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This Week's Citation Classic®

Kao K N & Michayluk M R. Nutritional requirements for growth of *Vicia hajastana* cells and protoplasts at a very low population density in liquid media. *Planta* 126:105-10, 1975.
[Prairie Regional Laboratory, National Research Council of Canada, Saskatoon, Saskatchewan, Canada]

Vicia hajastana Grossh. cells or protoplasts were not able to survive when cultured at a low population density in a mineral-salt medium unless the medium was supplemented with metabolic intermediates. A medium for cultivation of plant protoplasts at a density of one cell/ml was described. [The *SCI*® indicates that this paper has been cited in over 230 publications.]

K.N. Kao
Plant Biotechnology Institute
National Research Council
Saskatoon, Saskatchewan S7N 0W9
Canada

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I joined the Prairie Regional Laboratory (now Plant Biotechnology Institute), National Research Council of Canada, in 1969 as a cytogeneticist with a major in plant breeding. I was often told that in cell culture one must use a sufficient number of cells as inoculant to get them to grow in a liquid medium. However, at that time I needed a technique to culture a single isolated protoplast. At one of the American Tissue Culture Association's annual meetings (1971?), I learned that certain mammary cells were able to grow at a very low population density. I further learned that amino acids tended to leak out from plant cells in suspension culture.[1,2] It became clear to me that the inability of the plant cells to grow at a very low population density may have been caused by excessive diffusion of metabolic intermediates into the medium, resulting in their dilution in the cells to a level below that required for survival.[3] If this was the case, the cells should be able to grow at a very low initial population density in a medium enriched with the appropriate metabolic intermediates.

I was able to compose an adequate medium for culturing plant cells or protoplasts at a very low population density with relatively little effort because a simple assay method was used to determine the near optimum level of certain compounds to make the cell grow. I assumed that if the cells aged and died gradually, they were deficient in some essential compounds. If the cells turned brown in colour and died in a very short period of time, I assumed that certain of the compounds that I added into the medium had reached a toxic level. If a single cell did grow in a dishful of medium, I should be able to see the cell mass without a microscope eventually. No dry weight or growth rate was ever used in the experiment. It took me several years to develop such a medium. However, I did not put much effort into it in the first few years. In 1973 we developed a protoplast fusion technique.[4] I realized the importance of such a medium for cultivation of a single heterokaryocyte. Later on, we were able to grow a number of isolated intergeneric heterokaryocytes from fusion of protoplasts and to study their behaviour.[5]

I was surprised that this paper has become a *Citation Classic*. The reason that this paper was highly cited is perhaps that the medium could be used for cultivation of plant cells and protoplasts of many different species.[6,7] I wish to thank M.R. Michayluk for his help with our experiments.

1. **Stuart R & Street H E.** Studies on the growth in culture of plant cells. IV. The initiation of division in suspensions of stationary phase cells of *Acer pseudoplatanus* L. *J. Exp. Bot.* 20:556-71, 1969. (Cited 90 times.)
2. **Sargent P A & King J.** Investigations of growth-promoting factors in conditioned soybean root cells and in the liquid medium in which they grow: ammonium, glutamine, and amino acids. *Can. J. Bot.* 52:1747-55, 1974. (Cited 15 times.)
3. **Ham R G.** Dilution plating and nutritional considerations. A. Animal cells. (Kruse P F & Patterson M K, eds.) *Tissue culture methods and applications*. New York: Academic Press, 1973. p. 254-61. (Cited 15 times.)
4. **Kao K N & Michayluk M R.** A method for high-frequency intergeneric fusion of plant protoplasts. *Planta* 115:355-67, 1974. (Cited 370 times.) [See also: **Kao K N.** Citation Classic. (Barrett J T, comp.) *Contemporary classics in plant, animal, and environmental sciences*. Philadelphia: ISI Press, 1986. p. 118.]
5. **Kao K N & Wetter L R.** Advances in techniques of plant protoplast fusion and culture of heterokaryocytes. (Brinkley B R & Porter K R, eds.) *International cell biology, 1976-1977*. New York: Rockefeller University Press, 1977. p. 216-24. (Cited 5 times.)
6. **Binding H, Nehls R & Jörgensen J.** Protoplast regeneration in higher plants. (Fujiwara A, ed.) *Plant tissue culture*. Japanese Association for Plant Tissue Culture, 1982. p. 575-8. (Cited 5 times.)
7. **Lührs R & Lörz H.** Initiation of morphogenic cell-suspension and protoplast cultures of barley (*Hordeum vulgare* L.). *Planta* 175:71-81, 1988.

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