

This Week's Citation Classic™

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Bourgin J P & Nitsch J P. Obtention de *Nicotiana* haploïdes à partir d'étamines cultivées *in vitro*. (Production of haploid *Nicotiana* from excised stamens.)

Ann. Physiol. Vég. 9:377-82, 1967.

[Laboratoire de Physiologie Pluricellulaire, CNRS, Gif-sur-Yvette, France]

Stamens of various *Nicotiana* strains were cultured *in vitro*. Plantlets developed from the anthers and were raised to mature plants, most of which were haploid. [The SCI® indicates that this paper has been cited in over 140 publications since 1967.]

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"In 1966, the late Jean Paul Nitsch became associate professor of plant physiology at the Institut National de la Recherche Agronomique (INRA). As one of his first students, I spent one year in his Laboratory of Pluricellular Physiology at the CNRS Research Center at Gif-sur-Yvette to complete my undergraduate studies with an introduction to biological research. Nitsch was very interested in the possible practical applications of new plant cell culture techniques, so he asked me to try to extend to new species the phenomenon of somatic embryogenesis obtained in cultures of carrot tissues and a few other species. This technique was of particular interest since it could possibly lead to a method of vegetative propagation. Kindly guided by Luisa Rossini, an Argentinian scientist who had just arrived in Nitsch's lab, I thus received the routine but rapidly overwhelming project of trying to induce somatic embryogenesis in almost every old and new tissue strain in the lab. My only success remained confined to the well-known case of the carrot.

"Then, prompted by the confirmation by Guha and Maheshwari¹ of the pollen origin of the embryoids they had previously observed in cultured anthers of *Datura*, we decided to try to induce this

phenomenon in stamens from other species. In the case of two varieties of *Nicotiana tabacum* and of one of its presumed progenitor species, *N. sylvestris*, we were successful after long weeks of observing green plantlets growing out of the browned anthers. Nitsch showed me, with the fervor and care of an obstetrician, how to proceed to the transfer of the first subcultured plantlets to the nonaseptic environment of a growth chamber. Later on, Simone Hamon brought us the expected confirmation that at least the majority of these plants originated from pollen and not from somatic tissues, since most of them were haploid.

"Although our optimism was tempered by our failures in the culture of stamens from other species, we were aware of the promise opened up by these first extensions to other solanaceous species of pollen embryogenesis and the production of adult haploid androgenetic plants. However, Nitsch decided to publish our results in the rather low-profile journal of plant physiology of the INRA to which I owed a grant. A more decisive reason was probably that, for Nitsch, a good paper one that left no unanswered factual question. He was, rightly, convinced that the stage of pollen development in the excised anthers was a crucial factor in the triggering of pollen embryogenesis, a point that had not been explored either by Guha and Maheshwari's studies¹ or by our own.

"After I was appointed to an INRA position in Georges Morel's lab, Nitsch completed this work with the collaboration of his wife, Colette Nitsch, which led to the publication of an important paper in *Science*.² This paper, which among other results reported the success of pollen embryogenesis in more *Nicotiana* species, was among the first to pinpoint the potentialities of anther culture. It also revealed the existence of our preliminary report, as well as that of the contemporary work of Nakata and Tanaka,³ assuring them a high rate of citation due to the expansion of anther or pollen culture^{4,5} as a source of haploid plants, which are potentially useful tools in plant breeding and somatic cell genetics. It is plausible that the partial successes met by this technique prompted the research that has led to the production of haploid plants through ovary and ovule culture."⁶

1. Guha S & Maheshwari S C. Cell division and differentiation of embryos in the pollen grains of *Datura in vitro*. *Nature* 212:97-8, 1966. (Cited 130 times.)
2. Nitsch J P & Nitsch C. Haploid plants from pollen grains. *Science* 163:85-7, 1969. (Cited 295 times.)
3. Nakata K & Tanaka M. Differentiation of embryoids from developing germ cells in anther culture of tobacco. *Jpn. J. Genet.* 43:65-71, 1968. (Cited 80 times.)
4. Vasil I K. Androgenetic haploids. *Int. Rev. Cytol.* (Suppl. 11A):195-223, 1980.
5. Maheshwari S C, Rashid A & Tyagi A K. Haploids from pollen grains—retrospect and prospect. *Amer. J. Bot.* 69:865-79, 1982.
6. Yang H Y & Zhou C. In vitro induction of haploid plants from unpollinated ovaries and ovules. *Theor. Appl. Genet.* 63:97-104, 1982.