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BRIEF COMMUNICATION

Gametogenesis of Tobacco *in vitro*

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Abstract. Excised anthers of *Nicotiana tabacum* L. were cultured *in vitro* at the stage of pollen tetrads, which proved in our experiments to be the most suitable initial stage for cultivation, up to the stage of mature pollen grains, using Ito and Stern's nutrient medium, or this medium supplemented with uracil. Germinating capacity of the pollen grains formed and the lengths of pollen tubes were quantitatively evaluated.

During microsporogenesis, morphological changes taking place in the microsporocytes are accompanied by DNA synthesis. This basic synthesis occurs in three periods, namely always prior to the division of the microsporocyte nucleus (TAYLOR 1958). This fact enables one to study or affect the formation of pollen grains. Such studies usually employ the technique of *in vitro* cultivation of excised anthers. However, when the anthers of any plant species excised at premeiotic stages were set in culture no successful results concerning the formation of fertile pollen grains could be obtained (*e.g.* TAYLOR 1950, SPARROW *et al.* 1955, WALKER 1957). Only ITO and STERN (1967) and NAKATA and TANAKA (1968) succeeded in culturing meiotic cells, or anthers, in the test tube throughout the entire meiosis and in obtaining fertile pollen grains. This paper deals with *in vitro* cultivation of anthers excised at the stage of pollen tetrads up to the stage of mature anthers containing germinable pollen grains, involving attempts to affect the developing pollen grains with uracil.

Anthers of *Nicotiana tabacum* L., cv. Fixed A_2 426 ($2n = 48$) were employed for the experiments. At first we examined the anthers excised at various stages of the pollen grain development, namely at the stage of microsporocytes of the early meiotic prophase, or at that of pollen tetrads, or at the stage of uninucleate pollen grains. Anthers at the stage of pollen tetrads were chosen for the experiments themselves. Four anthers (a fifth serving as control for microscopic analysis) were placed onto the surface of the liquid nutrient medium according to ITO and STERN (1967), or, for experiments aimed at affecting microsporogenesis, onto this basic medium

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supplemented with uracil at a concentration of 57·5 mg l^{-1}. The cultures were kept in an incubator in the dark at 26° C for 8 to 14 days. After the cultivation was completed, the anthers were placed on a microscope slide and squashed in a drop of the nutrient medium for pollen cultivation (0·3 M sucrose in 0·001 per cent boric acid). All the tissues of the anther walls were removed, so that only the pollen grains were left. The slides thus prepared

TABLE 1

COMPARISON OF THE RESULTS obtained on culturing anthers in the basic medium and in that supplemented with uracil

Medium	Time of cultivation in days	Number of anthers set in culture	Anthers evaluated		Mean lengths of pollen tubes after 6 h [μm]
			Number	[%]	
Basic medium	10	44	9	20·5	592
		36	3	8·3	530
	11	60	8	13·3	507
		40	12	30·0	357
Basic medium + uracil	10	24	6	25·0	322
	11	44	3	6·8	272
Pollen grains grown in nature					356*

* After a 3 h cultivation

The effect of uracil is equivocal; however, the pollen tubes originating from the treated pollen grains did not seem to achieve the lengths of those from the controls.

TABLE 2

COMPARISON OF THE SIZE of pollen grains grown in nature with those developed in artificial media

Pollen grains	Diameter [μm]
Grown in nature	35—40
Developed *in vitro*	25
„Hypertrophic"	85—170

were kept in a moist chamber in an incubator at 26° C for 6 h. Then the preparations were stained with aniline blue in lactophenol. The germinated pollen grains were counted and the lengths of the pollen tubes were measured (Tabs. 1 and 2).

When using the cultivation technique mentioned, the anthers set in culture at the stage of pollen tetrads provided more reliable results than those cultured following an earlier stage of development, which, after two or three days of cultivation, became black and withered. Also when cul-

turing anthers at the stage of pollen tetrads, some of them withered after two or three days. However, some of them maintained their green colour up to the end of cultivation, a portion became yellow-green and maintained the colour, whereas only a small number of anthers became intensively yellow and resembled, in both colour and size, the anthers grown in nature. The reproducibility of the experiments was low, and thus the quantitative evaluation does not provide any evidence on the difference between the experiments, in which the effect of the basic medium alone was compared with that supplemented with uracil (Tab. 1). "Giant" pollen grains appeared in the cultures and germinated in some cases (Fig. 1). The occurrence of such "giant" pollen grains was described by NAKATA and TANAKA (1968) and called "hypertrophic pollen grains" (Fig. 1, Tab. 2). However, their viability (germinating capacity) was not mentioned. Like TAYLOR (1967) we too failed to repeat the results of VASIL (1957, 1959, 1963).

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Byly pěstovány prašníky *Nicotiana tabacum* L. *in vitro* ze stadia pylových tetrád, které se v našich pokusech osvědčilo jako nejspolehlivější výchozí stadium pro kultivaci *in vitro*, až do stadia zralých pylových zrn, jednak na živné půdě podle Ita a Sterna, jednak na této půdě s uracilem. Kvantitativně byly vyhodnoceny klíčivost vypěstovaných pylových zrn a délka pylových láček.

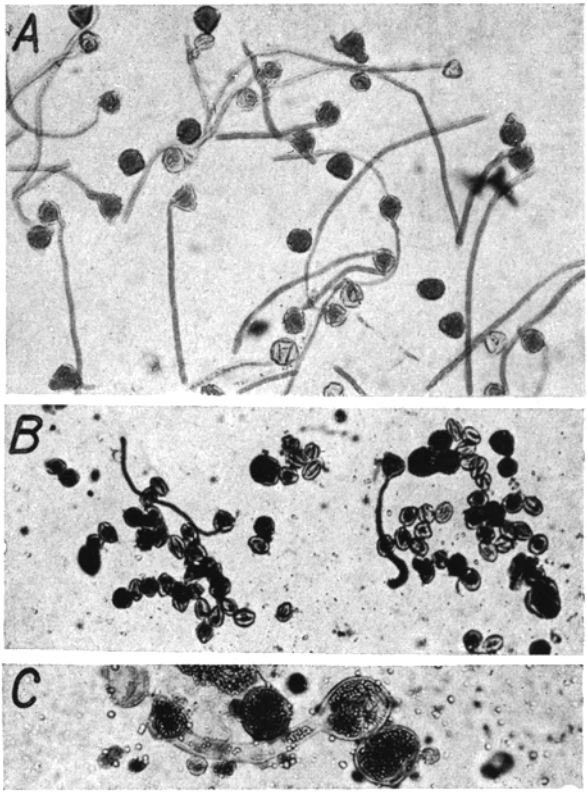

Fig. 1. Comparison of pollen grains and pollen tubes. *A*: pollen developed *in vivo* and cultivated *in vitro* for 3 h; *B*: pollen developed in the anthers cultured at the stage of pollen tetrads *in vitro* for 12 days, germinated *in vitro* for 6 h; *C*: "hypertrophic" pollen grains, one germinating. Magnification 120×.