

## In vitro Production of Haploid Plants from Pollen Grains of *Atropa belladonna* L.

The production of haploid plants in large numbers from pollen grains culture may become a suitable method for the cultivation of higher plants. The development of haploid plants from the male gametophyte has already been described for the following species: *Datura innoxia*<sup>1</sup>, several species of *Nicotiana*<sup>2-4</sup> and several varieties of *Oriza sativa*<sup>5</sup>. Here is a brief account concerning the formation of plants from the uninucleate pollen grains of *Atropa belladonna*.

Unopened buds of *A. belladonna* growing wild in the Botanical Garden, University, Poznań, were sterilized by dipping in 70% ethanol, immersing for 15 min in chlorine water and rinsing several times with sterile water. Anthers were removed aseptically and planted in the following nutrient media described by: 1. NITSCH<sup>6</sup> containing  $\text{FeSO}_4$  - 55 mg/l; 2. WHITE<sup>6</sup>; 3. LINSMAIER and SKOOG<sup>7</sup> containing kinetin - 4 mg/l and IAA - 2 mg/l. The inoculated anthers varied in maturity from the archesporial stage to the mature pollen grains. The youngest anthers did not grow in any medium and dried up after about 4 weeks. Those inoculated at the stage of binucleate pollen grains grew larger during the first 2 weeks of culture and after the next 6 weeks burst and dried up.

Anthers inoculated at the stage of uninucleate pollen grains when cultured on LINSMAIER and SKOOG medium

reacted differently. 5 weeks after plantation certain pollen grains increased in size, divided and formed spherical masses of cells (Figure 1). After that the normal stages of embryogenesis were observed. When embryos reached the 'torpedo' stage, the anthers burst and the embryos started to develop into seedlings (Figure 2). Some abnormalities were observed in the number and shape of cotyledons. This included the fusion of cotyledons or formation of polycotyledons. In certain embryos cotyledons were very short and did not grow larger during the further process of the development of seedlings. Mature embryos developed in about 18% of the inoculated anthers. In those anthers a various number of embryos was formed in each theca, usually 6-7, sometimes even 20 embryos in different stages of development were found. On NITSCH's medium only about 1% of the inoculated anthers produced mature embryos. On WHITE's medium embryos were never formed. The connective tissue of anthers did not produce callus or organ structures in any of the 3 media.

Embryos which were cultured on LINSMAIER and SKOOG medium produced a mass of different sized plantlets after the next 3 weeks (Figure 3). When separate shoots were transferred on the same medium they gave rise to new shoot buds with a poorly developed root system. In order to obtain fully developed plantlets, the shoot buds were transferred on NITSCH's medium where they continued to grow for the next 3 weeks. After the plantlets had reached the size of about 4 cm, and had developed a sufficient root system, they were removed and placed for several days on the wet bolting cloth with their roots submerged in water. Finally the plants were transferred to pots with soil (Figure 4).

Chromosome counts in the squashed root - tips and leaf primordia of plantlets revealed the presence of 36 ( $n$ ) chromosomes. In certain plants which developed from the same haploid embryo 72 ( $2n$ ) and 108 ( $3n$ ) chromosomes were found.

Further work is in progress to clarify this phenomenon of obtaining homozygous diploid and triploid plants from haploid embryos.

**Zusammenfassung.** Pollen aus Antheren von *Atropa belladonna* verschiedener Reifestadien können unter geeigneten in vitro Kulturbedingungen zu haploiden, diploiden oder triploiden Pflanzen heranwachsen.

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Poznań (Poland), 16 February 1971.

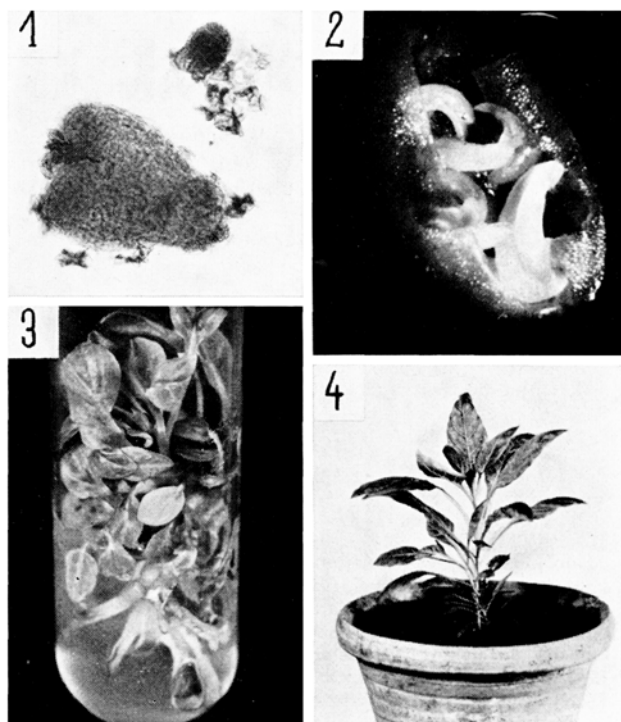


Fig. 1. Stages of embryonic development from pollen grains; globular and 'heart' stage embryos surrounded by pollen grains after 6 weeks of culture.  $\times 60$ .

Fig. 2. Embryos emerging from an anther after 8 weeks of culture.  $\times 10$ .

Fig. 3. Plantlets produced from pollen grains after 11 weeks of culture.  $\times 1/3$ .

Fig. 4. Haploid plant of *Atropa belladonna* raised from pollen grain ( $1/6$  of natural size).

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<sup>8</sup> Acknowledgments. I wish to thank the head of the Department of General Botany Prof. A. SZWEYKOWSKA for the facilities and critical reading of this report.