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# ISOLATION OF PHOTOSYNTHETICALLY ACTIVE MESOPHYLL CELLS AND PROTOPLASTS FROM LEAVES OF $C_3$, $C_4$ AND CAM PLANTS

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## ABSTRACT

The leaves of a $C_3$, $C_4$ and CAM plants yielded the intact mesophyll cells and protoplasts by enzymatic digestion of cell walls. The isolated mesophyll cells and protoplasts were photosynthetically active as indicated by an active $^{14}CO_2$ fixation.

## INTRODUCTION

SINCE the demonstration of the enzymatic isolation of protoplasts from root tips[1], there has been a rapid development in the isolation and utilization of plant protoplasts for diverse studies on plant metabolism[2–4]. Enzymatically isolated intact cells and protoplasts from leaves are particularly promising for photosynthetic studies[5,6].

In this study we report the isolation of intact mesophyll cells and protoplasts by enzymatic digestion of cell walls from leaves of $C_3$, $C_4$ and Crassulacean acid metabolism (CAM) plants. The photosynthetic carbon fixation capacity of thus obtained mesophyll cells and protoplasts was measured to test the physiological activity.

## MATERIALS AND METHODS

The plant materials selected for the present study were *Dolichos lablab* L., a $C_3$ plant (plant exhibiting the Calvin pathway); *Digitaria adscendens* Henr, a $C_4$ plant (plant exhibiting $C_4$ pathway) and *Bryophyllum calycinum* Solsib, a CAM plant. These plants were grown in the experimental plots of the University Botanical Garden under the natural photoperiod. The photoperiod was approximately 12 hours and the temperature 33° C day and 25° C night.

For the isolation of mesophyll cells, young and fully expanded leaves (*D. lablab and D. adscendens*, 3–5 weeks old; *B. calycinum*, 6–8 weeks old) were cut vertically across the leaf to give segments less than 1 mm in width. For *B. calycinum* the epidermis was removed from the two sides of the leaf with forceps before cutting segments. Leaf segments were vacuum infiltrated for about 5 min in 20 ml of an enzymatic digestion medium and incubated on a reciprocal shaker at room temperature. The enzyme medium at pH 5·8 contained 0·7 M mannitol, 5 mM $MgCl_2$, 2mM EDTA, 5 mM $K_2HPO_4$, and 2% macerase. After 15 min, the enzyme mixture was carefully removed, and the segments were incubated in a fresh medium for 2 hr at 30° C. A drop of incubating mixture was kept under light microscope to observe the separated leaf cells. The enzyme mixture was then filtered through cheese cloth, centrifuged at 100 g for 1 min, and washed three times with 0·7 M mannitol to obtain enzyme free sample of isolated mesophyll cells.

The cells thus obtained were incubated with the same enzyme mixture except 3% cellulase in the place of macerase for about 3 hr at 30° C. After incubation the enzyme mixture was centrifuged at 100 g for 2 min. The supernatant was removed and the protoplasts were cleaned three times with mannitol to remove the residual enzyme. The total chlorophyll content in cells and protoplasts was determined[7].

The isolated cells and protoplasts were used for photosynthetic $^{14}CO_2$ fixation. The assay mixture (2 ml) for $^{14}CO_2$ fixation contained 0·4 M mannitol, 1 mM $MgCl_2$, 2 mM EDTA, 50 mM Tricine–KOH (pH 7·5) and 5 mM $NaH^{14}CO_3$. The reaction vessels for *D. adscendens* and *B. calycinum* contained assay mixture, mesophyll cells (5–10 μg chlorophyll), protoplasts (5–10 μg chlorophyll) and 5 mM phosphoenolpyruvate (PEP). The assays were performed in the light with a total quantum flux of about 70 nanoeinsteins $cm^{-2}$ $sec^{-1}$ between 400–700 nm at 35° C. At specified intervals, aliquots were removed and the incorporation of $^{14}CO_2$ into acid stable products was determined by Liquid Scintillation counting.

Cellulase and macerase were obtained from Calbiochem Ltd., USA.

## RESULTS AND DISCUSSION

The leaves of the three species tested, *D. lablab*, *D. adscendens* and *B. calycinum* yielded the intact mesophyll cells and protoplasts as evidenced under the light microscope. The isolated mesophyll cells and protoplasts from the three species are photosynthetically active as indicated by an active $^{14}CO_2$ fixation. Photosynthetic $^{14}CO_2$ fixation by isolated cells and

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TABLE I

*Photosynthetic $^{14}CO_2$ fixation by isolated mesophyll cells and protoplasts (μ moles $mg^{-1}$ chlorophyll)*

| Plant species | Preparation | Time in minutes | | | |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 5 |
| *Dolichos lablab* ($C_3$ plant) | Mesophyll cells | 1·2 | 4·1 | 12·1 | 22·0 |
| | Mesophyll protoplasts | 13·5 | 36·4 | 73·0 | 84·3 |
| *Digitaria adscendens* ($C_4$ plants) | Mesophyll cells | 0·8 | 2·6 | 5·6 | 7·8 |
| | Mesophyll protoplasts | 10·2 | 23·7 | 30·3 | 50·1 |
| *Bryophyllum calycinum* (CAM plant) | Mesophyll cells | 1·4 | 3·2 | 3·3 | 3·5 |
| | Mesophyll protoplasts | 19·3 | 36·8 | 48·1 | 52·6 |

protoplasts determined at specified time intervals was shown in Table 1. All the three species exhibited higher rates of $^{14}CO_2$ fixation in mesophyll protoplasts than the mesophyll cells. The mesophyll cells and protoplasts of *Dolichos* exhibited a lag phase before attaining maximal rates of $^{14}CO_2$ assimilation. The cells of *Digitaria* and *Bryophyllum* did not show any lag phase during carbon fixation.

The fact that the isolated mesophyll cells and protoplasts could be a useful tool to examine the photosynthetic systems was demonstrated by the high rates of carbon fixation capacity of intact mesophyll cells and protoplasts of $C_3$, $C_4$ and CAM plants.

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