

ANATOMICAL CHARACTERIZATION OF PARASITIC PLANTS OF PAKISTAN

MIR AJAB KHAN¹, TAHSEEN SHARIF¹, MUSHTAQ AHMAD¹,
MUHAMMAD ZAFAR¹ AND RASOOL BAKHSH TAREEN²

¹Department of Plant Sciences, Quaid-i-Azam University Islamabad, Pakistan

²Botany Department, University of Balochistan, Quetta, Pakistan.

Abstract

The gross anatomical characteristics of 5 parasitic plant species, viz., *Cuscuta reflexa* Roxb. (Cuscutaceae), *Orobanche aegyptiaca* Pers. (Orobanchaceae), *Korthalsella opuntia* (Thomb.) Merrill, *Viscum album* L., and *Viscum cruciatum* Seib. Ex. Spreng. (Viscaceae) were investigated. The vascular bundles of all these parasitic plants were less developed having poorly developed phloem but well developed xylem. The most interesting features were the presence of terminal sclereids in *Viscum cruciatum* and brachysclereids in *Korthalsella opuntia*. Terminal sclereids have not been reported previously in the genus. These parasitic species show variation in the invasiveness of endophytic tissue of haustorium into the host vascular tissue. Haustorium of *Cuscuta reflexa* forms connection to the phloem of the host. The haustorium was consisting of few threads of xylem and phloem which penetrate only to the phloem tissue of the host. In *Korthalsella opuntia* and *Viscum cruciatum* haustoria form connection to the xylem of the hosts. The haustorium of *Korthalsella opuntia* is well developed and surrounds the host xylem in the form of cup.

Introduction

Angiosperm parasites in primary habitats are an integral part of an ecosystem. They behave as “prudent predators” and are adapted to life cycle of their principal hosts. Approximately 3,900 species of parasitic plants have been recorded (Nickrent, 2002), amounting to more than 1% of the flowering plants. These parasitic angiosperms belong to 22 families. Parasitic angiosperms have been recognized as an entity for over 2000 years. Almost half of the population of parasitic angiosperms belongs to family Scrophulariaceae. On basis of extent of autotrophism parasites can be classified as holoparasites and hemiparasites. On the basis of location of haustorial connection to the host, parasites can be divided into stem and root parasites, based on the presence of chlorophyll parasites are separated into achlorophyllous and chlorophyllous parasites. On the basis of selection of host parasites are divided into specialist plant parasites and generalist plant parasites. Generalist plant parasites like *Cuscuta* can attack several host species simultaneously (Govier *et al*, 1967). Interaction between the host and parasite occurs at the haustorium interface. The foreign parasite tissue grows into the host and forms a connection with the vascular system. Following that connection, competition between the host and parasite for resources begins. The presence of plasmodesmata between the haustoria and host cells is believed to assist the movement of food (Esau, 1948). The main stimulus for the development of haustorial tissue may simply contact with another surface. In some parasitic plants such as *Pedicularis*, haustoria can form dead plant material (Piehl, 1963). Parasites may affect the photosynthesis and nitrogen metabolism of the host. Most important is effect of parasite on the productivity and reproduction of the host (Silva & Rio, 1996). *Striga hermonthica* attacks the host sorghum; the predicted parasite induced weight loss of the host is 30 times the weight

gain of the parasite (Parker, 1984). Small amount of parasite biomass is required to cause a large effect on host biomass accumulation (Gurney *et al.*, 1999).

The present study was conducted to investigate the detailed anatomy of parasitic species of Pakistan. Attempts have been made to elaborate the importance of anatomy in understanding of different structures of parasitic plants as well as their physiology in relation to their cellular structures, especially haustorium.

Materials and Methods

Representative stem cuttings of the host at the point of the host-parasite association where the haustorium is connected to the host shoot system, leaves, stems and fruits of the parasitic plant under study were preserved in formalin acetic acid (FAA) for subsequent anatomical studies. Plant samples were soaked in water overnight under room temperature to remove the preservative. Clear sections were prepared, stained with safranin and mounted according to the method used by Bokhari (1971). For epidermal studies Shultze's method of maceration with improved techniques was followed (Subrahmanyam, 1996).

Results

In the present study detailed anatomical studies of 5 parasitic plants viz., *Cuscuta reflexa* Roxb. (Cuscutaceae), *Orobanche aegyptiaca* Pers. (Orobanchaceae), *Korthalsella opuntia* (Thomb.) Merrill, *Viscum album* L. and *Viscum cruciatum* Seib. Ex. Spreng. (Viscaceae) have been carried out. Their anatomical features are given as under:

1. *Cuscuta reflexa* Roxb.

The epidermal cells of stem are papillose. Cortex is made up of seven layers of parenchyma cells with small intercellular spaces. Six poorly developed vascular bundles are arranged in a ring. Pith is also made up of parenchyma cells. Medullary rays are not visible. The haustorium is a small thread of xylem vessels and phloem which penetrates only in the phloem tissue of the host (Fig.1-A).

2. *Orobanche aegyptiaca* Pers.

In the lower portion of the stem the epidermis is single layered, without cuticle and sparsely hairy. There is a wide zone of cortex made up of 20 layers of parenchyma cells with well developed intercellular spaces. There are many distinct vascular bundles arranged in the form of a ring around well developed pith. Each vascular bundle has xylem and phloem but xylem has number of fibers. Medullary rays are well developed and are between the vascular bundles (Fig. 1-B). In the inflorescence axis the stem anatomy is totally different from the basal portion of the stem. There is a continuous layer of xylem and phloem bundles surrounding hollow pith. There is also a ring of sclerenchyma cells in the cortex just outside the phloem ring. Cortex is made up of small cells, epidermis single layered and without cuticle. Epidermal cells appear to be different sized and some of the epidermal cells produce simple hairs. Leafy scales have an upper and lower epidermis which is a thin walled and without cuticle. Vascular bundles have small phloem but xylem is slightly more developed. Ground tissue is 4 celled in the middle and 2 celled in the margin of the scales and is undifferentiated (Fig. 1-C).

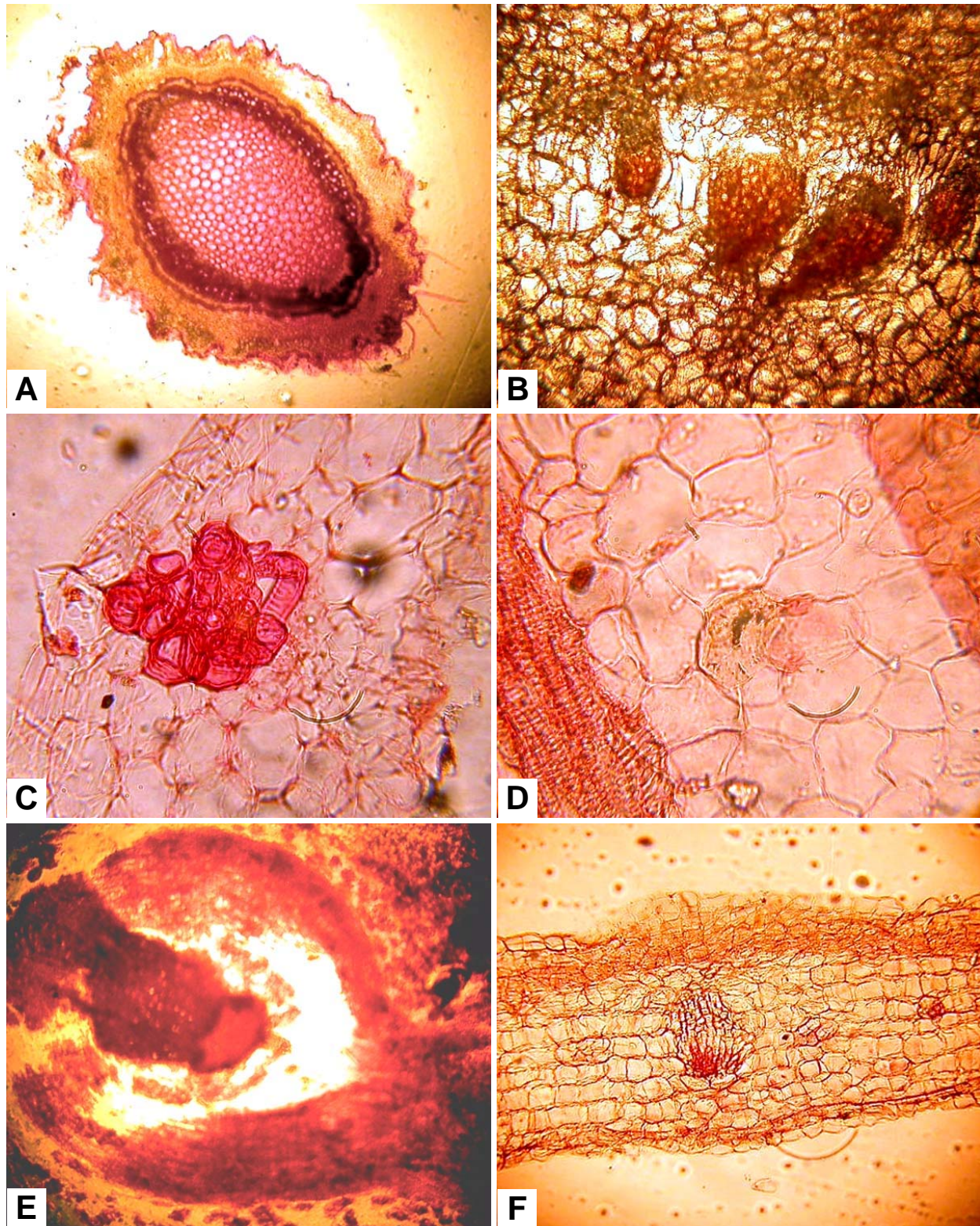


Fig. 1. Light micrographs of different portions of parasitic plants: (A)- L. S of endophytic tissue of haustorium of *Cuscuta reflexa* penetrating into the host phloem (100 x), (B)- T. S of stem of *Orobanche aegyptiaca* (100 x), (C)- T. S of scale of *Orobanche aegyptiaca* (400 x), (D)- L. S of stem of *Korthalsella opuntia* (100 x), (E)- L. S of endophytic tissue of haustorium of *Korthalsella opuntia* penetrating into the host xylem (100 x), (F)- T. S of leaf of *Viscum album* (100 x).

3. *Korthalsella opuntia* (Thomb.) Merrill

In the basal portion of the stem the epidermis is single layered and has well developed cuticle on the outer side. The vascular bundles are in the form of a ring. The phloem is poorly developed but xylem is well developed having a large number of fibrous tissue. Ground tissue is undifferentiated and present around the vascular bundles as between the vascular bundles. On the stem the stomata are slightly irregular but most of the stomata have parallel orientation. Stomata are of paracytic type. In the upper portion of the stem the vascular bundles are also arranged in the form of ring but individual vascular bundles are quite distant from one another. Xylem is also poorly developed and without sclernchyma. There are well developed brachysclereids on the periphery of vascular bundles in the ground tissue, these are also quite clear in the longitudinal section of the stem (Fig. 1-D). Ground tissue is differentiated into outer palisade like cells and inner spongy cells. Palisade like cells are outside the vascular bundles and sclereids. Haustorium has well developed vascular tissue and is made up of short tracheal cells. Most of the tissue is parenchymatous in nature (Fig. 1-E).

4. *Viscum album* L.

Epidermis cells in face view are polygonal in outline and are of different shapes. In Transverse section, epidermal cells are of different sizes and shapes on both the sides of the leaf. Few epidermal cells are papillose on both the sides. Cuticle is not present. Mesophyll is undifferentiated, made up of more or less polygonal cells with few intercellular spaces. Vascular bundles are bicollateral with very well developed phloem and few xylem vessels which are strongly lignified. There is also strong development of fibers associated with vascular bundles (Fig. 1-F). In petiole epidermal cells on the upper epidermis are smaller than the cells of the lower epidermis and there is a very well developed cuticle on both sides of the epidermis. Petiole is winged that is much narrow towards the margins. There are 6-7 vascular bundles arranged in an arc but the vascular bundles are unequal in size. As in the leaf, bundles are collateral with very developed phloem and few xylem vessels which are strongly lignified. The ground mesistem of the petiole is also undifferentiated (Fig. 2-A). The stem is irregular in outline and appears to be winged at many places. Epidermis is single layered with narrow lumen and a very thick cuticle on the outer side. The wall of the epidermis also appears to be lignified. Cortex is narrow with bundles of fibers just above the phloem of the vascular bundle. Vascular bundles are large about 9 in number and are arranged in a ring. Phloem is not well developed but xylem is extensively developed and strongly lignified. There are also group of fibers below each xylem portion of the bundle. Pith is small and made up of parenchyma cells. The modularly rays are very narrow made up of one or two layers of cells in between vessels. Crystals are totally absent from the stem (Fig. 2-B). Stomata in stem are arranged in longitudinal rows. In leaves stomata are irregular in arrangement and leaves are amphistomatic. In stem as well as in leaves stomata are mostly paracytic but a few anomocytic stomata were also observed. Fruit wall is many layered with outer having cuticle. It is two chambered berry. Spring wood is a ring porous type. In the spring wood vessels have broad lumen but in summer wood vessels are narrow lumened, interspersed in highly developed fibrous tissue. The arrangement of vessels varies; they are either in rows or in multiples (groups). All the vessels are round in outline and those in the spring wood are thin walled. Vessels have simple perforation plates. As seen in tangential longitudinal section rays are multiseriate (Fig. 2-C).

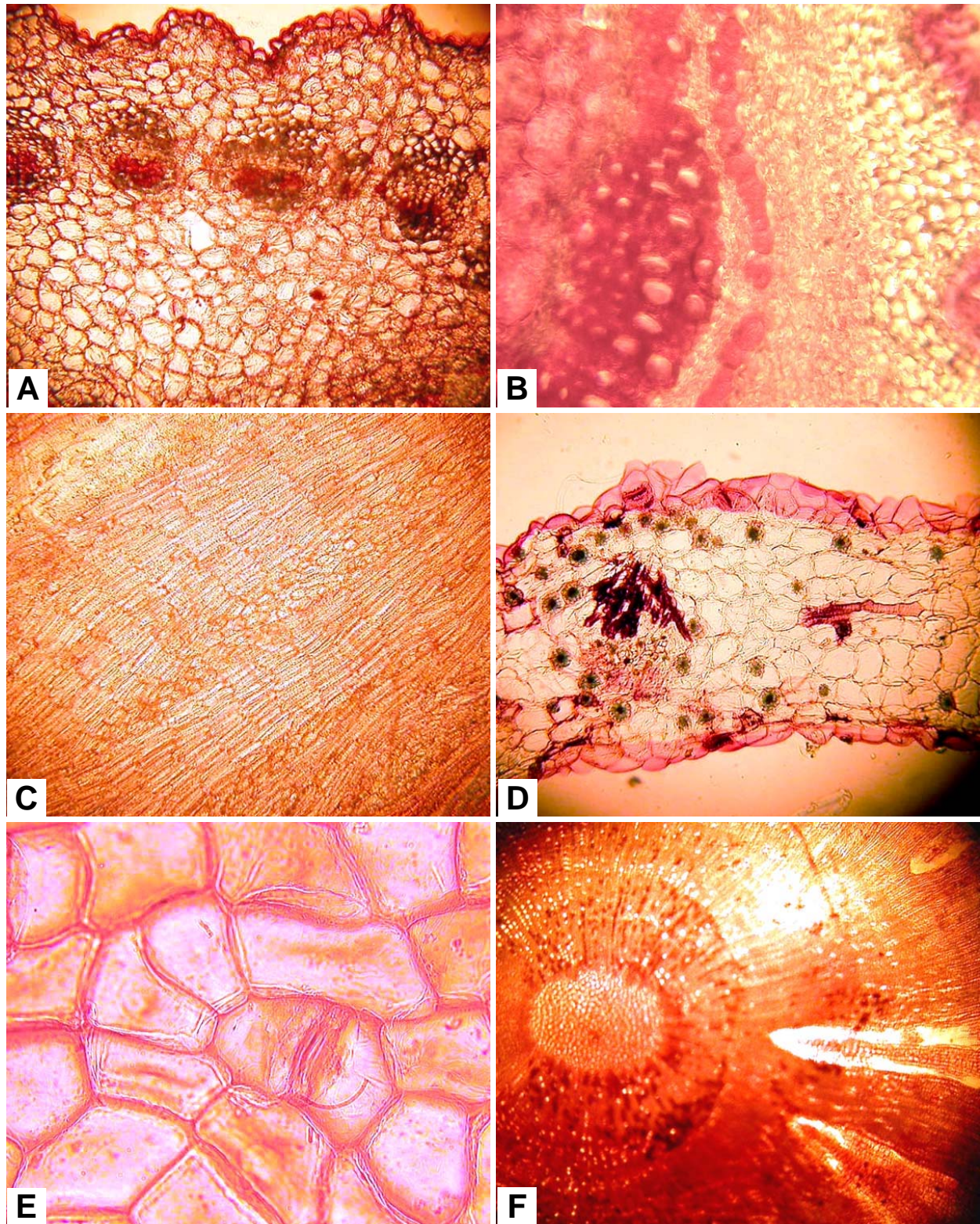


Fig. 2. Light micrographs of different portions of parasitic plants: (A)-T. S of petiole of *Viscum album* (100 x), (B)- T. S of stem of *Viscum album*(100 x), (C)- Tangential L. S of wood of *Viscum album* (100 x), (D)- T. S of leaf of *Viscum cruciatum* (100 x), (E)-Epidermis of *Viscum cruciatum* (400 x), (F)- L. S of endophytic tissue of haustorium of *Viscum cruciatum* penetrating into the host xylem (100 x).

5. *Viscum cruciatum* Sieb.ex Spreng.

The epidermal cells of leaves are similar on both sides. They are tubular in outline and have a thick layer of cuticle. Mesophyll is undifferentiated made up of more or less polygonal cells with small intercellular spaces. There are numbers of characteristic crystals present in many mesophyll cells. Each crystal has a central thick and black region surrounded by many polygonal small crystals. Vascular bundle is bicollateral; there are also lateral bundles which indicate that midrib is sparsely branched. The most interesting observation was the presence of terminal sclereids which can also be seen in transverse section (Fig. 2-D). They are directly attached to the vein endings. Terminal sclereids have not been reported previously in the genus. Phloem is made up of hyaline cells but xylem has very thick lignified walls. Stomata are distinctly of paracytic type (Fig. 2-E). Epidermal cells have straight walls and stomata mostly irregularly orientated. Leaves are amphistomatic. Stomata of stem are similar to leaves but unlike in leaves they are arranged in parallel rows. Petiole winged 6 to 7 vascular bundles arranged in the form of an arc. The vascular bundles are collateral. Xylem vessels are highly lignified. The ground tissue of petiole are of two types; the cells in wings are elongated appear palisade like while those in the other region are polygonal in outline. The Petiole photosynthetic. In T.S. stem appears to be smooth in outline with narrow cortex and small pith. There are strands of lignified fibers in the cortex. The vascular bundles are collateral with narrow region of phloem and very well developed xylem. Vascular bundles are separated by parenchymatous rays which run from cortex to the pith. Xylem vessels are arranged in radial rows. Crystals abundant in the cortex and in the phloem. The xylem is devoid of crystals. Epidermal cells have thick layer of cuticle on the outer side. In the longitudinal section the haustorium appears to be composed of number of cells in the periphery and tapering towards the inner side where it becomes to 2-3 celled, sending branches into the host xylem (Fig. 2-F). The vascular tissue is not observed in the haustorium. It appears that the haustorium absorbs lot of water from the host. The haustorium was not observed in the phloem region. In cross section of haustorium it is quite evident that it has large xylem cells and few phloem cells which indicate that haustorium absorbs water as well as food from the host.

Discussion

The parasitic plants studied during this investigation fall into two categories: holoparasites such as *Cuscuta reflexa*, *Orobanche aegyptiaca* and hemiparasites which include *Korthalsella opuntia*, *Viscum album* and *Viscum cruciatum*. Holoparasitic plants lack chlorophyll and are entirely dependant nutritionally on their host, weakening them and eventually results in the death of the host. Hemiparasites can partially manufacture their own food material and usually get very little direct food from the host. These parasites get water and minerals from the host and are in complete equilibrium with the host for a very long tome. So the host usually survives even when attacked by number of parasites.

In all of parasitic plants the vascular bundles were less developed having poorly developed phloem but well developed xylem. It means that parasitic plants can efficiently take water and prepare food from the host plant.

In holoparasitic plants, *Cuscuta reflexa* and *Orobanche aegyptiaca* the epidermis was without well developed cuticle, while in hemiparasitic plants, *Korthalsella opuntia*,

Viscum album and *Viscum cruciatum* the epidermis have well developed cuticle on both sides of the leaves and also on the stem. Leaves and stem of these parasitic plants had mostly of paracytic type of stomata however a few anomocytic types of stomata were also observed in *Viscum album*. Ehleringer *et al.*, (1985) have shown that stomata are the main pathway of evaporative water loss in mistletoes and that through stomatal action they can maintain a control over rates of water loss.

The leaves of the *Viscum album* and *Viscum cruciatum* were photosynthetic but their mesophyll was undifferentiated and was made up of polygonal cells. The vascular bundles were collateral. In *Viscum cruciatum* characteristic crystals were abundant. The petioles of the *Viscum album* and *Viscum cruciatum* winged and vascular bundles were arranged in the form of an arc. The petioles have well developed cuticle on both sides of the epidermis. The ground tissue is undifferentiated as in the leaves.

Epidermal cells of stem of *Cuscuta reflexa* were papillose. Endophytic tissue of haustorium was a small thread of xylem vessels and phloem. *Cuscuta* has been included in the family Convolvulaceae but the general occurrence of acotyledonous embryo and absence of internal phloem support its separation from the Convolvulaceae (Tiagi, 1951). Several contributions have been made to the understanding of the cytology (Fogelberg, 1938; Raghavan, 1957; Manitz, 1983) and haustorium formation (Tsivion, 1979) of *Cuscuta*.

Stem anatomy of *Orobanche aegyptiaca* was totally different. In the lower portion of the stem the vascular bundles were arranged in a ring around well developed pith but in the inflorescence axis, there was a continuous layer of xylem and phloem bundles arranged around hollow pith. There was a layer of sclerenchyma cells just outside the phloem ring. Host resistance mechanisms have rarely been reported in parasitic angiosperm–host associations but there are marked exceptions to this. A hypersensitive response has been observed in the *Striga gesnerioides*–Cowpea (Lane *et al.*, 1993) and the *Orobanche aegyptiaca*–vetch association (Goldwasser *et al.*, 2000): necrotic areas appeared at the site of parasite attachment due to localized cell death of the host tissue, resulting in degeneration of the parasite.

Stem anatomy of *Korthalsella opuntia* was quite different. In the lower portion of the stem the ground meristem was undifferentiated, while in the upper portion of the stem the ground tissue was differentiated into palisade like cells and spongy cells. Haustorium has well developed vascular tissue and was made up of short tracheal cells. Thoday (1958) reported that in the galls formed by *Korthalsella opuntia* on *Altingia exelsa* the haustorium forms a girdle round the host wood and perforates the host cambium by numerous papillae.

In *Viscum album* the stem was irregular in outline and appears to be lignified at many places while the stem of *Viscum cruciatum* was smooth in outline having thick cuticular layer on the epidermis. Crystals were abundant in phloem and cortex but xylem was devoid of crystals. *Viscum album* extracts have been classified as biological response modifiers. Its extracts have been described to exert immunomodulatory effects (Bloksma *et al.*, 1982; Gabius *et al.*, 1992 and Hajto, 1986) and a direct toxic effect on tumor cells (Janssen *et al.*, 1993 and Schaller *et al.*, 1996).

The most interesting features were the presence of terminal sclereids in *Viscum cruciatum* and brachysclereids in *Korthalsella opuntia*. The terminal sclereids were present at the vein endings while brachysclereids were scattered on the periphery of the vascular bundle in the ground tissue

These parasitic species show variation in the invasiveness of endophytic tissue of haustorium into the host vascular tissue. In *Cuscuta reflexa* haustorium forms connection to the phloem of the host more prominently than xylem. The haustorium consists of few threads of xylem and phloem which penetrate only to the phloem tissue of the host. In *Korthalsella opuntia* and *Viscum cruciatum* haustoria form connection to the xylem of the hosts. The haustorium of *Korthalsella opuntia* is well developed and surrounds the host xylem in the form of cup. The sinkers of *Viscum cruciatum* influence the direction of the host xylem elements, so that vessels meet them and end on (Thoday, 1958). Endophytic tissue of *Viscum cruciatum* consist of number of cells in the periphery and tapering towards the inner side, where it became 2-3 celled, sending branches into the host xylem (Fig. 2-F).

References

- Bloksma, N., P. Schimiermann., M. de Reuver., H. van Dijk and S. Willer. 1982. Stimulation of humoral and cellular Immunity by *Viscum* preparations. *Planta Medica* 46: 221-227.
- Bokhari, M.H. 1971. Morphology and Taxonomic Significance of Sclereids in *Limonium*. *Notes. R. Bot. Gbn. Edin.*, 30: 43-53.
- Ehleringer, J.R., E.D. Schulze, H. Ziegler, O.L. Lange, G.D. Farquhar and I.R. Cowan. 1985. Xylem-tapping mistletoes: water or nutrient parasites. *Science*, 227: 1479-1481
- Esau, K. 1948. Some Anatomical Aspects of Plant Virus Disease Problems. II. *Bot. Rev.*, 14: 413-449.
- Fogelberg, S.O. 1938. The cytology of *Cuscuta*. *Bull. Torrey Bot. Club*, 65: 631-645.
- Gabius, S., S. Joshi, K. Kayser and H. Gabius. 1992. The galactoside-specific lectin from mistletoe as biological response modifier. *International Journal of Oncology*, 1: 705-708.
- Goldwasser, Y., D. Plankhine, Y. Kleifeld, E. Zamski and B. Rubin. 2000. The differential susceptibility of vetch (*Vicia* spp.) to *Orobanchae aegyptiaca*: anatomical studies. *Annals of Botany*, 85: 257-262.
- Govier, R.N., M.D. Neldon and J.S. Pate. 1967. Hemiparasitic nutrition in Angiosperms. I. The transfer of organic compounds from host to *Odontites verna* (Bell). *Dum. New Phytologist*, 66: 285-297.
- Gurney, A.L., M.C. Press and J.D. Scholes. 1999. Infection time and density influence the response of sorghum to the parasitic angiosperm *Striga hermonthica*. *New Phytologist*, 146: 573-580.
- Hajto, T. 1986. Immunomodulatory effects of Iscador: a *Viscum album* preparation. *Oncology*, 43(Suppl. 1): 51-65.
- Janssen, O., A. Scheffler and D. Kabelitz 1993. *In vitro* effects of mistletoe extracts and mistletoe lectins: cytotoxicity towards tumor cells due to the induction of programmed cell death (apoptosis). *Fortschritte der Arzneimittel Forschung. Progress in Drug Research.*, 43: 1221-1227.
- Lane, J.A., J.A. Bailey, R.C. Butler and P.J. Terry. 1993. Resistance of cowpea (*Vigna unguiculata* (L.) Walp.) to *Striga gesnerioides* (Willd.) Vatke, a parasitic angiosperm. *New Phytologist*, 125: 405-412.
- Manitz, H. 1983. Die cytologie der Convolvulaceae und Cuscutaceae I. Zusammen-stellung der bekannten Chromosomomenzahlen. *Wiss. Ztschr. Friedrich-Schiller-univ. Jena*, 32: 915-944.
- Nickrent, D. L. 2002. Chapter 3. *Phylogentic Origion of Parasitic Plants*. (Eds.): J.A. Lopez-Saez, P. Catalan and L. Saez. Parasitic plants of the Iberian Peninsula and Balearic Islands. Mundi-Prensa, Madrid. pp. 29-56.
- Parker, C. 1984. The influence of *Striga* species on sorghum under varying nitrogen fertilization. pp. 90-98 In: *Proceedings of the 3rd international Symptom of Parasitic weeds*, (Eds.): C. Parker, L.J. Musselman, R.M. Polhill and A.K. Wilson. Aleppo, Syria. ICARD, Aleppo.
- Piehl, M.A. 1963. Mode of attachment, haustorium structure and hosts of *Pedicularis Canadensis*. *American Journal of Botany*, 50: 978-985.

- Raghavan, R. S. 1957. Chromosome number in Indian medicinal plants. *Proc. Indian Acad. Sci.*, 45: 294-298.
- Schaller, G., K. Urech and M. Giannattasio. 1996. Cytotoxicity of Different Viscotoxins and Extracts from the European Subspecies *Viscum album* L. *Phytotherapy Research.*, 10: 473-477.
- Silva, A. and C. Rio. 1996. Effects of the Mistletoe *Tristerix aphyllous* (Loranthaceae) on the Reproduction of its Cactus host *Echinopsis chilensis*. *Oikos*, 75: 437-442.
- Subrahmanyam, N.S. 1996. *Labortary Manual of Plant Taxonomy*. Vikas publishing house PVT. Ltd. New Delhi.
- Thoday, F.R.S. 1958. Modes of Union and Interaction between Parasite and Host in the Loranthaceae. III. Further Observations on *Viscum* and *Korthalsella*, *Proceedings of the Royal Society of London. Series B, Biological Sciences*, 188-206.
- Tiagi, B. 1951. A Contribution to the morphology and embryology of *Cuscuta hyaline* Roth and *C. Planiflora* Tenore. *Phytomorphology*, 1: 9-21.
- Tsivion, Y. 1979. Morphogenetic Sequences in the Formation of the haustorium of *Cuscuta campestris*. In: *Proceeding of the Second Symposium on Parasitic Weeds*. pp. 174-181. (Eds.): L.J. Musselman, A.D. Worsham and R.E. Eplee, North Carolina State University, Raleigh, NC. 296 pp.

(Received for publication 13 November 2008)