

This Week's Citation Classic

Kanai R & Edwards G E. Separation of mesophyll protoplasts and bundle sheath cells from maize leaves for photosynthetic studies. *Plant Physiol.* **51**:1133-7, 1973.
[Department of Horticulture, University of Wisconsin, Madison, WI]

Two cell types of C₄ plant leaves were separated by enzymatic digestion of cell wall. Mesophyll protoplasts were further purified by use of a polyethylene glycol-dextran two phase system. Conclusive evidence was obtained for intercellular division of the enzymes of C₄ pathway of photosynthesis. [The SC/® indicates that this paper has been cited over 105 times since 1973.]

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"The time of discovery in C₄ photosynthesis was exciting for all plant biologists. Waves of the renaissance in plant physiology reached me soon at a rural college in Japan. In the spring of 1971, I decided to go to the US to see what was going on in C₄ research. In my baggage I put cellulase and pectinase, products of a Japanese company. The capacity of these enzymes for the isolation of cells and protoplasts from some plant leaves was already shown by I. Takebe and his associates.¹ The enzyme samples, however, were stocked more than one year in a refrigerator, during my stay in C.C. Black's laboratory at the University of Georgia. At that time, I was concerned with a new type of C₄ plant having PEP carboxykinase as decarboxylating enzyme.² After I moved to my second visiting place, Gerry Edwards's laboratory at the University of Wisconsin, I offered to try the 'magic enzymes.' Most of the experiments were done with four hands according to the united idea of Gerry and myself.

"The first aim was to get crucial evidence on the then controversy on inter-

cellular distribution of enzymes of C₄ pathway between mesophyll cells and bundle sheath cells in maize leaves. From the beginning of the trials, the re-suits were promising for isolating meso-phyll cells as protoplasts, leaving the bundle sheath strands undigested, even from many C₄ plant species including maize. In our experience, the most important factors for reproducible results were to use leaves of young plants and to cut fine leaf segments (less than mm) with a sharp razor blade. Specific localization of RuBP carboxylase in bundle sheath cells and PEP carboxyl-ase in mesophyll protoplasts was shown in all C₄ plants tested.

"The second aim was to use isolated protoplasts as a tool for photosynthesis studies in general. Leaf protoplast would be advantageous for isolation of intact plant organelles, such as chloroplasts and mitochondria. Due to the shortness of my stay, I could only do a preliminary survey on intracellular localization of enzymes in C₄ mesophyll cells. This line of research was succeeded by colleagues at the laboratory. The number of citations to the article may indicate the potential of leaf protoplasts in various fields of study in plant physiology.

"In retrospect, I spent several happy months doing exciting research in Madison. The compartmentation and coordination of two cell types in C₄ leaves have been further extended to enzyme activities other than those of the C₄ pathway. Recently, differential expression of the chloroplast gene for RuBP carboxylase in maize leaf cell types was the subject of a study in molecular biology.⁴ However, some essential problems in C₄ photosynthesis still remain to be solved; transport mechanism of metabolites between two cell types, for example."

1. **Otsuki Y & Takebe I.** Isolation of intact mesophyll cells and their protoplasts from higher plants. *Plant Cell Physiol.* **10**:917-21, 1969.
2. **Edwards G E, Kanai R & Black C C.** Phosphoenolpyruvate carboxykinase in leaves of certain plants which fix CO₂ by the C₄-dicarboxylic acid cycle of photosynthesis. *Biochem. Biophys. Res. Commun.* **45**:278-85, 1971.
3. **Edwards G E & Huber S C.** C₄ metabolism in isolated cells and protoplasts. (Gibbs M & Latzko E, eds.) *Encyclopedia of plant physiology, new series.* Berlin: Springer-Verlag, 1979. Vol 6. p. 102-12.
4. **Link G, Coen D M & Bogorad L.** Differential expression of the gene for the large subunit of ribulose biphosphate carboxylase in maize leaf cell types. *Cell* **15**:725-31. 1978.