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Murashige T & Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* **15**:473-97, 1962.

The paper describes an improvised nutrient medium which enabled substantially greater growth of tobacco tissue cultures. Our experiments differed from previous studies in examining several nutrient components simultaneously. The new formulation was conspicuously high in all macronutrient salts, also included all micronutrients, and provided iron in the slowly but more readily available chelated form. Among organic substances, sucrose was increased from 2 to 3% and *myo*-inositol was made a standard addendum. When suplemented with suitable additions of auxin and cytokinin, the medium enhanced the monthly yield of tobacco callus from 5 g to 125 g/culture. It further ensured minimal interference from inorganic and common organic nutrients when used in bioassays of growth-promoting plant or animal extracts. [The *SCI*® indicates that this paper was cited 1203 times in the period 1963-1977.]

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"Tissue cultures are currently a major attraction among plant scientists, simply because of their special utility as a research tool and in commercial practice. Our paper contributes to basic tissue culture methodology, thus the frequent citation.

"The research described by our paper was an anticlimax of another project to which I had already devoted 3 years as a doctoral student. I began my dissertation work as Professor Skoog's student shortly after he and Professor C. O. Miller and their colleagues had discovered kinetin, the first of the cytokinin class of plant hormones. The auxin class was already common knowledge and the gibberellins were gaining familiarity in the western world. The discovery of kinetin inspired me greatly, and I was now determined to discover still other hormones that might remain hidden in plants. As an early reward of this determination, I discovered that an extract of tobacco leaves, when added in combination with auxin and kinetin to the culture medium of tobacco callus, increased tissue yield drastically, more than 500% over the controls.

"That autumn Professor Skoog hired several undergraduates to help me, and together we picked over 1,000 lbs of tobacco leaves from a field made available by Professor Ogden of Horticulture. We packed them in large plastic bags, normally used for turkeys, and hauled them to a large, rented food-storage locker where we froze them. Then, using facilities in Biochemistry and with the help of Professors Strong and Carver, we ground the leaves through a meat grinder that had seen better days, thawed them in a large stainless-steel vat, and squeezed out their juices with a handpress. We pooled, boiled, chilled and filtered the juices. We obtained 150 gals. of clear extract, which we ultimately concentrated to 5 gals of dark brown syrup in a Mojonnier evaporator.

"Innumerable fractionations of the extract followed. But after all the effort, I learned that much of the tobacco extract effect was located in the mineral fraction. Thus began the work to revise the composition of the nutrient formulation, which for many years had served as the basis of all plant tissue culture experiments.

"The revising took another 100 experiments and produced a new formula. But little enthusiasm remained for its publication. The blow of failing to discover a new hormone was still being felt. That is now past, and I am deeply appreciative of the recognition presently being given to the paper."