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Study of Phloem in The Haustoria of *Cuscuta reflexa* and *Santalum album*

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ABSTRACT
Santalum album a hemiroot parasitic tree and Cuscuta reflexa Roxb. a rootless, holoparasitic herb are the two important parasitic phanerogams belonging to the family Santalaceae and Cuscutaceae respectively. Lacmoid staining method along with fluorescence technique was adopted to localize the presence of phloem in the haustoria of the two selected species. The fluorescent microscopic observations on the haustorium of S. album revealed a well-developed phloem, it consist of sieve tubes and companion cells in the region of vascular core. Further the emission of fluorescence was observed in the upper portion of haustorium and endophyte. A well differentiated phloem was recorded in the region of endophyte which is in contact with the phloem of host in C. reflexa. During the present investigation distinct phloem was recorded in both the species which was confirmed by fluorescence microscopic studies.
Keywords: *Santalum album, Cuscuta reflexa, haustorium, phloem, holoparasitic,*

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INTRODUCTION

Angiosperms (Flowering plants) comprises of about 90% of the plant kingdom. These are one of the major groups of seed plants and arguably the most diverse group on the planet, with at least 2,60,000 living species distributed in 453 families. Parasitic plants are a small group of dicotyledonous which exhibit a great wealth of structural diversity that continues to fascinate Botanists. These constitutes only 2% of all the phanerogams, approximately 4700 species [23] and are quite distinct from other weeds in forming intimate attachments to their host crops either to roots or shoots. The parasitic phanerogams are distributed in about 20 distinctly different families. There are two classes of haustoria, depending on the formation site: lateral haustoria and terminal haustoria. Terminal haustoria develop at root tips by terminating the meristematic activity of radical tips, followed by cell expansion [26]. All parasitic flowering plants are dicotyledonous species without a single taxon from the monocotyledons. In gymnosperms, there is a solitary report of a possibly parasitic conifer from New caledonia *Podocarpus ustus* (*Parasitaxus ustus*) [12]. Parasitic plants have enthralled the naturalists for centuries due to the initiative fear off all things parasitic. For biologist parasites appear as a superbly successful species in the great mosaic of living things [14]. There are some parasites like *Agalinis* which often obtain their requirements from unsuspecting hosts, while more advanced parasites *viz*. species of *Striga* and *Orobanche* skilfully allure their hosts to assist in obtaining their nutrients. These features can easily surpass the skilled work of a craftsman. Phloem being the main food conducting tissue assumes special significance in parasitic phanerogams. References to the occurrence of phloem in these unique types of plants particularly in their haustoria are relatively infrequent, fragmentary and inconsistent. Interestingly, new information on the occurrence of phloem in the haustorium of only few parasitic angiosperms has thrown light for the discovery of the same in other parasites. With such an increase in the interest about parasitic flowering plants among botanist all over the

world. However, it is important to stress that the term 'haustorium' should not be confused with the term 'infection site', which by definition encompasses the adjacent tissue of the host[27]. Rajanna and Shivamurthy (2001) convincingly recorded and reported the occurrence of phloem in the haustorium of *Santalum album* for the first time [22]. *Pate et al* (2001) described the haustoria of *Olax phyllanthi* in relation to uptake, transfer and metabolism of xylem borne nitrogenous solutes derived from a host[19]. Among the various nutritional modes displayed by flowering plants, parasitism represents one of the most successful. During the present investigation, special method was adopted to record the occurrence and confirmation of phloem in the haustoria of selected parasitic plants.

MATERIAL AND METHODS

FLUORESCENCE MICROSCOPIC STUDY: Lacmoid Blue method [6]. To study the nature of phloem, fluorescence microscopic study was undertaken. Free hand sections of haustoria were taken and directly transferred to 0.25% lacmoid blue prepared in 30% ethanol for 48 hours (The Lacmoid blue stain was prepared by dissolving 0.13 g of Lacmoid blue powder in 17 ml of rectified spirit and made up to 50 ml with distilled water). Stained sections were then washed in tap water and differentiated in 1% Sodium carbonate in 50% alcohol for about 10 to 20 seconds. The section were dehydrated in alcohol series and placed in a mixture of absolute alcohol and xylene (1:1) for about 10 to 15 sec. Finally, the sections were cleared in xylene twice for about 5 min each and mounted with D.P.X. Later the sections were observed under fluorescence microscope.

RESULT

Cuscuta Reflexa: *Cuscuta reflexa* Roxb. is a rootless, holoparasitic herb with filiform stem attached to the host by numerous haustoria. It is commonly called doddera belonging to the family Cuscutaceae. It is one of more robust species with a vine 1 - 2 mm in thickness. The stem is reddish yellow in colour with green tinge the stem bears scaly leaves and a cluster of flowers. However photosynthesis is negligible, hence it is an obligate parasite. *C. reflexa* is a perennial plant on woody species including tree plants such as citrus, coffee, peach and litchi [18]. It spreads easily from one host to other and forms yellow patches, twins around all parts of host. At the point of contact it produces haustorial sinkers that contain special glandular cells (Figure.1) facilitating adhesion of the parasite to the host [1]. It finally penetrates the host tissue for drawing water and mineral nutrients. *Cuscuta reflexa* stem tightly coiled around the host stems more or less protruding haustoria were produced. As the haustoria matured, the meristem cells became organized into two cell types. Elongate cells in the distal region had a dense cytoplasm with large nuclei and enlarged nucleoli and mainly divided anticlinally, while cells at the proximal region actively divided anticlinally and periclinally and thus formed small derivatives arranged in radial files. Eventually, the mature upper haustorium was characterised by tissue consisting of two cell types; elongated digitate cells and smaller file cells. The tissue served as an endophyte primordium because it penetrated into the host tissue and formed the endophyte, the haustorial portion growing with in the host. The endophyte contains well differentiated xylem and phloem cells. The fluorescent microscopic studies on *Cuscuta reflexa* revealed that, the endophyte is in contact with the host phloem and the differentiation of phloem in the centre of haustorium was noticed. Further observations revealed that, the occurrence of well-developed phloem in the region of endophyte was confirmed by the emission of fluorescence (Figure 2).

***Santalum Album* L.**

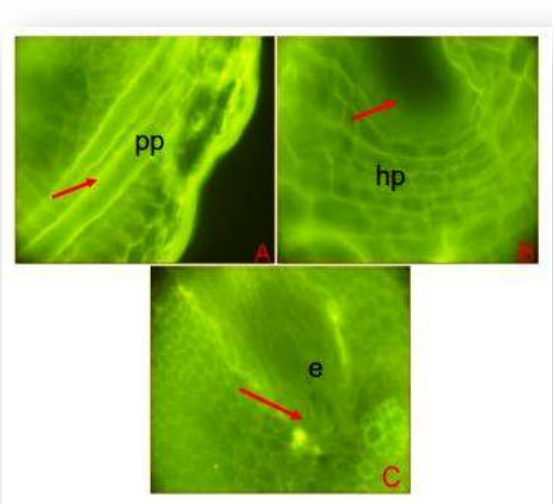
Santalum album L. a famous sandal wood tree is a member of family Santalaceae. The taxon grows as a hemiroot parasitic moderate sized, tall branched evergreen tree .

Structure of the mature haustorium: Mature haustorium of *Santalum album* shows a complex anatomical organization. During maturation changes occurs in the structure of endophyte and a number of tissues, develop within the haustorium. Xylem differentiation continues in the region of elongation of haustorium. The structure of endophyte varies depending upon the type of host. The host root cortex along with the cambium is separated from the stele and they appear to have been lifted away from the stele. This results in giving a saddle like appearance to the endophyte. There is a direct xylem to xylem contact between the two partners. Most of the treachery cells of the haustorium that are in contact with the host stele appear to lie directly against or in close proximity with the major elements. In addition to this a large number of haustorium parenchymatous cells called digitate cells are also seen in contact with xylem core. Sometimes these parenchymatous digitate cells are elongated, thick walled and densely protoplasmic. Many of these cells penetrate the host tracheary elements through the pits. The digitate cells exhibit both intracellular and intercellular growth. Sometimes digitate cell tips pressing the host root vessel cause the lumen of the host root vessel elements to become completely filled by the digitate cells. Most of the cells are in direct contact with host xylem and phloem elements. Distinct phloem comprising of sieve tube elements with simple sieve plates, companion cells and phloem parenchyma could be traced from parasite

root vasculature upto the host root stele. This is confirmed by the positive reaction of callose on the sieve plates to lacmoid stain (Figure 2). Regular sieve tube elements were seen in the developing haustoria and sieve plates are usually found on the end walls, which may be oblique or horizontal or intermediately placed were emitting fluorescence when observed under Fluorescent microscope. The fluorescent microscopic observations on the haustorium of *Santalum album* revealed a well-developed phloem, it consist of sieve tubes and companion cells in the region of vascular core (Figure 3). Further the emission of fluorescence was observed in the upper portion of haustorium and endophyte (Figure 4 and 5).

DISCUSSION

In most of the haustoria xylem is in contact with the host xylem and the phloem considered to be poorly represented or absent. Phloem is one of the most important food conducting tissue and this has been repeatedly said to be absent in the haustoria of parasitic Santalales[14,15] But some of the references revealed the occurrence of phloem in the haustoria, it is said to be confirmed only to the neck region or interrupted zone [25,10,20]. Further, Kuijt (1961) states that sieve elements are never a part of haustorial interphase of either holo or hemi parasites[13]. In *Santalum* a distinct sieve tube elements could be traced and recorded from the parent root up to the level of host root cortex [22]. Sieve elements were noticed consistently in both younger as well as in older ones. Further sporadic occurrence of phloem in the haustorium has been reported in the haustoria of *Castillija* [16,7] *Alectra vogelii* [4] in the primary haustorium [5] in *Areceuthobium globosum*. The fluorescent microscopic studies on the haustoria of Santalum *album* and *Cuscuta reflexa* revealed a well-developed phloem with sieve tube and companion cells have been clearly established without any doubt. However, in the present study, phloem could be traced and recorded both in younger and older ones in the haustorium of *Cuscuta reflexa.* In view of holoparasitic habit the Orobanchaceae members have received special attention with regard to the occurrence of phloem[17]. Juliano (1935) reported a reduction in xylem with a corresponding development of phloem in *Aeginetia indica* [11]. Kuijt and Toth (1985) could not observe phloem in the region of interphase of mature haustorial organ in *Boschniakia* [17]. Similarly, Baird and Riopel (1986) also failed to demonstrate phloem in *Conopholis* [2]. However, an interesting series of work earlier [3,8,9] on *Orobanche* had led to the discovery of phloem conduit system in the haustorium. This system involves well developed sieve tube elements with sieve plates in the haustorium, followed by transitional sieve elements with some features of typical sieve tube elements and distal conduct cells which have less sieve element like lying directly adjoining to the host sieve tube element. With light microscopic studies on *Orobanche racemosa* [21] were not able to observe the cell types described by [3]. Shivamurthy and Niranjana (1996), Rajanna and Shivamurthy (2001) have demonstrated the occurrence of phloem in the haustorium of *Sclerophyrum wallichianum and Santalum album* respectively [24,22]. However they observed phloem from parent root to lower region of vascular core in this taxon. In *Santalum album* and *Cuscuta reflexa* of the present investigation revealed a well differentiated phloem in the haustoria confirmed with fluorescence microscopic observations.



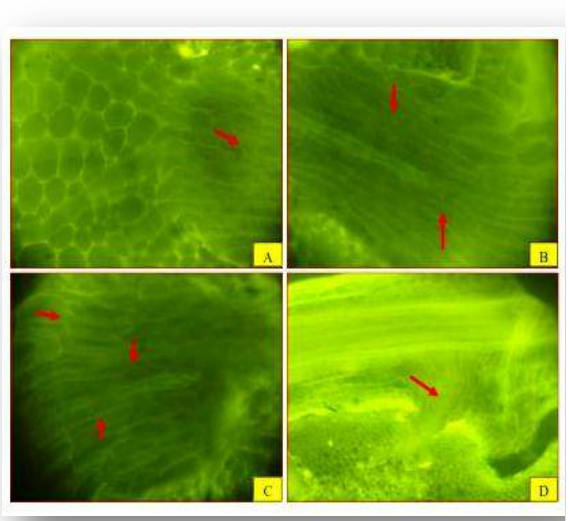
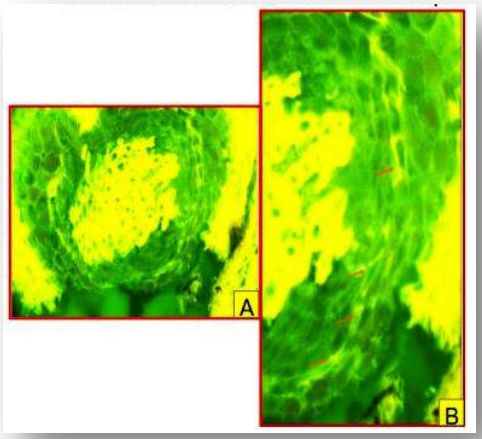


Figure 1
A. Section of the young haustorium tested for parasite phloem (pp)(Arrows)
B. V/S of the haustorium showing the endophytes in contact with phloem(hp) (Arrows)
C. V/S of the haustorium tested for phloem.
Note: The differentiation of phloem in the centre of the haustorium (Arrows)

Figure 2
A-D Section of the haustorium tested for the localization of phloem by using lacomoid blue (Arrows)



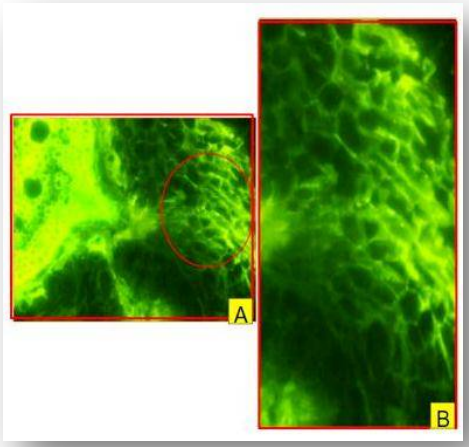


Figure 3
A. L/S of the haustorium showing vascular core tested for the localization of phloem observed under fluorescent microscope.
B. A portion of (A) is enlarged to show phloem (Red arrows)

Figure 4
A. A portion of haustorium showing phloem observed under fluorescent microscope
B. A portion of (A) is enlarged

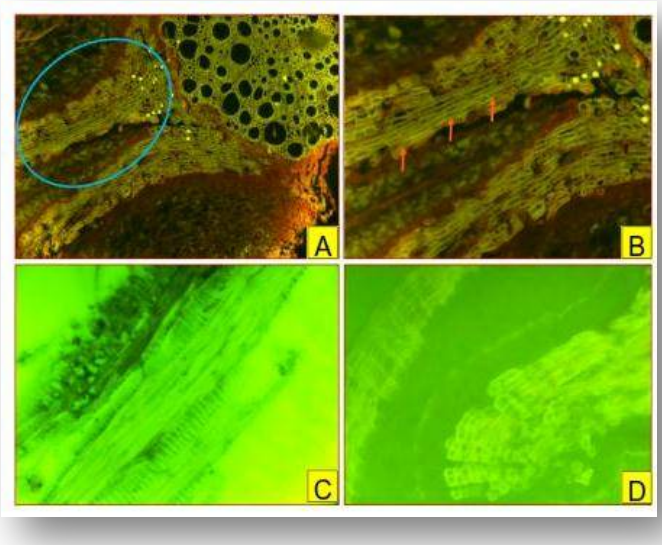


Figure 5
A. V/S of haustroium showing vascular core tested using Lacomoid blue for the localization of phloem and observed under fluorescent microscope
B. A portion of (A) is enlarged
C. A portion of haustorium showing phloem (Arrows) observed under fluorescent microscope
D. Upper portion of the haustorium showing phloem (Arrows)

CONCLUSION

Cuscuta reflexa Roxb. is a rootless herb with a filiform stem attached to the host by numerous haustoria. It is an obligate parasite, spreading easily from one host to another and forming yellow patches around the host. It produces haustorial sinkers with glandular cells, facilitating its adhesion to the host. As the haustoria mature, the meristem cells become organized into two cell types: elongated digitate cells and smaller file cells. The mature upper haustorium serves as an endophyte primordium, containing well-differentiated xylem and phloem cells. The mature haustorium of *Santalum album* exhibits a complex anatomical organization, with changes in endophyte structure and tissue development. Xylem differentiation continues in the elongation region. The endophyte structure varies depending on the host type, with a saddle-like appearance and direct xylem-to-xylem contact. Treachery cells and digitate cells are found in contact with the xylem core, often elongated and densely protoplasmic. Distinct phloems consist of sieve tube elements, simple sieve plates, companion cells, and phloem parenchyma, traced from parasite root vasculature up to the host root stele. Fluorescent microscopic observations reveal a well-developed phloem consisting of sieve tubes and companion cells in the vascular core region.

DECLARTION OF COMPITING INTEREST

The authors declare that they don't have any known competing financial interests or personal relationships that could have appeared to influence the work in this paper.

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