccensis) and without host species. After 180 days of establishment, morphological traits were recorded and noted that plant height, collar diameter and root length was higher in Dalbergia sissoo and Melia dubia as compared to other host species. Significant enhancement in morphological traits was recorded with external supply of Hoagland nutrient medium. Similarly, higher shoot biomass and root biomass were noted in Dalbergia sissoo and Melia dubia under control as well as with externally supplied nutrient medium. While, maximum haustorial connections were formed with Dalbergia sissoo over studied host species. On the other hand physiological trait such as chlorophyll content was abundant in sandalwood grown with Dalbergia sissoo. Physiological parameters such photosynthetic rate, transpiration rate were higher in sandalwood cultivated with Dalbergia sissoo. Macronutrient such as nitrogen, phosphorus and potassium content was also higher in Dalbergia sissoo and Melia dubia. The results revealed that sandalwood could be grown successfully with Dalbergia sissoo and external supply of nutrients might enhance the growth and physiology of sandalwood.

Key words: Sandalwood, Host species, Haustoria, Nutrient medium, Chlorophyll content

INTRODUCTION

Due to adverse safety issues along with prior official prohibitions on sandalwood, India doesn't yet have the extensive propagation of "Chandan," despite sandalwood (*Santalum album L.*), the second-costliest wood in the world (Verma *et al.,* 2023; Bunney *et al.,* 2022). However, the federal system has modified its regulations to permit citizens and landowners to cultivate sandalwood on their private property and farms. These days, NITI AYOG has placed special emphasis on growing sandalwood on agricultural land. Farmers and other stakeholders around India are very interested in this miraculous tree because of the financial benefits that sandalwood plantations provide (Soundarajan *et al.,* 2017; Verma *et al.,* 2023b). The moderately sized, perennial, semi-parasitic sandalwood tree possesses several fragrant and therapeutic qualities, including antioxidant, neural, and anticancer effects (Liu *et al.,* 2023). Due to parasitism, sandalwood retrieves 70 percent of the water and nutrients from the roots of its host species through xylem/phloem connections made by the Haustorium organ (Rocha *et al.,* 2017; Mohapatra and Avni, 2022). Sandalwood absorbs nutrients such as Nitrogen, Phosphorus, Potassium and Calcium from roots of host plant (Srikantaprasad *et al.,* 2022). The literature revealed that over 300 plant species could serve as hosts in tropical and sub-tropical environments (Srikantaprasad *et al.,* 2022). Literature also reported three types of host species for sandalwood viz., pot level host; mid-term host and long term host (Rocha *et al.,* 2017). However, considering environmental fluctuations, there are still no long-term acceptable host species for sandalwood.

It was believed that the relationship between hemi-parasites and their hosts was influenced by mineral nutrients and water, the two main below-ground abiotic resources (Zhang *et al.,* 2017; Mishra *et al.,* 2018; Doddabasawa and Chittapur, 2021). Certain research indicated that external supply of Hoagland nutrient medium provided essential mineral nutrients, including potassium, magnesium, calcium, zinc, boron, nitrogen, and phosphorus to plants (Xu *et al.,* 2020). Thus, it was necessary to normalize

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the long-term selection of appropriate host species as well as management practices for sandalwood cultivation. Thus, the current investigation aimed to figure out the suitable sandalwood-host combination that would enable it to sustain itself and supply sufficient nutrients for long-term growth.

MATERIALS AND METHODS

Experimental setup and treatment details

A randomized block design experiment with five replications was conducted in 2022 and 2023 at the Central Soil Salinity Research Institute (ICAR) in Karnal, using 20-kilogram plastic pots. 1.0–1.5 years old sandalwood seedlings were transplanted together with five distinct types of host plants: *Aquilaria malaccensis* (Agarwood), *Azadirachta indica* (Neem), *Melia dubia* (Dek/Malabar neem), *Alternanthera sp*. (nursery host), and *Dalbergia sissoo* (Shisham). Treatments were imposed viz., T_1 (Best available water) and T_2 (Best available water + Hoagland nutrient medium) after 60 days of successful establishment of sandalwood plants in the pots. After 180 days of imposed treatments, plants were uprooted to record various morphological and physiological traits of sandalwood planted with different host species.

Morphological traits

The effects of nutrient medium and host species on sandalwood morphological parameters were assessed. Plant height was measured using meter scale (centimeter) and diameter was measured using digital vernier caliper (millimeter). Fresh shoot/root biomass (gram) was taken at the time of harvesting and dry shoot/root biomass (gram) was taken after proper drying in oven using weighing balance. Numbers of leaves and haustoria were counted manually.

Physiological traits

The total amount of chlorophyll was measured in mg g^{-1} fresh weight and determined using the Arnon (1979) method. The gas exchange parameters such as Photosynthetic rate (Pn) and Transpiration rate (E) were recorded on leaves through portable gas exchange system Infra-red gas analyser (LICOR–6800, LICOR Inc., Lincoln, NE) (Kumar *et al.,* 2023; Verma *et al.,* 2023b).

Macronutrients Uptake

After 180 days of seedling establishment, nutrients were analyzed in leaves of sandalwood. 50 mg well –dried leaf sample were digested with 80% nitric acid on hot plate then made the volume up to 50 ml with distilled water. Potassium content was analyzed using with flame photometer (Systronics flame photometer 128). The ammonium vando-molybdate phosphoric acid yellow color method was used to measure the percentage of phosphorus in 5 ml of digested plant extract. On the other hand, Nitrogen (N) content was estimated by Kjeldahl method.

Statistical analysis

The two-way factorial analysis of variance (ANOVA) was performed using IBM SPSS statistics software (Version 27.0) on measured and computed data that was collected in triplicates. Dunkan`s Multiple Range Test (DMRT) was used to compare mean differences at a 5% level of probability.

RESULTS AND DISCUSSION

Because it is semi-parasitic, sandalwood demands a plant to serve as its host that can grow in tandem with it and supply nutrients and water to promote better development. Consequently, to understand the developmental aspect, the present study was conducted on sandalwood cultivated with different host plants with or without external supply nutrient medium.

Morphological and Physiological traits

The morphological traits were significantly influenced by different host plants. It was shown that sandalwood cultivated with *Dalbergia sissoo* and *Melia dubia* had higher collar diameters (4.85 and 4.59 mm) and plant heights (77.42 and 68.40 cm) among studied host species as shown in table 1. Despite this, sandalwood cultivated in the absence of a host plant survived but it grew very slowly, measuring just 13.67 cm in plant height and 1.59 mm in collar diameter. These results were consistent with earlier research that revealed that, out of the ten host species during study, *Dalbergia sissoo* and *Melia dubia* had the greatest growth in terms of plant height, diameter, and biomass (Verma *et al.,* 2023a). It was also noted that external supply of nutrient medium showed

23.58% and 20.31% increment in mean plant height and mean diameter of studied sandalwood-host combinations. Sandalwood absorbs all essential nutrients and water from roots of host species through modified organ called haustoria (Verma *et al.,* 2023b; Zhang *et al.,* 2022). So, root length is another important morphological parameter that plays important role in nutrient uptake (Rocha *et al.,* 2017; Srikantaprasad *et al.,* 2022). Present results revealed that external supply of Hoagland nutrient medium showed increment of 17% in root length over studied sandalwood-host combinations (Table 1). Significant improvement was also noted in root length when sandalwood grown with *Dalbergia sissoo* (42.12 cm) and *Melia dubia* (33.52 cm) among studied host species. Maximum number of leaves formation indicates the highest photosynthetic activity in plants. Present results revealed that maximum number of leaves was formed when sandalwood grown with *Aquilaria malaccensis* (325) and *Dalbergia sissoo* (306.33) as compared to other host species. Conversely, sandalwood grown in the absence of a host plant has the fewest leaves. Significant increment of 22.33% was noted in sandalwood-host combinations when Hoagland nutrient medium was supplied externally over studied treatments (Table 1). On the other hand, sandalwood planted with *Dalbergia sissoo* (22.82g Fresh Weight and 12.50g Dry Weight) and *Melia dubia* (27.64g Fresh Weight and 14.49g Fresh Weight) showed highest shoot biomass in terms of fresh weight and dry weight as compared to other host species (Table 1).

Table 1: Sandalwood's morphological characteristics with various hosts under the supply of nutrient medium

Treatments/Traits	Plant Height (cm)	Collar Diameter (mm)	Root Length (cm)	Number of Haustoria	Number of Leaves	Shoot Fresh Weight (g)	Shoot Dry Weight (g)	Root Fresh Weight (g)	Root Dry Weight (g)
Sandalwood response with different host species									
Sandalwood (alone)	13.67^{d}	1.59^{d}	9.50^{d}	0.00	33.50^{d}	6.06^{e}	2.61^{e}	3.29^{d}	1.91^{d}
Alternanthera sp. (Nursery host)	48.40^{c}	2.48^{c}	27.93^{c}	17.00^{b}	292.33^{c}	11.01^{d}	6.13^{d}	8.60^{c}	3.84^{c}
Azadirachta indica (Neem)	66.50^{b}	3.50^{b}	29.50^{bc}	17.50^{b}	285.17^{c}	10.03^{d}	5.67^{d}	13.48^{a}	6.46^{ab}
Dalbergia sissoo (Shisham)	77.42^{a}	4.85^{a}	42.12^{a}	24.67^{a}	306.33^{b}	22.82^{b}	12.50^{b}	15.10^{a}	7.42^{a}
Melia dubia (Malabar Neem)	68.40^{b}	4.59^{a}	33.52^{b}	16.67^{b}	281.33^{c}	27.64^{a}	14.49^{a}	14.92^{a}	6.91^{ab}
Aquilaria malaccensis (Agarwood)	66.95^{b}	2.92^{bc}	32.83^{b}	17.00^{b}	325.00^{a}	17.07^{c}	9.18^{c}	11.58^{b}	6.40^{b}
LSD @ 5% (H: Host)	6.30	0.69	4.11	2.41	12.84	2.49	1.00	1.74	1.00
P-value	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
F- value	115.51	27.69	59.29	98.19	615.69	94.05	169.99	58.40	38.69
Sandalwood response under different stress treatments									
T_1 [Best Available Water (BAW)]	50.89^{b}	2.90^{b}	26.94^{b}	13.56^{b}	228.44^{b}	13.97^{b}	7.38^{b}	9.81^{b}	4.36^{b}
T_2 (BAW + Hoagland Nutrient Medium)	62.89^{a}	3.75^{a}	31.52^{a}	17.39^{a}	279.44^{a}	17.57^{a}	9.48^{a}	12.50^{a}	6.63^{a}
LSD @ 5% (T: Treatment)	3.64	0.40	2.37	1.39	7.41	1.44	0.58	1.00	0.58
P-value	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
F-value	46.41	19.14	15.84	32.29	201.56	26.70	55.61	30.52	64.57
LSD @ 5% (H×T)	NS	NS	5.82	3.41	18.16	NS	NS	NS	NS
P-value	0.10	0.21	0.004	0.012	0.00	0.81	0.62	0.47	0.35
F-value	2.09	1.55	4.69	3.72	8.63	0.45	0.71	0.95	1.16

Means with at least one letter common are not statistically significant ($p<0.05$) using Dunkan`s LSD test

Substantial increment was noted in shoot biomass of sandalwood- host combinations when nutrient medium was supplied externally. However, as Table 1 illustrates, sandalwood planted without a host species grew poorly in terms of biomass (6.06g Fresh Weight and 2.61g Dry Weight). Haustoria are particular structures that aid in the transportation of water and nutrients from the host plant to the parasitic plants. They are derived from the posterior extension of both primary and lateral roots (Doddabasawa and Chittapur, 2021; Zhang *et al.,* 2022). Number of haustoria is another important morphological trait that plays important role in eco-physiology of sandalwood parasitism. It was observed that sandalwood planted with *Dalbergia sissoo* and *Azadirachta indica* showed maximum haustoria formation (24.67 and 17.50) as shown in Table 1. More number of haustoria formation showed abundant transfer of mineral nutrientsand water from host plant to parasitic pl ant (Rocha *et al.,* 2017; Kumar *et al.,* 2021). Notable advancement of 28.2% in haustoria formation was also noted when nutrient medium supplied externally. The relationships between sandalwood and its host species, resulting in improved microenvironment conditions, increased nutrient availability, and decreased competition, may be responsible for these improvements (Gomes and Adnyana, 2017; Smith *et al.,* 2017). Hemi-parasitism, which primarily relies on the molecular basis of both parasitic and hosts plant, is an efficient way for them to obtain water and nutrients from the plants they invade without performing a significant amount of biomass to roots (Moncalvillo and Matthies, 2023). Fresh weight and dry weight of root was highest when sandalwood grown with *Dalbergia sissoo* (15.10g Fresh Weight and 7.42g Dry Weight) and *Melia dubia* (14.92g Fresh Weight and 6.91g Dry Weight) as compared to other hosts over studied treatments. When nutrient medium was supplied externally, significant increment of 27.42% in fresh weight and 52.01% in dry weight of root was observed as shown in Table 1.

Sandalwood grew without host plant showed poor growth as compared to sandalwood planted with different host species. Plant improvement and growth are eventually facilitated by photosynthesis, mineral nutrition, and overall physiological performance. Chlorophyll content is an important physiological trait in sandalwood parasitism. Present results revealed that chlorophyll content was higher when sandalwood grown with *Dalbergia sissoo* and *Melia dubia* as shown in Figure 1. Addition of nutrient medium enhanced the chlorophyll content of sandalwood-host interactions.

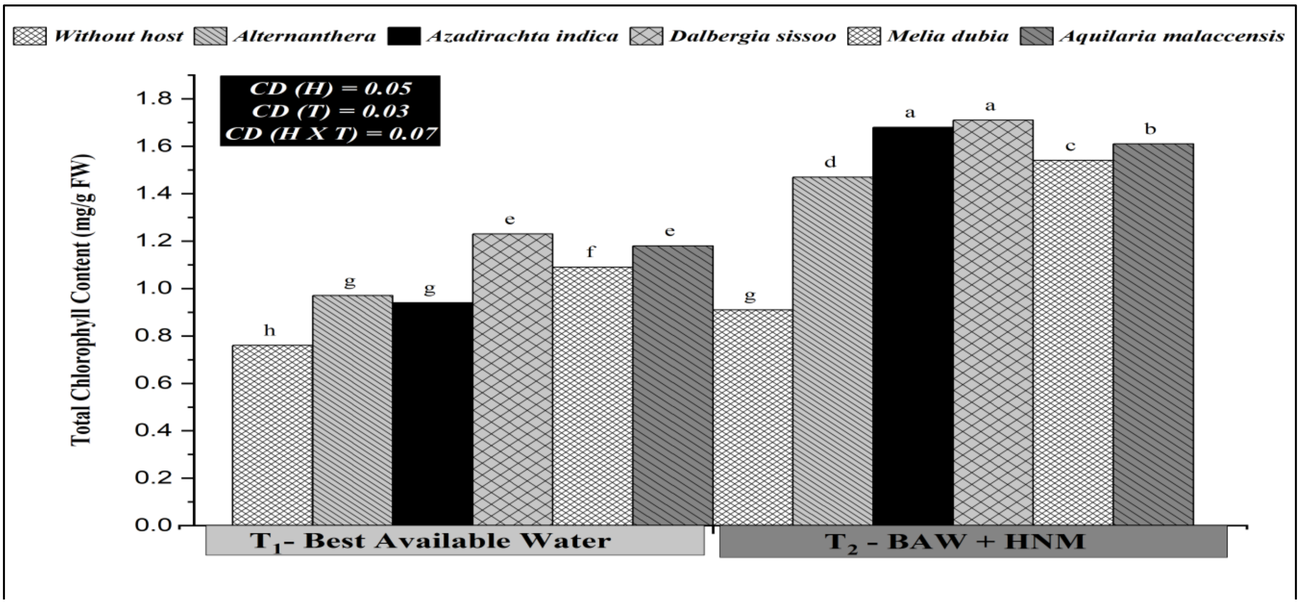
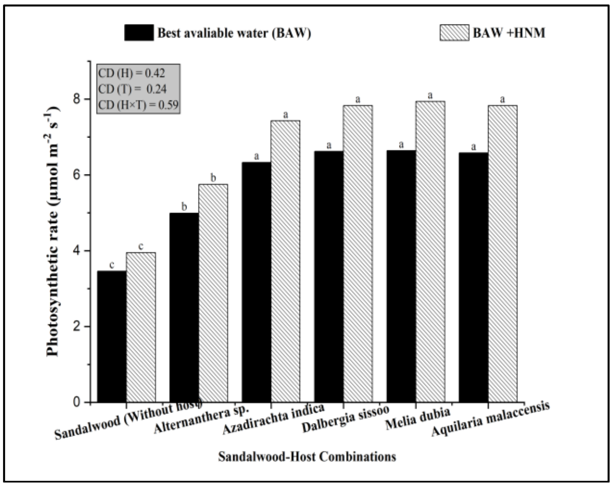


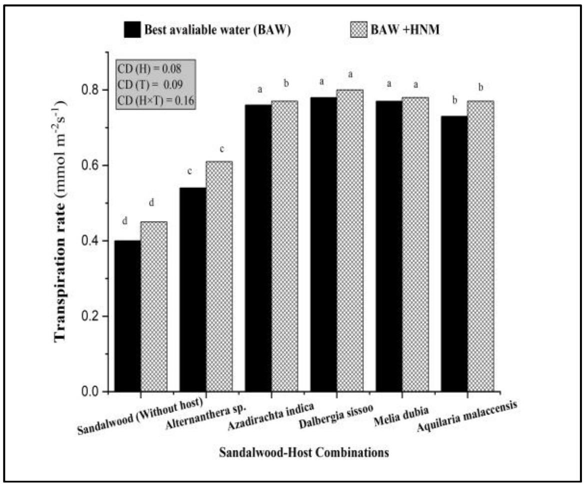
Figure 1: Effect of host species and Hoagland nutrient medium on chlorophyll content of sandalwood. Means with at least one letter common are not statistically significant ($p<0.05$) using Dunkan`s LSD test

Physiological Functioning

Photosynthesis, mineral feeding, respiration, transportation, and eventually plant growth and development all support a plant's physiological functioning (Kumar *et al.,* 2023). Among these, photosynthesis is essential to plants because it allows them to absorb light energy and convert it into sugars. Host plant regulates sandalwood photosynthetic rate and observed that sandalwood grown with *Dalbergia sissoo* and *Melia dubia* showed maximum photosynthetic rate compared to other studied host under different conditions (Figure 2A). When nutrient medium was supplied externally it showed significant increment in photosynthetic rate activity of sandalwood planted with different host species. Low water content and osmotic balance disrupt turgor, which influences the conductance of stomatal cells, evaporation, and photosynthesis. It also impacts root water uptake, which is influenced by the possibility of difference between leaf and soil water. Transpiration rate was also higher in sandalwood cultivated with *Dalbergia sissoo* and *Melia dubia* over studied hosts (Figure 2B). These factors make it difficult for plants to draw water from the soil (Rocha and Santhoskumar, 2022). Reduced photosynthetic rate and transpiration rate put disturbance in water uptake and the elevated intrinsic water use efficiency may be a compensatory mechanism to reduce water loss (Smith *et al.,* 2017).



(A)



(B)

Figure 2: Effect of host species and Hoagland nutrient medium on Photosynthetic rate (A) and Transpiration rate (B) of sandalwood. Means with at least one letter common are not statistically significant ($p<0.05$) using Dunkan`s LSD test

Macronutrients Uptake

Sandalwood performance is influenced by the demand for subsurface elements and the host's capacity to supply both water and mineral fertilizers (Doddabasawa and Chittapur, 2021). Literature revealed that sandalwood absorbs nutrients Nitrogen, Phosphorus and Potassium from host's root through haustorium. The current study clarified how various host species can have a favorable or detrimental impact on the nutrients that are transferred from the host to the sandalwood. It was noted that sandalwood grown with *Dalbergia sissoo* and *Melia dubia* showed higher nitrogen (0.287% and 0.285%) and phosphorus content (0.623% and 0.605%). While sandalwood grown in the absence of host showed lowest nitrogen and phosphorus content as shown in Table 2. The host benefited from the higher P levels in host tissues, and the parasite's detrimental effects were greatly reduced (Rocha *et al.,* 2017; Gomes and Adnyana, 2017).

Table 2: Mineral nutrient uptake in sandalwood with various hosts under the supply of nutrient medium

Treatments/Traits	Nitrogen (%)	Phosphorus (%)	Potassium (%)
Sandalwood response with different host species			
Sandalwood (alone)	0.197^{c}	0.292^{e}	2.23^{b}
Alternanthera sp. (Nursery host)	0.262^{b}	0.518^{d}	2.43^{b}
Azadirachta indica (Neem)	0.280^{a}	0.513^{d}	1.79^{f}
Dalbergia sissoo (Shisham)	0.287^{a}	0.623^{a}	1.91^{e}
Melia dubia (Malabar Neem)	0.285^{a}	0.605^{b}	2.16^{c}
Aquilaria malaccensis (Agarwood)	0.265^{b}	0.533^{c}	2.04^{d}
LSD @ 5% (H: Host)	0.011	0.015	0.062
P-value	0.00	0.00	0.00
F-value	88.52	550.29	119.69
Sandalwood response under different stress treatments			
T_1 [Best Available Water (BAW)]	0.208^{b}	0.519^{a}	2.04^{b}
T_2 (BAW + Hoagland Nutrient Medium)	0.317^{a}	0.509^{b}	2.15^{a}
LSD @ 5% (T: Treatment)	0.006	0.009	0.036
P-value	0.00	0.017	0.00
F-value	1358.03	6.56	40.95
LSD @ 5% (H×T)	0.015	0.021	NS
P-value	0.00	0.00	0.78
F-value	30.24	47.45	0.50

Means with at least one letter common are not statistically significant ($p<0.05$) using Dunkan`s LSD test

K^+ is well known for its physiological involvement in plants, where it regulates transpiration and upholds osmotic and ionic homeostasis (Kumar *et al.,* 2021). Present results revealed that sandalwood cultivated with *Azadirachta indica* and *Dalbergia sissoo* showed lowest K^+ content (1.79% and 1.91%) in leaves of sandalwood. On the other hand, external supply of nutrient medium showed significant enhancement in nutrient transfers from host to sandalwood (Table 2). It was found that the nutrient status was considerably increased by the interaction between chelated iron and nutrient availability (Kumar *et al.,* 2021).

CONCLUSIONS

The cultivation and commercialization of sandalwood in semiarid regions has not been very successful, despite its enormous potential for commercial production. In the present study, we focused on the nutrient intake and physiology of sandalwood in economically feasible hosts. The most beneficial effects on sandalwood development were shown by *Dalbergia sissoo* and *Melia dubia,* which showed improved nutrient uptake, chlorophyll content and morphological traits over studied treatments. In addition, external supply of nutrients might be beneficial for sandalwood growth.

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