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ACKNOWLEDGEMENTS. Necessary facilities provided by the Dean, College of Forestry and Hill Agriculture, are acknowledged. The work was carried out utilizing grants received under the Uttaranchal Diversified Agriculture Support Project.

Received 19 March 2005; revised accepted 27 August 2005

Histochemical and biochemical studies of parasite–host interaction of *Cassytha filiformis* Linn. and *Zizyphus jujuba* Lamk.

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Parasite–host interaction of *Cassytha filiformis* and *Zizyphus jujuba* through histochemical studies revealed the presence of specialized glandular cells facilitating adhesion of parasite to the host and further specialization to obtain nutrients from phloem tissue of the host. Histoenzymological studies indicated the presence of high acid phosphatase activity of the parasite, which revealed the digestion of macromolecules/energy transfer and intercellular transport of parasite. Partial photosynthesis activity of parasite was noticed by accepting hydrogen released from photolysis of water through Hill reaction.

Keywords: *Cassytha filiformis*, haustoria, Hill reaction, parasite–host interaction, *Zizyphus jujuba*.

PARASITES are unusal plants, well adapted to their mode of life. More than 2500 species of higher plants are known to live parasitically on other plants. These parasitic plants produce flowers and seeds and belong to several widely separated botanical families[1]. They vary greatly in their dependence on their host plants. For example, *Viscum* (mistletoes) have chlorophyll but no roots and therefore depend on their hosts only for water and minerals. *Cuscuta* (dodders) and *Cassytha* (amarbeli) have little chlorophyll and no true roots. Hence they depend on their hosts for water, food and minerals. The most common and serious parasites belong to the following botanical families and genera.

Cuscutaceae: *Cuscuta* (dodders); Scrophulariaceae: *Striga* (witch weed); Orobanchaceae: *Orobanche* (broom rapes); Cassythaceae: *Cassytha* (ambarbeli); Loranthaceae: *Elytranthe*, *Korthalsella* and *Loranthus*, Viscaceae: *Arceuthobium* (dwarf mistletoes); *Phoradendron* (American true mistletoes) and *Viscum* (European true mistletoes).

Cassytha filiformis Linn. Cassythaceae (Figure 1 *a*) is a twining parasitic, perennial angiosperm which adheres to the host by suckers (haustoria). The plant is characterized by leaves reduced to minute scales, small flower, hermaphrodite, sessile, spicate, perianth tube short and globose, stamens six, ovary globose and fruit a drupe enclosed in the enlarged inflated perianth tube. During winter *Cassytha* seeds grow along with the seeds of the host plant (*Zizyphus*) in infested fields. During the growing season, the

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seeds germinate and produce a slender yellowish shoot, but no roots. When in contact with a susceptible host, the shoot encircles the host plant, sends haustoria into it and begins to climb up the plant. The haustoria penetrate the stem or leaf and reach into the vascular tissues, from which they absorb food and water. Soon after contact with the host is established, the base of the *Cassytha* parasite shrivels and dries up, so that the parasite loses all connection with the ground and becomes completely dependent on the host for food and water. The parasite continues to grow and expand while the growth of host plants is suppressed, which may even die. In the meantime, the parasite plant has developed flowers and produced seeds[1]. Many host–parasite interaction studies of *Cuscuta*, *Viscum*, *Orobanche* and *Striga* have been cited in the literature[1–16]. However, for *Cassytha* only little information is available. Hence histochemical and biochemical studies of parasite–host interaction of *C. filiformis* and *Zizyphus jujuba* were undertaken.

C. filiformis Linn. Cassythaceae (voucher specimen no. 3976)[17], the parasitic plant with the host *Z. jujuba* Lamk. Rhamnaceae (voucher specimen no. 2557)[17] were collected from Bharathidasan University, Tiruchirapalli campus and used for histochemical and biochemical studies. Microtome

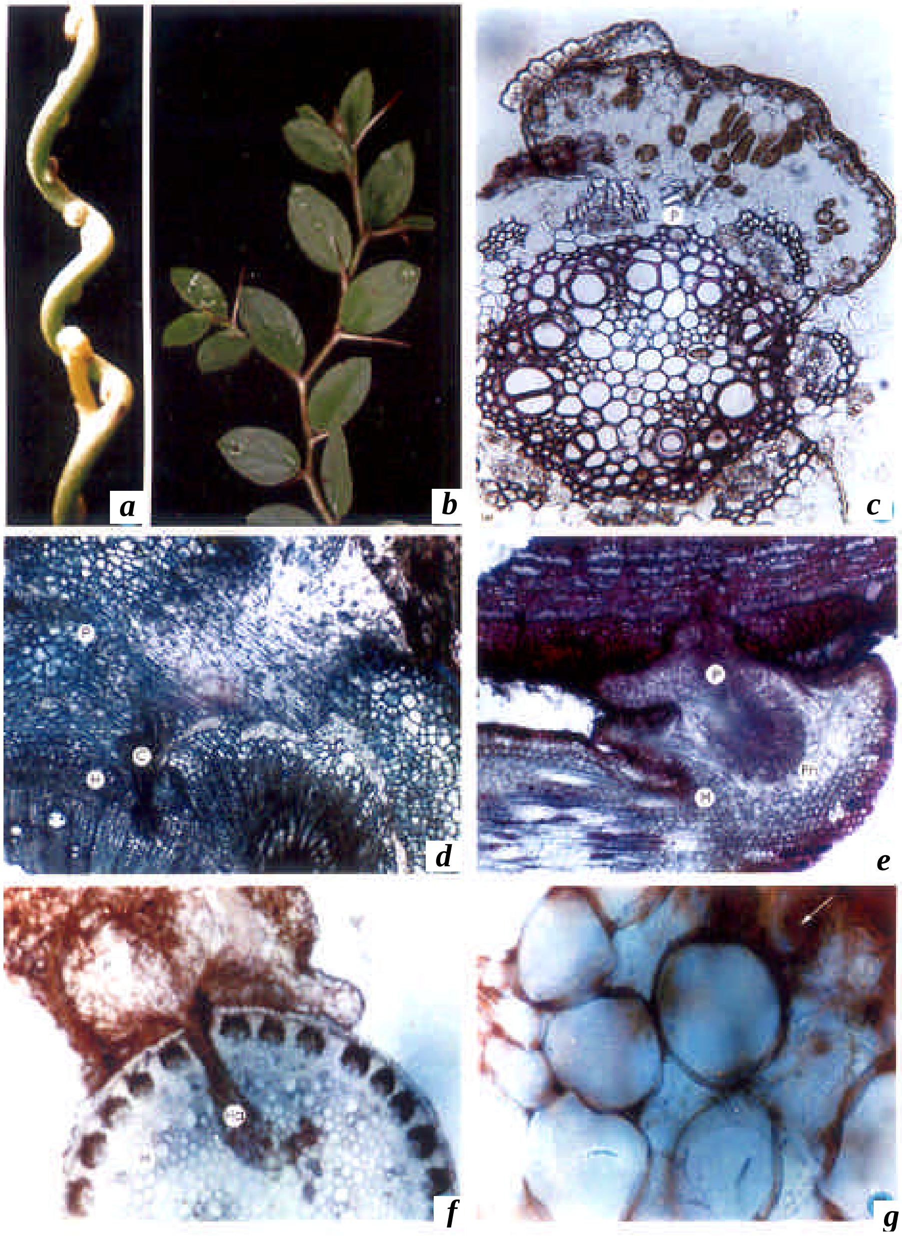

Figure 1. Parasite–host interaction of *Cassytha filiformis* Linn. and *Zizyphus jujuba* Lamk. ***a***, *C. filiformis* parasite (P), 20x; ***b***, *Z. jujuba* host (H); ***c***, Stem anatomy of *C. filiformis*, 200x; ***d***, *C. filiformis* (P) form a collar around the host (H), 100x; ***e***, Parasite penetrates the phloem (Ph) of host, 100x; ***f***, Phosphatase and peroxidase activity along haustoria (Ha), 100x; ***g***, Digestion of host cells, 400x.

Table 1. Chlorophyll estimation in *Zizyphus jujuba* (host)

Zizyphus jujuba (host)	(mg/g fresh weight)		
	Chlorophyll *a*	Chlorophyll *b*	Total Chlorophyll
Host without parasite interaction	8.92	12.03	20.95
Host with parasite interaction	7.41	9.28	16.69
Chlorophyll reduction (%)	16.93	22.99	20.33

sections of the parasite and host with parasite (suckers) haustoria complex were taken according to the standard procedure[18].

For histochemical studies, sections of host–parasite complex were studied in phloroglucinol-HCl[19] and toluidine blue 'O'[20] and photographed.

For histoenzymological studies, sections of the host–parasite complex were studied in acid phosphatase – metal salt method[21], alkaline phosphatase – tetrazolium method[22], succinate dehydrogenase reaction method[23] and peroxide reaction method[24].

For biochemical studies, quantitative estimation of chlorophyll *a*, *b* and total chlorophyll[25] and photosynthetic activity by Hill reaction studies[26] were made.

Stem anatomy of *C. filiformis* has shown a central cylinder of collateral vascular bundles (Figure 1 *b*). Inner cortex is wide and represented by enlarged palisade-like chlorenchymatous cells.

Phloroglucinol-HCl and toluidine blue 'O' histochemical studies revealed the presence of specialized glandular cells, facilitating adhesion of parasite to the host. The glandular *C. filiformis* cells continue to divide and spread like a collar around the host plant (Figure 1 *c*); they then penetrate the phloem (Figure 1 *d*). The results are comparable with earlier reports[4,27]. Dorr and Kollmann[4] reported the presence of similar cells in hemp parasitized by *Orobanche ramose*. Lee and Lee[27] reported the same in *Cuscuta australis*.

Haustoria of the parasite showed elongated sieve elements. Penetration of *Z. jujuba* (host) tissue by *C. filiformis* (parasite) is not accompanied by crushing of host cells. Histochemical studies revealed high acid phosphatase and peroxidase activities along haustoria of parasite (Figure 1 *e*). Cortical or vascular tissues of the host indicate digestion of macromolecules (Figure 1 *f*) or energy transfer and intercellular transport for the parasite as indicated by studies[5,9,16] in different species of *Cuscuta*.

Biochemical studies revealed reduction in chlorophyll *a*, *b* and total chlorophyll pigments of the host due to host–parasite interactions (Table 1), although the parasite (*Cassytha*) is itself partly autotrophic. Estimation of Hill reaction in parasite ensures partial phytosynthetic activity by acceptance of hydrogen released from phytolysis of water, as reported by Dinelli *et al.*[13] in the case of *Cuscuta campestris*. This was demonstrated by the initial reading of blue colour (1.301) changing to pale blue colour (1.097) when DCPIP (Dichloro Phenol Indo Phenol) market dye for oxidized chlorophyll extract indicating photosynthesis.

The present work indicates that the parasite does not attack the tissues of the host beyond those that are necessary to establish a dynamic relationship with the host vascular tissue. Apparently, *C. filiformis* acquires the needed metabolites from the host vascular system rather than from a combination of host vascular translocates and nutrients gained by the destruction of host tissue.

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ACKNOWLEDGEMENTS. We thank DST-FIST, New Delhi for providing infrastructure facilities for the Department of Botany, National College, Tiruchirapalli. We also thank Mr K. Santhanam, Patron Secretary, Mr K. Raghunathan, Secretary, Dr M. Nagarajan, Principal and Dr V. T. Sridharan, Head, Department of Botany, National College for encouragement.

Received 4 February 2005; revised accepted 15 September 2005

Long-term lowland rice and arable cropping effects on carbon and nitrogen status of some semi-arid tropical soils

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Samples of surface (0–30 cm) soils were collected from eight sites in the semi-arid tropical regions of India to evaluate and compare the long-term effects of lowland rice or paddy and non-rice or arable systems on soil organic C (SOC), soil inorganic C (SIC) and total N status. The results showed that soil samples from sites under lowland rice double cropping system had greater organic C and total N content than those from soils under rice in rotation with upland crop or under other arable systems. The SOC : N ratio was wider in soil samples from sites under lowland rice compared to those under other arable systems, which had lower C : N ratios. Samples from soils under lowland rice system tended to have a narrower SIC : N ratio than those under arable systems, indicating a better pedoenvironment under paddy rice. Our results support earlier findings that sites under continuous wetland rice cropping accumulate organic matter and contain higher soil organic matter compared to the sites under other arable systems.

Keywords: Arable systems, lowland rice, natural vegetation, nitrogen, pedoenvironment.

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MAINTENANCE of fertility in soils of the tropics and semi-arid tropics (SAT), a prerequisite for sustainable increase in agricultural productivity, is a major challenge to farmers and researchers alike. Soils, especially those in the SAT are low in organic matter and nutrient reserves. High temperature in the tropics, which results in rapid decomposition and loss of organic matter, is the primary cause of the low organic matter status of soils. Moreover, under dryland agriculture, application of nutrients and organic matter through external inputs is generally low due to socio-economic conditions of the farmers and other factors. Under low or no external input management practices, agricultural productivity depends on the inherent fertility of soils, which is generally low[1].

Jenny and Raychaudhari[2] studied the effect of climate and cultivation on the nitrogen (N) and organic matter reserves in Indian soils. The approach used was based on collection of soil samples across the country from cultivated fields and forested soils in relation to a climatic grid in which mean annual temperature and mean annual precipitation appeared as independent variables. Based on the analysis of 500 soil samples for organic carbon (C) and total N across India, these authors showed that the climatic effects on soil organic matter and N status are pronounced. Soil N and C increased with increasing mean annual precipitation and decreased with increasing mean annual temperature. Soils in the drier regions had low reserves of organic matter and N compared to those in the humid and sub-humid zones of the country[2].

Velayutham *et al*.[3] and Bhattacharyya *et al*.[4] carried out studies on carbon stocks in Indian soils. Analysis of thousands of soil samples in the course of these studies helped prioritize research on C sequestration potential in soils of the SAT regions in India. Recently, the concept of quasi-equilibrium values of soil organic C relative to organic C status of forest soils as reference or control has been found