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Photosynthetic Potential and Accumulation of Assimilates in the Developing Chloroembryos of *Cyamopsis tetragonoloba* (L.) Taub[1]

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ABSTRACT

The photosynthetic potential of leaves and chloroembryos of *Cyamopsis tetragonoloba* (L.) Taub as measured by ^{14}C-bicarbonate fixation, Hill activity, and *in vivo* fluorescence transients is compared. On a chlorophyll basis, dark fixation of $NaH^{14}CO_3$ in chloroembryos was 1.5 times higher than that of the leaf, whereas carbon fixation under illumination was threefold higher in the leaf than in the embryos. Rates of O_2 evolution were four times more in embryo than in leaf chloroplasts. Shading of developing fruits on the day of anthesis for 10 days induced a 65% reduction in dry matter accumulation in the etiolated embryos, as compared to the normal green embryos of the same fruit half covered by a transparent Polythene sheet. The reduction in dry weight, size of the embryos, and levels of assimilates after shading the developing fruits may be ascribed to partial autotrophy of the chloroembryos.

Chlorophyll-bearing embryos (chloroembryos) occur in some Angiosperms (27). They may be physiologically active during development, although they reside deep inside the fruit wall and seed coat, and sometimes are surrounded by the endosperm (12, 20). Light is necessary for continued synthesis of Chl pigments by the embryos (11). The photosynthetic potential as well the *in vivo* function of these embryos is not known. This paper examines the possible roles of chloroembryos during *in vivo* seed development of *Cyamopsis tetragonoloba* and measures their photosynthetic capabilities.

MATERIALS AND METHODS

Pods of *Cyamopsis tetragonoloba* (L.) Taub cv Nowbagar were obtained from plants that were raised in the university botanic garden in red loam soil with 50 to 60% relative humidity and 27 to 32°C temperature and were irrigated once in 2 d. Other details of growth conditions were described previously (7). The flowers were tagged on the day of anthesis. The growing embryos were grouped into four stages based on the DAA,[3] *viz.*, stage I: embryos of 8 to 10 DAA; stage II: 15 to 18 DAA; stage III: 26 to 28 DAA; and stage IV: 36 DAA. These embryos stages constituted an average of 11 30, and 41% of the fresh weight of the seeds, respect Embryos were isolated from the developing fruits man using scalpel and forceps..

Shading Treatments

Developing fruits at different growth stages were sh with dark Polythene sheet made as a small compact tu sleeve half of the intact fruit and the other half was co with a colorless transparent Polythene sheet. After 10 these fruits were sampled for isolation of etiolated em from the shaded and green embryos from the unsh regions of the same fruit.

Hill Activity

Active chloroplasts were prepared according to Mills Joy (16) in sorbitol medium so as to contain 20 to 30 μg per ml. The rate of O_2 evolution in isolated chloroplasts monitored polarographically at 20°C using a Hansatec electrode. Saturating actinic light at an irradiance of 80 m^{-2} was provided by a 200 W tungsten lamp from a proj The reaction mixture contained 330 mM sorbitol, 50 Tricine KOH (pH 7.9), 2 mM EDTA, 5 mM NH_4Cl, a mM $MgCl_2$, chloroplasts equivalent to 20 to 30 μg Chl pe and 50 μM dichlorophenol indophenol. Chl content wa termined by the method of Arnon (1) using 80% aqu acetone.

Dry Weight Determination

Green and etiolated fruit wall and embryos of respe growth stages were isolated from green and etiolated fr The preweighed fresh samples were dried in an oven at 1°C until a constant dry weight was obtained. The value expressed as mg dry matter per gram fresh weight.

Determination of Assimilates

Green and etiolated embryos were ground thoroughly prechilled pestle and mortar with a known volume of methanol, and the extract was centrifuged at 5000 rpm f min at 5°C. The supernatant was saved, the pellet was e tracted, and the supernatants were pooled. The pigment-supernatant (removed by petroleum ether) was used fo

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[3] Abbreviations: DAA, days after anthesis; PEPCase, phosphoenolpyruvate carboxylase.

mining total soluble sugars by the method of Dubois *et al.* using glucose as standard and free amino acids by the thod of Troll and Canan (26) with glycine as standard. e resulting pellet after methanol extraction was used for termining total soluble starch after digestion with 52% ClO_4 by the method of McCready *et al.* (15) using glucose standard. Total soluble proteins were determined by the thod of Lowry *et al.* (14) and that of lipids by the method Barnes and Blackstock (3) using cholesterol as standard. the values were multiplied with a lipid factor of 0.8.

Vivo Fluorescence Transients

In vivo Chl fluorescence induction was followed in intact en embryos of stage III and leaves from the 8th node of *opsis*, after excitation with a broad band blue light (400–nm, Corning 5113) at an irradiance of 700 $\mu E/m^2/S$. Leaf embryo samples were mounted individually between two glass frames and placed diagonally in a 4 mL standard cuvette so as to receive the actinic light at 45°C. The tation light provided by a 150 W halogen bulb was focused to the cuvette by a pair of lenses. The photomultiplier matzu, R 375) placed at 90° to the excitation beam was ected by in interference filter (max 690 nm, half bandwith nm, Schott). The signal from the photomultiplier was tly displayed on a storage oscilloscope.

$^{14}CO_3$ Fixation

af discs of 1 cm diameter were excised and washed in led water. The materials were then incubated in 1 mL of ed $NaHCO_3$ solution (LCC 162, ^{14}C-sodium bicarbonate, Bombay) of 5.0 μCi/mL activity and exposed to an iance of 160 W/m^2 through a 6 cm water filter for 15 and the corresponding controls were kept in dark. After n of incubation, 0.05 ml of 1 N HCl was added to e excess $[^{14}C]NaHCO_3$ from solution and washed ly with cold $NaHCO_3$ (5 mM) followed by distilled water. arly, the excised green embryos were also subjected to ame kind of experiment in 10 mL capacity beakers. To the level, the beakers were filled with mercury up to just the beak. The beakers could then be covered with the perm, seed coat, and fruit wall to simulate a condition arable to the position of embryos inside the fruit (24), he rest of the procedure was similar to that described for ses. After termination of carbon fixation, the tissues ashed thoroughly and homogenized in ethanol (80%) entrifuged at 3000*g* for 5 min. The supernatant was ed and made to a final volume of 10 ml with ethanol. mL of this supernatant, 9.8 ml Brays scintillation l were added and ^{14}C incorporation by respective sam- counted in a liquid scintillation counter (ECL, with 84% efficiency. The rate of ^{14}C absorption is ed as μmol/mg Chl·h.

RESULTS AND DISCUSSION

Shading and Dry Matter Accumulation

ding the development fruits of *Cyamopsis* with dark ene sheet for 10 d resulted in etiolation of not only the fruit wall but also the embryos, and the embryos were poorly developed with thin cotyledons, as compared to the normal green embryos in fruits covered by clear Polythene sheet. As shown in Table I, shading the young fruits of *Cyamopsis* just a day after anthesis for 10 d caused about a 65% reduction in dry matter of the etiolated embryos compared to control. However, the etiolated part of fruit wall showed only 12% weight reduction. Similarly, in stage II fruits, reduction as a result of shading was 40% and 9% in the embryos and fruit wall, respectively, and reduction in the dry matter of the embryos of stages III and IV fruits was 16% and 7%, respectively, without causing significant reduction in the dry matter of the fruit wall. In comparison, Hole and Scott (9) observed that shading of pea fruits reduced the yield per fruit by 24% over the unshaded control.

In *Cyamopsis* embryos, the high percentage of reduction in dry matter upon shading may be due to a breakdown of chloroplast pigments in the embryos which otherwise might have contributed through their own photosynthesis to dry matter production. The yield component most affected by shading was average weight of the embryo per fruit, and neither the number of seeds nor fruit size was affected, unlike the previous report of Hole and Scott (9) that shading reduced the number of seeds per fruit without affecting seed weight. Prevention of radiant energy reaching the embryo in the black Polythene shaded part of the fruit probably resulted in limited synthesis of assimilates in the embryo, whereas the other half covered by clear Polythene sheet synthesized their own assimilates. Although Khanna and Sinha (12) and Sinha and Sane (24) reported the relative importance of fruit wall toward the supply of assimilates to the developing seeds in pea and beans,

Table I. *Effect of Shading on Dry Matter Accumulation in Embryos and Fruit Wall of Cyamopsis at Different Stages of Growth*

Etiolation of fruit wall and embryos occurred in the region of fruit shaded with dark polythene sheet, whereas fruit wall and embryos of the region covered with clear polythene sheet remained green. Values are mean of three different experiments. The data in parentheses indicate the range of values.

Stage of Growth		Control	Shaded	Inhibition
		mg/g fresh wt		%
I	Embryo	82 (78–86)	29 (27–31)	64.6
	Fruit wall	152 (148–155)	133 (130–136)	12.2
II	Embryo	125 (118–132)	75 (71–78)	40.4
	Fruit wall	209 (197–220)	219 (171–266)	4.8
III	Embryo	254 (251–256)	216 (203–229)	14.8
	Fruit wall	214 (205–222)	220 (208–232)	(+)3.0
IV	Embryo	349 (338–360)	323 (321–334)	7.4
	Fruit wall	219 (214–224)	220 (212–228)	(+)0.5

in *Cyamopsis* the reduction in dry matter of fruit walls upon shading was very meager (Table I), which suggests little importance of the fruit wall in supplying assimilates to developing chloroembryos.

Fruit Shading and Levels of Assimilates

Since stage II embryos were found to contain the maximum amount of pigments (data not shown), stage II fruits were half shaded for a period of 10 d, and when the fruits reached stage III (under shading), the effect of loss of Chl from the stage II to stage III on the accumulation of assimilates in the embryo was studied (Table II). A moderate to significant reduction was observed in all the biochemical constituents investigated, in the etiolated embryos compared to control except for free amino acids. The increased level of free amino acids in the etiolated embryo may be ascribed to its restricted capacity to incorporate all the free amino acids into proteins. Significant reduction in the levels of basic biochemical constituents such as total soluble sugars, total soluble proteins, total soluble starch and total lipids, as well as dry matter production in the peak pod filling stage (stage III) upon shading is indicative of the fact that the majority of these constituents are possibly synthesized in the embryos autotrophically and stored in the embryo, and therefore, any interference in the embryo photosynthesis may result in reduction of dry matter accumulation in the seed as well.

Photosynthesis of Chloroembryos

The photosynthetic potential of the chloroembryos was investigated. Hill activity was highest (133 μmol O_2/mg Chl·h) in the isolated chloroplasts of the stage II embryos, whereas in subsequent stages the rate of O_2 evolution declined (Table III). The rate of O_2 evolution of leaf chloroplasts was only 26 μmol O_2/mg Chl·h, which is about five times lower compared to the stage II embryo chloroplasts. The observed high Hill activity of embryonal chloroplasts as compared to the leaf is in complete agreement with the earlier report (2) that cotyledonary embryonal chloroplasts of *Lathyrus latifolius*, *Pisum sativum*, and *Vicia faba* showed 340%, 144%, and 280% higher activity, respectively, than chloroplasts of the corresponding leaves.

The transients of Chl *a* fluorescence in the leaf and embryos of *Cyamopsis* differed markedly in O-I and I-D phase (Fig. 1). The I-D phase was almost eliminated in the leaf, indicating that electron transport between the two photosystems was either blocked or partially reduced (21, 22). The bimonophasic (I-D elimination) changes in fluorescence induction were shown to occur when the electron transport between the two photosystems is either blocked or partially reduced (22). The prominent I-D transient in leaf is closely related to the rapid oxidation of the quencher, Q, the secondary acceptor pool A which is reoxidized by photosystem I (21, 22). The half rise time ($t_{\frac{1}{2}}$) to reach the steady state was 220 ms in leaf and 100 ms in embryo indicating the presence of larger plastoquinone pools in leaf chloroplasts than in the embryo chloroplasts. These observations are analogous to those reported for sun and shade leaves (4, 5), upper and lower sides (13), and palisade and spongy chloroplasts of the same leaf (25) and are consistent with the view that leaves

Table II. *Effect of Shading Developing Fruits of Cyamopsis* on Levels of Assimilates in Stage III Embryos

Stage II embryos were grown to stage III under shading and the biochemical constituents of the embryos were determined. Values are mean of three different experiments. The data in parentheses indicate the range of values.

Nature of Embryo	Total Soluble Sugars	Total Solublar Starch	Total Free Amino Acids	Total Soluble Proteins	Total Lipids
			mg/g fresh wt		
Green	43 (41–44)	45 (42–48)	6.00 (5–7)	112 (107–117)	12.5 (12–13)
Etiolated	31 (30–32)	35 (31–39)	9.00 (8–10)	76 (71–80)	7.1 (7–7.26)

Table III. *Hill Activity in Chloroembryos of Cyamopsis at Various Stages of Growth*

Chloroplasts were isolated and O_2 Evolution was measured polarographically using dichlorophenol indophenol. Values are mean of three different experiments and the range of values is given in parentheses.

Stage of Embryo Growth	O_2 Evolution
	μmol O_2/mg Chl·h
I	106 (99–112)
II	133 (118–147)
III	124 (112–136)
IV	86 (80–93)
Leaf	16 (22–29)

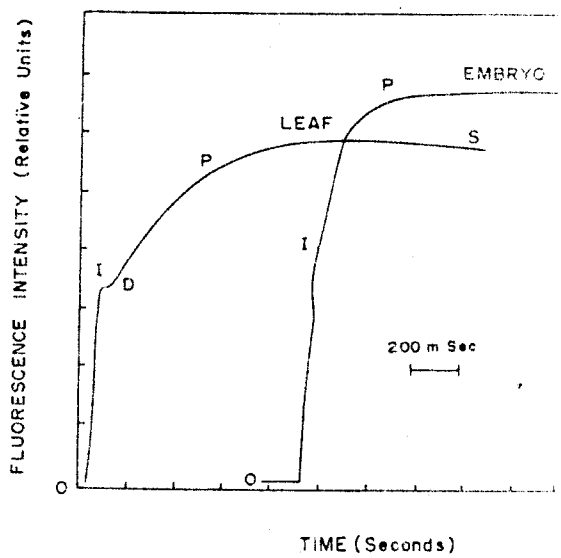

Figure 1. *In vivo* fluorescence transient measurements of embryo and leaf of *Cyamopsis* (O, level of stationary fluorescence; I, rise; D, dip; P, peak; S, steady state fluorescence.

Table IV. *^{14}C Bicarbonate Fixation in Embryos (Stage III) and Leaf of Cyamopsis*

Data are the mean of three different experiments ± SE

Tissue	^{14}C-Bicarbonate Fixation
	μmol/mg Chl·h
Embryo (light)	510 ± 11.9
Embryo (dark)	76 ± 2.2
Leaf (light)	558 ± 10.6
Leaf (dark)	18 ± 0.7

as the embryos are adjusted to direct or filtered light as the case may be.

^{14}C-Fixation Studies

The results of ^{14}C-bicarbonate fixation in the chloroembryos of the stage III fruits and leaf are compared in Table IV. ^{14}C fixation by green embryos in light was quite comparable to leaf fixation. However, dark ^{14}C fixation was about twofold higher in the embryos as compared to the leaf. A critical review of the data in Table IV indicates that there is a significant light independent fixation of $^{14}CO_2$ in the embryo. The increased dark fixation by embryos agrees with the results obtained by Sinha and Sane (24) that, in the developing seeds of pea, 75% $^{14}CO_2$ fixation occurred in dark. The embryo might fix carbon through PEPCase besides the major fixation by ribulose bisphosphate carboxylase. This is further supported by the earlier work on *Cyamopsis* (19) and partly by the finding that the fruit wall of chick pea possessed considerable PEPCase activity indicating its role in refixation of respired CO_2 liberated from the fruiting structures right from the developing stage up to maturity (23).

It is well established in literature (5, 8, 25) that low light intensity (6–9 W/m^2) is quite sufficient to promote photosynthetic activity. Similarly, in chloroembryos of *Cyamopsis*, photosynthesis might take place *in vivo* because of the possibility of sufficient sunlight (23–30%) reaching the embryos (11) and the presence of photochemical reaction centers as measured by Hill activity, *in vivo* Chl *a* fluorescence, as well as carbon fixation enzymes (19, 23, 24) coupled with the ability for ^{14}C-bicarbonate fixation and decrease in dry weight of fruits and their organs as evidenced by shading experiments. Therefore, it may be surmised from the present study that the chloroembryos may be involved in refixation of CO_2 lost during respiration possibly through PEPCase as well, as it is known that respired CO_2 is recaptured for fixation by the impervious fruit wall (23).

The chloroembryos can be considered to be partially autotrophic through *in vivo* photosynthesis enabling a self-sustained growth of the embryo. Growth and development of the green embryos of *Cyamopsis* and accumulation nutrients in them are possibly from two different sources—photosynthate contribution by foliage (import) as well as by chloroembryos themselves (synthesis).

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