Development of hybrid plants from ovules of *Nicotiana tabacum* pollinated in vitro with pollen grains of *Nicotiana knightiana*

A. Ślusarkiewicz-Jarzina and M. Zenkteler[1]

Institute of Plant Genetics, Polish Academy of Science, Strzeszyńska 30/36, 60-479 Poznań (Poland), April 5, 1983

Summary. Hybrid embryos and endosperm were developed in ovules of *Nicotiana tabacum* after pollination in vitro with pollen grains of *Nicotiana knightiana.* Embryos produced calluses which after transferring to fresh medium differentiated into shoots which later formed plants. In 8 plants among 14 fully-developed plants growing in pots the chromosome numbers were 2n = 36; the remaining 6 plants were aneuploids. All plants were male and female sterile.

Test-tube pollination of ovules may be useful in overcoming certain barriers of incompatibility which lie in stigma and style. The method of sexual in vitro hybridization has already been successfully applied to obtaining interspecific hybrids among some species of *Caryophyllaceae*[2]. Lately this method has been applied for the development of hybrids between *Nicotiana alata* × *N. debney*[3] and *N. tabacum* × *N. rustica.* Among 86 species of *Nicotiana* there are only 9 known interspecific hybrids developed by means of pollination of stigmas in vivo[5–8]. Maliga et al.[9] obtained hybrid plants from fused protoplasts of *Nicotiana tabacum* with *N. knightiana*; however, all the plants possessed a

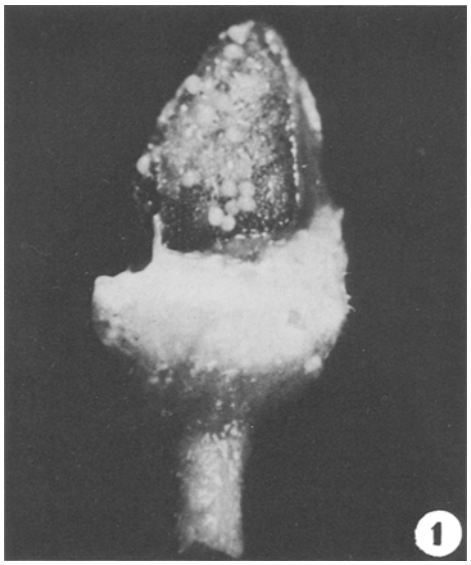

Figure 1. 4-day-old culture; developing ovules of *Nicotiana tabacum* situated on the placenta after pollinating in vitro with pollen grains of *N. knightiana.* (× 7).

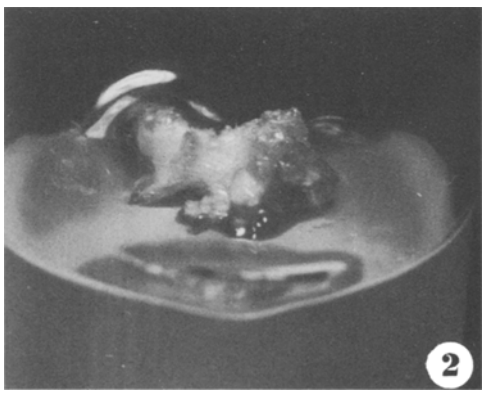

Figure 2. Callus tissue produced from hybrid embryo after 3 weeks of culture on MS medium supplemented with 2 mg/l 2,4-D. (× 3).

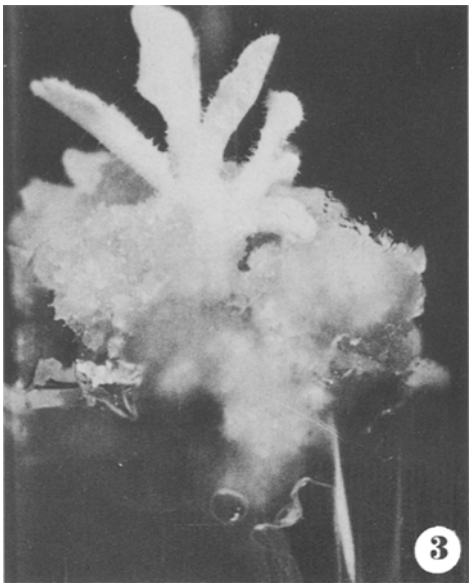

Figure 3. Differentiation of shoots in callus tissue developed from hybrid embryo on MS medium supplemented with 4 mg/l kinetin and 2 mg/l NAA. (× 2).

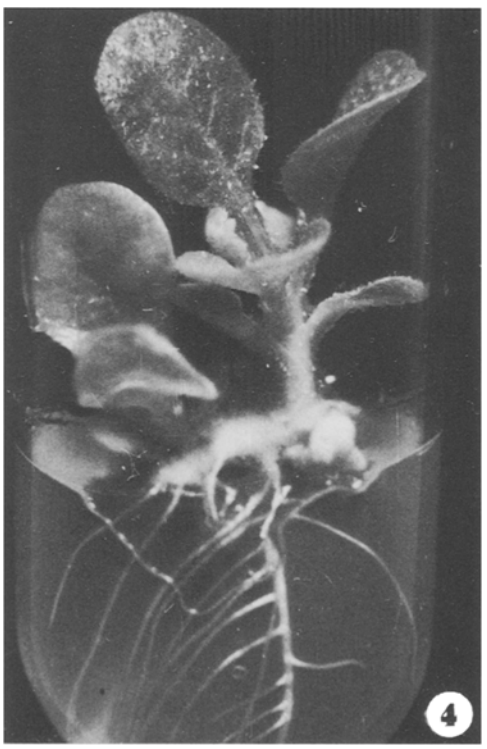

Figure 4. Fully developed hybrid plant in test-tube. (× 2).

Figure 5. *A* and *C* Parental plants; A, *Nicotiana tabacum*; C, *Nicotiana knightiana*. (×0.5). *B* Hybrid plant and the somatic number of chromosomes 2n = 36. (×2500).

varying number of chromosomes, usually ranging between 44 and 131. The present report concerns the development of hybrids derived from the ovules of *N. tabacum* cv. Samsun pollinated in vitro with pollen grains of *N. knightiana*. Goodsp.

Flower buds, from which ovules were to be obtained for culture work, were bagged 2 days before pollination. Pistils were briefly surface-sterilized with 70% ethanol, then with 0.1% mercuric chloride for 3 min. Following surface-sterilization the pistils were rinsed 3 times in sterile water. Later the style and the ovary wall were removed and the ovules along with the placenta were inoculated on the medium prescribed by Nitsch[10]. The same day, anthers were excised from the still-closed flower buds and kept for 2–4 h in the sterile inoculation chamber. Later the pollen grains were scooped out and spread on the surface of the cultured ovules. All the experimental material was cultured in the dark at a temperature of 22–26 °C.

After the 1st 3 days of culture some of the pollinated ovules enlarged (fig. 1). Dissection of those ovules revealed normally developed proembryos and endosperm. Enlarged ovules, 7 or 8 days after pollination, were transferred to the nutrient medium prescribed by Murashige and Skoog[11] (MS) supplemented with 2 mg/l of 2,4-dichlorophenoxyacetic supplemented with 2 mg/l of 2,4-dichlorophenoxacid (2,4-D). Three weeks later about 10% of the inoculated ovules burst and a mass of white calluses started to develop from the embryos (fig. 2). Small fragments of calluses were again transferred on the MS medium supplemented with 4 mg/l of kinetin (K) and 2 mg/l of naphtaleneacetic acid (NAA). During the next 3 weeks of culture calluses produced shoots (fig. 3) which, after transferring on to MS medium containing 2 mg/l of indoleacetic acid (IAA), developed into fully formed plants (fig. 4). The 8-week-old plants, after thorough washing in water, were transferred to soil in pots and raised in the greenhouse. After another 6 weeks plants started to produce flowers. Thus 14 plants fully formed with flowers were obtained.

Caryological investigations of squashes of the root tips revealed in 8 plants the presence of 36 chromosomes (fig. 5, B). In the remaining 6 plants there was an aneuploid number of chromosomes. The preliminary analysis of meiosis in anthers and the cytoembryological analysis of ovules revealed the occurrence of various abnormalities, the consequence of which were a complete male and female sterility of hybrid plants (2n = 36). All the 8 plants showed an intermediate phenotype between the parental plant species (figs. 5, A–C), particularly in the shape and size of leaves, flowers, ovaries and anthers and in the color of leaves and flowers.

1 Reprint requests to M.Z., Department of General Botany, Institute of Biology, Adam Mickiewicz University, PL-61-713 Poznań (Poland).

2 Zenkteler, M., Int. Rev. Cytol. suppl. *11B* (1980) 137.
3 Zenkteler, M., Slusarkiewicz-Jarzina, A., and Woźna, J., Bull. Soc. Amis Sci. Lett. Poznań Sér. D*21* (1981) 79.
4 Marubashi, W., and Nakajima, T., 5th Int. Congr. Plant Tissue and Cell Culture, Tokyo 1982, Abstract No. 250.
5 Goodspeed, T.N., Chronica Botanica Waltham, Mass. USA 1954.
6 Lunyeva, M.Z., Poddubnaja-Arnoldi, V.A., Butenko, R.G., and Bhandari, N.N., Phytomorphology *3* (1970) 270.
7 Lunyeva, M.Z., Butenko, R.G., and Poddubnaja-Arnoldi, V.A., Citołogia Genetika *4* (1972) 291.
8 Lunyeva, M.Z., Poddubnaja-Arnoldi, V.A., and Dzhalilova, Kh.Kh., Bot. Zurnal *59* (1974) 874.
9 Maliga, P., Kiss, Z.R., Nagy, A.H., and Lazar, G., Molec. gen. Genet. *163* (1978) 145.
10 Nitsch, J.P., Am. J. Bot. *38* (1951) 566.
11 Murashige, T., and Skoog, F., Physiologia Pl. *15* (1962) 473.

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