Occurrence of Phloem in the Haustorium of *Aeginetia pedunculata* Wall. – A Root Holoparasite of Orobanchaceae

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ABSTRACT: In the present investigation, the occurrence of phloem and callose deposition on sieve plates in the haustorium of *Aeginetia pedunculata* Wall., a root holoparasitic herb of Orobanchaceae, is demonstrated. Our studies reveal that the haustorium of *A. pedunculata* has highly specialized phloem tissue, comprising of sieve tube elements and companion cells found associated occasionally with the xylem strands. More often, they are found as isolated strands in the lobes of the endophyte and the cortex of parasite root. Distinct sieve tube strands can be traced from the parasite root vasculature up to the region of the entry of the haustorium cells into the host stele. The sieve elements show the normal pattern of callose deposition. The callose deposited around the sieve pores show positive staining to lacmoid blue and fluorescence was observed, confirming their phloic nature.

KEY WORDS: *Aeginetia pedunculata,* phloem, callose, fluorescence, haustorium, Orobanchaceae.

INTRODUCTION

The search for authentic phloem in the haustoria of parasitic vascular plants has been rather unproductive in the past. The presence of phloem in the haustoria of parasitic flowering plants has attracted the attention of anatomists in recent years. Occurrence of phloem in the haustoria of only a few parasitic angiosperms has been convincingly demonstrated. In *Arceuthobium* (Viscaceae), Calvin *et al.,* (1984) showed highly specialized phloem tissue. The sieve elements have transverse to oblique, usually simple sieve plates and small but numerous diffuse lateral pores. The sieve elements show the normal pattern of callose deposition. In case of *Viscum* (Loranthaceae), (Salle, 1976) the sieve tube members exhibiting simple sieve plates, P-proteins, endoplasmic reticulum, plastids and mitochondria. Kuijt and Dobbins (1971) documented the presence of phloem in the haustorium of *Castilleja* (Scrophulariaceae). It was found to occur in the internal portion of the endophyte and on the periphery adjacent to cortical cells of the host. Dörr *et al.,* (1979) have also reported the occurrence of phloem in the primary haustorium of *Alectra* (Scrophulariaceae). In *Orobanche,* Dörr and Kollmann (1975) also recorded the occurrence of phloem. In *Scleropyrum* (Santalaceae), very distinct sieve tube elements have traced from the parent root to the lower regions of vascular core (Shivamurthy and Niranjana, 1996) and distinct phloem was recorded in the endophyte region of the haustorium of *Santalum* (Santalaceae) (Rajanna and Shivamurthy, 2001). *Aeginetia pedunculata*, a member of Orobanchaceae, has not been studied earlier from this point of view until the present study.

MATERIALS AND METHODS

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Specimens of *Aeginetia pedunculata* Wall. with haustoria parasitizing *Themeda triandra* (Poaceae) were collected from Wynad ghats, Kerala State, India, soon after the first few showers of monsoon (June-July). Young and old haustoria were carefully dug out from the soil along with the host roots, and fixed immediately in F. A. A. (formalin 40% - glacial acetic acid and 70% ethanol) in the proportion 1:1:18. Fixed materials were then dehydrated in tertiary butanol series and embedded in paraffin, and sectioned at 10-14 µm thick using rotary microtome. Some sections were stained with tannic acid ferric chloride combination with aniline blue in clove oil as counterstain for routine anatomical observations (Johansen, 1940; Cheadle *et al.*, 1953). Other sections were stained for localizing the presence of callose on the sieve plates using lacmoid blue and/or aqueous aniline blue (Krishnamurthy, 1988). The fluorescence was observed when viewed under Leitz fluorescence microscope using appropriate filters (Currier and Strugger, 1956).

RESULTS

Aeginetia pedunculata is a leafless, fleshy root holoparasitic herb (Fig. 1A) with underground-branched rhizome. Flowers borne on elongated branched peduncles, large portions of which remain underground. Flowers are large and attractively coloured. Calyx spathaceous, reddish, split in the front nearly to the base and loaded with mucilaginous substances. Corolla tube yellowish, externally with bright blue coloured throat. Stamens four, didynamous, unilocular ovary bearing a large number of ovules on multilobed parietal placentae. Stigma broadly cordiform, peltate and white in colour. Ovoid capsule with a very large number of brownish minute seeds.

This obligate parasite possesses numerous white, ellipsoidal rhizomatous, brittle and delicate underground roots producing numerous tubercles or swellings, which attach themselves to the host roots by means of sucking organs or haustoria (Figs. 1B & 1C). The parasite has a small tuber like structure at the site of attachment (Primary haustorium) of radicular tip (Fig. 1B) with the host root. From this tuber a large number of secondary roots arise which establish contacts with the host roots (Secondary haustorium). The secondary roots of the parasite closely interweave with that of its host. In such a close net working of root systems of the two individuals a large number of secondary haustorial contacts are established (Fig. 1C). Each point of contact restricted to a small area on the host root. However, such contacts may be seen one after the other between the roots of the two individuals at short intervals. The parasite root is slightly enlarged at the regions of its contact with the host root. The host root gets separated from the parasite root even with very slight pressure indicating the fragile nature of haustorial contacts.

The secondary haustoria are exogenous in origin. They arise by the meristematic activities of parenchymatous cells in the cortex of parasite root. The mature haustorium of *A. pedunculata* appears like an extension of the root itself. The entrance into the host root is affected by the pressure exerted by the enlarging epidermal and cortical cells of the tubercle facing the host root. After reaching the surface of the host root, the cells of the haustorium penetrate into the stele of the host root at several points (Fig. 1D), which may be all along the length of the stele, or all along the circumference of the stele (Fig. 1D). The cortical cells of the host root that come in the way are completely replaced by the endophyte of the haustorium. However, the remaining portions of the host root cortex are left undisturbed.
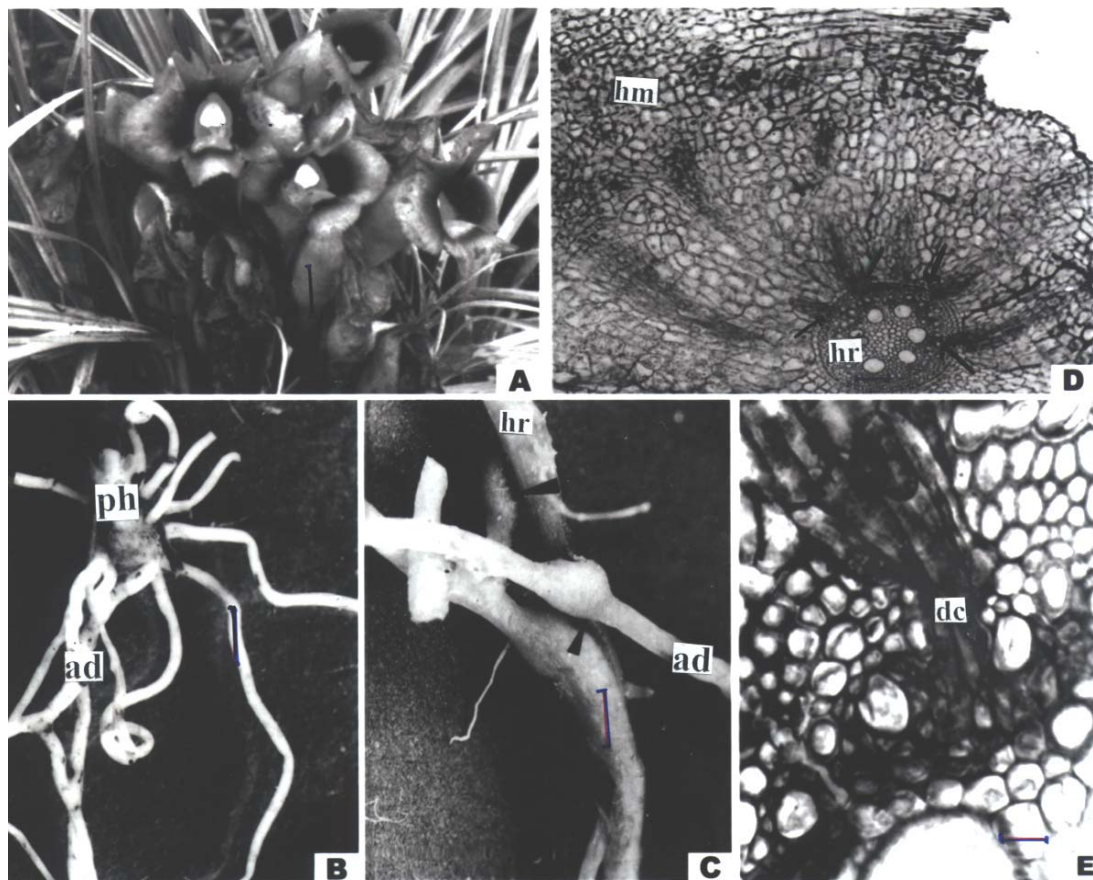
Fig. 1. A: Habit of *Aeginetia pedunculata* on *Themeda triandra* Forsk. B: Primary haustorium (ph), detached from host root, with lateral roots (ad, adventitious root) in all directions. C: Secondary haustorial attachments (arrowheads) between parasite and host roots (hr). D: Section of secondary haustorium (hm) with the host root (hr) cut transversely. Note the parasite digitate cells have penetrated host root stele (arrowheads) at many points all along the latter's perimeter. E: A portion of D enlarged to show digitate cells (dc) entry in to the host root stele. Bars = 1 cm (A); 0.5 cm (B); 0.4 cm (C); 100 µm (D); 2 µm (E).

The xylem strands linking the parasite root stele with that of the host root are normally uni- to biseriate (Fig. 2A). Each of the connecting xylem strands comprises of one or two rows of tracheary elements. The secondary wall thickening is usually of scalariform type. Surrounding the xylem strand is found one to three layers of parenchymatous digitate cells with darkly staining cytoplasm and conspicuous nuclei (Figs. 2A & 2B).

Phloem, comprising of sieve tube elements, companion cells and phloem parenchyma, is occassionally found associated with the xylem strands in the lobes of the endophyte and cortex of the parasite root. Sieve tube elements are endowed with sparse cytoplasmic contents and the callose material is deposited around the sieve pores on the sieve plates (Fig. 2B). Such distinct sieve tube strands can be traced from the parasite root vasculature upto the region of the entry of the haustorium digitate cells (Fig. 1E) into the host root stele. These sieve tube strands again run obliquely within the haustorium consequent to which they appear as isolated strands in sections of haustoria (Fig. 2C). Sieve areas are evenly distributed on lateral walls. The transverse walls show sieve plates. The callose deposited around sieve pores show positive staining to lacmoid blue confirming their phloic nature.
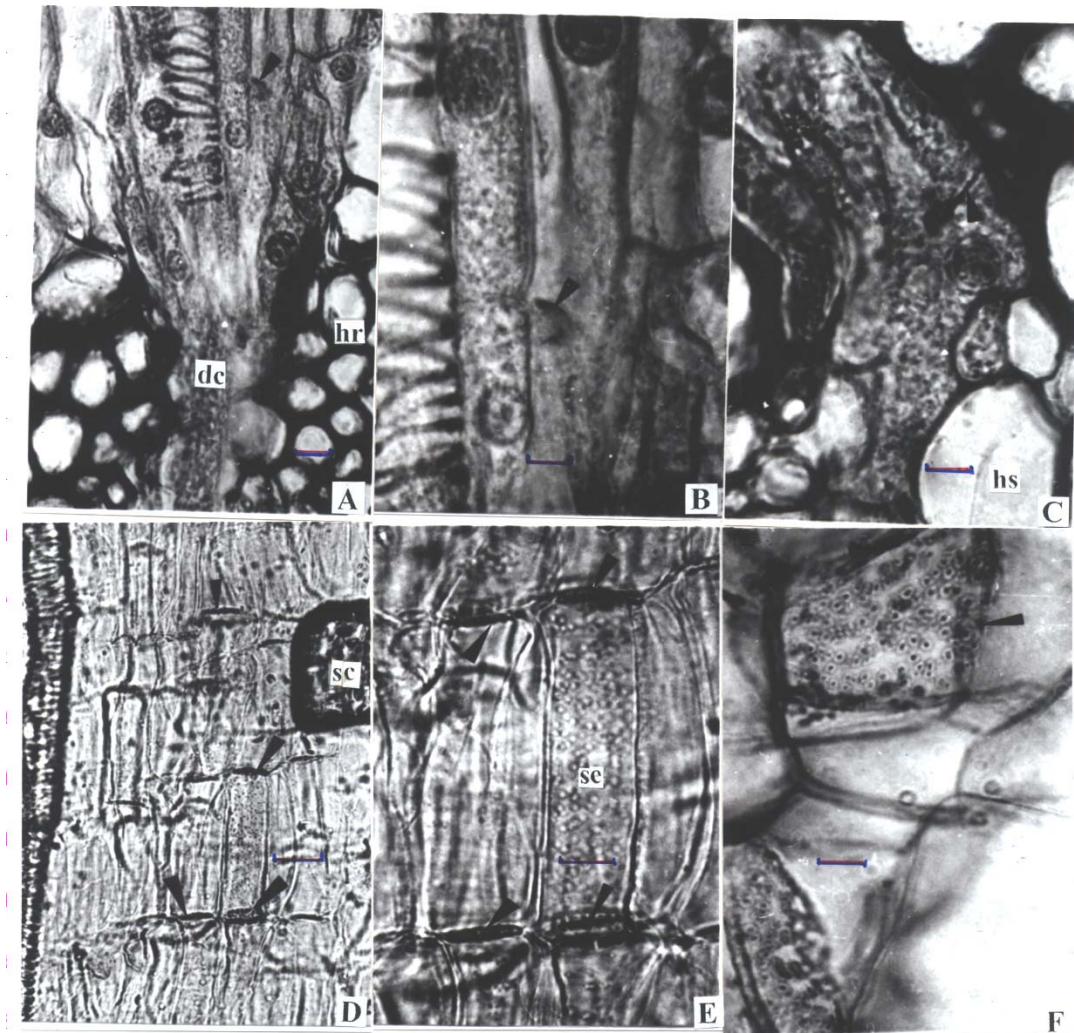
Fig. 2. A: A portion of haustorium at the region of entry of digitate cells (dc) into host root (hr) stele. Section stained with lacmoid blue. Transverse wall (arrowhead) of a cell showing positive reaction to lacmoid blue indicating its callose composition. B: Part of A enlarged to show the sieve plate (arrowhead). Note sparse content of cell above transverse wall. C: Transverse wall (arrowhead) of a parasite cell showing positive reaction to lacmoid blue indicating the presence of callose wall material. Nucleus seen just below the transverse wall belongs to another digitate cell (hs, host sieve tube element). D-F: Sections of basal part of flowering stalk stained with aqueous aniline blue to demonstrate phloem. Typical sieve plates are seen on end walls (arrowheads) in D (sc, Sclereid). Part of D is magnified in E to show sieve plates (arrowheads) and the presence of sieve area all over the sieve tube element (se). Surface view of sieve plates (arrowheads) in F in different focus, sieve pores appear as black dots. Bars = 1 μm (A); 1 μm (B); 1 μm (C); 0.8 μm (D); 0.8 μm (E); 1 μm (F).

On the other hand, phloem in the root proper and flowering stalk is distinctly well developed with sieve tube elements and companion cells. Sieve plates are found on the end walls of the sieve tube elements. This became very evident in sectioned materials stained with aqueous aniline blue (Figs. 2D-2F). Often, sieve areas are observed all over the lateral walls of some sieve tube elements (Figs. 2D & 2E). The callose cylinder around the sieve pores and the callose plugs takes a clear blue staining with aniline blue. In the developing sieve tubes, the sieve pores are not plugged with callose. However, in mature sieve tube elements, the sieve pores are plugged with the callose (Fig. 2F).

DISCUSSION

Hemiparasitic angiosperms are said to be dependent only on the xylem of their hosts for mineral and certain organic solutes and water. This has led to the general belief that there is no necessity of development of phloem tissue in the haustorium (Rajanna and Shivamurthy, 2001). But there is a necessity for phloem borne assimilates from the parasite to supply carbon and energy for haustorial growth and for development. Further, there has also been a general consensus among investigators in the field that the phloem elements are never a part of the haustorial interface of either holo- or hemiparasites (Rajanna and Shivamurthy, 2001). Nevertheless the occurrence of phloem in the haustorium of hemiparasitic phanerogams has been well documented in recent years (Shivamurthy and Niranjana 1996; Rajanna and Shivamurthy, 2001), while the search for its presence in the root holoparasitic angiosperms has received special attention (Kuijt and Toth 1985), so much, so that the observation of phloem even in the aerial stem of *Epifagus* (Orobanchaceae) warranted a report (Walsh and Popovich, 1977). Juliano (1935) reported a reduction in xylem with a corresponding development of phloem in *Aeginetia indica*. However, his report is not specific about the occurrence of phloem in the haustorium. Kuijt and Toth (1985) could not observe phloem in the region of interface of mature haustorial organ in *Boschniakia* (Orobanchaceae). Similarly, Baird and Riopel (1986) also failed to demonstrate phloem in *Conopholis* (Orobanchaceae). However, an interesting series of works earlier by Dörr and Kollmann on *Orobanche* (1974, 1975 and 1976) had led to the discovery of specialized phloic 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 contact cells which are less sieve element like lying directly adjoining the host sieve tube element. But Pennypacker *et al*. (1979) in their light microscopic studies on *Orobanche ramosa* were not able to observe the cell types described by Dörr and Kollmann (1975). The phloem observed in *Castilleja* (Scrophulariaceae) is near the periphery of the crest, along the contact surface, and also in more interior tissue. The sieve pores are bordered by callose and have strands of proteinaceous material (P-protein) (Kuijt and Dobbins,1971). The most notable feature of phloem in *Arceuthobium* is the apparent scarcity of sieve elements (Calvin *et al*., 1984). Callose was absent in regions of non-functional phloem (Calvin *et al*., 1984).

In *A. pedunculata* of the present study, well-differentiated phloem could be observed in the root, rhizome and scape. Typical sieve tube elements are always present in the upper regions of haustorium. Normal phloem could be traced in the haustorium of *A. pedunculata* upto the region of endophyte entry into host root. The occasional observation of transverse wall of a digitate cell of the haustorium with in the host stele responding positively to critical lacmoid staining for callose, indicates that the three-tiered system of phloem described for *Orobanche* by Dörr and Kollmann (1975) appear to be present in *A. pedunculata* of the present study also. Though Dörr and Kollmann (1975) and Kuijt and Toth (1976) felt that the specialized phloic conduit system reported in *Orobanche* can be distinguished only under the electron microscope, the employment of lacmoid staining for callose and critical observations during the present study revealed such structures even under light microscope. The existence of such phloic conduit in the haustoria of other members of Orobanchaceae cannot be ruled out. Though direct sieve tube to sieve tube contact beween the parasite and host could not be

observed, an unusually large number of sieve areas were observed at the regions of contact between the mass of digitate cells and host sieve tubes. Whether these sieve areas belong to haustorial cells or to the host sieve elements and if it is latter, whether their formation is induced by the entry of parasite needs to be investigated further.

ACKNOWLEDGEMENTS

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根寄生植物 *Aeginetia pedunculata* (列當科) 吸足具韌皮部之研究

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摘　　要

　　本研究中證實了一種野菰屬根全寄生植物 (root holoparasite) *Aeginetia pedunculata* 的吸足 (haustorium) 確有韌皮部的存在和其篩板具胼胝質的堆積。*A. pedunculata* 的吸足具極為特化的韌皮部組織，包含了有時與木質部相伴出現的篩管和伴細胞，這些構造更常單獨地出現在內生植物與宿主植物根部皮層相接的凹處，此明顯的篩管束由寄生植物的根部維管束可上溯至吸足的細胞進入宿主植物之中柱區域。篩管的胼胝質位篩板的周圍，呈現一般的正常堆積形式，對 lacmoid blue 染劑呈螢光反應，可確定其屬韌皮部。

關鍵詞：*Aeginetia pedunculata*、韌皮部、胼胝質、螢光、吸足、列當科。

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