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Anatomy and Functional Status of Haustoria in Field Grown Sandalwood Tree (*Santalum album* L.)

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Abstract

To study the anatomy and functional status of sandal tree haustoria, two treatments of six year old field grown sandal tree growing with and without host *Casuarina* were investigated. Sandal tree was observed to form haustoria with host *Casuarina* and the wild grass grown around it. Sandal tree planted without host formed haustoria with the roots of nearby trees. However, maximum numbers of haustoria were observed in the sandal tree with host growing in the same pit. Anatomical studies of haustoria with host *Casuarina* reveals that vascular connections between the host and the sandal tree became so intimate that the host root and the parasitic root became almost a single physiological unit catering to the nutritional requirement of sandal tree. Furthermore, our investigations revealed that direct lumen-lumen xylem connections between the xylem of the host and the parasite were absent. Functional status of Sandal-haustoria was also studied by observing the translocation of radio-labelled phosphorus (^{32}P) from host to sandal tree by labelling of hosts and wild grass with ^{32}P and tracing it in sandal tree. After 2 h and 4 h of labelling *Casuarina* with ^{32}P, no notable counts were observed. Higher counts of translocated ^{32}P were observed in sandal tree after 6 h of labelling the host plant. There were marginal increase in ^{32}P count in sandal tree with time and this increase continued upto 8 days and thereafter observed a reduction up to 16 days, which indicated the decay of already translocated ^{32}P after 8th day. ^{32}P count also observed in sandal tree when wild grass was inoculated with ^{32}P. Translocations from the host plants other than *Casuarina* planted in the same pit were also investigated and its translocation observed varied with host species. The translocations from cocoa to sandal tree and *Casuarina* to sandal tree were the most efficient. The possible reverse translocation from sandal tree to host plant was also observed when ^{32}P applied to sandal tree. The results from the radiotracer studies indicated that sandal tree forms a network of roots, connected through haustoria, between sandal tree and different hosts including the grass species growing around it. The implication of the these results is that the host plants need not be present in the same pit of sandal tree as it can extend its root to distance of 1.5 to 3 m to form haustoria on neighbouring plants.

Keywords: Sandal tree; Host; Haustoria; Radio-labelled phosphorus

Introduction

Sandal tree (*Santalum album L.*) is a precious tree well known for its fragrant heart wood (East Indian Sandalwood) and the scented oil derived from it (East Indian Sandal tree oil). It is commonly known as sandal tree or chandan and is a semi root parasite tree of the family Santalaceae. *S. album* is indigenous to India covering an area of 9600 sq. km [1] and more than 90% lies in south Indian states of Karnataka and Tamil Nadu [2]. In India, the annual production of sandal tree has declined from 4000 Mg heartwood per year in the 1950s to 500 Mg in 2007 as against the global annual demand of about 5000 to 6000 Mg wood and around 100 to 120 Mg oil [1]. The depletion of sandal tree forest is attributed to factors like illicit felling, disease and smuggling, which are very rampant and is the major problem in the entire sandal tree growing states [3].

Considering the growing demand and the diminishing supply of sandalwood from its natural habitat, there is a great potential for raising sandal tree in not only forest areas but also in private land like home gardens and other agroforestry systems. The regeneration and establishment of sandal tree has been problematic because of the poor understanding of host-parasite relationships [4]. Production of sandal tree wood can be increased by extensive plantation of this species after properly understanding the host-parasite relationship, proper production of planting materials and knowledge of silviculture of this species. At the same time, only a few literatures are available indicating the relation of host in field grown sandal tree. Understanding of the haustorial anatomy is also important as sandal tree takes up food materials from the host plants through this specialized tissue. Considering the above, investigations were carried out to understand the anatomy and functional status of haustoria in the field grown sandal tree.

Materials and Methods

The investigations were carried out at the six year old sandal tree field plot available in College of Forestry, Kerala Agricultural University, Vellanikkara, Thrissur district, Kerala during 2009-2011. To understand the influence of the host plant on sandal tree grown in field, the experiment were conducted in two treatments viz, T_1- Sandal tree growing without host (*Casuarina*) (Host plant dead naturally within 2 years after establishment of sandal), T_2- Sandal tree with host (*Casuarina*) growing in same pit. Two sample trees, one with and the other without host (*Casuarina*) were excavated to investigate the haustorial physical association. Soil of one quarter of the area around sandal tree was carefully removed by loosening the soil with water spray and number of functional and nonfunctional haustoria on the host roots was recorded.

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Anatomical studies were conducted to understand the functional status of sandal-haustoria association. Thin (2-5 μm) microscopic sections of sandal-haustoria were taken following standard procedures of fixing, tissue processing and staining. Functional status of sandal-haustoria was also studied by observing the translocation of mineral nutrient P from host to sandal tree by using ^{32}P. The translocation through the haustoria from host plant to sandal tree and back were studied by labeling host plant (*Casuarina*) and wild grasses growing around sandal tree with ^{32}P and observing the translocation of the radio-label to sandal tree. The first treatment of labeling host plant with ^{32}P was done in two different ways. One by labelling the host plants (*Casuarina*) without sandal tree in the same pit but growing between the rows and other in the host plants (*Casuarina*) growing with sandal in the same pit. Sandal trees as well as the *Casuarina* growing around to the labelled plants were traced for ^{32}P.

The diluted ^{32}P sample applied to the host plant by root feeding. The feeder roots of the host plant were excavated and were inserted into a polyethene tube of size (2 x 15 cm^2). ^{32}P solution at the rate of 1.2 mCi in 20 ml, used for labelling one host (*Casuarina*) plant, was discharged to the polyethene tube with the root tip (After filling the bag it was sealed with cello tape). For labelling grass species growing around sandal tree, only 0.06 mCi, made up to 1 ml was used. Fresh leaf samples were collected from both host and sandal tree at 1 h, 2 h, 6 h, 2 days, 8 days and 16 days after ^{32}P application and were assayed for ^{32}P activity. The radioactivity was determined in a computer controlled liquid scintillation system (Hidex-Triathler) using Cerenkove Counting mode and the activity was expressed as Counts per minute (cpm g^{-1}) [5].

Results and Discussion

Kujit et al. [6] reported that parasitic plants access their hosts' resources through a key organ called the haustorium, which provides a physical as well as a physiological bridge between the parasite and host. The presence of functional haustoria (Plates 1 and 2) indicates the translocation of water and nutrients between host and sandal tree. Number of functional and non-functional haustoria in host roots is shown in Table 1. Maximum number of haustoria was observed in the sandal tree growing with host. Sandal tree without host also formed haustoria with the roots of host growing in the adjacent pit. Annapurna et al. [7] observed maximum number of haustorial formation with good host and significantly enhancing the growth and nutrient status of sandal seedlings. The haustorial connections were not easily detached during the excavation due to the tissue graft between the host root and the sandal-haustoria.

Anatomy of sandal-haustoria attached with the host (*Casuarina*) (Plate 3), showed a close vascular connections between the sandal tree and the host. Taide, Varghese and Singh et al., [8-10] also observed vascular connections between the host and the sandal tree through haustoria. The authors opined that the vascular connections between the host and the sandal tree became so intimate that the host root and the parasitic root became almost a single physiological unit catering to the nutritional requirement of sandal tree. Furthermore, our investigations revealed that direct lumen-lumen xylem connections between the xylem of the host and the parasite are absent (Plate 4). This infers that movement of xylem sap from host could only occur principally via pits of host xylem elements.

The counts of ^{32}P in sandal tree, translocated from the host plant at different time intervals, after labelling the host plant with ^{32}P are showed in Figure 1. After 2 h and 4 h of labelling *Casuarina* with ^{32}P, no notable counts were observed in sandal tree but higher counts were observed after 6 h of labelling the host plant. This indicates that the rate of translocation of radio-labelled phosphorus from host to sandal tree is very rapid. The peak count of ^{32}P in sandal tree was observed on eighth day of the labelling, showing that translocation of ^{32}P progressed upto eighth day. The reduction trend after the eighth day may be due to the decay of ^{32}P.

Radio-labelled phosphorus translocated from the host to sandal tree growing at different distance are shown in the Table 2. The ^{32}P in the labelled *Casuarina* grown with sandal tree in same pit was more as compared to *Casuarina* grown alone. Sandal tree in the same pit as *Casuarina* showed a ^{32}P count of 283 cpm.$g^{-1}$and sandal tree which was 1.5 m away from *Casuarina* showed a count of 216 cpm.g^{-1} and a count of 260 cpm.g^{-1} when *Casuarina* and sandal tree was in separate pits (1.5 m away). Sandal tree growing 1.5 m away from the host plant showed more or less same ^{32}P count as that of labelled *Casuarina* (260 cpm.g^{-1} and 263 cpm.g^{-1}). The ^{32}P count in sandal tree which was 2.5 m and 3 m away from labelled *Casuarina* also showed appreciable count. The anatomical studies showing the vascular connections between the host plant and sandal tree permits translocation of ^{32}P.

Casuarina being a drought adapted species with needles in place of leaves, dilution effect is less and most of the ^{32}P absorbed is translocated

Plate 1: Excavated sandalwood tree root.

Plate 2: Sandal-haustorium formed on host *Casuarina* root.

	Functional haustoria	Non functional hustoria
Sandal+*Casuarina*	44	6
Sole sandal	12	6

Table 1: Number of sandal-haustoria on the excavated roots of host.

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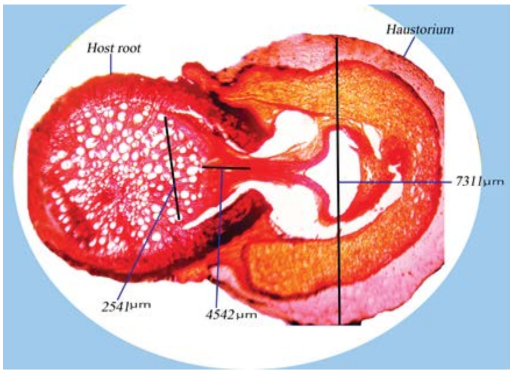

Plate 3: LS of sandal-haustorium with host *Casuarina* (10X).

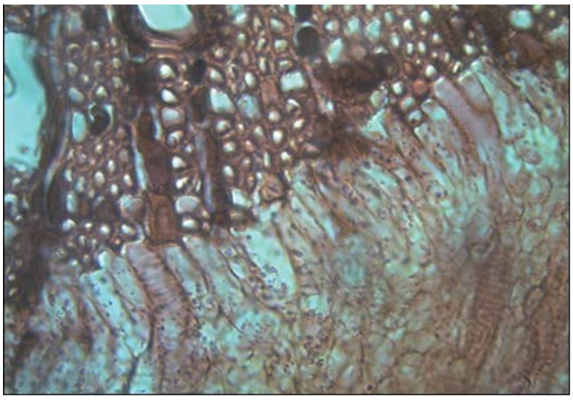

Plate 4: LS of sandalwood haustoria showing xylem-xylem connection between sandalwood and host root (40 X)
HX: Host xylem SX: Sandal-haustorial xylem.

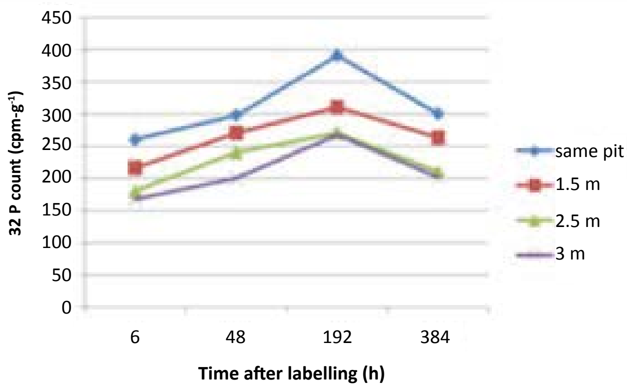

Figure 1: Count of ^{32}P translocated from host, casuraina to sandal trees growing at different distances on different time intervals.

Treatments	^{32}P count (cpm.g^{-1})				
	Labelled *Casuarina*	Sandal in same pit	Sandal at 1.5 m from host	Sandal at 2.5 m from host	Sandal at 3 m from host
*C+S	360	283	216	180	-
*C	263	-	260	248	200

Table 2: Translocation of ^{32}P from labelled host plant to sandal tree at different distances.

to sandal tree. The ^{32}P count in sandal tree translocated from wild grass growing around the sandal tree is shown in Figure 2. There was significant transfer of ^{32}P from the labelled wild grasses to sandal tree. The ^{32}P from hosts were translocated to sandal tree in both cases. The possible reverse translocation from sandal tree to host plant is evident from the data on ^{32}P count translocated from labelled sandal tree to host plants (Table 3). The percentage of translocation from sandalwood tree to host *Casuarina* was 26 and to teak was 34.89. From the data, it is evident that translocation from sandal tree to host is also equally efficient.

Translocations from the host plants other than *Casuarina* planted in the same pit were also investigated (Table 4). The variations observed in the ^{32}P translocated from host plant to sandal tree depends on the species of the host, may be due to the difference in the number of the haustoria formed by sandal tree on the host, preference of host species by sandal tree, and the efficiency of translocation from host to sandal tree depending on the host species. The percentage of translocation from hosts to sandal varied from 27.6% to 78.5%. The percentage of the total ^{32}P count detected in sandal tree and host plant also varied depending on the species of the host plant and the number of the host species present in the same pit as that of sandalwood tree. Percentage varied from 27.65, when rubber was host to 71%, when cocoa was the host. The second and third plant present in same pit as sandalwood tree also showed ^{32}P count translocated from the labelled host plant. As the host plants cannot have root connections, translocation from labelled host to other host plants in the pit or in the adjacent pit may be mediated through sandalwood tree which might have formed functional haustorial connections in all the host plants surrounding it.

The sandal tree can form a network of roots, connected through haustoria, between sandal-*Casuarina*-sandal tree and even with the grasses growing around it. The implication of the result from the radiotracer studies is that the host plants need not be present in the same pit as that of sandal tree. It can extend its root to distance of 1.5 to 3 m (based on the data available from the present study) to form haustoria on host plant. A best field host tree sandal tree would be that with more functional haustoria, but at the same time offers minimum

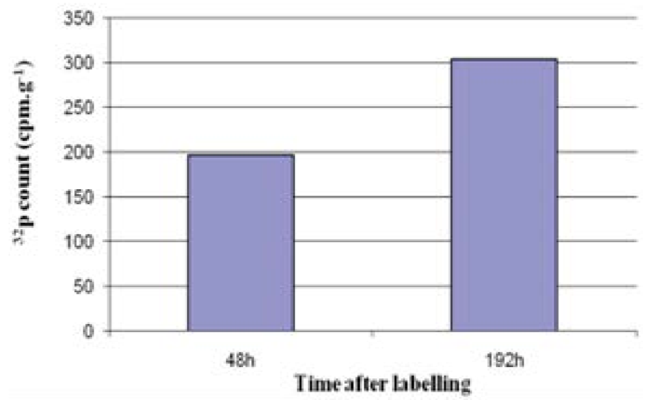

Figure 2: Count of translocated ^{32}P from wild grass to sandal tree.

Treatments	^{32}P counts (cpm g^{-1})		
	Sandal	***Casuarina*	**Teak
*Sandal + *Casuarina* + Teak	513	183	275

*Indicate ^{32}P labelled plant
**All the host plants were planted in the same pit as that of sandalwood tree

Table 3: Translocation of ^{32}P from sandal tree to host trees.

Treatments	^{32}P counts (cpm.g^{-1})				Total count of sandal and treated host (cpm.g^{-1})	Percentage count in sandal with treated host (%)
	Sandal	**Host 1	**Host 2	**Host 3		
Sandal + Cocoa*	251	102			353	71.10
Sandal + Cashew*	^{32}0	275			595	53.78
Sandal + Cashew* + *Casuarina* +	198	224	170		422	46.91
Sandal + Teak*	542	376			918	59.04
Sandal + Teak* + *Casuarina*	^{32}1	479	119		800	40.12
Sandal + Coconut* + *Casuarina*	120	289	126		406	29.55
Sandal + *Casuarina**	458	161			619	73.20
Sandal + Coconut* + *Casuarina* + Rubber	215	527	1^{32}	120	742	28.9
Sandal + *Casuarina** + Rubber	483	1^{32}	124		615	78.53
Sandal + *Casuarina** + Teak	196	155	316		351	55.84
Sandal + Rubber*	142	372			514	27.62
Sandal + Rubber* + *Casuarina*	217	436	99		653	33.23

* Indicate ^{32}P labelled plant

** All the host plants were planted in the same pit as that of sandalwood tree.

Table 4: Translocation of ^{32}P from host trees to sandal tree.

competition for above ground resources. Planting sandal tree as an intercrop in suitable distances from the main crop can be considered. These crop plants will give periodical returns whereas sandal tree planted in field may yield significant income in the long term.

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