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The Isolation of Tissue Culture of *Populus alba L. 'pyramidalis'*

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Abstract. Tissue culture was isolated from the stem of *Populus alba* L. '*pyramidalis*'. Callus formation was observed since November till March (1974), *i.e.* till the formation of calluses suitable for further subcultivation. The most vigorous growth was obtained with the callus culture cultivated on the nutrient medium of DIAZ-COLON *et al.* (1972) on which more than 11 g of fresh matter was produced after 30 d at the end of the first year of cultivation in darkness, with inoculum weight 1.5—1.8 g. A mild decrease in growth rate of the tissue culture was observed after the first year of cultivation. When illuminated, the originally yellow calluses turned green. The morphological and anatomical structure of the callus culture is also described and cell shape and cell size evaluated.

Cell cultures, cultivated *in vitro*, have recently been used with increasing frequency as model material for plant physiological studies (cell growth studies, cell differentiation studies *etc.*), as well as for the investigation of cell metabolism (STREET 1974). They were also used in timber research, when studying the possibility of enhancing growth, or the possibility of improving timber quality (DURZAN and CAMPBELL 1974).

In this paper the isolation and the basic biological characteristics of tissue culture isolated from the stem of *Populus alba* L. '*pyramidalis*' are described.

Material and Methods

Isolation and Cultivation

4 to 6 cm long and 8 to 12 mm thick internodal cuttings from the shoots of *Populus alba* L. '*pyramidalis*' were used for the isolation of tissue culture. The cuttings were sterilised by immersing them in ethanol and by treating them several times with flame. The cuttings were then cut into 3—6 mm segments, explanted onto the nutrient medium of MURASHIGE and SKOOG (1962), containing per litre 30 mg of glycine, 5 mg of nicotinic acid, 1 mg of thiamine, 1 mg of pyridoxine and 0.1 mg of 2,4-dichlorophenoxyacetic acid (ERDELSKÝ 1971), and kept in the dark. The isolated callus culture was further cultivated on nutrient according to MURASHIGE and SKOOG (1962) containing per litre 2 mg of glycine, 0.5 mg of nicotinic acid, 0.1 mg of thiamine, 0.5 mg of pyridoxine, 1 mg of indole-3-acetic acid, 0.5 mg of naphthalene acetic acid, 0.1 mg of 2,4-dichlorophenoxyacetic acid (DIAZ-COLON *et al.* 1972).

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The explants were cultured in 100 ml Erlenmeyer flasks containing 25 ml of agar (0.8%) nutrient medium, at 26 ± 1 °C, in the dark or in diffuse incandescent light with the intensity of 800 lx and 8 h long light period.

Evaluation of the Obtained Material

In the experiments in which callus formation was to be obtained, the percentage of callus-forming explants was followed as well as the morphological appearance of the formed callus. Fresh weight of the formed material, percent increase in fresh weight (with the weight of inoculum being 100%) and percent dry weight were determined when following growth characteristics of the tissue culture. Morphological appearance and anatomical structure of the calluses were followed in sections of paraffin embedded tissue, and cell dimensions and cell shape were followed in squashes (1000 cells grown in the light and the same number of cells grown in the dark were measured), when the tissue culture was investigated from the morphological and anatomical points of view. When sections were made, the material was fixed in Navashin's fixation solution modified by Taylor (Němec *et al.* 1962) and further prepared by means of usual preparation techniques.

Results and Discussion

Seven different nutrient media were used during our attempts to isolate tissue culture from the stem of *Populus alba* (in the period from November 1973 to March 1974). Positive results were achieved with the nutrient medium of Murashige and Skoog (1962) as modified by Erdelský (1971). Despite a sharp decrease in the frequency of callus-forming explants (Fig. 1) in December and January (from 50% callus-forming explants at the beginning of November to 5.8% in January), callus formation could be observed during the entire experimental period. That is in agreement with the results of Chardenon and Taris (1960) and of Bychenkova (1963), who also observed an all-year round activity of poplar explants. Calluses formed since November till February were hard and lignified (Fig. 2a), difficult to separate from the original explant and unsuitable for further cultivation. Only the calluses formed at the end of March (Fig. 2b) were soft, with a friable structure, easily separable from the original explant, viable and capable of further cultivation. The percentage of callus-forming explants again rose to 58.3% at this time.

Tissue culture, maintained on the same medium on which it was isolated, produced only minute weight increments. For this reason a suitable nutrient medium was sought which would enable a sufficiently vigorous growth of the culture. The nutrient medium according to Diaz-Colon *et al.* (1972) proved to be the best of several media which represented different modifications of the original Murashige and Skoog (1962) nutrient medium [*i.e.* after Brown and Lawrence (1968), with the addition of organic compounds according to Harvey (1967), and according to Erdelský (1971) with kinetin (1 mg l^{-1}) and with succinic acid (1 mg l^{-1}) added by us]. Callus culture cultivated on this medium produced at the end of the first year of cultivation in darkness in 30 d more than 11 g of fresh weight with inoculum weight of 1.5–1.8 g. From the data presented on growth dynamics in the course of 40 d (Fig. 3) it follows that vigorous growth occurred between days 10 and

30 after inoculation. Growth practically stops after 30 d of cultivation. The percentage of dry weight increases in the first growth stages which indicates that cell metabolism in the tissue culture is enhanced at this time. By contrast a highly significant decrease occurs between days 10 and 20 which shows that the high increments in fresh weight in this period are connected with the uptake of water by the cells.

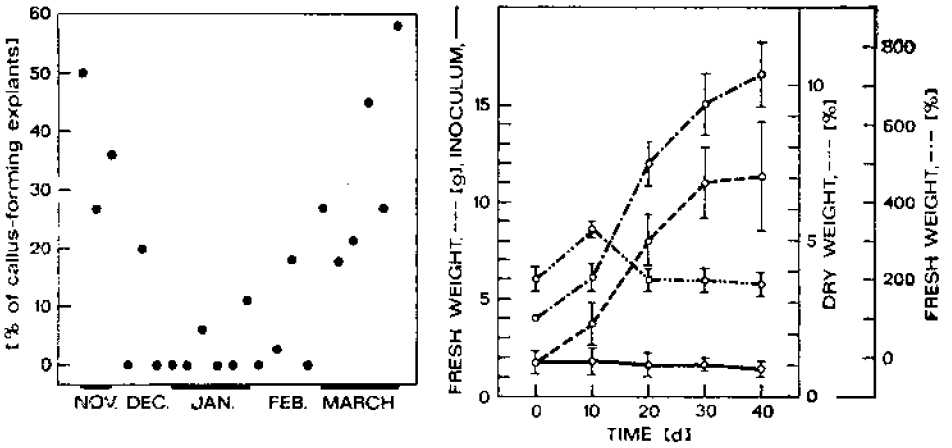

Fig. 1. The rate of callus formation on the explants of *Populus alba* '*pyramidalis*' in the period since November to March (expressed in per cent of callus-forming explants).
Fig. 3. Growth rate of tissue culture of *Populus alba* cultivated in darkness.

Fluctuations in growth rate were observed in the course of the first year (1974) of cultivation. Growth steadied after this period. However a decrease in growth rate could be observed simultaneously. The percentage of increase in fresh weight, with passage of 20 d, was in December 1974 450.6% in comparison to 365.3% a year later (Fig. 4). Despite the fact that this decrease is not significant statistically, we observed it in all our experiments. A similar observation was recorded by WINTON and MATHES (1973) with *Populus tremuloides* cultures.

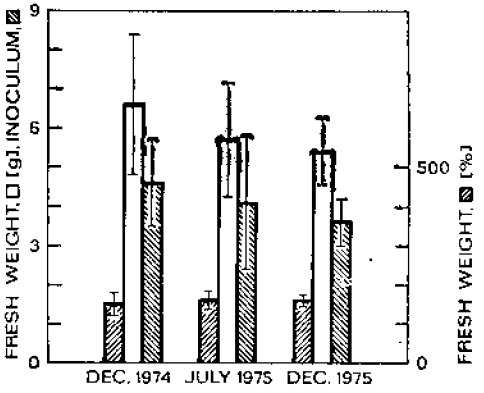

Fig. 4. Growth stability of tissue culture of *Populus alba* cultivated in darkness in the course of one year. Dashed column: inoculum; empty column: fresh weight; dotted column: % of fresh weight.

The influence of light (800 lx) on the growth of poplar tissue culture is only minute and statistically insignificant (Table 1). The difference in dry weight content which is higher in the cultures cultivated in the light in comparison with the cultures kept in darkness is also on the margin of statistical significance ($t = 2.2190$ for $N = 16$). We suppose this to be the result of low irradiance intensity.

TABLE 1

A comparison of growth rate of tissue culture of *Populus alba* '*pyramidalis*' cultivated either in the light or in the dark for 24 d after inoculation

	Inoculum		Fresh weight		% fresh weight		% dry weight	
	$\bar{x}$	$s_{\bar{x}}$	$\bar{x}$	$s_{\bar{x}}$	$\bar{x}$	$s_{\bar{x}}$	$\bar{x}$	$s_{\bar{x}}$
Dark	1.62	0.03	5.42	0.28	365	18.8	3.76	0.10
Light	1.70	0.04	5.92	0.27	374	14.7	4.03	0.06

$n = 15$

The calluses of the tissue culture of *Populus alba*, cultivated in darkness, are light yellow in colour, with a clod-like structure, crumbling under pressure, with the crumbling increasing in calluses older than 28 d. When cultivated in light, the calluses turn green due to the differentiation of chloroplasts (Fig. 5). These calluses are slightly harder and more compact in comparison with calluses of the same maturity grown in the dark. Development of a green colour in poplar calluses grown in the light was also described by WINTON and MATHES (1973) and by CHALUPA (1974).

As far as anatomical features are concerned, the callus tissue of *Populus alba* is formed by isodiametrical, easily separable, undifferentiated cells (Fig. 6), with individual tracheal cells (Fig. 7). The sections of 24-day-old calluses show that the cells are situated closely next to each other without large intracellular spaces (Fig. 8). An internal disruption occurs in older calluses (35 d after inoculation) accompanied by the formation of large intercellular spaces (Fig. 9). This fact explains the crumbling of older calluses. A higher number of small, concentrically arranged cells could be observed in the surface parts of the callus. They usually developed round the tracheal

TABLE 2

Cell size in tissue culture of *Populus alba* '*pyramidalis*'

Interval [μm]	Cell number [%] light	darkness
to 20	2.60	1.39
21 — 40	37.94	46.44
41— 60	45.20	43.78
61— 80	12.03	6.47
81—100	1.64	0.59
over 100	0.54	1.39

cells (Fig. 10). Similar cell aggregates were observed in callus culture of *Ginkgo biloba* by BARDINSKAYA and MOSKALEVA (1960) who took them for meristematic centres.

The size of most cells of *Populus alba* tissue culture (Table 2) fluctuates between 20 and 60 μm. Cells of smaller or larger dimensions were observed only sporadically. HRICOVÁ *et al.* (1974) reported similar cell dimensions (40 to 60 μm) in cell culture of *Picea excelsa* L.

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Figures at the end of the issue.

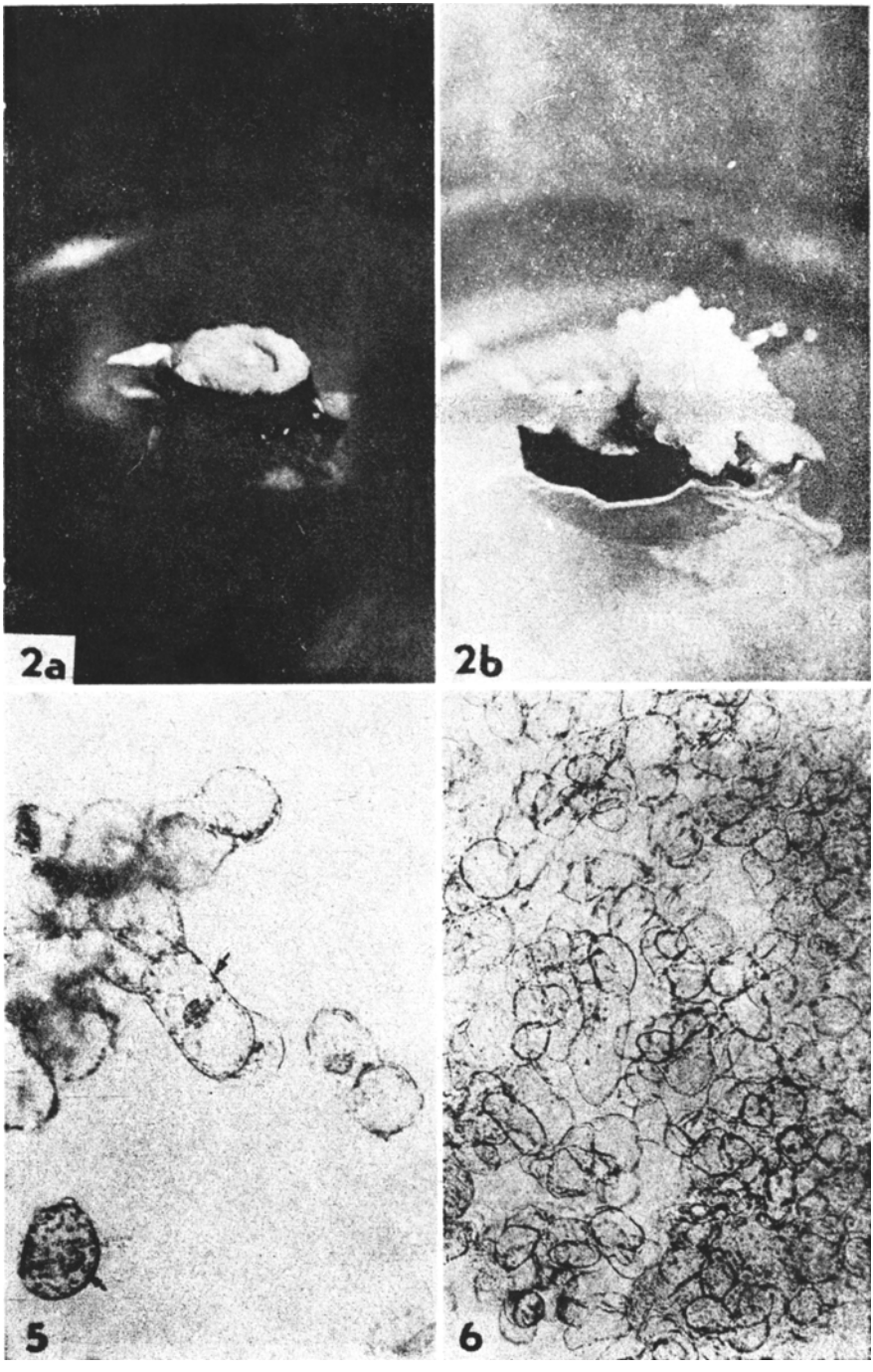

Fig. 2. Callus formation on a primary explant from the stem of *Populus alba*.
Fig. 5. Cells of tissue culture of *Populus alba* cultivated in the light. (↑) chloroplasts. Magn. 285×.
Fig. 6. Cells of tissue culture of *Populus alba* cultivated in darkness. Magn. 285×.

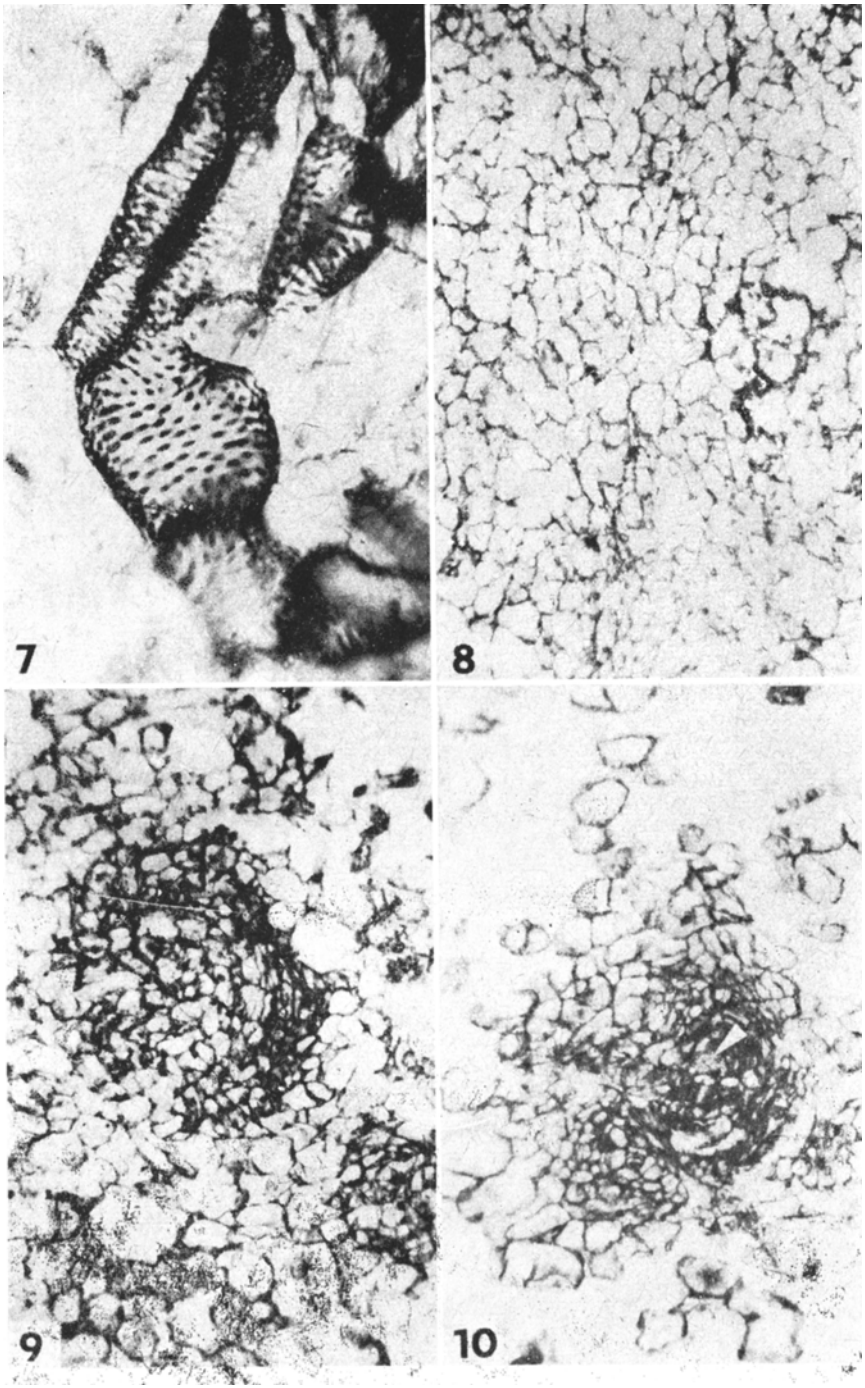

Fig. 7. Tracheal cells in tissue culture of *Populus alba*. Magn. 600×.
Fig. 8. A section across callus tissue of *Populus alba*, 24 d after inoculation. Magn. 100×.
Fig. 9. A section across callus tissue of *Populus alba*, 35 d after inoculation. Magn. 100×.
Fig. 10. A section across callus tissue of *Populus alba*, 35 d after inoculation. (↑) tracheal cells. Magn. 100×.