The importance of the architecture of the host plant was revealed in an experiment with regard to obstacles. Obstructions in the form of sticks were introduced in a cage of dimensions 21 cm × 21 cm × 18 cm. The dome, which was usually built at the centre of the area in a control cage, was now shifted to one side and was smaller in size.

Prey capture efficiency in *C. cicatrosa* is lesser than a sticky trap of comparable size. Prey availability is known to influence web-site selection in several spiders, but is unlikely to do so in this case for the following reasons: (1) Inefficient web in terms of prey capture, compared to an equivalent-sized sticky web. (2) The spider is unlikely to abandon its web easily, since a large amount of energy has been spent during web construction.

Prey considerations are likely to be secondary to architecture considerations, as the web structure is affected by space limitations, i.e. the web has to be built first before the quality of the site (in terms of prey availability) can be determined.

Dispersion measurements indicate that there is a high degree of clumping. Even though *C. cicatrosa* is solitary, it is found in aggregations. Hanschel and Lubin studied web-site selection in a desert spider and concluded that *Seothyra hanscheli* does not actively choose sites, but has a restricted dispersal. Site tenacity may be a result of the spider’s inability to predict site quality coupled with high costs of relocation. They inferred that spiderlings tend to remain near the mother’s site, that had a previous record of success. This explanation could be the reason for a high aggregation in *C. cicatrosa* as well. Dispersal could be affected by the wind, but since the webs are found in sheltered areas, this is minimized. A possible implication of aggregation is that *C. cicatrosa* is on the way towards the evolution of sociability, as all the other members of this genus have done.

Structural requirements are the most important factor for the web-site selection in *C. cicatrosa*. A suitable way to classify plants according to their structural complexity is through the use of fractal dimensions, as laid out by Lawton. A further analysis of the web structure could lead to better understanding of the spider’s needs and its interaction with the environment.


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**Restriction of mitochondrial oxidative metabolism leads to suppression of photosynthetic carbon assimilation but not of photochemical electron transport in pea mesophyll protoplasts**

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The present study examines and establishes the primary effect of mitochondrial inhibitors, oligomycin, antimycin A and salicylhydroxamic acid (SHAM), on the photosynthetic carbon assimilation and photochemical electron transport activities, monitored in intact mesophyll protoplasts. These inhibitors caused a marked restriction of malate (+ glutamate)-dependent O₂ uptake in mitochondria (53–73%) isolated from pea leaves. When mesophyll protoplasts were illuminated in the presence of mitochondrial inhibitors, there was a significant decrease (> 45%) in HCO₃⁻-dependent O₂ evolution, while the decrease in O₂ evolution was marginal (< 10%) in the presence of benzoquinone (BQ) (photosystem PSII-mediated) and NO₃⁻-dependent on PSII + PSI as electron acceptors. 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), a typical photosynthetic inhibitor decreased drastically all the three reactions: HCO₃⁻ or BQ or NO₃⁻-dependent O₂ evolution in mesophyll protoplasts. Our results indicate that mitochondrial oxidative metabolism (through both cytochrome and alternative pathways) is essential for maintenance of photosynthetic carbon assimilation, but not for PSI or PSII-dependent photochemical electron transport activities in mesophyll protoplasts of pea.

APART from meeting energy requirements in dark, mitochondrial respiration plays an essential role in maintenance of photosynthesis. Restriction of mitochondrial metabolism decreased the rates of photosynthetic carbon assimilation while increasing the lag. Mitochondrial oxidative metabolism through both oxidative

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electron transport and oxidative phosphorylation, is essential for maintaining high rates of photosynthesis during not only short cycles of darkness and illumination, but also under stress conditions like photoinhibitory light or low temperature. Mitochondria optimize photosynthesis by fulfilling two important functions in a plant cell: (i) preventing over-reduction of chloroplastic redox carriers and (ii) supply of ATP for sucrose synthesis.

In all the above studies, the role of mitochondrial respiration in optimizing chloroplastic photosynthesis is documented by the usage of typical mitochondrial inhibitors. For example, oligomycin was used as an inhibitor of oxidative phosphorylation, antimycin A to inhibit the cytochrome oxidase pathway, while salicylhydroxamic acid (SHAM) was used to inhibit an alternative pathway. Different authors used either whole leaves or intact protoplasts or chloroplasts to indicate the effect of mitochondrial inhibitors on different components of photosynthetic carbon assimilation.

A major question which was not examined in detail, was whether the mitochondrial inhibitors affected photosynthetic carbon fixation or photochemical activities, or both. It is essential that this question be examined not with chloroplasts but with intact cells or protoplasts, since the interaction between different organelles is exhibited only in intact cells/protoplasts. The present study was undertaken to analyse the effect of mitochondrial inhibitors on photosynthetic reactions of carbon fixation and photochemical activities.

Three different reactions were identified to monitor photosynthesis in intact protoplasts. Using the system of mesophyll protoplasts of pea, the following components of photosynthesis were determined: BQ-dependent O₂ evolution (photosystem II-mediated), NO₂-dependent O₂ evolution (mediated by PSI and PSII), and HCO₃-dependent O₂ evolution (reflects carbon assimilation capacity). The effects of three mitochondrial inhibitors were compared with that of DCMU, a well-known inhibitor of PSI. The mitochondrial inhibitors were oligomycin (inhibitor of oxidative phosphorylation), antimycin A (inhibitor of cytochrome pathway) and SHAM (inhibitor of an alternative pathway of mitochondrial electron transport chain). We demonstrate that these three mitochondrial inhibitors restricted photosynthetic carbon assimilation, but have no effect on PSI or PSII-dependent photochemical reactions.

Mesophyll protoplasts were isolated from fully-expanded first and second pairs of leaves from pea plants grown under a natural photoperiod of approximately 12 h and average daily temperature of 30°C day/20°C night using a mixture of cellulase and macerozyme as already described. The protoplasts were collected and purified by step-wise-filtration, according to the method of Devi et al. The viability and intactness of the protoplasts were routinely checked using neutral red and Evans’ blue. The isolated protoplasts were 90–97% intact. Chlorophyll was estimated according to the method of Arnon.

The capacity of carbon fixation of the isolated mesophyll protoplasts was monitored as HCO₃⁻→O₂ evolution. The reaction medium of 1 ml for the assay of carbon fixation contained 0.4 M sorbitol, 1 mM CaCl₂, 1 mM MgCl₂, 1 mM NaHCO₃ in 25 mM Hepes-KOH, pH 7.5 and protoplasts equivalent to 20 µg chlorophyll. Protoplasts were illuminated at 1000 µmol m⁻²s⁻¹ using a 35 mm slide projector at 25°C and O₂ evolution was measured by using a Clark-type O₂ electrode (model DW2, Hansatech, King’s Lynn, UK).

The mesophyll protoplasts were incubated with 1 mM BQ or 10 mM NaNO₂ in place of NaHCO₃ to measure the PSI activity or whole chain (PSII + PSI) activity, respectively. Also, the assay medium while measuring NO₂⁻ dependent O₂ evolution (the whole chain activity) contained 5 mM glycolaldehyde (inhibitor of CO₂ fixation), to make sure that the observed O₂ evolution was due to photochemical activity and not due to carbon fixation. NaNO₂ was added to the reaction medium as soon as the light was switched on.

Mitochondria were isolated by a modified procedure of Day et al. Pea leaves (10 g) were disrupted in a mixer for 20 to 30 s with 60 ml of ice-cold medium containing 0.3 M sucrose, 20 mM Hepes-KOH (pH 7.4), 2 mM EDTA, 4 mM cystein, 0.1% BSA (w/v), 0.5% PVP-40 (w/v) and 5 mM glycine. The homogenate was filtered through three layers of cheesecloth and centrifuged for 5 min at 2000 g. The supernatant was centrifuged at 10,000 g for 20 min and the pellet washed by resuspending in 60 ml of 0.3 M sucrose containing 10 mM KH₂PO₄-KOH (pH 7.2), 1 mM EDTA, 0.1% BSA (w/v) and 5 mM glycine and recentrifuged at 10,000 g for 20 min. The final suspension of mitochondria was made in 2 to 3 ml of washing medium.

O₂ consumption was measured polarographically in 1.0 ml reaction medium containing 0.3 M sucrose, 10 mM KH₂PO₄-KOH (pH 7.2), 10 mM KCl, 5 mM MgCl₂ and 0.1% BSA (w/v) in presence of malate (+ glutamate). In test samples, the different respiratory inhibitors, as required, were added to the incubation medium while monitoring O₂ consumption.

The integrity of the mitochondria was assessed by measuring ascorbate-Cyt c oxidoreductase activities in intact and osmotically-burst mitochondria, by a slightly modified procedure of Douce et al. The percentage of mitochondrial integrity was > 90.

Cellulose (Onozuka R-10) and macerozyme R-10 (pectinase) were procured from Yakult Honsha, Japan. Antimycin A, oligomycin, SHAM, DCMU and glycolaldehyde were from Sigma, USA. The data presented are the average values (± SD) of results from three to four experiments conducted on different days.
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Figure 1. Effect of mitochondrial inhibitors, oligomycin (0 to 500 ng ml⁻¹), antimycin A (0 to 500 nM) and SHAM (0 to 500 μM) on malate (+ glutamate) oxidation of isolated mitochondria. The reaction medium of 1 ml contained mitochondria equivalent to 30–40 μg protein. The rate of O₂ uptake was 521 ± 25 nmol O₂ mg⁻¹ protein min⁻¹. Data are averages of two separate experiments done on different days.

Figure 2. Change in rate of oxygen evolution when mesophyll protoplasts (20 μg ml⁻¹) are incubated with different concentrations of DCMU (0–100 nM). O: Rate of oxygen evolution when electrons are transferred from H₂O to NO₂⁻ (representing PSII and PSI activity). Δ: Rate of oxygen evolution when electrons are transferred from H₂O to BQ (indicates PSI activity, excluding PSII). In controls, the rate of O₂ evolution in μmol mg⁻¹ Chl h⁻¹ under different conditions were: 154 ± 6 (HCO₃⁻ dependent O₂ evolution); 445 ± 20 (BQ-dependent O₂ evolution); 52 ± 6 (NO₂⁻ dependent O₂ evolution). Other details are as described in the text.

The effects of oligomycin, antimycin A and SHAM on the coupled rates of mitochondrial respiration were assessed by examining the effect of test compounds on mitochondrial respiratory activity (Figure 1). At the range of concentrations used in the present study, oligomycin (500 ng ml⁻¹) and antimycin A (500 nM) decreased the rate of malate (+glutamate)-dependent O₂ uptake (a typical respiratory activity representing state 4/state 3) in isolated mitochondria by 53% and 73% of control, respectively, while SHAM (500 μM) decreased these coupled rates of mitochondrial respiration by 57%.

DCMU, a typical inhibitor of PSII, decreased all the three reactions in pea mesophyll protoplasts: NO₂⁻ dependent (>60%) as well HCO₃⁻ dependent O₂ evolution (<90% activity of control) along with BQ-dependent O₂ evolution by 60–90% (Figure 2). The presence of oligomycin (500 ng ml⁻¹) or antimycin A (500 nM) inhibited HCO₃⁻ dependent O₂ evolution by 35–45% of control. These two compounds did not affect either NO₂⁻ dependent O₂ evolution or BQ-dependent O₂ evolution (Figure 3 a and b). Figure 3 c shows the effect of SHAM on photosynthetic carbon assimilation and photochemical activities. The bicarbonate-dependent O₂ evolution was inhibited significantly (<45%) when the concentration of SHAM was raised from zero to 500 μM, while the NO₂⁻ dependent and BQ-dependent O₂ evolution was marginally affected (<10% of control). However there was a stimulation of less than 10% in PSII activity at low concentrations of oligomycin, antimycin A or SHAM.

Although there are several reports emphasizing the significance of beneficial effects rendered by mitochondrial metabolism to photosynthetic activity, they do not show whether the mitochondrial oxidative metabolism optimizes photosynthetic carbon assimilation through photochemical activities or independent of photochemical activities. This report describes the effect of restricted mitochondrial metabolism on the photochemical activities as well as the carbon assimilation capacity in intact plant cells. The pattern of decrease in mitochondrial respiratory activity by the three inhibitors (Figure 1) was quite similar in case of photosynthetic O₂ evolution in presence of bicarbonate, but not of either BQ or NO₂⁻ (Figure 3 a–c). The novelty of the present work is the choice of photosynthetic reactions which could be determined with intact protoplasts. Whole-chain activity (involving both PSII and PSI) is measured as NO₂⁻ dependent O₂ evolution, where electrons are transferred from H₂O to ferredoxin (Fd). PSI activity is measured as BQ-dependent O₂ evolution, where electrons are transferred from H₂O to BQ. Photosynthetic carbon fixation was measured as HCO₃⁻ dependent O₂ evolution.

Technically, it is difficult to measure all the photosynthetic reactions using intact cells or protoplasts directly. For example, ferricyanide is impermeable across the plasma membrane. Further, to suppress catalase activity, sodium azide is included during measurements of methyl viologen-dependent O₂ uptake, which itself is a mitochondrial inhibitor.

The NO₂⁻ dependent O₂ evolution measured in the present study reflects the activity of both PSI and PSII, as electrons are transported from H₂O to reduce NO₂⁻ via Fd. The decrease in NO₂⁻ dependent O₂ evolution in
the presence of DCMU (Figure 2) confirms that blocking the flow of electrons from PSII to PSI interferes with NO$_3^-$ reduction. There were several reports expressing the whole-chain electron transport activity in terms of NO$_3^-$ dependent O$_2$ evolution$^{19,20,31}$. We inhibited the photosynthetic carbon fixation with glycolaldehyde and added NaN$O_2$ to measure the photochemical activities accurately, independent of carbon fixation. The rates of NO$_3^-$ dependent O$_2$ evolution reported in the present study are low, but similar to those of earlier reports$^{21,30,31}$.

DCMU is a well-known inhibitor of photosynthetic electron transport and subsequently CO$_2$ fixation$^{19,25}$. As expected, all the three types of photosynthetic reactions were suppressed by DCMU. In contrast to the effect of DCMU, the three mitochondrial inhibitors, SHAM, antimycin A and oligomycin, did not exert any significant effect on photochemical activities of the whole chain and PSI, but strongly inhibited photosynthetic carbon fixation (Figures 3 a–c).

While it is clear that the essentiality of mitochondrial oxidative metabolism is linked with the capacity of photosynthetic carbon assimilation, attempts have already been made to study this aspect further. The dependence of photosynthetic carbon assimilation on mitochondrial respiration varied with different light intensities and CO$_2$ concentration$^{15}$. Further, the cytosolic redox status, mediated by malate-OAA (oxaloacetate) shuttle appears to be strongly modulated by the alternative pathway of mitochondrial electron transport chain$^{10}$.

Restriction of mitochondrial oxidative metabolism by typical mitochondrial inhibitors, oligomycin or antimycin A or SHAM, leads to the inhibition of only bicarbonate-dependent oxygen evolution. These inhibitors did not affect the electron transport activities of PSI, PSI or photophosphorylation. Our results therefore suggest that oxidative metabolism (through cytochrome pathway or an alternative pathway) is essential for optimization of photosynthetic carbon assimilation, but not for photochemical activities.

Respiratory enzyme activities in the oxygen-deficient waters of the Arabian Sea

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Denitrification in the oxygen-poor waters at intermediate depths in the Arabian Sea is very intense. Depth profiles of nitrite and the activities of the electron transport system (ETS) and dissimilatory enzymes such as nitrate reductase and nitrite reductase within the nitrite-bearing waters, display depth-mismatched maxima. There are indications of contribution by both dissimilatory nitrate reduction and nitrification to the nitrite enrichment of the secondary nitrite maximum (SNM) between 150 and 350 m. The waters below the SNM, not bearing nitrite but within the oxygen minimum zone, are also potentially denitrifying.

Analyses of the relationships between various parameters indicate the existence of a layered ecosystem in which nitrification and denitrification activities are present in several closely-spaced layers. This is probably caused by the inflow of different water masses into this hypoxic region, as suggested by the strong maxima and minima in enzyme activities at equivalent depths.

A thick layer of low-oxygen water exists between ~150 and 1100 m in the Arabian Sea. It is associated in its upper part with large nitrite accumulation and nitrate deficit, denoting intense denitrification. The secondary nitrite maximum (SNM) layer around 150–600 m is characterized by high metabolic activities1 and maximum concentrations of both suspended particles and particulate protein2. The reasons for the acute oxygen depletion are unclear. So also the carbon source sustaining the intense denitrification remains to be demystified. Recent studies on transparent exopolymer particles (TEP)3,4 provide a clue as to how the carbon demand could be met. The bacterial activities associated with these waters have yet to be fully identified, although respiration measured as the electron transport system (ETS) activity in suboxic waters containing >0.2 µM NO3 has been assumed to be predominantly due to denitrification5,6. However, nitrifiers are also known to be intimately involved in the nitrogen metabolism of such regimes7. And, the numerical model of Anderson et al.8 incorporating oxidation of ammonium and nitrite by nitrifiers at the boundaries of the oxygen-deficient waters has been largely substantiated by the work of Lipschultz et al.9 in the eastern tropical south Pacific. Similar analyses are lacking for the Arabian Sea.

The carbon and nitrogen cycles operating in the oxygen minimum zone (OMZ) of the Arabian Sea have global implication, since these waters serve as strong sinks for fixed nitrogen on a global scale10 and a significant source of N2O (which is produced by both nitrification and denitrification) to the atmosphere11,12.

The objective of this study was to look at the fine details of distribution of metabolic activities within the OMZ of the Arabian Sea, specifically around the SNM, in order to produce some significant ecological insights