IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

COMPARITIVE ACCOUNT OF SUCCINIC DEHYDROGENASE LOCALIZATION IN THE HAUSTORIA OF *CUSCUTA REFLEXA ROXB.*AND *SANTALUM ALBUM L.*

SURESHA N.S*, SANDHYA RANI D,and DEEPA R. HEBBAR

Department of Botany, Maharani's Science College for Women, Mysore-570 005

Abstract

In ***Cuscuta reflexa.*** succinic dehydrogenase activity was very poor in young haustorium but more pronounced when the endophyte penetrated the host cortex and the enzyme accumulated more in the matured endophyte. In young haustorium of ***Santalum album L.***, the enzyme activity was very less but it was more pronounced in matured haustorium at the region of parasite contact with the host and also in the vascular core.

Keywords; Endophyte, Haustorium, succinic dehydrogenase, vascular core.

INTRODUCTION

Cuscuta reflexa Roxb. is a total stem parasitic angiosperm belonging to the family Convolvulaceae (Stefanovic *et al*, 2002) but some investigators classified the genus as a member of family Cuscutaceae (Swift 1996). It is one of more robust rootless herb with filiform tem measure 1 - 2 mm in thickness when fresh. The stem is reddish yellow in colour with green tinge and bears scaly leaves and a cluster of flowers. However photosynthesis is negligible hence it is an obligate parasite it is attached to the host by numerous haustoria . It is one of the most robust species, it is found to develop haustorial contacts only on living tissues and not on dead pith tissue. The haustorium initials developed in the cortex of the *Cuscuta* stem at the attachment site and it gave rise to main haustorial body. The haustorium initials developed into a group of meristem cells , during these events, the cortical cells between the meristem and the stele began to expand towards the contact side and the stem was tightly coiled around the host with more or less protruding haustoria produced on the side of the parasite stem contacting the host.

Santalum album L. a famous sandal wood tree is a member of family Santalaceae. The Sandal wood trees confined to Karnataka, Tamilnadu and Kerala in India. Mysore is famous for sandal wood trees because in Karnataka majority of the plants grows in this area. Other parts in Karnataka State includes Chamarajanagar and Coorg district. The taxon grows as a hemiroot parasite. It is moderate sized, branched evergreen tree .

In Santum album the shape and size of the haustoria varies and are usually whitish, appears as a small hemispherical outgrowth. The older ones become brownish and slightly spread over the host root. The haustoria may completely enclose the host root. Single root of the parasite may form haustoria on diverse hosts, when the haustoria may be formed at any angle with reference to the host root. Haustoria are initiated by the parasite at its sub apical region and also arise laterally on the roots. The young haustorium is developed by the cortical cells of the parasite root.

The haustorium consists of narrow neck a cushion shaped body and a broad apex. The body consists of a massive parenchymatous tissue; the apex of the haustorium plays an active part in the penetration of the host root cortex. Before penetration of the host has begun, the haustorial cells forming the contact surface gets modified into columnar cells, which presumably secrete an adhesive, reaching from the margin of the mantle part way up the flanks of the haustorium. The young parenchymatous core at an early stage begins to divide actively in planes parallel to the host surface. 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. The haustorium and the host are held together by the clasping folds or the mantle formed during the early stages of development .

Most of the biochemical reactions in a living cell are governed through the enzymes. These are unstable molecules; they are synthesized and degraded very rapidly. Enzyme localization in cells and tissues is a challenging goal in the field of Biology. Localization of enzymes study offers a procedure for tissue characterization and the importance of enzymes in the developing plant tissues help to study tissue differentiation.

Enzyme reactions are also activated in the cells when the tissue is sectioned. The end product of enzyme reaction is also detected. The enzymes like catalases, peroxidases, esterases etc., may become activated when tissue is damaged. The enzyme activity is partially or completely independent of cellular stability, but the enzymes like dehydrogenases activity is associated with the cellular integrity, because these enzymes are destroyed partially or completely when the cell undergoes lysis. Enzyme reactions are also activated in the cells when the tissue is sectioned. The end product of enzyme reaction is also detected. The enzymes like catalases, peroxidases, esterases etc., may become activated when tissue is damaged. The enzyme activity is partially or completely independent of cellular stability, but the enzymes like dehydrogenases activity is associated with the cellular integrity, because these enzymes are destroyed partially or completely when the cell undergoes lysis.

In the present study qualitative localization of enzymatic activity during the development of haustorium of Stem parasite and root parasite were obtained through histochemical tests , because of their importance in cellular reactions.

MATERIALS AND METHODS

Haustoria were collected at various stages of development, The collected materials were immediately brought to the laboratory along with the host . Root systems of *S. album* were carefully dugout of the soil along with the host roots, the soil particles and unwanted materials were carefully washed and cautiously separated.

The thin free hand sections of both the species were taken by following free hand sectioning method (Ruzin 1999). For the histochemical localization of succinic dehydrogenase fresh plant materials were used separately and the sections were tested at room temperature. The following method is standardized and adopted to detect histochemical localization of succinic dehydrogenase (Nachlas *et al*.,1957 method)

The sections were incubated about 30 mins at room temperature in a substrate solution made up of 5 ml of 0.2m phosphate buffer at pH 7.6 , { prepared by dissolving **A**) 27.8 g of Sodium phosphate monobasic in 1000 ml of distilled water and **B**) 53.65 grams of Sodiumphosphate dibasic in 1000 ml of distilled water. Mix 13 ml of solution **A** and 87 ml solution **B** and make it 200 ml by distilled water} and 5 ml of 0.2m Sodium succinate (Prepared by dissolving 1.35 g of the reagent in 500 ml of distilled water) and 10 ml of Nitro BT solution (Prepared by dissolving 10 mg of reagent in 10 ml of distilled water) . Finally the sections were washed in distilled water and mounted in glycerine. The sites of Succinic dehydrogenase activity appears deep blue in colour.

Observations and discussions

Two taxa were selected for the present investigation, of which *Santalum album L.* is a hemi root parasitic tree and *Cuscuta reflexa* Roxb. is a rootless sprawling holo stem parasitic vine.

Both the taxa were found to develop haustorial connections widely on different dicotyledonous and monocotyledonous host species and exihibited great variation in the extent of parasitism. Sandal tree grows better with luxuriant green foliage when associated with some hosts belonging to Fabaceae such as *Crotalaria juncea, Erythrina indica, Clitoria tarnetia, Pongamia glabra, Dalbergia sissoo and Abrus precatorius.* While with others the growth is slightly retarded, this might be associated with nitrogen metabolism there is no indication of any sort of host specificity. In some cases the occurrence of hyperparasitism has been reported, this clearly demonstrates that the angiosperm parasites do not have any host specificity (Rajanna *et al*., 2010). Self parasitism was common in *Cuscuta reflexa.*

In general the facultative root parasites such as *Rhinanthus* and *Odontites* have extremely broad host range. A possible exception to this generalized in *Seymeria* which is apparently a facultative parasite has a very limited host range. Some stem parasitic angiosperms have been recorded only on some particular host. *Dendrophthoe falcata* is an exception to this. Haustorium is one of the most important specialized organ among parasitic angiosperms. It forms a structural and physiological bridge between the parasite and its host.

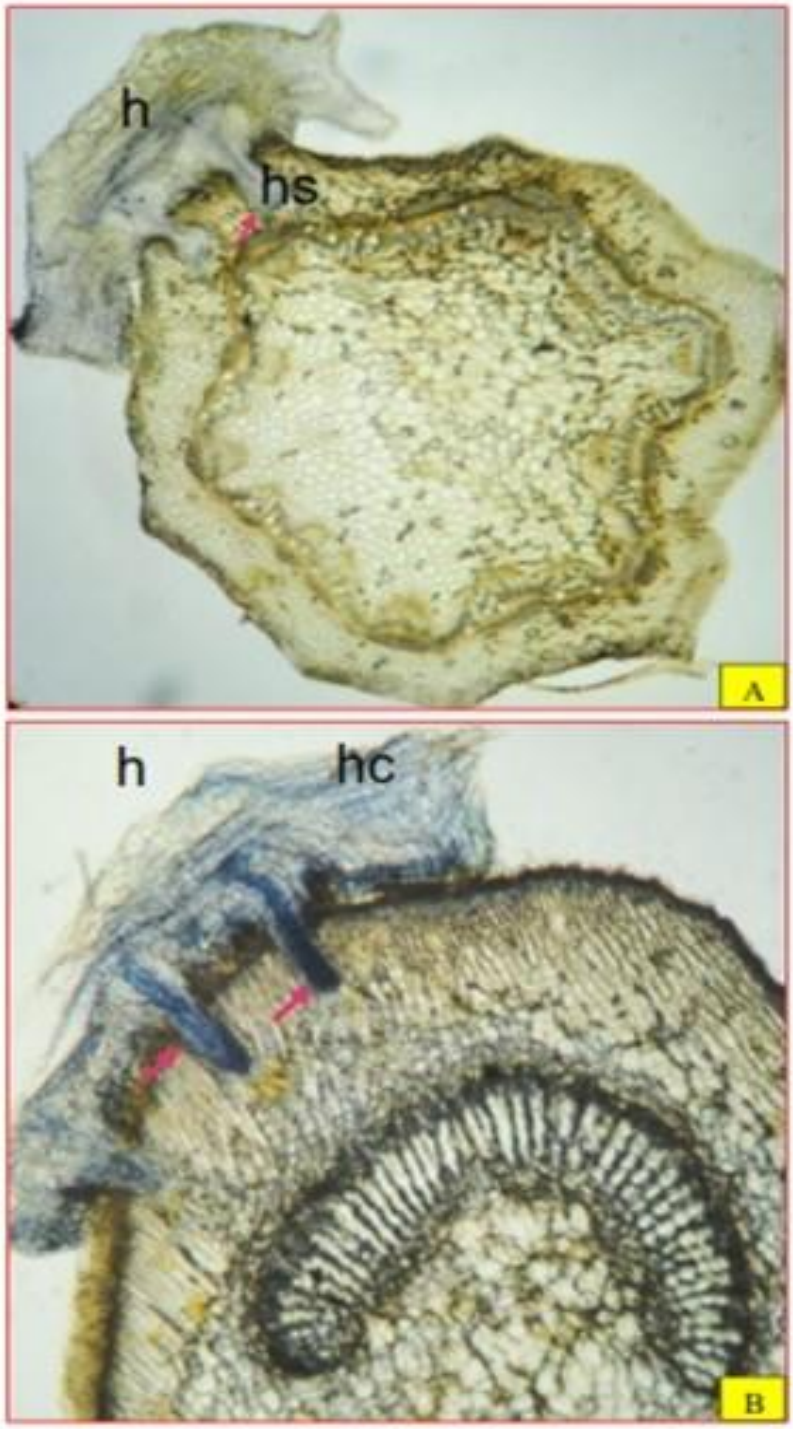
Normally, two kinds of haustoria are recognised on the basis of their origin (Kuijt 1977). If the root apical meristem of the embryo gets transformed into a haustorium, it is referred to as "primary haustorium". This is observed among the members of Orobanchaceae, Loranthaceae, Viscaceae and *Striga orobanchoides* of Scrophulariaceae. On the other hand, haustoria developing from regions other than the radicular apex of embryo are called "Secondary haustoria", both the parasitic taxa selected for the present study developed only secondary haustorial contacts with their hosts.

The size of the haustoria of root parasitic angiosperms normally very small organs, measuring in mm or at the most few cms in diameter. The contact between the haustorium and host organ is very delicate in *Cuscuta reflexa.* But in *Santalum album the* haustoria are firmly anchored to the host plant.

The haustoria are normally globose, conical, oval and hemispherical or button shaped structures in *Santalum album.* Similar shapes of haustoria have been observed in *Exocarpus* (Fineran 1963), *Comandra umbellata* (Kuijt 1969) of Santalaceae, *Gaiadendron punctatum* and *Atkinsonia ligustrina* (Fineran and Hocking 1983) both are root parasites of Loranthaceae and *Olax phyllanthi* (Pate *et al* 1990) of Olacaceae.

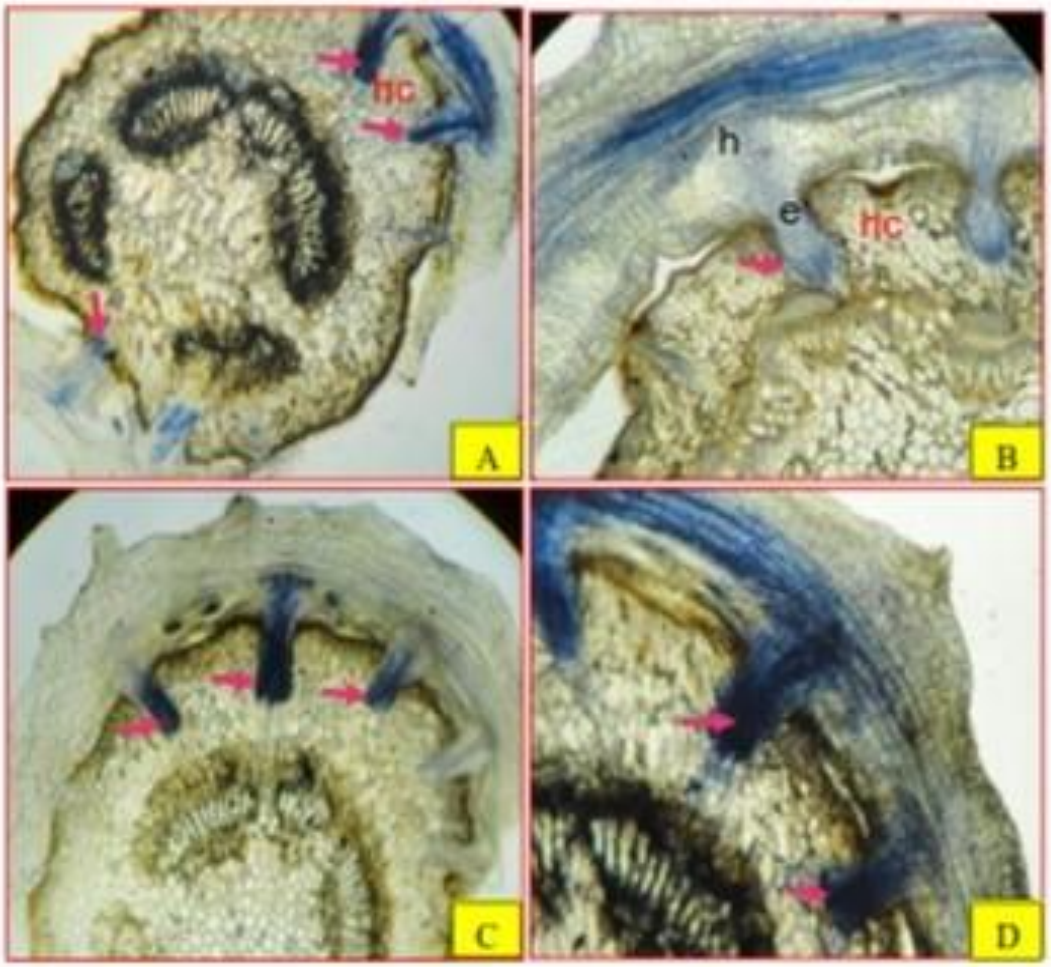
In *Santalum* the haustoria are initiated just behind the apex of growing roots. Such observations have been made by Fineran (1965) in *Exocarpus bidwillii*, Tooth and Kuijt (1976) in *Comandra umbellata*, A well marked gland is organized within the developing haustorium just before the penetration of the host root in *Santalum album* but in *Cuscuta reflexa* gland formation was not observed. This unique feature is reported in the developing haustorium of only *Santalum* members so far. A structure similar to gland organization and function has not been described in any other parasitic angiosperms outside the santalales. The gland is reported to be absent in the aerial members of Loranthaceae (Kuijt 1969). The first proof of direct involvement of enzymatic activity during the invation process came from the work by Goshen *et al*, (1998).

Figure 1
***Cuscuta reflexa* Roxb.**
Test for Succinic dehydrogenase activity



A-B. Cross sections of different stages of haustorium which penetrate the host cortex and stele
Note: Multiple entry of the haustoria shows Succinic dehydrogenase activity (arrows)

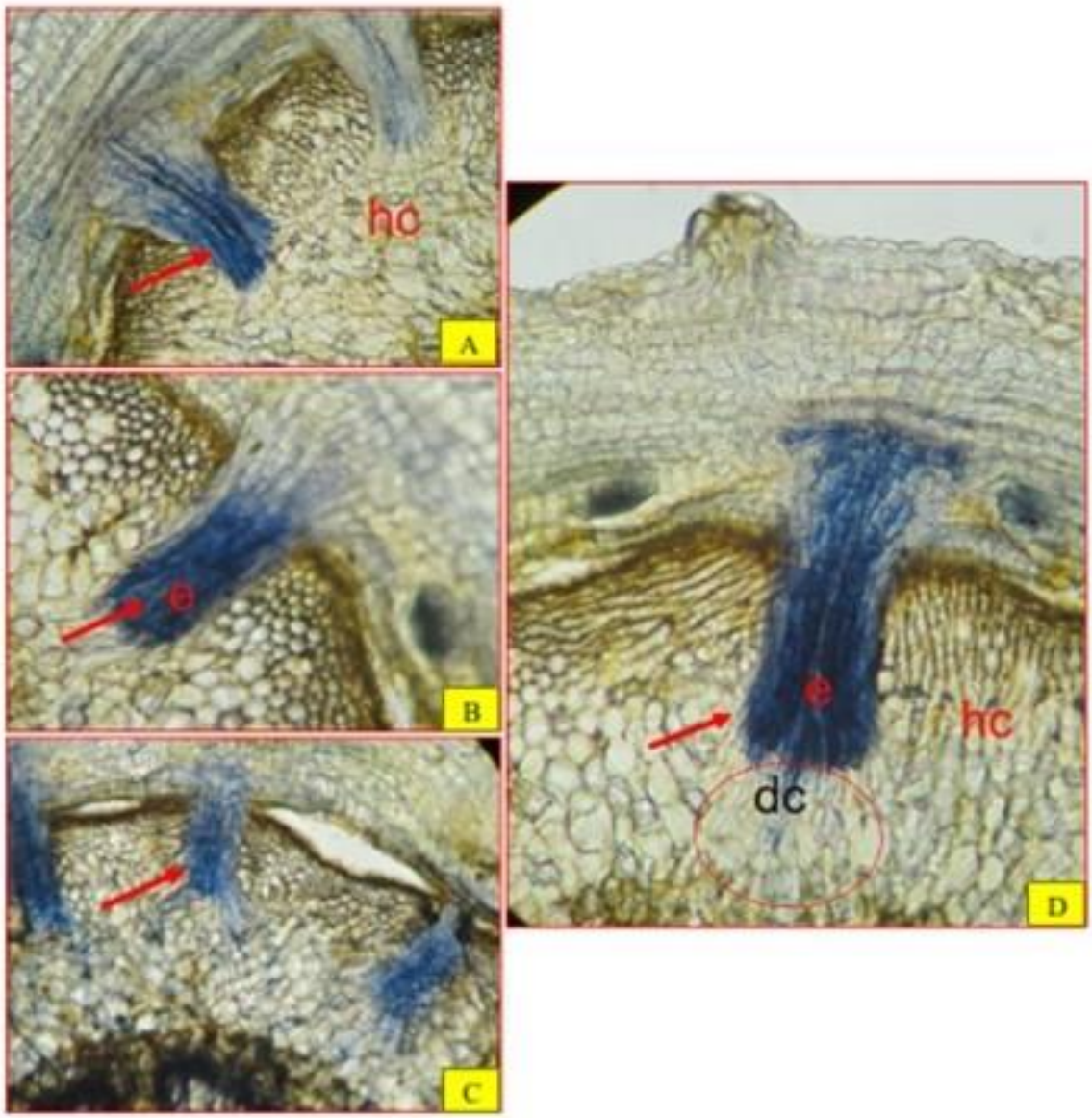
Figure 2
***Cuscuta reflexa* Roxb.**
Test for Succinic dehydrogenase activity



A&B. V/s of the haustorium with multiple entry (arrows) into the host stem cut transversely Note: the endophyte (e) penetrate the host cortex (hc)

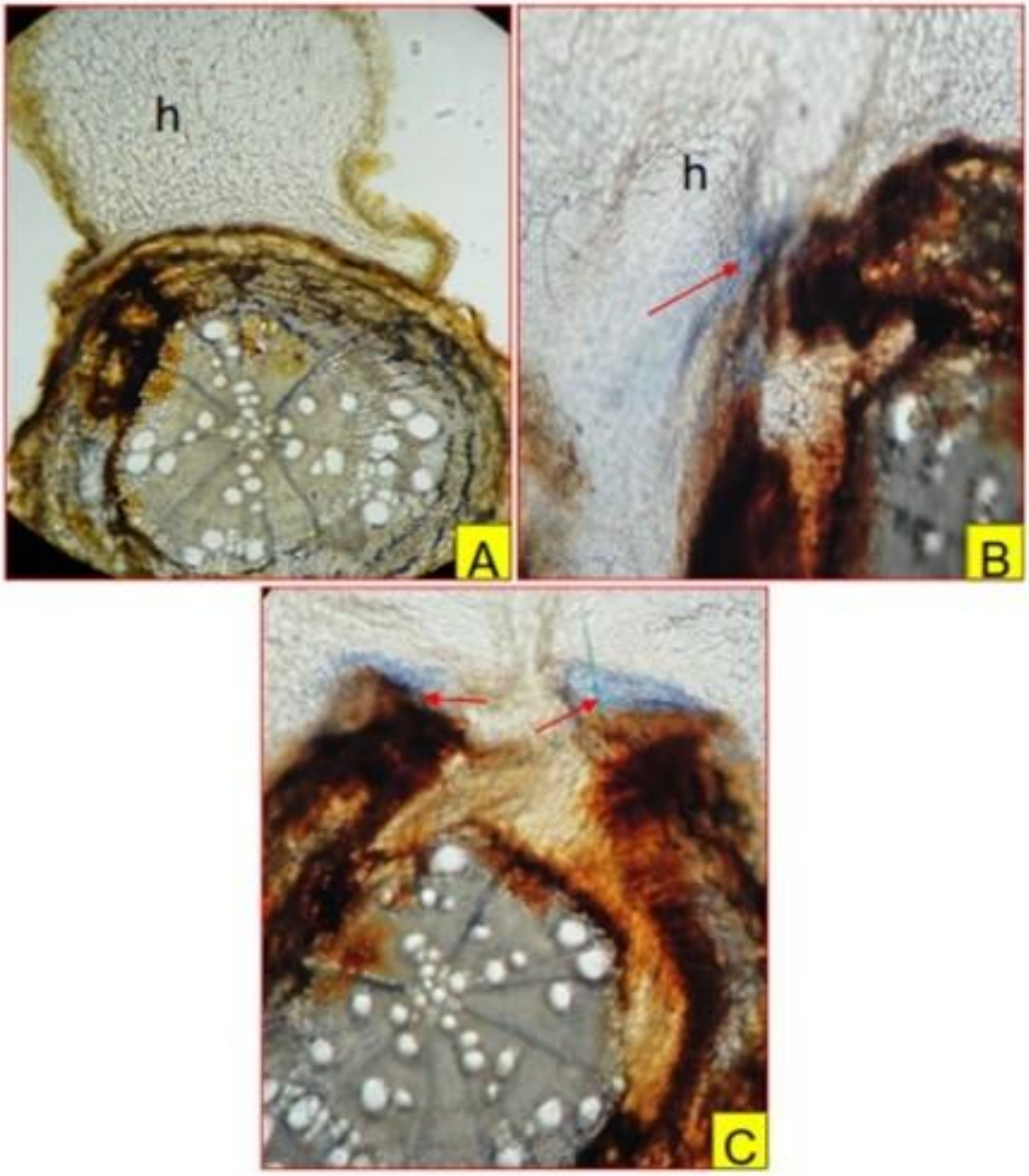
C&D. Multiple entry of haustoria shows Succenic dehydrogenase activity (arrows)

Figure 3
***Cuscuta reflexa* Roxb.**
Test for Succinic dehydrogenase activity



A&B. V/s of the haustorium with multiple entry (arrows) into the host stem cut transversely Note: the endophyte (e) penetrate the host cortex (hc)
C. Multiple entry of haustoria shows Succinic dehydrogenase activity (arrows)
D. A portion of the haustorium enlarged to show digitate cells (dc)

Figure 4
***Santalum album* L.**
Test for Succinic dehydrogenase activity



A, B & C. V/s of young haustorium (h) showing successive stages of penetration to the host tissues showing succinic dehydrogenase activity (arrows)

It has been previously postulated that degenerating endophyte cells may be releasing autodigestive enzymes (Tooth and Kuijt 1977) which distrupt host cells. No such degenerating cells were observed during the present study. Therefore, we conclude that any enzymatic weakening of the host is a primary function of the endophyte . The endophyte then moves through the host tissue by mechanically damaging the host cells, the enzymatic weakening being necessary preconditioning for endophyte penetration.

Similar kind of enzyme localization tests were conducted in angiosperms apart from the parasites byKumar and Mathur (2009) They noticed that very high activity of peroxidise in the cortex, vascular region and pith cells of normal stem in *Terminalia arjuna*. Occurrence of enzymatic studies on the haustorium of parasitic phanerogams are infrequent and fragmentary, Hence the present study represents for the first time the localization of succinic dehydrogenase in the haustorium *Cuscuta reflexa* and *Santalum album.* In both of the paraites Succinic dehydrogenase activity appears blue.

The enzyme activity in *Cuscuta reflexa* noticed only in the parasite. During the early stage of haustorial development the enzyme activity is very poor both in the haustorium as well as in the region of endophyte (Fig. 1 A). As the development of the endophyte proceeds it penetrate the host cortex, the enzyme activity gradually increases (Fig. 1 B). Further it is noticed that as the endophyte matures the secretion of enzyme is more and it only accumulated at the region of endophyte forther its activity does not appear in the region of haustorium (Fig. 3 B, C& D).later it is noticed that from the matured endophyte the digitate cells comes in contact with the host vascular tissue (Fig. 3 D).

In *Santalum album* enzyme activity is not pronounced in the host, in mature haustoria, the enzyme secretion is more pronounced when compare to that of young hautoria. In early stage the enzyme secretion is very poor and it appears only at the point of contact. It is usually synthesized by the cortical cells of the haustorium (Fig. 4 B). Before the act of penetration, the young haustorium does not contain this activity (Fig. 4 A). But as the development of haustorium proceeds, the enzyme activity gradually increases (Fig. 4 B). The gross anatomy of the haustorium in the later stages of development showed the activity and is restricted to the clasping region (Fig. 5 A), vascular core and the cortex portion of the endophyte (Fig. 5 B & C).

SUMMARY AND CONCLUSIONS

Two taxa were selected for the present investigation, of which *Santalum album L.* is a hemiroot parasitic tree and *Cuscuta reflexa* Roxb. is a rootless sprawling holo stem parasitic vine. 4In both the parasites of present investigation, the haustorial formation initiated by the meristematic activities of cortical cells. A well marked gland is organized within the developing haustorium just before the penetration of the host root in *Santalum album* but in *Cuscuta reflexa* gland formation was not observed.

Occurrence of enzymatic studies on the haustorium of parasitic phanerogams is infrequent and fragmentary. During the present study histochemical localization of succinic dehydrogenase was reported first time in the haustoria of *Cuscuta reflexa* and *Santalum album*. High enzyme activity was observed in the parasitic tissues, especially in the parts of the haustorium in contact with newly formed xylem tissues of the host and the parts of the haustorium close to the site of entry.

In both the taxa the enzyme activity is more pronounced in the parasite only , it is noticed that the juvenile haustoria secret less quantity of enzymes but as the growth proceeds the concentration gradually increases , activity appears at the region of vascular core and the tip of the endophyte, hence in both the

taxa the enzyme activity is same. The haustorium of both the parasitic taxa in the present study shows positive reaction .The enzyme localization appears blue.

REFERENCES

1. Fineran, B. A. (1963). Studies on the root parasitism of *Exocarpus bidwillii* Hook. F- I. Ecology and root structure of the parasite. *Phytomorph*, 12, 339-55.
2. Fineran, B. A. (1963a). Studies on the root parasitism of *Exocarpus bidwillii* Hook. F-II. External morphology, distribution and arrangement of haustoria. *Phytomorph*, 13, 30-41.
3. Fineran, B. A. (1965). Studies on the root parasitism of Exocarpus bidwillii Hook. f. VI. Haustorial attachment to non-living objects and the phenomenon of self-parasitism. *Phytomorph*ol, 15, 387-99.
4. Fineran, B. A., & Hocking, P. J. (1983). Features of parasitism, morphology and haustorial anatomy in Loranthaceous root parasites. The biology of mistletoes. *Academic Press*: Sydney, etc, 205-27.
5. Goshen, L. D. A. L. I. A., Portnoy, V. H., Mayer, A. M., & Joel, D. M. (1998). Pectolytic Activity by the Haustorium of the Parasitic PlantOrobancheL. (Orobanchaceae) in Host Roots. *Annals of Botany*, 81(2), 319-326.
6. Kuijt, J. (1969). The biology of parasitic flowering plants. University of California Press, Berkeley. The biology of parasitic flowering plants. University of California Press, Berkeley.
7. Kuijt, J. (1977). Haustoria of phanerogamic parasites. Annual Review of Phytopathology, 15(1), 91-118.
8. Nachlas, M. M., Tsou, K. C., De souza, Cheng, C. S., & Seligman, A. M. (1957). Cytochemical demonstration of succinic dehydrogenase by the use of a new p-nitrophenyl substituted ditetrazole. *Journal of Histochemistry & Cytochemistry*, 5(4), 420-436.
9. Pate, J. S., Kuo, J., & Davidson, N. J. (1990). Morphology and anatomy of the haustorium of the root hemiparasite *Olax phyllanthi* (Olacaceae), with special reference to the haustorial interface. *Annals of Botany*, 65(4), 425-436.
10. Pate, J. S., Pate, S. R., Kuo, J., & Davidson, N. J. (1990). Growth, resource allocation and haustorial biology of the root hemiparasite *Olax phyllanthi* (Olacaceae). *Annals of Botany*, 65(4), 437-449.
11. Rajanna . L . and Suresha.N.S.2010. Hosts of *Cuscuta reflexa* . J. Swamy Bot . Club. 27; 39- 42.
12. Stephens, E. L. (1912). The Structure and Development of the Haustorium of *Striga lutea* With Plate XCIII. Annals of Botany, (4), 1067-1076.
13. Swift, C. 1996. Cuscuta and Gramica species dodder a plant parasite USA: Colorado State University
14. Toth, R., & Kuijt, J. (1976). Anatomy and ultrastructure of the young haustorial gland in *Comandra* (Santalaceae). *Canadian Journal of Botany*, 54(20), 2315-2327.
15. Toth, R., & Kuijt, J. (1977). Anatomy and ultrastructure of the haustorium in *Comandra* (Santalaceae). *Canadian Journal of Botany*, 55(4), 455-469.