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.)

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 mammmary 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 cultivating 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 cells 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.


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