Xylem formation in isolated stem segments of *Pinus silvestris* L.

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A new technique for studying the morphogenesis of xylem in conifers has been recently developed by Brown and Wodzicki (1969). The authors used isolated stem segments of *Larix leptolepis* Endl., and *Pinus densiflora* Sieb. and Zucc. aseptically inserted into latex tubing connected to a glass reservoir with nutrient medium at the upper end and a receiver flask at the bottom. The method allows the investigation of xylem formation under natural physical conditions of undisturbed tissue, supplied directly with nutrient medium of any desired composition. The experiments proved the possibility of normal xylem formation within a period of 28 days. The extension of studies beyond this period seems to be justified, if all stages of xylem morphogenesis, especially the constant reproduction of normal cambial initials is to be studied. The time may be limited when the effect of various constituents of the medium is investigated, because the stem segments may contain a reserve of substances essential for initiation or normal differentiation of xylem over shorter periods.

The obvious advantages of the method for investigation of factors controlling xylem morphogenesis prompted the author to try it on isolated stem segments of *Pinus silvestris* L. — the main timber species in Poland.

MATERIAL AND METHODS

The youngest terminal shoots of four-year-old *Pinus silvestris* L. trees were sectioned and aseptically introduced into culture system described by Brown and Wodzicki (1969). The nutrient medium recommended by these authors was considerably diluted and modified as given in Table 1. Culture was initiated on February 20, 1969 before the spring initiation of cambial activity occurred (Fig. 1). Three groups of 5 segments each were collected after 21, 37, or 65 days of culture. The medium of two other groups was exchanged after 37 days for medium deficient in IAA or GA. The segments of these two groups were collected after additional 28 days. No contamination of the tissue was observed during the whole period of experiment.

Figs 1—3. Xylem formation in isolated stem segments of *Pinus silvestris* L. Transverse sections of stems before culture (fig. 3), after 21 days (fig. 2) and 37 days in culture (fig. 1)

Transverse, radial, and tangential free-hand sections of all segments were stained with safranin and light-green S. F., or phloroglucinol — HCl and examined under a Zeiss optical microscope.

Table 1

Composition of medium used for culture of isolated stem segments

Major elements	Mg/liter	Minor elements	Mg/liter	Organic supplements	Mg/liter
NH ₄ NO ₃	82.5	H ₃ BO ₃	2.48	Inositol	10.0
KNO ₃	95.0	MnSO ₄ ·4H ₂ O	8.92	Ascorbic acid	10.0
CaCl ₂ ·2H ₂ O	22.0	ZnSO ₄ ·4H ₂ O	3.44	Nicotinic acid	0.05
MgSO ₄ ·7H ₂ O	18.5	KI	0.33	Pyridoxin — HCl	0.05
KH ₂ PO ₄	8.5	Na ₂ MoO ₄ ·2H ₂ O	0.10	Thiamin — HCl	0.01
		CuSO ₄ ·5H ₂ O	0.01	L — arginine	25.0
		CoCl ₂ ·6H ₂ O	0.01	Sucrose	20000
		FeSO ₄ ·7H ₂ O	0.74	Indole-3-acetic acid	5.0
		Na ₂ — EDTA	0.56	Kinetin	0.2
				Gibberellic acid	1.0

RESULTS AND DISCUSSION

Production of fully differentiated tracheids was observed in segments collected as early as after 21 days (Fig. 2). Only one of these segments revealed no cambial activity. During the following days, xylem rapidly formed and the number of cambial xylem derivatives after additional 16 days reached 20 in some radial files (Fig. 3). However, the production of xylem in various stem segments differed significantly. There were no new mature tracheids formed in one of the stem segments collected 37 days after the experiments started. Formation of xylem ceased soon after its rapid differentiation. No new cambial xylem derivatives were found after additional 28 days (Table 2). Neither did the segments with excluded IAA or GA during this part of the experiment produce additional xylem. The xylem produced during the first period was microscopically normal. The cell wall of mature tracheids was lignified. The medullary rays consisted of parenchyma and ray tracheids with folded inner surfaces of cell wall characteristic for *Pinus silvestris* L. Multiseriate rays with radial resin canals were observed in addition to the uniseriate rays on tangential sections. A few longitudinal resin canals were also newly differentiated.

The method proved to be suitable for investigation of xylem formation in *Pinus silvestris* L. to the same degree as for *Larix leptolepis* Endl. and *Pinus densiflora* Sieb. and Zucc. known from the literature. Moreover, it confirmed the supposition that the time of xylem formation under the conditions of the experiment is still greatly limited. At this step of investigation it is impossible to say what is the cause preventing xylem formation over a longer periods. The possibility of exhaustion

Table 2

Number of new tracheids formed in isolated stem segments cultured in sterile conditions. Averages of 5 stem segments

Number of days	Treatment	Average number of new tracheids formed under experimental conditions		
		Radially enlarging xylem*	Maturing and mature xylem*	Total
21	Full medium	0.70	0.85	1.55
37		2.30	4.15	6.45
65		2.20	2.35	4.55
65	Medium deficient in GA	2.30	3.75	6.05
65	Medium deficient in IAA	2.60	1.35	3.95

* According to terminology proposed by Wilson et al. (1966).

of the reserve of some substances essential for continuous xylem formation and absent in the medium as well as the production of some by products of the altered metabolism of the cell which could inhibit the formation of xylem should be taken into account. However, also possibility of the interference of other factors disturbing the normal process of xylem differentiation such as the imposed transport of nutrients through the mature xylem or the disturbance of the normal osmotic regime of tissues may not be overlooked.

SUMMARY

Stem segments of *Pinus sylvestris* L. were cultured in a system supplying the liquid nutrient medium containing vitamins, plant growth substances and sucrose directly to the mature xylem in sterile conditions. Normally differentiated xylem is formed within 37 days under the experimental conditions described. The method does not so far permit formation of xylem over longer periods.

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LITERATURE

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Tworzenie się drewna w izolowanych odcinkach pędów Pinus sivestris L.

STRESZCZENIE

Izolowane odcinki pędów *Pinus silvestris* hodowano w warunkach sterylnych na płynnej pożywce zawierającej witaminy, substancje wzrostowe i sacharozę dostarczaną bezpośrednio do w pełni zróżnicowanego drewna. Stwierdzono tworzenie się nowych normalnie zróżnicowanych cewek w ciągu 37 dni trwania doświadczenia. W późniejszym okresie nie zaobserwowano dalszego przyrostu liczby cewek.