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This Week's Citation Classic

Kao K N & Michayluk M R. A method for high-frequency intergeneric fusion of plant protoplasts. *Planta* **115**:355-67, 1974.
[Prairie Regional Laboratory, National Research Council, Saskatoon, Saskatchewan, Canada]

The paper described a method for fusion of free plant protoplasts with a high molecular weight polyethylene glycol solution. The fusion was enhanced by Ca^{2+} ions and inhibited by Na^{+} or K^{+} ions. Heterokaryocytes from fusion of unrelated species were viable and able to undergo cell division. [The *SCI*® indicates that this paper has been cited over 195 times since 1974.]

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"In the late 1960s and early 1970s, a number of laboratories were investigating plant somatic cell hybridization. The specialists in this field knew what essential steps (namely, isolate free protoplasts, induce them to fuse and regenerate plants from hybrid protoplasts) we should take in order to achieve this goal. In 1972, a few somatic hybrid plants had been produced by protoplast fusion.[1] However, a reliable fusion method was still lacking and I was just as desperate as the others trying to find a good fusion method. One day in June, 1973, I thought that perhaps I could induce protoplast fusion by microsurgical methods which were successfully used in mammalian cell fusion. This method did not work for me. However, while working on this procedure I incorporated polyethylene glycol into one of the solutions. When I added some protoplasts to this solution, much to my delight and wonder, I observed that the protoplasts immediately adhered tightly to each other. A method for high frequency fusion of protoplasts was developed within a month. Soon it became obvious that polyethylene glycol was an ideal compound for fusion of plant protoplasts. I would like to thank L.R. Wetter and W.F. Steck in this laboratory, and C.C. Lee, department of chemistry, University of Saskatchewan, Saskatoon, for helping me describe the structure and chemical properties of this compound.

"The method is as follows: (1) Place a drop of a mixture of two different types of protoplasts on a cover slip. (2) Slowly add three drops of polyethylene glycol solution to the mixed protoplasts. (3) Elute the polyethylene glycol solution with a protoplast culture medium. Later on I learned that Keller and Melchers[2] were able to fuse free protoplasts with a high pH and high Ca^{2+} solution. Further increase in fusion frequency became possible when the PEC was eluted away with Keller's solution.[3] The heterokaryocytes were readily distinguishable from the parental types when the protoplasts of one of the parental types were obtained from green leaves and the others from colorless cultured cells.

"The experiment was designed by myself and most of the experimental work was carried out by myself and Morris R. Michayluk, my technician. The frequency of citation may be attributed to the following reasons: (1) Polyethylene glycol was employed for the first time as a fusogenic agent. (2) The method is simple. (3) Polyethylene glycol is odorless, nontoxic, and cheap. (4) Soon after this method was described, many scientists found that polyethylene glycol could also be used to fuse mammalian cells, insect cells, fungal, algal, and bacterial protoplasts. The polyethylene glycol soon earned the reputation of being an universal fusogen."

1. **Carlson P S, Smith H H & Dearing R D.** Parasexual interspecific plant hybridization. *Proc. Nat. Acad. Sci. US* **69**:2292-4, 1972.
2. **Keller W A & Melchers G.** Effect of high pH and calcium on tobacco leaf protoplast fusion. *Z. Naturforsch. C* **28**:737-41, 1973.
3. **Kao K N, Constabel F, Michayluk M K & Gamborg O L.** Plant protoplast fusion and growth of intergeneric hybrid cells. *Planta* **120**:215-27, 1974.