Indian Journal of Agricultural Sciences **86** (7): 946–50, July 2016/Article
https://doi.org/10.56093/ijas.v86i7.59850

A comparative study of morphology and pathogenesis of *Cuscuta* and *Orobanche* – the two devastating parasitic plants

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Received: 7 April 2015; Accepted: 5 May 2016

ABSTRACT

Orobanche and *Cuscuta* are two completely different types of holoparasitic plants that behave as devastating weeds on many economically important crops and pose serious challenges in their management. They are phaneroamic and non-photosynthetic plants which require other host plants for their nutrient requirement. In the present study both *Orobanche* and *Cuscuta* were compared in terms of their morphology, anatomy and pathology. The study helps in understanding the differences in the target site of the parasites, viz. leaf and stem for *Cuscuta* and roots for *Orobanche* and the difference in their ecology. The seeds of the two parasitic weeds showed morphological differences, viz. in their seed size, coat structure and texture. The conditions for germination of *Orobanche* was very stringent and followed certain defined sequence in terms of water absorption, pre-conditioning, sensitivity to germination stimulants. However, the germination of *Cuscuta* was more spontaneous and moisture was the major requirement. Post-germination development of both the parasitic weeds was result of the chemotropic response of the receptors in the parasites to the stimuli from the host. *Cuscuta* and *Orobanche* represent different lineages of evolution in their parasitic nature and have different haustorial anatomy. Parasitization of the host was very rapid in *Cuscuta* (27-31 days) with multiple haustorial connections with the host stem, leaf, petiole, fruits and even on its own stem when compared to *Orobanche* with mostly single point of entry into the host and about 45-60 days to emerge above ground to produce a floral stalk.

Key words: *Cuscuta*, Germination, Histology, *Orobanche*, Parasitization

Among the different parasitic plants, *Orobanche* spp. and *Cuscuta* spp. are considered a major threat to the cultivation of different economically important crops. Economically important host plants of *Orobanche* include mustard, potato, tomato, egg plant (brinjal), tobacco, sunflower and faba bean. The weedy *Orobanche* spp. are annuals that reproduce by seeds which are dark brown, oval, minute (0.35 by 0.25 mm) and can remain viable in the soil for up to 20 years (Puzzilli 1983). The minute seeds germinate only if induced by certain compounds called germination stimulants, released by host and certain non host plants in the root exudates (Kadry and Tewfic 1956). A single flowering stalk of *Orobanche* can produce more than few million seeds (King 1952). Some species of *Orobanche* exhibits extreme selectivity, for example the *O. cernua* populations infesting sunflower does not infect tobacco and vice versa (Musselman 1994). There are several species of *Orobanche* reported from all over the world and the most common in India are *O. cernua, O. crenata, O. ramosa* and *O. aegyptiaca*, distributed in the states of Madhya Pradesh, Karnataka, Andhra Pradesh, Tamil Nadu, Gujarat, Maharashtra, Rajasthan and Haryana mainly infesting tomato, tobacco, potato, brinjal, and mustard. Losses of about 20-80% in the solanaceous vegetables (tomato, tobacco and brinjal) and about 30-35% in mustard by *Orobanche* were reported in India (Ramachandraprasad *et al*. 2008).

Cuscuta (the dodders, family Convolvulaceae), are obligate stem parasites, with long, multi-branched, yellow thread like modified stem called twines, attacking a wide range of mostly dicotyledenous host plants, including pulses and vegetables. *Cuscuta* maintains very intimate relationship with the host plants and a two-way transfer of genetic material between the host and the parasite was reported (Mower *et al*. 2004). Upon germination *Cuscuta* reaches the host, starts twining, develops haustoria and after 10 to 15 days starts flowering. Matured flowers form seeds after drying, fall on the ground and seeds germinate to form new plants. *Cuscuta* spp. is a major problem in the states of Andhra Pradesh, Chhattisgarh, Gujarat, Odisha, West Bengal and Madhya Pradesh on oilseed crops like niger, linseed, pulses, viz. blackgram, greengram, lentil, chickpea (prominently in rice-fallows) and fodder crops including lucerne and berseem (Moorthy *et al.* 2003).

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Among the 12 different species of *Cuscuta* in India, *C. campestris* and *C. reflexa* are the most common and under favorable conditions causing yield losses of about 54.7 to 98.7 % in chickpea by 1-10 plants/m^2 of *C. campestris* (Mishra 2009). *Cuscuta* can be successfully managed at their initial stages of infestation using herbicides, but management of *Orobanche* is very difficult because of its underground development. However, the management of both these parasitic weeds poses huge challenges to the agricultural workers all over the world and there appears to be no successful method to manage these weeds so far (Parker 2012). The current study attempts to compare and contrast their biology, pathogenic process, development and life cycle, to enable the readers to appreciate the basic difference between these two different parasites that have different target areas of infection and different ecosystem of occurrence and development apart from their morphology and pathogenesis. This would help in developing suitable management methods against them.

MATERIALS AND METHODS

Seeds of *Cuscuta* were collected from chickpea in a farmer's fields in Mandla district of Madhya Pradesh (latitude: $23^0$31'51.54"N, longitude: $80^0$27'55.49"E, altitude 456.60 m) and of *Orobanche* from tomato in a farmer's field in Gwalior (latitude: 26°17'N, longitude: 78°13'E, altitude: 617.0 m), Madhya Pradesh, India, during March 2012. The seeds were sun dried and stored in air tight plastic containers at room temperature (30±2^0C). Identity of species of *Cuscuta* and *Orobanche* was confirmed as *C. campestris* and *O. crenata* based on the standard characters given by Costea and Tardif (2005) and Foley (2004), respectively.

The experiments were conducted under controlled conditions in the containment facility (23±2^0C maximum and 12±2^0C minimum) at Directorate of Weed Research, during 2012 to 2014.

Inoculation of Orobanche on tomato: 1 g seeds of *O. crenata* were thoroughly mixed in pots with 10 kg soil and sand (1:1) mixture and watered periodically up to 20 days to facilitate preconditioning of seeds. Afterwards tomato seeds were sown in the pots to induce germination of the conditioned seeds. *Orobanche* emergence was observed 45-50 DAS of tomato.

Inoculation of Cuscuta on chickpea: Chickpea seeds were sown in pots with 10 kg soil and sand (1:1) mixture and upon germination of chickpea, 10 seeds of *C. campestris* were sown near each host plants.

Fresh samples of *Cuscuta* and *Orobanche* including twines, the host attachment portion and tubercle, were collected at different stages of the growth, washed gently under running water and using a soft brush to avoid damage in the connection. These were cut into small pieces of 1- 2cm length and immediately fixed in FAA solution (5 ml 40% formaldehyde, 5 ml glacial acetic acid, 90 ml 70% ethyl alcohol) for 24 hr. Thin hand sections were taken from these samples and stained using 0.5 % toludine blue. Observations were taken under stereo binocular and compound microscope with camera attachments.

RESULTS AND DISCUSSION

Seed morphology, physiology and germination

O. crenata seeds were ultra-minute, dust like and cannot be seen by naked eyes. When observed under stereo microscope, the seeds appeared dark brown, oval in shape, about 3-5µ in size and with conspicuous surface reticulations. *C. campestris* on the other hand have relatively bigger sized seeds of size about 0.16 cm, light yellow colored and oval in shape and can be seen through naked eyes. The seeds of *C. campestris* under microscope, showed less prominent surface reticulations compared to the seeds of *O. crenata,* where the reticulations were relatively deep and showed a clear pentagonal/hexagonal pattern. Seed structure of these parasites have been reviewed by several authors earlier (Agnew and Agnew 1994, Plaza *et al*. 2004).

The process of seed germination of these parasites were different *O. crenata* seeds essentially required a preconditioning period of at least 20 days in soil at temperature of 23±2^0C and alternate day of watering followed by introduction of the host plant. The pre-conditioning requirement of *Orobanche* seed germination was reported *in vitro* by Aditi and Kannan (2014). However, the temperature requirement during the preconditioning period varies between different species of *Orobanche* (Nickrent and Musselman 2004). It is well established that *Orobanche* seeds do not germinate without reception of volatile stimulants called strigolactones that are released from the host root (Mangus *et al*. 1992). This is an evolutionary adaptation to ensure the availability of host plants in the immediate vicinity of the germinated seed of *Orobanche*, which are very minute and with very limited resources for long time survival without the specific host (King 1952). Several synthetic analogues of these natural stimulants (Strigolactones) such as GR24, Nijmegen-1 (Zwanenburg and Mwakaboko 2011) have been developed.

Cuscuta seeds do not require any kind of pre-conditioning or stimulation by the host as in the case of *Orobanche*. Germination takes place as and when temperature and moisture conditions are favorable, even immediately after harvest. However, they do have a dormancy period and the seeds stored for a long period require scarification with concentrated sulphuric acid for their germination. This may be due to the fact that *Cuscuta* has a broad host range and after germination can parasitize any plant in their vicinity, unlike the often host specific *Orobanche*. It has been reported that even self parasitization is also very common phenomenon in *Cucuta* (Furuhashi *et al*. 2011).

Conditioning is a natural adaptation of these parasites to ensure that their seed germination coincides with the host plant cycle. Aditi and Kannan (2014) reported that the preconditioning of *Orobanche* seeds at 23±2^0C with 70%

RH for a period of 10 days was required for germination, production of germ tube and reaching the host root. However, under simulated conditions in soil, 10 days of conditioning was not sufficient and instead about 25-30 days of preconditioning was required. This may be due to the different edaphic factors like temperature, moisture, pH and microbial activities. Similar observations on the factors affecting the duration of conditioning period like temperature, origin, and age of the seeds were given by Westwood *et al*. (2012). The fresh seeds without preconditioning did not germinate even after a period of 40 days. It was observed that the seeds which belong to the category of Orthodox seeds, do not metabolize during the pre-conditioning period and often remain inactive, which helps the seed to conserve its limited nutrition and wait for the arrival of the host plants (Mayer and Bar-Nun 1994). In contrast the seeds are active and metabolize during the conditioning period. They absorb moisture and the seed coat softens to receive the germination stimulants, which results in swelling and softening of the seed coat to enable rupture of testa and produce the germ tube (Matusova *et al*. 2004). Color of the seeds coat changed and became dark brown to light brown or slightly orange type during preconditioning may be due to removal of hard seed coat. Germination of the seeds initiates with the seeds becoming swollen by absorbing water and the seed coat becoming light orange in color. Germ tube is a transparent radicle of the seed which grows up to 3 mm in search of host after that dies out of unavailability of nutrient reserve.

However *Cuscuta,* which has a very broad host range and temperature range does not have the requirement for this preconditioning period and the seeds germinate whenever there is moisture available in the vicinity (Kannan *et al*. 2014). However, in older seeds the seed coat becomes tough/hard and needs scarification with H_2SO_4 for 5-20 min for germination. Further it was observed that some portion of the seeds with improper development of testa, still in the mother plant, may germinate directly after maturity (Costea and Tardif 2005). *Cuscuta* seeds stored for long time may stay dormant for several years until their testa become rotten and becomes soft to absorb moisture. The radicle is yellow in color and the pointed transparent tip is filled with enzymes to degrade cell wall of the host after finding a suitable one.

These factors of seed germination have great impact in the management strategies of the parasitic weeds. "Suicidal germination" is the strategy to induce germination of parasitic weed seeds by application of germination stimulants/favorable conditions in the absence of the host and thus reduce the seed bank. In the case of *Orobanche* it requires application of natural or synthetic germination stimulants to mimic the presence of host, it may be a simple irrigation before one month of sowing the main crop to induce suicidal germination in *Cuscuta*.

The germination of *Orobanche* seeds leads to the development of a root like organ known as the procaulome. The procaulome attaches stretches for a short distance, not more than 3 mm and has to attach immediately within about 3 to 4 hr (Kadry and Tewfic 1956) to the root and develops haustoria to penetrate and establish in the host. This is because of the very little reserve nutrient in the minute seeds of *Orobanche*. There exists only one 'haustorial bridge' between the parasite and a procaulome, however a single host plant can have multiple tubercles.

Cuscuta, upon germination form the epicotyls which can grow up to 10 cm in search of host. After attachment to the host the connection with the ground breaks and it starts coiling and circumnutating the host stem. *Cuscuta* seedlings can survive generally independently up to about 8-10 days before attaching to the host. It is reported that in these days, *Cuscuta* can synthesize some basic food required for its survival. Thus in case of *Cuscuta,* the phenomena of suicidal germination can be achieved by a simple irrigation before the sowing of the host crop to induce germination and in absence of the host plants. The germinated seedlings will die within 8-10 days (Kannan *et al*. 2014). The radicle forages towards the host and develops the vegetative portion which consists of stem and scale leaves. The proliferation of stem cortex cells forms haustoria which are multiple throughout the stem.

Thus the process of germination and development of germ tube in *Orobanche* and foraging in the direction of the host is a result of the chemotropic response of the receptors in the parasites to the stimuli from the host.

Haustorium and its development in the host

The term haustorium (plural; haustoria) was coined by *de* Candolle 1813 to describe the connection between *Cuscuta* and its hosts. The primary function of haustorium is to absorb nutrient from the host and transfer to the parasite. The parasite develops a haustorial cushion to adhere on the surface of host and. This helps in creation of a mechanical pressure on the host cell wall, which is aided by the lytic enzymes present in the pre-haustorial cells at the tip of the cushion leads to the gradual penetration of the host cell wall.

Finding haustorial connection in *Cuscuta* was easy as the haustorium of *Cuscuta* was produced as an outgrowth of the stem cortex. It appeared as parenchymatous cells surrounding xylem vessels and stele region. The stem of parasite surrounded the host organ and fixed its haustoria at different aerial site of host plant at different level. After fixing, haustorium developed and elongated absorbent hair like structure and penetrated the host cell wall. The perfascicular sclerenchyma, the phloem, the xylem and, sometimes, the pith. Whereas, on *Orobanche* finding the attachment site is relatively difficult because of their underground connection and finding of haustorial connection with nutrient bridge is still very difficult because of numerous false root connections around the host roots. *Orobanche* was attached to secondary and tertiary roots of the host plant for easy penetration through their thin and soft cell wall. Nutrient bridge of the parasite was possessing deposition in the cross section as well as

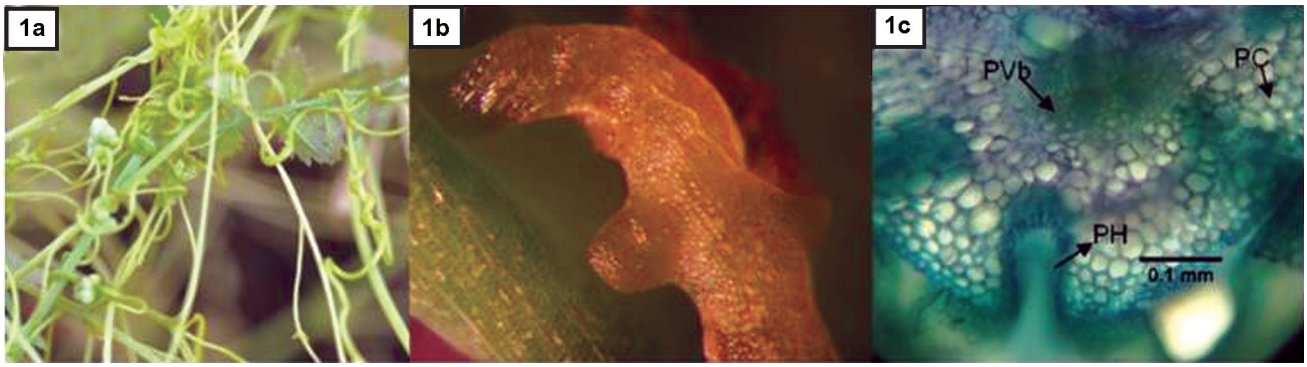

Fig 1 *a. Cuscuta* leaf, stem and self parasitization *b.* Lateral outgrowth from the twine which further develops inside the host to become haustoria and sometimes pseudohaustoria, *c.* Cross section of showing *Cuscuta* parasitizing on its own stem and haustorial penetration. PH- Parasite haustoria, PC- Parasite cortex, PVb- Parasite Vascular bundles

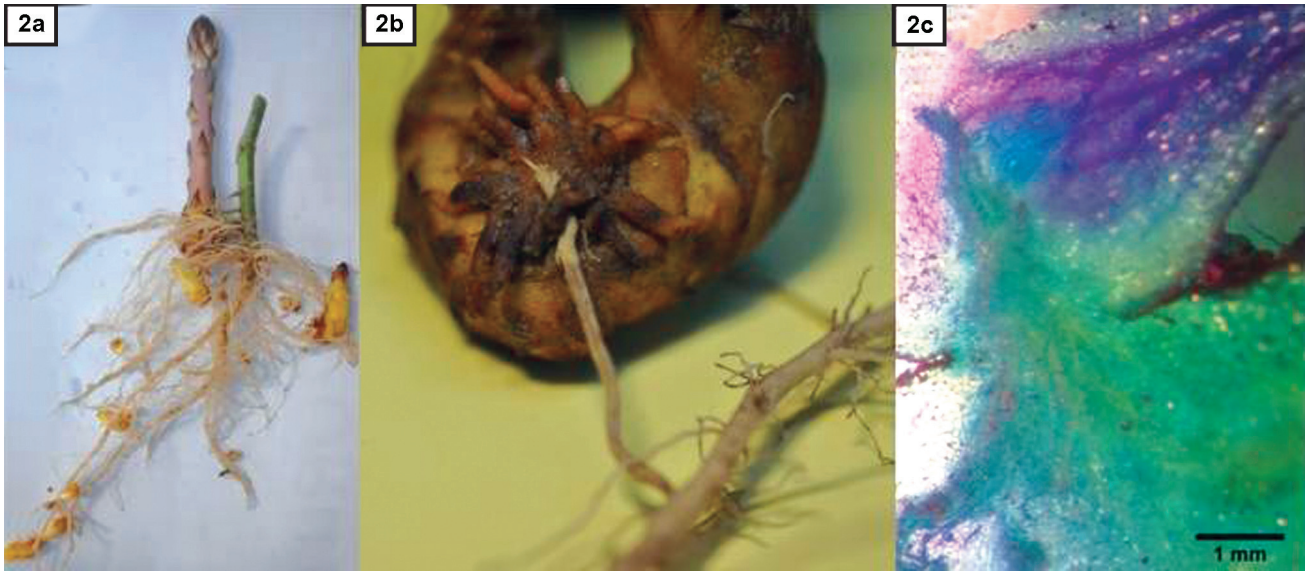

Fig 2 *a.* Multiple attachments of *Orobanche* in different stages of growth at different sites, *b.* Pseudo haustoria and false roots engulfing the host root at one point, *c.* Cross section showing *Orobanche* parasitizing in tomato sharp and pointed entry towards vascular bundles

longitudinal section which was dark colored and prominent.

Orobanche achieves approximately 0.2% of its C supply from its host's xylem, while the amount of N measured in the xylem sap of the host could supply only 5% of that accumulated by the parasite (Hibberd *et al*. 1999). *Cuscuta* and *Orobanche* represent different lineages of parasitic plant evolution, and have different haustorial anatomy. Although both species are able to absorb macromolecules from host phloem, the upper limits of molecule differ.

Penetration of the host

After reaching the host root, *Orobanche* germ tube adheres to the root surface, develops haustoria the search hyphae are pointed and sharp and pierce the host vascular bundle till it enters the pith with to form a dark double layered nutrient bridge. Entry through the epidermis is by mechanical pressure that pushes portions of the cell wall aside and by dissolution of the middle lamella (Joel and Losner-Goshen 1994). Dissolution of the cell walls could be traced in the vascular cylinder of the host roots. The vascular tissues which are formed in the host root become connected with a fleshy mass of tissues called the 'tubercle'. After the vascular tissues are formed completely, the tubercle produces numerous root like outlets known as crown roots. Haustoria from the crown roots attack other host roots and produce secondary tubercles. After the production of crown roots, a bud is produced on the tubercle which will subsequently form the main axis of the flowering stalk. Cellulase and polygalacturonase in the roots are involved in degrading proteins and lipoproteins of the cell walls and membranes of the host tissues to initiate haustorial connection (Singh and Singh 1993).

Cuscuta upon entering the host develops secondary haustoria which spread rapidly to produce search hyphae. These hyphae are fingerlike and grow horizontally alongside the pith of the host to enter the vascular bundle and form the nutrient bridge. The epicotyl develops until it reaches to the host and after stabilizing the grip, develops haustorium to enter inside the host stem.

Parasitization of the host was very rapid in *Cuscuta* when compared to *Orobanche.* After attachment to the host, *Cuscuta* covered the entire potted chickpea plant in a period of 27-31 days (Kannan *et al*. 2014) by developing multiple haustorial connections with the host stem, leaf, petiole, fruits and even on its own stem (Fig 1). In case of *Orobanche* the connection with the host root is through a single haustorium, initially at one point of the secondary or tertiary root of the host plant. *Orobanche* also produces multiple pseudohaustoria which does not penetrate the host root, but act like false root for supporting the flowering stalk *of Orobanche* (Fig 2).

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