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Enhancing sandalwood growth: Host plant selection and salinity stress tolerance

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Abstract

Being a semi-root parasitic nature, sandalwood steals water and nutrients from host species. Therefore, it is crucial to research the influence of salinity stress on host-parasite relationships both theoretically and practically, for the successful establishment of sandalwood plantations. To find out the best suited host species for sandalwood, a RBD experiment was conducted to identify the effect of salt stress on sandalwood planted with two selected host species (*Alternanthera sp.* and *Azadirachata indica*) and without host species. After 300 days of establishment, morphological traits were recorded and noted that plant height, collar diameter and root length was higher in *Azadirachta indica* as compared to other host species. Significant reduction in morphological traits was recorded under salt stress condition. Similarly, higher root biomass was noted in *Azadirachta indica* under control as well as in saline conditions. On the other side, shoot biomass was higher in sandalwood grown with *Alternanthera sp*. Sandalwood grown in the absence of host plant showed no survival under salinity stress. Plant water traits were higher when sandalwood cultivated with *A. indica* while lowest in sandalwood grown in the absence of host species. Na^+/K^+ ratio and other nutrients (*viz.*, nitrogen, phosphorus and potassium) was higher sandalwood planted with *A. indica* while poorer in sandalwood planted without host species. The results revealed that *A. indica* could be best host for sandalwood under stress as well as control conditions which enhances the growth and physiology of sandalwood.

Key words: Sandalwood, host species, traits, salinity, Na^+/K^+ ratio

Introduction

Sandalwood is most costly wood across the globe due to fragrant and therapeutic qualities including antioxidant, neural, and anticancer effects (Liu *et al*., 2023; Kumar *et al*., 2015). Farmers and other stakeholders around India are very interested in this miraculous tree because of the financial benefits that sandalwood plantations provide (Rocha *et al*., 2017; Kumar *et al*., 2012; Kumar *et al*., 2021). Due to prior official prohibitions on sandalwood, India doesn't yet have the extensive propagation of sandalwood (Viswanath, 2022; Zhang *et al*., 2022). Now, the federal system has modified its regulations regarding permission for cultivation of sandalwood on their private property and farms. Due to hemi-parasitism, sandalwood steals 70 percent of the water and nutrients from the roots of its host species through xylem/phloem connections (Rocha *et al*., 2017; Mohapatra and Anil, 2022; Yang *et al*., 2014). Sandalwood steals macronutrients such as nitrogen, phosphorus, potassium, calcium and water from host plants via a modified structure (Srikantaprasad *et al*., 2022). The literature revealed that over 300 plant species could serve as hosts in tropical and sub-tropical environments (Nagaveni and Vijayalakshmi, 2003; Srikantaprasad *et al*., 2022). However, considering environmental fluctuations like abiotic stresses, there are still no long-term acceptable host species for sandalwood. Salinity is one of the major abiotic stresses around the world that limit growth and productivity of sandalwood (Dhansu *et al*., 2021). In plants, salinity primarily causes hyper-ionic and hyper-osmotic stress, which disrupts cellular processes, such as transpiration, photosynthesis, and function of plasma membrane (Hasanuzzaman and Fujita, 2022; Kumar *et al*., 2023). There is no information available in the literature regarding influence of salinity on sandalwood. In literature, there is no long term- host species recommended for sandalwood under stress conditions. So, the selection of best suited long term host species is necessary for better growth and development of sandalwood under salinity stress because sandalwood takes 15-20 years for maturation and heartwood cultivation. Keeping the above in view, the present study was conducted to find out the best suited sandalwood host combination under stress.

Materials and methods

Experimental setup and treatment details: A randomized block design experiment with three replications was conducted in 2022 and 2023 at the Central Soil Salinity Research Institute (ICAR) in Karnal, using 20-kilogram plastic pots. Sandalwood seedlings (1.0-1.5 years old) were transplanted together with two different types of host plants: *Azadirachta indica* (Neem) and *Alternanthera sp*. (nursery host). Treatments *viz*., T1 (Best available water) and T2 (Salinity stress EC_{iw}~8ds/m) were imposed after 60 days of successful establishment of sandalwood seedlings in the pots. After 300 days of imposed treatments, plants were uprooted to record various morphological and physiological traits of sandalwood planted with different host species.

Morphological traits: The effect of salinity stress and host species on sandalwood's morphological parameters was assessed. Plant height was measured using meter scale (cm) and diameter was measured using digital vernier caliper (mm). Fresh shoot/root biomass (gm) was taken at the time of harvesting and dry shoot/root biomass (gm) was taken after proper drying in oven using weighing balance. Numbers of leaves and haustoria were counted manually.

Physiological traits: Utilizing 5 µL of sap from leaves of sandalwood, the osmolality (mmol kg^{-1}) was measured applying the digital reading of a vapor pressure osmometer (Model 5600, ELITech Group, Belgium). The osmotic Potential (ψs) was then calculated using the Vant'Haff equation in –MPa (Kaur *et al*., 2022b). Using the chilled mirror dew point approach, the water potential (-MPa) was determined (Soni *et al*. 2021a). The relative water content (%) was estimated by using Barr and Weatherley (1962) method. The total quantity of chlorophyll was calculated using Arnon (1979) method and given in mg g^{-1} fresh weight.

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. Na^+ and K^+ content were 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 (Richards, 1954). On the other hand, nitrogen content was estimated by Kjeldahl method (Jackson et al., 1991).

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. Duncan`s Multiple Range Test (DMRT) was used to compare mean differences at a 5% level of probability.

Results and discussion

Sandalwood relays on host species for nourishment due to hemi-parasitism. Salinity stress negatively impacts the growth and development of sandalwood tree. Hence, present study was conducted to fill the knowledge gap on suitable host species for sandalwood under salt stress.

Morphological and physiological traits: Salinity stress negatively affects the morphological traits of sandalwood planted with different host plants. It was shown that sandalwood cultivated with *Azadirachta indica* had higher collar diameter and plant height 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 6.17 cm in plant height and 0.74 mm in collar diameter. These findings were in line with past research that showed *A. indica* had the highest growth in terms of plant height and collar diameter during the study among the ten host species (Kumar *et al*., 2021). Salinity stress (ECiw~8ds/m) resulted in no survival in sandalwood grown without host species, as shown in Table 1. Sandalwood steals all the essential nutrients and water from roots of host species via xylem to xylem connections made between host plant and sandalwood (Verma *et al*., 2023; Zhang *et al*., 2022). The literature suggests that a higher number of haustorial connections between sandalwood and its host species leads to a significant transfer of nutrients from the host to the parasite (Rocha *et al*., 2017; Srikantaprasad *et al*., 2022). The current results indicate that salinity stress (ECiw ~8 dS/m) caused a 34.21% reduction in root length and a 55.85% decrease in haustoria formation across the examined sandalwood-host combinations (Table 1). 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). Notable enhancement was also noted in root length and haustoria formation when sandalwood grown with *A. indica* among studied host species. Higher number of leaves formation revealed the highest photosynthetic activity in plants. Similarly, maximum number of leaves was formed when sandalwood grown with *A. indica* as compared to other host species. Conversely, sandalwood grown in the absence of a host plant has the fewest leaves. On the other side, sandalwood grown with *Alternanthera sp.* showed higher shoot biomass while root biomass was higher with *Azadirachat indica* (8.87 g Fresh weight and 2.04 g Dry weight) as shown in table 1. Present results showed A. indica was best host species as it has higher salinity tolerance ability. Sandalwood is valuable tree species due to its high therapeutic values (Rocha *et al*., 2017). Considering its therapeutic value, present result provides valuable information regarding host species for sandalwood cultivation and salinity tolerance level of sandalwood.

Salt stress showed notable reduction in shoot and root biomass of sandalwood grown with different host species. Sandalwood grown in the absence of host showed poorer growth in terms of shoot biomass and root biomass as shown in Table 1. Research indicates a decrease in root and shoot biomass of sandalwood cultivated with various host species under saline stress (Kumar *et al*., 2021). Previous studies showed that sandalwood gains higher biomass in the presence of suitable host species (Balasubramanian *et al*., 2021).

Physiological traits: Sandalwood exhibited reduced growth under salt stress, highlighting the essential role of adequate water supply for its proper development. Davies *et al*. (2018) further emphasized that effective water management strategies are necessary for sandalwood to grow well. Present results suggested that salinity stress caused decrease in plant water traits (ψw, ψs and RWC) of sandalwood plants irrespective of host plants (Fig. 1A & 1B). This reduction in sandalwood's ψw, ψs and RWC may be the result of significant nutritional disturbances in the early stages, which is evident in discoloration wilting, and leaf loss, along with a decreased supply of water caused by stressors or by a malfunctioning haustoria linkage (Rocha *et al*., 2014). Among host species, sandalwood had the minimum values of water potential (-1.74 MPa) and osmotic potential (-2.03 MPa) with *A. indica* under salt treatment (Fig. 1A & 1B), which showed towards the better water status of sandalwood through water absorption via xylem connectivity of the host.

Relative water content (RWC), another significant plant water characteristic, explains how plants regulate or maintain the ideal degree of cell hydration (Pooja *et al*., 2019; Dhansu *et al*., 2021) in a stressful environment. Chlorophyll content was higher in sandalwood cultivated with *A. indica* over studied hosts (Figure 2A and 2B). Sandalwood grown in the absence of host species showed no survival under salinity stress conditions.

Macronutrients uptake: Na^+/K^+ remained higher under saline conditions that indicated selectivity of sandalwood in decreasing the

Table 1. Sandalwood's morphological characteristics with various hosts under salinity stress.

Treatments/Traits	Plant height (cm)	Collar diameter (mm)	Root length (cm)	Number of haustoria	No. of leaves	Shoot fresh weight (gm)	Shoot dry weight (gm)	Root fresh weight (gm)	Root dry weight (gm)
Sandalwood response with different host species									
Sandalwood (without host)	6.17^{c}	0.74^{c}	4.00^{c}	0.00	11.83^{b}	2.20^{c}	0.81^{c}	1.36^{c}	0.63^{c}
Alternanthera sp. (Nursery host)	36.50^{b}	1.79^{b}	23.50^{b}	10.33^{b}	217.50^{a}	17.61^{a}	4.82^{a}	5.52^{b}	2.04^{b}
Azadirachta indica (Neem)	51.50^{a}	2.46^{a}	26.67^{a}	12.00^{a}	222.50^{a}	16.24^{b}	4.59^{b}	8.87^{a}	4.21^{a}
LSD @ 5% (H)	3.76	0.47	2.73	0.51	11.24	0.55	0.12	0.80	0.65
Sandalwood response under different stress treatments									
T_1 [Best Available Water (BAW)]	38.22^{a}	2.17^{a}	21.78^{a}	10.33^{a}	187.56^{a}	14.07^{a}	4.42^{a}	7.47^{a}	3.19^{a}
T_2 (Salinity stress EC_{iw}~8ds/m)	24.55^{b}	1.16^{b}	14.33^{b}	4.56^{b}	113.67^{b}	9.96^{b}	2.40^{b}	3.03^{b}	1.39^{b}
LSD @ 5% (T)	3.07	0.39	2.23	0.42	9.15	0.45	0.10	0.66	0.53
LSD @ 5% (H×T)	NS	NS	NS	0.73	15.89	NS	0.17	1.14	NS

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

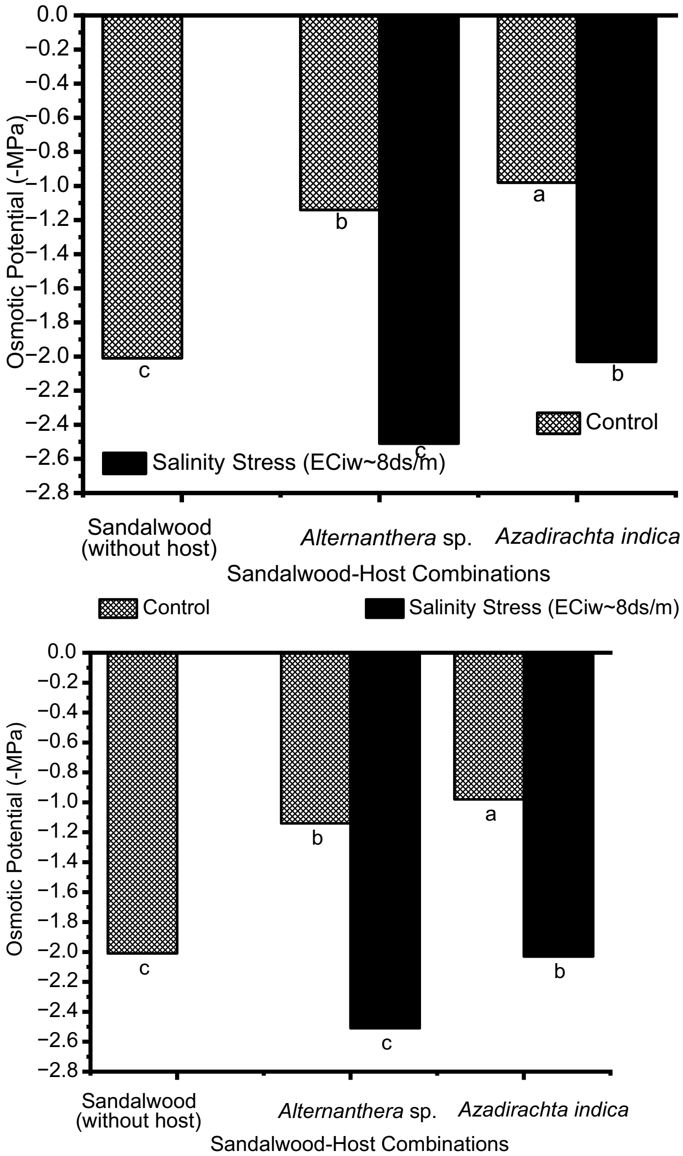

Fig. 1. Effect of host species and salinity stress (EC_{iw}~8ds/m) on Osmotic potential (A) and Water potential (B) of sandalwood. Means with at least one letter common are not statistically significant (P<0.05) using Duncan`s LSD test.

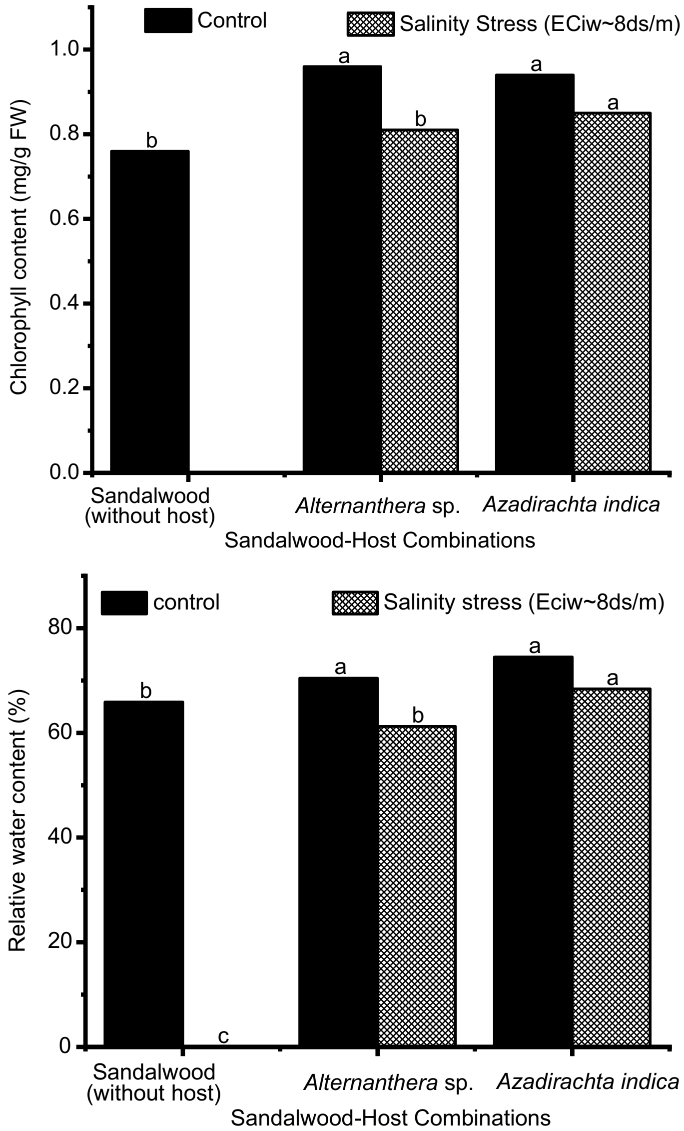

Fig. 2. Effect of host species and salinity stress (EC_{iw}~8ds/m) on Relative water water (A) and Chlorophyll content (B) of sandalwood. Means with at least one letter common are not statistically significant (P<0.05) using Duncan`s LSD test.

uptake and transport of K^+ to mitigate the injurious effects of salinity (Roy and Chowdhary, 2020). Moreover, higher Na^+/K^+ levels can contribute to osmotic adjustment compared to the accumulation of organic (compatible) solutes like proline (Lata *et al*., 2022).

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; Nagaveni and Vijayalakshmi, 2003). Literature revealed

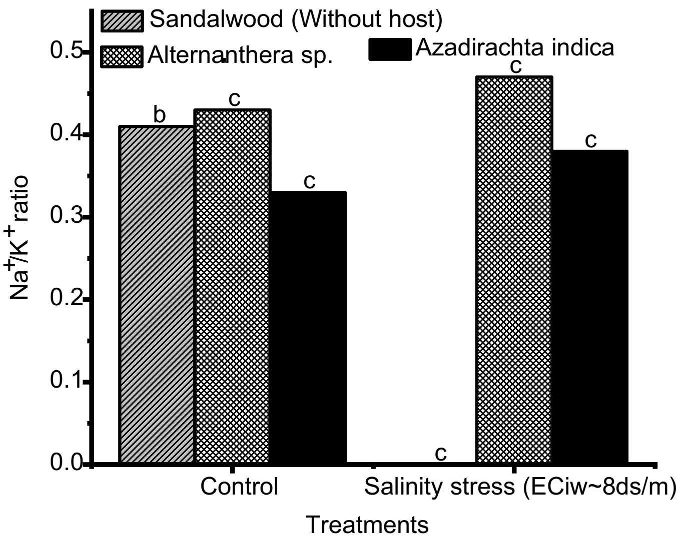

Fig. 3. Effect of host species and salinity stress (EC_{iw}~8ds/m) on Na^+/K^+ ratio of sandalwood. Means with at least one letter common are not statistically significant (P<0.05) using Duncan`s LSD test.

that sandalwood absorbs nutrients N, P and K 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 *A. indica* showed higher nitrogen (0.20%) and with *Alternanthera sp.* showed higher phosphorus content (0.45%). 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 (Glatzel and Geils, 2009; Gomes and Adnyana, 2017). The present results revealed that salt stress negatively impact the transfer of mineral nutrients like N, P and K from host species to sandalwood. 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 *A. indica* showed lowest K^+ content (1.72%) in leaves of sandalwood. On the other hand, Salinity stress showed significant reduction in nutrient transfers from host to sandalwood (Table 2). It was

Table 3. Correlation matrix of control

Parameter	PH	PD	RL	NH	NL	SFW	SDW	RFW	RDW	P	N	K	WP	OP	RWC
PH	1														
PD	0.91***	1													
RL	0.96***	0.84**	1												
NH	0.93***	0.76*	0.98***	1											
NL	0.90***	0.73*	0.96***	0.99***	1										
SFW	0.91***	0.73*	0.96***	0.99***	1.00***	1									
SDW	0.91***	0.74*	0.97***	0.98***	1.00***	1.00***	1								
RFW	0.95***	0.95***	0.93***	0.85**	0.82**	0.81**	0.83**	1							
RDW	0.87**	0.86**	0.88**	0.77*	0.74*	0.72*	0.75*	0.963***	1						
P	0.74*	0.50	0.84**	0.93***	0.95***	0.95***	0.94***	0.609	0.52	1					
N	0.93***	0.76*	0.94***	0.97***	0.96***	0.96***	0.96***	0.806**	0.72*	0.88**	1				
K	-0.52	-0.75*	-0.36	-0.20	-0.15	-0.15	-0.17	-0.66	-0.66	0.15	-0.23	1			
WP	0.86**	0.66	0.93***	0.98***	0.99***	0.99***	0.99***	0.76*	0.67	0.98***	0.94***	-0.05	1		
OP	0.94***	0.77*	0.97***	1.00***	0.99***	0.99***	0.99***	0.86**	0.78*	0.92***	0.96***	-0.23	0.98***	1	
RWC	0.93***	0.79*	0.98***	0.99***	0.99***	0.99***	0.99***	0.87**	0.79*	0.91***	0.95***	-0.23	0.98***	0.99***	1
CHL	0.84**	0.67*	0.92***	0.95***	0.96***	0.95***	0.95***	0.75*	0.70*	0.91***	0.93***	-0.07	0.94***	0.93***	0.95***

Table 3. Correlation matrix of salinity stress

	PD	RL	PH	NH	NL	SFW	SDW	RFW	RDW	CHL	WP	OP	RWC	Na	K	Na/K	P
PD	1																
RL	0.97***	1															
PH	0.99***	0.99***	1														
NH	1***	1.0***	0.99***	1													
NL	0.99***	0.98***	0.95***	0.98***	1												
SFW	1***	1***	0.98***	1***	0.99***	1											
SDW	0.97***	0.94***	0.90***	0.95***	0.99***	0.97***	1										
RFW	0.99***	1***	1***	0.99***	0.97***	0.99***	0.92***	1									
RDW	0.95***	0.98***	0.99***	0.97***	0.92***	0.96***	0.86**	0.99***	1								
CHL	0.99***	0.99***	0.93***	0.97***	1***	0.98***	0.97***	0.95***	0.90**	1							
WP	-0.94***	-0.91***	-0.86**	-0.92***	-0.97***	-0.94***	-0.99***	-0.88**	-0.80**	-0.98***	1						
OP	-0.98***	-0.96***	-0.92***	-0.97***	-0.99***	-0.98***	-0.99***	-0.94***	-0.88**	-1.00***	0.99***	1					
RWC	0.97***	0.94***	0.90**	0.95***	0.99***	0.96***	1***	0.92***	0.85**	0.99***	-1.00***	-1.00***	1				
Na	0.91***	0.87**	0.81**	0.88**	0.95***	0.90***	0.98***	0.84**	0.75*	0.96***	-0.99***	-0.97***	0.99***	1			
K	0.90***	0.86**	0.80*	0.87**	0.94***	0.89**	0.98***	0.82**	0.73*	0.96***	-0.99***	-0.97***	0.98***	1***	1		
Na/K	0.97***	0.95***	0.90***	0.95***	0.99***	0.97***	1***	0.92***	0.86**	1***	-0.99***	-0.99***	1***	0.98***	0.98***	1	
P	0.97***	0.94***	0.90***	0.95***	1***	0.96***	1***	0.92***	0.85**	0.99***	-0.99***	-0.99***	1.00***	0.98***	0.98***	1.0***	1
N	0.98***	0.96***	0.92***	0.97***	1***	0.98***	1***	0.94***	0.88**	1***	-0.99***	-1***	0.99***	0.97***	0.97***	1.0***	0.99***

PH: Plant Height; PD: Plant Diameter; RL: Root length; NH: Number of haustoria; NL: Number of leaves; SFW: Shoot fresh weight; SDW: Shoot fresh weight; RFW: Root fresh weight; RDW: Root dry weight; P: Phosphorus; N: Nitrogen; K: Potassium; WP: Water potential; OP: Osmotic potential; Na: Sodium; Na/K: Sodium-potassium ratio; RWC: Relative water content; CHL: Chlorophyll content. ## *** Correlation is significant at 0.001 level (two tailed). ## ** Correlation is significant at 0.01 level (two tailed). ## * Correlation is significant at 0.05 level (two tailed) found that the nutrient status was considerably increased by the interaction between chelated iron and nutrient availability (Rocha *et al*., 2014).

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)	0.09^b	0.17^c	1.09^c
Alternanthera sp. (Nursery host)	0.19^a	0.45^a	2.30^a
A. indica (Neem)	0.20^a	0.43^b	1.72^b
LSD @ 5% (H)	0.007	0.009	0.055
Sandalwood response under different stress treatments			
T_1 [Best Available Water (BAW)]	0.20^a	0.46^a	2.09^a
T_2 (Salinity Stress EC_{iw}~8ds/m)	0.13^b	0.24^b	1.31^b
LSD @ 5% (T)	0.005	0.008	0.045
LSD @ 5% (H×T)	0.009	0.013	0.078

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

Correlation analysis: Pearson`s correlation analysis was performed on studied traits under control as well as stress conditions and noted significant variability between traits (Fig. 4). All the studied traits showed significant positive correlation with each other under control condition (Table 3). While under salinity stress, studied traits showed different pattern as WP and OP is negatively correlated with all the studied traits (Table 4).

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 salt stress, nutrient intake and physiology of sandalwood in economically feasible hosts. The most beneficial effects on sandalwood development were shown by *A. indica,* which showed improved nutrient uptake, Na^+/K^+ ratio and morpho-physiological traits over studied treatments.

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Conflicts of Interest: The authors declare no conflict of interest.

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