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Histochemical localization of peroxidase activity in the haustoria of *Santalum album* L. and *Cuscuta reflexa* Roxb

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Abstract
During the early stages of the haustorium development in *Cuscuta reflexa* Roxb. The enzyme is found to be accumulated at the region of contact between host and parasite. As the haustorium matures, the enzyme concentration appears to be less in the upper region of the haustorim. In *Santalum album* L. the enzyme activity was more pronounced in the early stages at the region of contact between the host and parasite and also in the vascular core of the endophyte. Further the concentration gradually decreases as the haustorium becomes older. Guaiacol and Benzidine are used to localize peroxidase activity.

Keywords: Histochemical, Peroxidase, Haustorium, Benzidine, Guaiacol

1. Introduction
In a living cell many chemical reactions are controlled by some specialized substances called enzymes. The term enzyme was coined by Kuhne (1878) [5]. These are also called as biocatalysts, because during chemical reaction they combine with the substrate, after completing the reaction they are set free. Most of the biochemical reactions are governed through the enzymes. These are unstable molecules and are synthesized and degraded very rapidly. Enzyme localization in cells and tissues is a challenging goal in the field of biochemistry and enzymology. Localization of enzymes study offers a procedure for tissue characterization. *Cuscuta reflexa* Roxb. is a leafless twining total stem parasitic vine belonging to the family Cuscutaceae and *Santalum album* L. a semiroot parasite commonly called as sandal wood tree is a member of the family Santalaceae. The haustoria forms an anatomical and physiological bridge between the host and parasite. Histochemical localization of enzymes evidenced their role in the process of entry of haustoria in to the host system. Enzyme localization in the haustoria are poorly studied in parasitic angiosperms. Hence the present study describes the localization of peroxidase activity in the haustorium of two important parasites viz., *Cuscuta reflexa* Roxb. and *Santalum album* L.

2. Materials and Methods
Haustoria of *C. reflexa* and *S. album* along with their hosts were collected and immediately thin free hand sections were taken and placed in 0.1M phosphate buffer at pH 7. Later the sections were incubated at room temperature for about 5 minutes in a substrate solution (5 ml of 1% Benzidine solution, 1 ml of 5% of Ammonium chloride solution and 5 ml of 0.1m Hydrogen peroxide) and mounted in glycerin. The sites of peroxidase activity appears blue [3]. If Guaiacol is used instead of Benzidine the peroxidase activity indicated by brown colour [11].

3. Results and Discussion
C. reflexa is a common perennial parasitic phaneropgam plant growing on different host species including tree species such as *Citrus*, Coffee, Peach and Litchi [16]. It grows by means of thin stem and forms haustorial connections to the plant coming into contact. *S. album* a famous sandal wood tree is a member of the family Santalaceae, a taxon grown as a hemi root parasite. It is moderate sized tall branched evergreen tree. This parasitic tree found to develop haustorial connections on the roots of unrelated taxa. The haustorium forms an anatomical and physiological bridge between the host and parasite. Tissue compatibility and the haustoria of parasitic angiosperms were studied by Kuijt (1983) [8]. Haustorial initials in the cortex of the *Cuscuta* stem at the site of attachment give rise to main haustorial body. The mode of penetration at this stage is mechanical rather than enzymatic. In the host cortex, haustorial surface cells elongated inside the host tissue, forming haustorial hyphae, some hyphae reached the conducting tissue of the host. In *S. album*, when the young parasitic root comes in contact with the host, the hypodermal parenchyma cells produce a protruberence at the site haustorium initiation.
Peroxidase activity appears both in the host and parasite. Its activity appears blue or brown when the sections are treated with Benzidine or Guaiacol respectively. In *C. reflexa* during the early stage of haustorium development the peroxidase activity appears to be more both in the region of haustorium and endophyte (Figure 1 A), the concentration gradually increases as the haustorium matures and appears at the region of vascular core of the endophyte and in the upper part of the haustorium (Figure 1 B). Further peroxidase concentration decreases as haustorium becomes old (Figure 1 C).
In *S. album* during the early stage of haustorium development the enzyme activity appears only at the region of attachment of host and parasite, the peroxidase enzyme

was released from the haustorium and deposited at the region of contact to the host tissue (Figures 2 A, 2 B, 3 A & 3 B). As the development proceed, the enzyme gets accumulated in the vascular core of the haustorium (Figure 2C). In matured haustorium the activity is restricted to the peripheral sides of the haustorium (Figures 2C & 3C). The microscopic investigations clearly revealed that the peroxidase activity appears to be more in parasite than in the host tissues. In the initial stage of penetration the concentration of peroxidase activity is more and gradually decreases as the haustorium matures. Haustorium penetrates the host by deformation and disruption of the host tissues, may be lytic process are involved in the degradation of the cell wall by the enzyme peroxidase.

Studies on the development, structure and histochemistry have been investigated in the haustorium of many parasitic angiosperms. Normally two kinds of haustoria are recognized on the basis of origin [6]. The haustoria developing from the region other than the radicular parts are called the secondary haustoria. During the present investigation the haustoria of both the taxa developed only secondary haustoria. The haustoria of various species of *Cuscuta* have been studied by early workers [4, 19, 20]. In *Santalum* the haustoria are initiated just behind the apex of growing roots. This is confirmed by the work of Fineran (1965) [2] in *Exocarpus bidilli*. High enzyme activity was observed in the parasitic tissue at the site of haustorial contact and entry with the host vascular system. The fact that the enzyme is also found in considerable amount near the site of entry and at the advancing front of the haustorium may implicate it the enzymatic action that has been postulated as one of the mechanisms of host penetration by the parasite haustoria [7, 15]. Nandhakumar *et al.*, (1976) [14] showed that holoparasites that have access to supply of organic nitrogen compounds from the host phloem as well as xylem nitrogen, have lack of the enzyme serine/threonine dehydratase, making them heterotrophic for the amino acid isoleucine. Histochemical studies on the haustorial cells of *Striga hermonthica* have shown to exhibit high enzyme activities [1], while, McNally *et al.*, (1983) [13] reported that the activities of the ammonia-assimilating enzymes glutamine synthetase and glutamate synthase are relatively low in parasitic plants. *Cuscuta* constantly establishes new contacts to the host plant so that the development of haustorium form endogenously arising from meristematic tissues. It is confirmed with the work of Lee and Lee (1989) [10]. Whereas Goshen *et al*., (1998) [12] recorded pectin methyl esterase with specific antibodies in the cytoplasm and cell walls of *Orobanche* intrusive cells and in adjacent host apoplast. The host parasite interphase of *Boschniakia hookeri* walpers (Orobanchaceae) was studied by Kuijt and Tooth (1985) [9]. Rajanna *et al*., (2013) [17] recorded for the first time histochemical localization of succinic dehydrogenase activity in peripheral zones of the penetrating haustorium into the host tissues of two important parastic angiosperms viz., *Cassitha filiformis* and *Cuscuta reflexa*. Futher, histochemical localization of acid phosphatase in the haustoria of two stem parasites such as *Cassytha filiformis* and *Cuscuta reflexa* was reported by Rajanna and Suresha (2014) [18]. However the enzyme may be mostly associated in the absorption of host material derived from cells damaged by mechanical invation of host tissue. But no work has been done on the localization of peroxidase. Hence the present study records for the first time localization of peroxidase in the haustorium of *C. reflexa* and *S. album*.

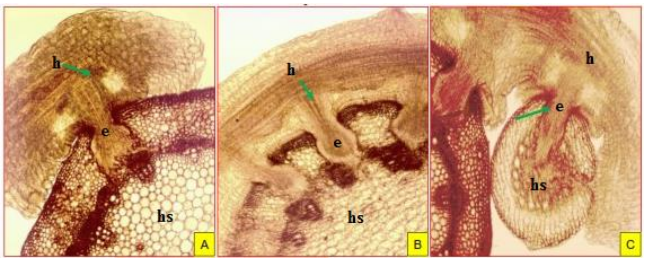

Fig 1: Test for Peroxidase activity in *Cuscuta reflexa* Roxb. with Guaiacol

A. V/S of the haustorium (h) with host stem (hs) cut transversely tested for peroxidase activity (green arrow). B. V/S of haustoria tested for localization of peroxidase (green arrow) (Note: Multiple entry of the haustoria (white arrows) in to the host tissue). C. V/S of mature haustorium showing entry of endophyte (e) into the host xylem.

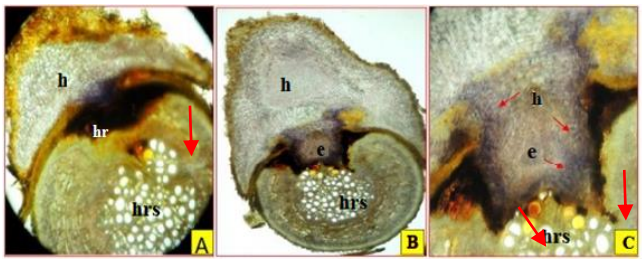

Fig 1: Test for Peroxidase activity in *Santalum album* L. using Benzedine

A. V/S of young haustorim (h) showing peroxidase activity (red arrow) during penetration into the host root (hr). B. V/S of mature haustorium showing peroxidase activity (red arrows). C. V/S of the mature haustorium showing enzyme activity (red arrows) in endophyte (e) region.

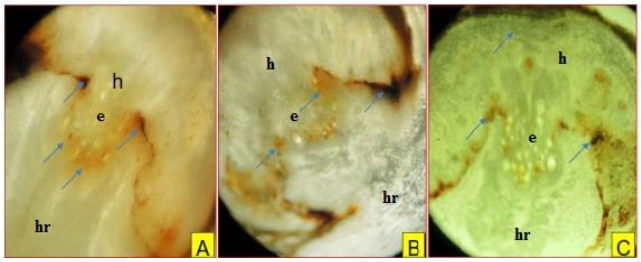

Fig 3: Test for Peroxidase activity in *Santalum album* L. using Guaiacol

A. V/S of young haustorim (h) showing peroxidase activity (blue arrows) during penetration into the host root (hr). B-C. V/S of mature haustoria showing peroxidase activity (blue arrows) (Note the decrease in the activity in C at endophyte region as the hasutorium matures further).

4. Conclusion

During the present investigation histochemical localization of peroxidase enzyme were studeid for the first time in the haustorium of *Cuscuta reflexa* and *Santalum album* using Benzedine and Guaiacol. High enzyme activity was observed in the parasitic tissues, especially in the parts of

the haustorium in contact with the host tissue and the parts of the haustorium close to the site of entry.

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