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REVIEW

Photosynthesis in *Cuscuta reflexa*: A total plant parasite

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Abstract

Photosynthetic properties of *Cuscuta* species, such as chloroplast ultrastructure, contents of chlorophylls, carotenoids, and plastid proteins, photosystem and CO_2 fixation activities, and photosynthetic genes composition are reviewed.

Additional key words: carotenoids; chlorophyll; chloroplast ultrastructure; CO_2 fixation; genes; photosystems 1 and 2; plastid proteins.

Introduction

Cuscuta reflexa is a parasitic flowering plant. It is a total stem parasite, completely devoid of functional leaves and roots. The golden yellow stem exhibits luxuriant growth on the host. The stem remains attached to the host through haustoria and absorbs nutrients from the host with the help of these haustoria. The leaves are minute, scale like, and are of the same colour as the stem. The plants develop rapidly and the stem can grow as much as 8 cm within a day (Dawson *et al.* 1984, Panda and Choudhury 1992a).

Cuscuta is traditionally regarded as an obligate parasite as it has little capacity to assimilate carbon and nitrogen. However, after the report of Zimmermann (1962) that *Cuscuta* is chlorophyllous and could be induced to grow autotrophically in laboratory (Lab), many researchers studied its photosynthetic characteristics. Analyses of different photosynthetic parameters such as chloroplast ultrastructure, photosynthetic pigment contents, photochemistry (electron transport activities of photosystems PS1 and PS2), and ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) activity suggest the possibility of photosynthesis in the parasite.

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Abbreviations: Chl - chlorophyll; DCPIP - 2,6-dichlorophenol indophenol; Lab - laboratory; LI - low irradiance; MV - methylviologen; PS - photosystem; RuBPCO - ribulose-1,5-bisphosphate carboxylase/oxygenase.

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Chloroplast ultrastructure

Machado and Zetsche (1990), with the help of electron photomicrographs, have shown the presence of chloroplasts in the cells of *Cuscuta* twigs. Similarly as normal chloroplasts, they possess starch grains and ribosomes in the stroma, stroma thylakoids, and a few grana with small number of thylakoid stacks. Dodge and Lawes (1974) have also shown presence of stroma thylakoids in *C. europaea*. Laudi *et al.* (1974) report formation of elaborate thylakoid system in the plastids of *C. australis* under low irradiance (LI).

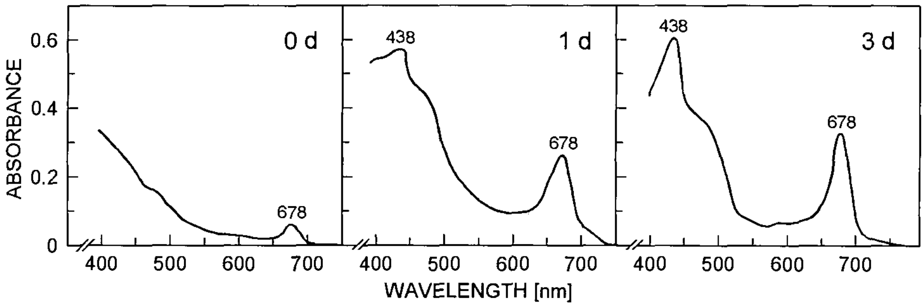

Fig. 1. Absorption spectra of chloroplasts isolated from *Cuscuta* twigs growing on host in the field (0 d) and from twigs incubated in the laboratory for 1 to 3 d in distilled water under an irradiance of 6 W m^{-2}.

Chloroplasts isolated from *Cuscuta* twigs growing on the host in the field do not show structural characteristics similar to those of mature chloroplasts of higher plants (Laudi *et al.* 1974, Panda 1990). Absorption spectra of chloroplasts prepared from such samples are distorted (Fig. 1). The red band is very much reduced and the blue (Soret) band is not at all resolved. On the other hand, when the twigs are grown in the Lab in distilled water under LI, the chloroplasts show an improved absorption spectrum, with a distinct red (678 nm) and blue (438 nm) band. The heights of the peaks are also higher than in the field-grown sample (Panda 1990, Sahu 1997). There is further improvement in the spectrum when the twigs are kept in the Lab for 3 d (Fig. 1). Then the spectrum closely resembles the absorption spectra of chloroplasts of higher plants (Panigrahi and Biswal 1979, Choudhury and Biswal 1984, Panda 1990).

Pigment composition

Chlorophyll (Chl): Though functional leaves are absent, Zimmermann (1962), Pizzolongo (1964), and Laudi *et al.* (1974) noted the presence of appreciable amount of Chl in young stem tissue and suggested that, contrary to general belief, *Cuscuta* may not be a total parasite. Subsequently, several other workers have quantified the Chl *a*, Chl *b*, carotenes, and xanthophylls in *C. reflexa* (Janardhanarao *et al.* 1984, Machado and Zetsche 1990, Panda and Choudhury 1992a,b, Sahu and Choudhury

1997). The ratio of Chl *a/b* is usually 2. During the elongation of a young rapidly growing branch (on host), this ratio increases (Panda and Choudhury 1992a, Sahu and Choudhury 1997).

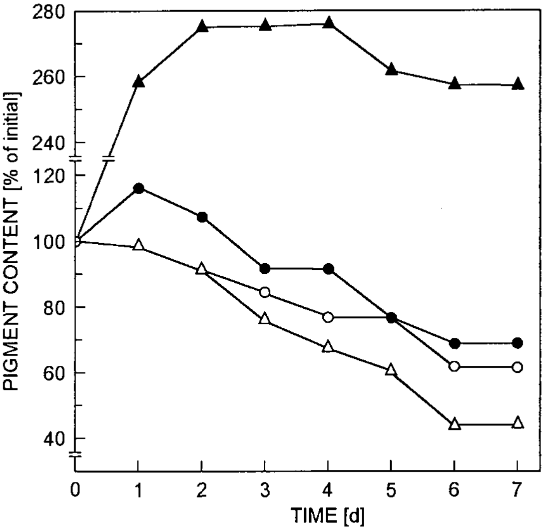

Fig. 2. Changes in chlorophyll (Δ, ▲) and carotenoid (O, ●) contents of *Cuscuta* twigs growing on host in the field (Δ, O) and of twigs incubated in the laboratory in distilled water under an irradiance of 6 W m^{-2} (▲, ●). The values (100 %) at 0 d are to 0.12 g kg^{-1}(f.m.) for both pigment types.

When the apical portions of *Cuscuta* branches are incubated in Lab in distilled water under only 6 W m^{-2}, the Chl content increases manifold (Fig. 2) and this difference is retained during the 7 d of Lab incubation in distilled water. The total Chl content in the laboratory grown twigs under LI was 0.65 g kg^{-1}(f.m.) (Panda and Choudhury 1992a,b) which is close to the pigment content of monocot leaves. The Chl *a/b* ratio in the field-grown plants is higher than in the twigs grown under LI (Sahu 1997). Thus *Cuscuta* growing in the field on host and exposed to natural irradiance suffers photodestruction of pigments, especially of Chl *b*, and possibly of other pigments too (Lichtenthaler *et al.* 1989, Demmig-Adams 1990, Choudhury *et al.* 1994, Webb and Melis 1995, Preiss and Thornber 1995).

Carotenoids: Macleod (1961, 1962) and Baccarini (1966) have shown that the carotenoid content in *C. reflexa* is comparable to the amount in the leaves of autotrophic plants. Baccarini (1966) reported the presence of similar amounts of α- and β-carotenes and xanthophylls in *C. reflexa* and nonparasitic plants. High pressure liquid chromatography (HPLC) detected β-carotene, zeaxanthin, neoxanthin, violaxanthin, lutein, *etc.* in the field-grown *Cuscuta* branches (Fig. 3). The contents of β-carotene, lutein, violaxanthin, and neoxanthin (but not zeaxanthin) significantly increased after incubation of the twigs in Lab under LI (Sahu 1997).

However, the zeaxanthin content, which was high in the field-grown twigs, was reduced significantly (Fig. 3).

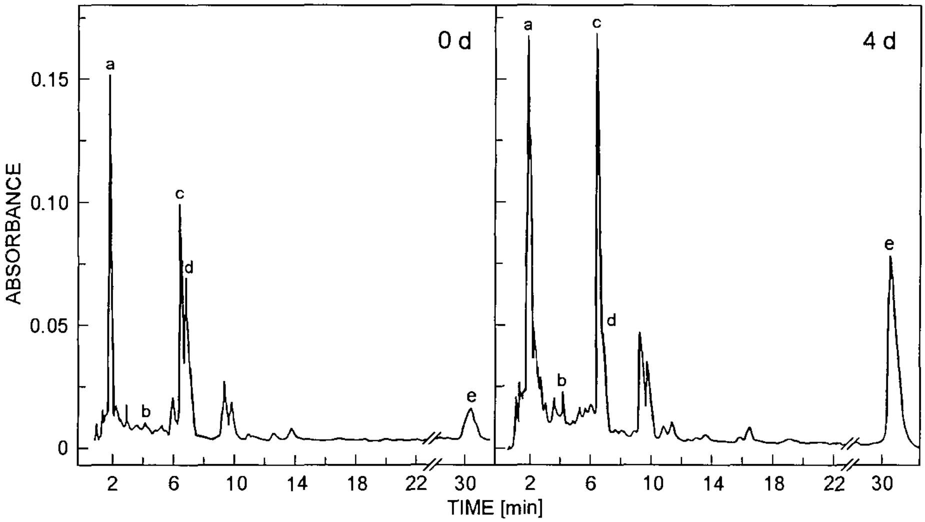

Fig. 3. High pressure liquid chromatograms of carotenoid extracts of *Cuscuta* twig growing on host in the field (0 d) and of twigs incubated in the laboratory in distilled water under an irradiance of 6 W m^{-2} (4 d). The peaks are: *a* - neoxanthin, *b* - violaxanthin, *c* - lutein, *d* - zeaxanthin, *e* - β-carotene. Other peaks were not identified (Sahu 1997).

High irradiance induces the synthesis of zeaxanthin by deepoxidation of violaxanthin through the xanthophyll cycle (Bilger and Björkman 1990, Demmig-Adams 1990, Demmig-Adams and Adams 1992, Choudhury *et al.* 1994). Formation of zeaxanthin from violaxanthin is a photoprotective strategy in plants if the leaves are exposed to an irradiance higher than they can process for photosynthesis (Choudhury and Choe 1996, Anderson *et al.* 1997, Gilmore 1997). As observed in the chromatograph profile (Fig. 3), the zeaxanthin content was significantly high in the branch growing on the host in the field. When the twigs are incubated in laboratory under 6 W m^{-2}, the content of zeaxanthin becomes almost zero. This suggests that the twigs growing in the field on host are under irradiation stress. This may lead to photooxidation resulting in low concentrations of photosynthetic pigments.

Plastid proteins

The total protein content of chloroplasts isolated from the twigs is the highest in the apical region and declines towards the mature haustorial regions (Sahu 1997). The content of membrane proteins (insoluble proteins) is 2.5 times that of the stroma proteins (soluble proteins). This value is comparable to that for wheat chloroplasts (Choudhury *et al.* 1993).

The total protein content of chloroplasts in the Lab-grown *Cuscuta* is 20 % higher than in the field-grown sample (Sahu 1997). The contents of both soluble and membrane proteins increase when the plants are incubated in the Lab under LI, nevertheless, the membrane/soluble protein ratio declines during the initial phase of Lab incubation (Sahu 1997). Thus in the field, the amount of RuBPCO (which is the major soluble protein of the chloroplasts) is low which may be the cause for the low CO_2 fixation ability of the plant (Machado and Zetsche 1990) consequently leading to the parasitic mode of nutrition.

Photosynthetic activities

Cuscuta may grow *in vitro* in the Lab (see review by Malik and Singh 1980) for several months until flowering. In the branches grown under starved and LI condition, Macleod (1962) found a fairly high amount of Chl and the photosynthetic efficiency (on Chl basis) approaching that of higher plants. Zimmermann (1962) incubated *Cuscuta* twigs on sterile agar media under different irradiances and observed LI induced photosynthetic activities in the obligate parasite.

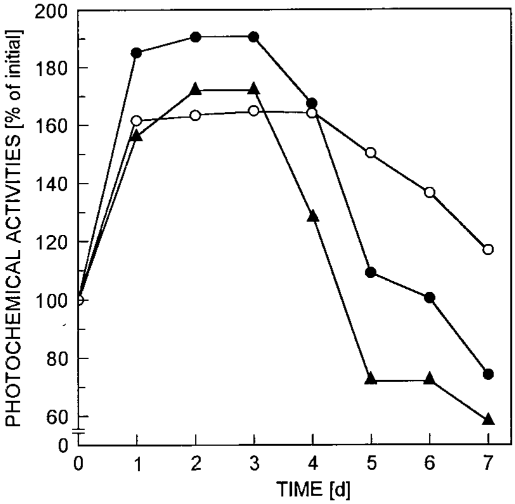

Fig. 4. Changes in the rates of oxygen evolution (PS2, ▲) and methylviologen reduction (PS1, ○; PS1+PS2, ●) activities of isolated chloroplasts of *Cuscuta* twigs growing on host in the field (0 d) and twigs incubated in the laboratory for 1 to 7 d in distilled water under an irradiance of 6 W m^{-2}. The values (100 %) at 0 d are equal to 2.58 mmol(O_2 evolved) kg^{-1}(Chl) s^{-1} for PS2; 28.62 mmol(methylviologen reduced) kg^{-1}(Chl) s^{-1} (for PS1), and 3.05 mmol(methylviologen reduced) kg^{-1}(Chl) s^{-1} (for PS1+PS2) (Sahu and Choudhury 1997).

The Hill reaction and methylviologen (MV) reduction activities of isolated chloroplasts reflect the photochemical potential of thylakoid membrane (Fig. 4). Significant DCPIP photoreduction activity by the chloroplasts isolated from the branches growing on host in the field has been reported by Panda and Choudhury

(1992a,b). However, Lab incubation of the detached branches, which brought about more than a five-fold increase in total Chl content, was not associated with a proportionate increase in the electron transport activity of isolated chloroplasts. In isolated chloroplasts of *C. reflexa* a remarkable increase in the MV reduction (PS1 and PS2 activities) and oxygen evolution was found after Lab-incubation under LI of stems grown in the field (Sahu 1997, Sahu and Choudhury 1997). However, the increase in photochemical activities was restricted upto 3 d of Lab incubation.

CO_2 fixation activity

Experiments with $^{14}CO_2$ show that different species of *Cuscuta* are able to carry out photosynthesis, though in a reduced rate (for reviews see Malik and Singh 1980, Stewart and Press 1990). Machado and Zetsche (1990) observed in purified RuBPCO from the stem homogenate of *C. reflexa* that although the amount of the enzyme was low in the tissue, it could drive the Calvin cycle at a slow but measurable rate. These observations and the photochemical analyses of chloroplasts indicate that under suitable conditions *Cuscuta* develops the ability for CO_2 fixation.

According to Singh *et al.* (1970), proximal and haustorial regions of *Cuscuta* filament are more active in saccharide metabolism than the apical region. The movement of saccharides from autotrophs to parasites has been reviewed by Smith *et al.* (1969). The nutrient transport from the host to the parasite occurs through a passive filtration process (Malik and Singh 1980). With the help of ^{14}C labelling, Wolswinkel (1974) has shown that *Cuscuta* can draw the entire assimilates from the host leaves in which they normally move to the growing organs or developing fruits.

Photosynthetic genes

Machado and Zetsche (1990), Haberhausen *et al.* (1992), Bömmer *et al.* (1993), Subramaniam *et al.* (1994), and Haberhausen and Zetsche (1994) have cloned and sequenced the plastid DNA of *C. reflexa*. These plastids contain almost all the photosynthesis related genes such as genes for PS1 (*psaA*), PS2 (*psbA, psbB, psbC, psbD*), cytochrome *b/f* complex (*petB, petG*), ATP synthase (*atpA, atpB, atpE*), *etc.* However, there is a functional loss of *ndhB*, the gene for NADPH-dehydrogenase (Bömmer *et al.* 1993). The arrangement of genes of the sequenced area is the same as in plastid DNA of higher plants. Sequence homologies between the photosynthesis related genes of *Cuscuta* and corresponding genes from higher plants are in the range of 90 % (Haberhausen *et al.* 1992). The only significant difference is that the transcript level of the genes for the large subunit of RuBPCO (*rbcL*) in *Cuscuta* is strongly reduced compared to photoautotrophic plants. The reduced *rbcL* transcript level may ultimately be responsible for the lower soluble protein content (RuBPCO) in the chloroplasts. It may also result in a lower RuBPCO activity as measured by Machado and Zetsche (1990). However, *C. reflexa*, though a holoparasite, has conserved all the photosynthesis related genes necessary for the organization of the electron transport chain in the thylakoid membrane and operation of the Calvin cycle (Machado and Zetsche 1990, Haberhausen *et al.* 1992). In spite of the presence of all photosynthesis genes, *Cuscuta* has adapted to a parasitic mode of life which could be due to the poor transcription of these genes (Haberhausen *et al.* 1992, Haberhausen and Zetsche 1994) or for some other unknown physiological reasons (Sahu 1997).

Conclusions

In *Cuscuta* growing on the host in the field appreciable amounts of Chl and carotenoids as well as a few thylakoids in plastids have been found. Though the amount of RuBPCO, the key enzyme for carbon dioxide fixation, is low, it can drive the Calvin cycle at a slow rate. On Lab incubation of a detached branch in distilled water under LI, the pigment content in stem remarkably increases. High pressure liquid chromatograph analyses of carotenoids of the field growing sample show the presence of β-carotene and various xanthophylls such as lutein, violaxanthin, neoxanthin, zeaxanthin, *etc.* The Lab incubation of the branches under LI causes a dramatic reduction in the zeaxanthin content whereas the concentrations of other carotenoids increase. The total plastid protein contents including thylakoid and stroma proteins in the field-grown branches are low. The Lab incubation of the branches results in the increase of different proteins. However, the ratio of membrane proteins to soluble proteins decreases. Chloroplasts isolated from the field-grown twigs do not show a well organized thylakoid structure as observed by electron photomicrographs or from the appearance of absorption spectra. However, transfer of the twigs to LI in the Lab brings about a qualitative change in the absorption spectra suggesting improvement in the thylakoid organization. Chloroplasts isolated from the field-grown samples show a low photochemical potential in terms of oxygen evolution and MV reduction. However, when the branches are incubated in the Lab, the rate of the photochemical activities increases substantially.

In recent years, several workers have tried to map the plastid genome of *C. reflexa*. According to their observations all the plastid genes necessary for the formation of electron transport chain and operation of the Calvin cycle are present in the parasite in the functional form. However, in spite of the presence of all photosynthesis related plastid genes, *Cuscuta* has adapted to a parasitic mode of life which may be due to poor expression of the genes or due to some unknown physiological reasons.

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