ACTA SCIENTIARUM POLONORUM *Acta Sci. Pol., Hortorum Cultus 11(2) 2012, 3-13*

DEVELOPMENT OF ROYAL PAULOWNIA (*Paulownia tomentosa* Steud.) *in vitro* SHOOT CULTURES UNDER THE INFLUENCE OF DIFFERENT SACCHARIDES

Wojciech Litwińczuk, Edyta Bochnia
University of Rzeszów

Abstract The influence of chosen saccharides on the growth and development of the *in vitro* shoot cultures of *Paulownia tomentosa* – valuable and multi-purpose tree species. The mainly sucrose (10–50 g dm^{-3}), glucose and fructose (15.8 and 30 g dm^{-3}) were tested in three subsequent passages. Then the usefulness of saccharides in different applications of *in vitro* cultures was assessed. The basic MS medium supplemented with BA (1 mg dm^{-3}), NAA (0.1 mg dm^{-3}), and GA_3 (0.1 mg dm^{-3}) was used. Saccharides in tested concentrations influenced development of *in vitro* cultures. Proliferation of axillary shoots remained comparable whereas the development of adventitious shoots significantly and the frequency of vitrification decreased while the concentration of sucrose increased from 20 to 40 g dm^{-3}. Glucose (15.8 g dm^{-3}) stimulated both proliferation and elongation of axillary shoots. Sucrose (10 g dm^{-3}), and fructose (15.8 g dm^{-3}) favoured development of adventitious shoots and vitrification of cultures. The media supplemented with sucrose (30–40 g dm^{-3}) or glucose (15.8 g dm^{-3}) may be recommended for micropropagation of true-to-type plants, whereas sucrose (50 g dm^{-3}), and fructose (15.8 g dm^{-3}) for long term storage, and plant breeding, respectively.

Key words: empress tree, micropropagation, axillary shoots, adventitious shoots, sucrose, glucose, fructose

INTRODUCTION

Paulownia tomentosa Steud. (royal paulownia, empress or princess tree) is species native to China. Nowadays risen in popularity, paulownia is grown in many countries including U.S.A western and south Europe not only as ornamental tree but also multi-purpose species used among others as medicinal plant, source of renewable energy as well for reforestration and reclamation of mine sites [Rao et al. 1996, Ozaslan et al.

Corresponding author – Adres do korespondencji: Wojciech Litwińczuk, The Chair of Plant Physiology and Biotechnology, Faculty of Biology and Agriculture, University of Rzeszów, 2 Ćwiklińskiej St., 35-601 Rzeszów, Poland, tel.: (+48) 17 785 53 05, e-mail: wlitw@univ.rzeszow.pl

2005, Barton et al. 2007]. Paulownias could be propagated by seeds and root cuttings [Rao et al. 1996, Ozaslan et al. 2005]. Each method, as in the cases of the other species, has well-known advantages and disadvantages. The micropropagation seems to be a prospective method of mass-production of valuable cultivars. Some authors have already reported the method of paulownia micropropagation. It's generally based on multiplication of shoot explants on MS medium supplemented with BA

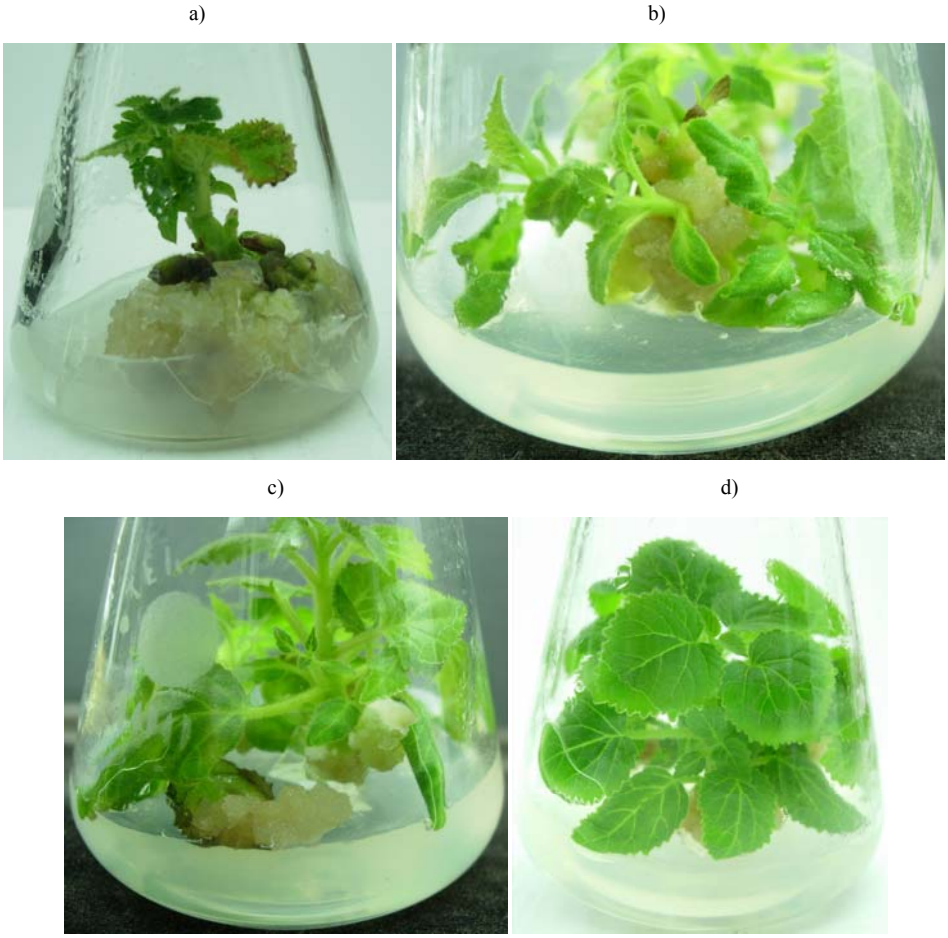


Fig. 1. Some disadvantages of *in vitro* culture of paulownia: a) excessive callus growth at the base of explants what weaken axillary shoot proliferation; b) adventitious shoots developed on callus at the explant base; c) explant pushed out the medium by emerging leaves; d) big leaves make difficult observation of cultures

Ryc. 1. Wybrane wady kultur *in vitro* paulowni: a) nadmierny wzrost kalusa u podstawy eksplantatu, co hamuje proliferację pędów kątowych; b) pędy przybyszowe powstałe na kalusie u podstawy eksplantatu; c) eksplantat wypchnięty z pożywki przez rozrastające się liście; d) duże liście utrudniają obserwację kultur

(1.0–3.0 mg dm^{-3}), NAA, IAA or IBA and GA_3 (all at 0.1 mg dm^{-3}) [Marcotrigiano and Stimart 1983, Rao et al. 1996, Kumar et al. 1998, Ipekci et al. 2001, Rout et al. 2001, Ozaslan et al. 2005, Rajbahak and Sah 2010]. However, some negative effects of propagation in such conditions could be observed. Marcotrigiano and Stimart [1983] first mentioned excessive growth of callus at the base of explants, and development of adventitious shoots on callus. We encountered the same phenomena as well as symptoms of vitrification, and formation too large leaves which weaken axillary shoot proliferation and make difficult observation of cultures (fig. 1). The authors of available articles concerning micropropagation of paulownias used mainly media supplemented with sucrose (30 g dm^{-3}) [Marcotrigiano and Stimart 1983, Rao et al. 1996, Kumar et al. 1998, Rout et al. 2001, Ipekci and Gozukirmizi 2003, Ozaslan et al. 2005, Rajbahak and Sah 2010]. The only Bergmann and Heung-Kyu [1997] and Taha et al. [2008] used sucrose in other concentration (20 and 25 g dm^{-3}, respectively). However, they did not informed why they chose different doses of sucrose. Burger et al. [1985] and Ipekci et al. [2001] did not give what concentration of sucrose they used. It's well-known that the saccharides can modify development and quality of *in vitro* cultures of many species. However, to our best knowledge the influence of different saccharides on *Paulownia* sp. was not described. Therefore, the aim of the present study was to test chosen saccharides, and assess their usefulness in different applications of *in vitro* cultures of this species.

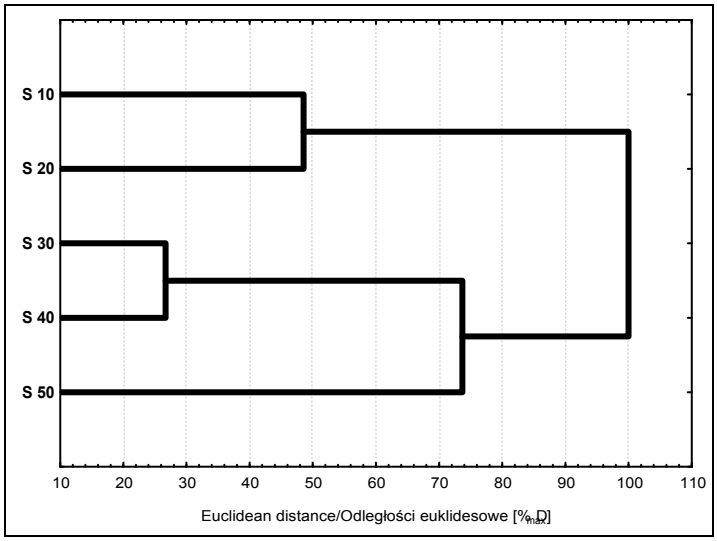
MATERIAL AND METHODS

The experiments were carried out on *in vitro* cultures of *Paulownia tomentosa* Steud. The influence of different saccharides was investigated through three subsequent one-month long subcultures. In the first experiment the sucrose in five concentrations (10–50 g dm^{-3}) was tested. In the second one – different saccharides were used. There were: glucose, fructose (15.8 and 30 g dm^{-3}). The lower concentration of monosaccharides were the molar equivalent of sucrose (30 g dm^{-3} = 88 mM, control). The preliminary experiments concerning other saccharides: mannitol and sorbitol (16.04 and 30 g dm^{-3}), galactose (15.8 g dm^{-3}), maltose (30 g dm^{-3}) were set also. They were carried out through the only one subculture as the obtained results were not promising. The basic MS medium [Murashige and Skoog 1962] supplemented with BA (1 mg dm^{-3}), NAA, and GA_3 (0.1 mg dm^{-3} both), pH 5.7, solidified with Biocorp AB 12 agar (7.0 g dm^{-3}), was applied. In the second experiment the agar concentration was increased to 8.0 g dm^{-3}. Light was provided by cool white fluorescent lamps (OSRAM) at approximately 26 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with a 16/8 hr day/night photoperiod. Temperature was set on 26ºC. Cultures were grown *in vitro* in glass jars (350 cm^{-3}) with ventilated polypropylene twist lids, filled with medium (50 cm^{-3}) and with 8 nodal explants (about 0.5 cm long and at least 2 nodes) of axillary origin per jar. In the second experiment the number of explants per jar was decreased to 5. Each treatment was represented by at least 4 jars. At the end of passage cultures were took out from jar and the number of axillary (AX) and adventitious (AD) shoots was determined. Then the ratio of AX shoots was calculated according to following formula: 100% × number of AX shoots/number of shoots (both AX and AD ones).The length and width of the biggest

leaf blade and the size of callus as well as number of cultures developed exclusively AX shoots and number of vitrified cultures were recorded also. Collected data were subjected to an ANOVA, LSD mean separation test at $\alpha = 0.05$ and analysis of regression (first experiment) using Statistica 9.0 or Statgraphics Centurion XVI computer software. Data presented as percentage were analyzed after arcsin transformation (ratio of AX shoots) or subjected to test on difference between two proportions (number of cultures developed exclusively AX shoots, number of vitrified cultures). Cluster analysis based on Ward's method and Euclidean distance was used to evaluate similarity of cultures grown on modified media. It was based on specified traits: number of axillary shoots (3–14 mm), number of axillary shoots (> 15 mm), number of cultures which developed exclusively axillary shoots, number of adventitious shoots (3–14 mm), number of adventitious shoots (> 15 mm), width of leaf blade, length of leaf blade, diameter of callus, and number of vitrified cultures.

RESULTS

Sucrose influenced development of shoot *in vitro* cultures of paulownia. In general, cluster analysis revealed that the reaction of cultures on sucrose given in lower concentrations doses (10 and 20 g dm^{-3}) was quite different than on the other treatments (fig. 2). The reaction of cultures on sucrose (40 g dm^{-3}) was close to control (30 g dm^{-3}).



S 10, S 20, S 30, S 40, S 50 – media supplemented with sucrose (10, 20, 30, 40, 50 g dm^{-3}, respectively.
S 10, S 20, S 30, S 40, S 50 – pożywki z dodatkiem sacharozy, odpowiednio w stężeniu 10, 20, 30, 40, 50 g dm^{-3}.

Fig. 2. Similarity of development of paulownia *in vitro* cultures on media supplemented with different doses of sucrose

Ryc. 2. Podobieństwo rozwoju kultur *in vitro* paulowni na pożywkach z dodatkiem różnych dawek sacharozy

Proliferation of axillary shoots was significantly weakened on the media supplemented with sucrose in the highest and lowest doses while compared to other treatments (tab. 1). However, production of long axillary shoots was highest in the presence of sucrose in doses of 20–30 g dm^{-3}, and lowest in the case of highest concentration. In general, the development of adventitious shoots decreased along with rising sucrose concentration (tab. 1, 2). The frequency of cultures, which developed the only axillary shoots increased in such conditions also. The size of leaves depended on sucrose concentration. They were smaller on media augmented with extreme doses of sucrose in comparison to intermediate (20–40 g dm^{-3}) ones. The growth of callus became more intense towards the sucrose concentration close to 30 g dm^{-3}, then slowed down and stabilized (tab. 1, 2). The vitrification of cultures was strongly suppressed while sucrose in doses higher than 30 g dm^{-3} was applied (tab. 1).

Table 1. Development of paulownia *in vitro* cultures depending on the concentration of sucrose
Tabela 1. Rozwój kultur *in vitro* paulowni w zależności od stężenia sacharozy

Analysed traits Analizowane cechy	Concentration of sucrose, g dm^{-3} Stężenie sacharozy, g dm^{-3}				
	10	20	30 control	40	50
Number of axillary shoots (3–14 mm) Liczba pędów kątowych (3–14 mm)	2.4 a [a]	2.5 a	2.6 a	2.8 a	2.6 a
Number of axillary shoots (> 15 mm) Liczba pędów kątowych (> 15 mm)	1.0 b	1.6 c	1.5 c	1.2 b	0.3 a
Total number of axillary shoots (> 3 mm) Łączna liczba pędów kątowych (> 3 mm)	3.4 a	4.1 b	4.2 b	4.1 b	2.9 a
Number of adventitious shoots (3–14 mm) Liczba pędów przybyszowych (3–14 mm)	1.3 b	0.8 b	0.6 b	0.4 a	0.4 a
Number of adventitious shoots (> 15 mm) Liczba pędów przybyszowych (> 15 mm)	0.1 b	0.2 b	0.1 ab	0.1 ab	0.0 a
Total number of adventitious shoots Łączna liczba pędów przybyszowych	1.4 c	0.9 b	0.7 ab	0.5 a	0.4 a
Total number of shoots Łączna liczba pędów	4.9 b	5.0 b	4.9 b	4.5 b	3.4 a
Ratio of axillary shoots in culture [b], % Udział pędów kątowych w kulturze, %	72.8 a	84.2 b	88.7 bc	90.7 c	90.5 c
Number of cultures which developed exclusively axillary shoots, % Liczba kultur zawierających wyłącznie pędy kątowe, %	48.1 a	67.6 b	72.8 bc	80.6 c	80.4 c
Width of leaf blade, mm Szerokość blaszki liściowej, mm	4.8 b	6.6 c	7.3 c	7.1 c	3.7 a
Length of leaf blade, mm Długość blaszki liściowej, mm	6.6 b	8.4 c	9.0 c	9.2 c	5.7 a
Diameter of callus, mm Średnica kalusa, mm	5.1 a	8.8 b	13.7 d	11.8 c	11.7 c
Number of vitrified cultures, % Liczba szklistych kultur, %	41.7 c	31.2 b	3.5 a	8.7 a	2.1 a

[a] different letters in the rows indicate significant differences among means for $\alpha < 0.05$; [b] with regard to total number of shoots
[a] odmienne litery wskazują na istotne różnice przy $\alpha < 0.05$; [b] w stosunku do łącznej liczby pędów

Table 2. Relationship among chosen traits of paulownia *in vitro* cultures and concentration of sucrose (results of regression analyses)

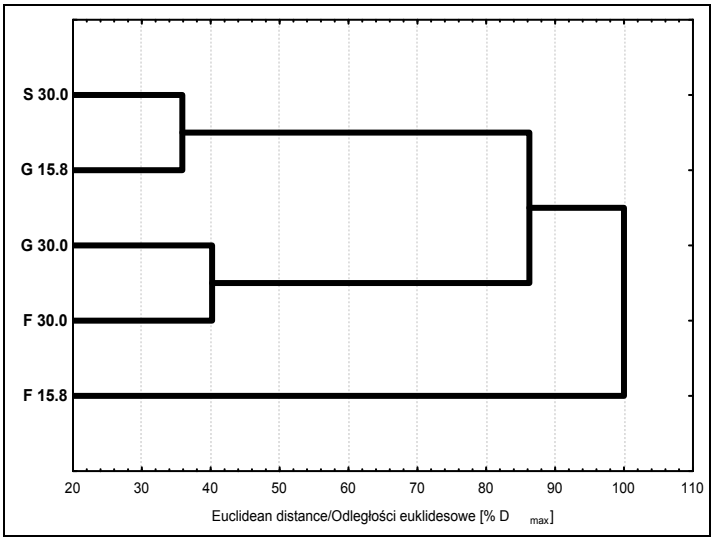
Tabela 2. Związek między wybranymi cechami kultur *in vitro* paulowni a stężeniem sacharozy (wyniki analizy regresji)

Analysed traits Analizowane cechy	Linear model Model liniowy	R^2	r_{xy}	SL
Number of axillary shoots (3–14 mm) Liczba pędów kątowych (3–14 mm)	nf	---	---	ns
Number of axillary shoots (> 15 mm) Liczba pędów kątowych (> 15 mm)	nf	---	---	ns
Total number of axillary shoots (> 3 mm) Łączna liczba pędów kątowych (> 3 mm)	nf	---	---	ns
Number of adventitious shoots (3–14 mm) Liczba pędów przybyszowych (3–14 mm)	y = exp(2.02 – 0.760*ln(x))	95.7%	-0.98	**
Number of adventitious shoots (> 15 mm) Liczba pędów przybyszowych (> 15 mm)	y = 0.21 – 0.004*x	76.5%	-0.91	*
Total number of adventitious shoots Łączna liczba pędów przybyszowych	y = exp(2.29 – 0.809*ln(x))	96.4%	-0.99	**
Total number of shoots Łączna liczba pędów	---	---	---	ns
Ratio of axillary shoots in culture, % Udział pędów kątowych w kulturze, %	y = exp(3.99 + 0.139*ln(x))	89.6%	0.96	**
Number of cultures which developed exclusively axillary shoots, % Liczba kultur zawierających wyłącznie pędy kątowe, %	y = exp(3.17 + 0.324*ln(x))	99.0%	0.99	**
Width of leaf blade, mm Szerokość blaszki liściowej, mm	nf	---	---	ns
Length of leaf blade, mm Długość blaszki liściowej, mm	nf	---	---	ns
Diameter of callus, mm Średnica kalusa, mm	y = exp(0.44 + 0.560*ln(x))	74.2%	0.89	*
Number of vitrified cultures, % Liczba szklistych kultur, %	y = 48.0 – 1.02*x	74.1%	-0.90	*

R^2 – coefficient of determination; r_{xy} – correlation coefficient; SL – significance level; nf – not fitted; ns – not significant; x – concentration of sucrose

R^2 – współczynnik determinacji; r_{xy} – współczynnik korelacji; SL – poziom istotności; nf – brak dopasowania; ns – brak istotności; x – stężenie sacharozy

The kind of saccharide and its concentration strongly influenced development of shoot *in vitro* cultures of paulownia. Cluster analysis revealed that the reaction of cultures on fructose (15.8 g dm^{-3}) was distinctly different than on other treatments (fig. 3). The development of cultures grown on medium supplemented with glucose 15.8 g dm^{-3} was most similar to control (sucrose 30 g dm^{-3}). Reaction of cultures on monosaccharides given in higher molar doses was comparable as well (fig. 3). Glucose and fructose in higher concentration significantly suppressed both proliferation and elongation of axillary shoots (tab. 3). The significant differences in axillary shoot production on media supplemented with such compounds in lower concentration and sucrose were not confirmed. However, glucose (15.8 g dm^{-3}) improved the elongation of axillary shoots (tab. 3). The development of adventitious shoots was similar for all treatments except



S 30.0, G 15.8, G 30.0, F 15.8, F 30.0 – media supplemented with sucrose (30 g dm^{-3}), glucose (15.8 and 30 g dm^{-3}), and fructose (15.8 and 30 g dm^{-3}), respectively.
S 30.0, G 15.8, G 30.0, F 15.8, F 30.0 – pożywki z dodatkiem sacharozy (30 g dm^{-3}), glukozy (15.8 i 30 g dm^{-3}) i fruktozy (15.8 i 30 g dm^{-3}).

Fig. 3. Similarity of development of paulownia *in vitro* cultures on media supplemented with different saccharides
Ryc. 3. Podobieństwo rozwoju kultur *in vitro* paulowni na pożywkach z dodatkiem różnych cukrów

for fructose (15.8 g dm^{-3}), which strongly stimulated such phenomenon. As a result the production of shoots (regardless of their origin) was the highest in such conditions whereas generally the lowest on media augmented with higher doses of fructose and glucose (tab. 3). In comparison to other cultures, explants treated with fructose (15.8 g dm^{-3}) developed more often cultures consisted both of axillary and adventitious shoots. Cultures grown on media supplemented with sucrose or glucose (15.8 g dm^{-3}) developed bigger leaves than the other ones (tab. 3). The formation of callus was much lower in the presence of fructose than sucrose and glucose. The vitrification of cultures was not observed on media supplemented with sucrose and fructose (30 g dm^{-3}). Such phenomenon was clearly intensified in the case of application of fructose in lower dose (tab. 3).

Cultures of paulownia didn't grow the presence of sorbitol (16.04 and 30 g dm^{-3}) and galactose (15.8 g dm^{-3}). Cultures fed on maltose (30 g dm^{-3}) grew much slower than on media supplemented with sucrose, glucose, and fructose. All measured vegetative traits of cultures were visibly lessened whereas considerably more cultures were vitrified (data not presented). Cultures grew on media supplemented with mannitol (16.04 and 30 g dm^{-3}) developed only very short axilarry shoots which looked as vitrified (details not presented).

Table 3. Development of paulownia *in vitro* cultures depending on the kind and concentration of saccharides

Tabela 3. Rozwój kultur *in vitro* paulowni w zależności od rodzaju i stężenia cukrów

Analysed traits Analizowane cechy	Kind and concentration of saccharide, g dm^{-3} Rodzaj i stężenie cukru, g dm^{-3}				
	sucrose sacharoza (30) control	glucose glukoza (15.8)	glucose glukoza (30)	fructose fruktoza (15.8)	fructose fruktoza (30)
Number of axillary shoots (3–14 mm) Liczba pędów kątowych (3–14 mm)	3.4 a[a]	3.7 a	3.0 a	3.5 a	3.2 a
Number of axillary shoots (> 15 mm) Liczba pędów kątowych (> 15 mm)	1.5 b	2.0 c	1.1 a	1.8 bc	1.1 a
Total number of axillary shoots (> 3 mm) Łączna liczba pędów kątowych (> 3 mm)	4.9 ab	5.7 b	4.1 a	5.3 b	4.3 a
Number of adventitious shoots (3–14 mm) Liczba pędów przybyszowych (3–14 mm)	0.2 a	0.6 a	0.3 a	2.2 b	0.2 a
Number of adventitious shoots (> 15 mm) Liczba pędów przybyszowych (> 15 mm)	0.03 a	0.2 a	0.1 a	1.1 b	0.0 a
Total number of adventitious shoots Łączna liczba pędów przybyszowych	0.3 a	0.8 a	0.4 a	3.3 b	0.2 a
Total number of shoots Łączna liczba pędów	5.1 ab	6.5 b	4.5 a	8.6 c	4.5 a
Ratio of axillary shoots in culture, % Udział pędów kątowych w kulturze, %	96.8 b	91.9 b	92.9 b	75.5 a	95.9 b
Number of cultures which developed exclusively axillary shoots, % Liczba kultur zawierających wyłącznie pędy kątowe, %	90.6 b	83.0 b	77.5 b	52.8 a	85.7 b
Width of leaf blade, mm Szerokość blaszki liściowej, mm	10.3 b	10.0 b	7.5 a	7.5 a	5.7 a
Length of leaf blade, mm Długość blaszki liściowej, mm	12.7 c	12.6 c	9.9 ab	10.0 b	8.1 a
Diameter of callus, mm Średnica kalusa, mm	18.2 c	12.4 b	17.5 c	6.5 a	8.0 a
Number of vitrified cultures, % Liczba szklistych kultur, %	0.0 a	1.9 ab	2.0 ab	7.5 b	0.0 a

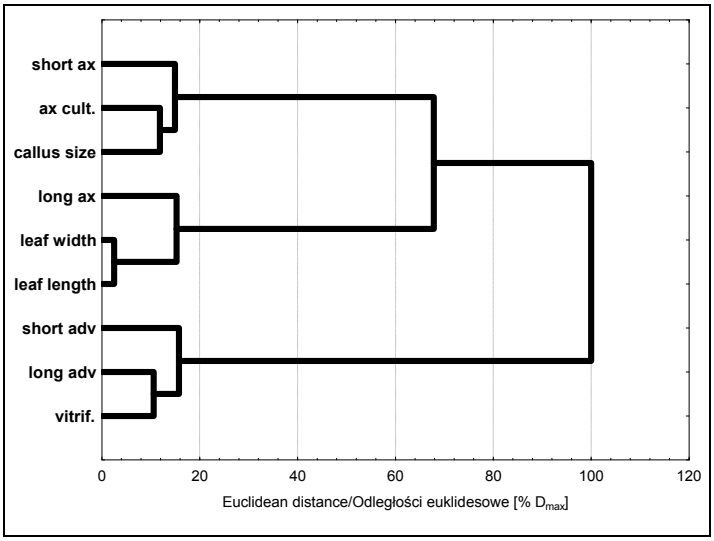
[a] explanations, see tab. 1; [a] objaśnienia, patrz tab. 1

Cluster analysis revealed close correlation between development of adventitious shoots and culture vitrification as well as between frequency of cultures composed from the only axillary shoots and callus size (fig. 4, 5). Such relationship was observed both for media supplemented with sucrose in different doses and for media augmented with various saccharides. It was also found that the size of leaves was related to elongation of axillary shoots (fig. 4, 5). However, such relation was slightly modified in two experiments.

DISCUSSION

The aim of the present study was to test the influence of chosen, often used saccharides on development of *in vitro* shoot cultures of *Paulownia tomentosa*. The authors of

available articles concerning micropropagation of paulownias used mainly media supplemented with sucrose (30 g dm^{-3}) [Marcotrigiano and Stimart 1983, Rao et al. 1996, Kumar et al. 1998, Rout et al. 2001, Ipekci and Gozukirmizi 2003, Ozaslan et al. 2005, Rajbahak and Sah 2010]. In addition they didn't describe the phenomenon of occurrence of adventitious shoots which is common in cultures of many species [Boxus 1999, Litwińczuk and Wadas 2008]. Adventitious shoots are suspected to be the main source of somaclonal variation [De Klerk 1990] and therefore should be eliminated from micropropagation process. It's well-known and documented that saccharides can modify development and quality of *in vitro* cultures of many species [George et al. 2008]. The media of various compositions should be used to increase the effectiveness of *in vitro* techniques in plant propagation, germplasm preservation or breeding of new cultivars. Thus the second aim of the present study was to assess usefulness of tested saccharides in different applications of *in vitro* cultures of paulownia.

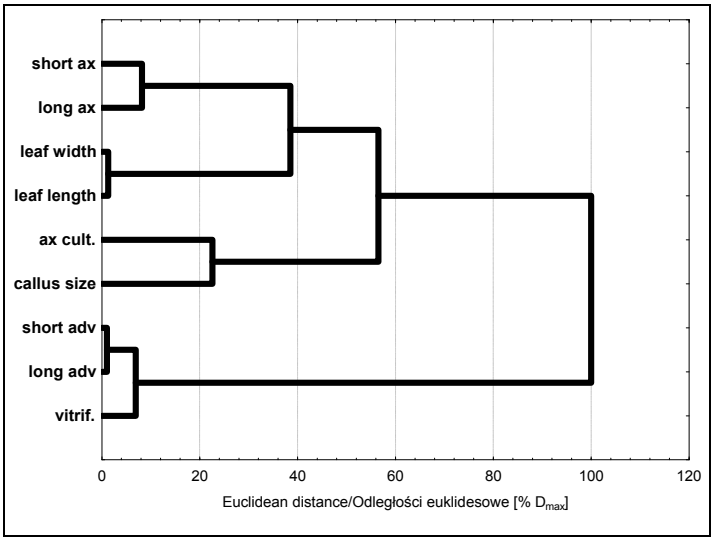


short ax – Number of axillary shoots (3–14 mm); long ax – Number of axillary shoots (> 15 mm); ax cult. – Number of cultures which developed exclusively axillary shoots [%]; short adv – Number of adventitious shoots (3–14 mm); long adv – Number of adventitious shoots (> 15 mm); leaf width – Width of leaf blade [mm]; leaf length – Length of leaf blade [mm]; callus size – Diameter of callus [mm]; vitrif. – Number of vitrified cultures [%]

short ax – liczba pędów kątowych (3–14 mm); long ax – liczba pędów kątowych (> 15 mm); ax cult. – liczba kultur zawierających wyłącznie pędy kątowe [%]; short adv – liczba pędów przybyszowych (3–14 mm); long adv – liczba pędów przybyszowych (> 15 mm); leaf width – szerokość blaszki liściowej [mm]; leaf length – długość blaszki liściowej [mm]; callus size – średnica kalusa [mm]; vitrif. – liczba szklistych kultur [%]

Fig. 4. Relationship among chosen traits of paulownia *in vitro* cultures grown on media supplemented with different doses of sucrose

Ryc. 4. Związek między wybranymi cechami kultur *in vitro* paulowni rosnącymi na pożywkach z dodatkiem różnych dawek sacharozy



explanations, see fig. 4; [a] Legenda: objaśnienia, patrz ryc. 4.

Fig. 5. Relationship among chosen traits of paulownia *in vitro* cultures grown on media supplemented with different saccharides

Ryc. 5. Związek między wybranymi cechami kultur *in vitro* paulowni rosnącymi na pożywkach z dodatkiem różnych różnych cukrów

The results of presented work proved that paulownia *in vitro* cultures, can utilize both sucrose and its components, as cultures of many other species [George et al. 2008]. However, it seems they cannot metabolize mannitol and sorbitol as could do some plants from Apiaceae, Rosaceae or Oleaceae. Sucrose influenced development of *in vitro* cultures. Interestingly in spite of the proliferation of axillary shoots in the presence of sucrose in 20–40 g dm^{-3} doses remained comparable, the development of adventitious shoots significantly decreased (tab. 1, 2). The frequency of vitrification was lowered also. Thus the sucrose in 30–40 g dm^{-3} concentration seems to be suitable for axillary shoot multiplication. The glucose (15.8 g dm^{-3}) could also be used with this purpose. It should be recommended especially in the last subculture before rooting of shoots because it increases elongation of axillary shoots (tab. 3). It should make easier manipulation of such shoots and production of plantlets of improved size/quality.

For long term storage of paulownia cultivars *in vitro* sucrose (50 g dm^{-3}) should be recommended. In such condition cultures grew slower and were consisted mainly of axillary shoots (tab. 1). The adventitious shoots occured in low quantity, were much smaller and easy to distinguish from axillary ones. In addition, diminished leaf blades facilitated observation of cultures.

In order to stimulate production of adventitious shoots, useful in plant breeding, sucrose (10 g dm^{-3}) and fructose (15.8 g dm^{-3}) could be applied. However, in the case of sucrose a lot of cultures were vitrified (tab. 1). As vitrified shoots are difficult to root,

and acclimatization *in vivo*, the application of fructose seems to be better solution. The close correlation between development of adventitious shoots and culture vitrification was revealed by cluster analysis (fig. 4, 5). Similar event was observed by Litwińczuk and Wadas [2008] in the case of highbush blueberry. It corresponds with statement of Gaspar et al. [1992] about epigenetic basis of phenomena created by *in vitro* conditions, as redifferentiation, rejuvenalization, habituation and vitrification. Surprisingly negative relationship between frequency of cultures composed from the only axillary shoots and callus size was also revealed by cluster analysis (fig. 4, 5). It is possible that fast growing callus loses its organogenetic ability. The growth of callus was closer related to proliferation of axillary than adventitious shoots. It seems moderate callus plays positive role, and stimulate growth of axillary shoots by increasing culture contact area of explants with the media and facilitate taking media components. Similar effect was found by Litwińczuk and Debergh [1995] in the case of cultures of white mulberry (*Morus alba*).

Summerizing, the present work has proved that development and quality of paulownia *in vitro* cultures are modified by kind and concentration of saccharides in the medium. The media supplemented with sucrose (30–40 g dm^{-3}) or glucose (15.8 g dm^{-3}) may be recommended for micropropagation of true-to-type plants, whereas sucrose (50 g dm^{-3}), and fructose (15.8 g dm^{-3}) for long term storage, and plant breeding, respectively.

CONCLUSIONS

1. *In vitro* cultures of *Paulownia tomentosa* can utilize sucrose and its components in contrast to mannitol and sorbitol.
2. Development and quality of paulownia *in vitro* cultures are modified by kind and concentration of saccharides in the medium.
3. Depending on the kind and concentration of saccharides in the medium *in vitro* cultures of paulownia consisted of various number of axillary and adventitious shoots.
4. Proliferation of axillary shoots was similar whereas the development of adventitious shoots and the frequency of vitrification significantly decreased while the concentration of sucrose increased from 20 to 40 g dm^{-3}.
5. Glucose (15.8 g dm^{-3}) stimulated both proliferation and elongation of axillary shoots.
6. The media supplemented with sucrose (30–40 g dm^{-3}) or glucose (15.8 g dm^{-3}) may be recommended for micropropagation of true-to-type plants.
7. For long term storage of paulownia *in vitro* cultures sucrose (50 g dm^{-3}) should be advised as cultures grew slower and were consisted mainly of axillary shoots.
8. In order to stimulate production of adventitious shoots, useful in plant breeding, fructose (15.8 g dm^{-3}) may be proposed.

REFERENCES

Barton I.L., Nicholas I.D., Ecroyd C.E., 2007. Paulownia. The Forest Research Bull., 231, 5–68.

Bergmann B.A., Heung-Kyu M., 1997. *In vitro* adventitious shoot production in paulownia. Plant Cell Rep., 16, 315–319.

Boxus P., 1999. Micropropagation of strawberry *via* axillary shoot proliferation. [In:] Methods in Molecular Biology. Plant Cell Culture Protocols, R.D. Hall (ed.). Humana Press Inc., Totowa NJ, 111, 103–114.

Burger D.W., Liu L., Wu L., 1985. Rapid micropropagation of *Paulownia tomentosa*. HortScience, 20, 760–761.

De Klerk G.J., 1990. How to measure somaclonal variation. Acta Botanica Neerlandica, 39(2), 129–144.

Gaspar T., Kevers C., Crevecoeur M., Penel C., Foidart J.M., Greppin H,. 1992. Habituation and Vitrification of Plants Cultured *In Vitro*: A Reciprocal Relationship. Wissenschaftlische Zeitschrift der Humboldt-Universitat zu Berlin. Reihe Mathematik, Naturwissenschaften, 41 (3), 35–40

George E.F., Hall M.A., De Klerk G.J., 2008. Plant propagation by tissue culture. 3rd Edition. The Backround. Springer, 124–130.

Ipekci Z., Altinkut A., Kazan K., Bajrovic K., Gozukirmizi N., 2001. High frequency plant regeneration from nodal explants of *Paulownia elongata*. Plant Biol., 3, 113–115.

Ipekci Z., Gozukirmizi N., 2003. Direct somatic embryogenesis and synthetic seed production from *Paulownia elongata*. Plant Cell Rep., 22, 16–24.

Kumar P.P., Rao C.D., Goh C.J., 1998. Influence of petiole and lamina on adventitious shoot initiation from leaf explants of *Paulownia fortunei.* Plant Cell Rep., 17, 886–890.

Litwińczuk W., Debergh P., 1995. Zależność pomiędzy wzrostem pędów i tkanki kalusowej kultur morwy białej (*Morus alba* L.) w świetle badań nad wykorzystaniem przez nie węgla oraz fosforu i azotu z pożywki. Mat. I Ogólnopol. Konf. „Zastosowanie kultur *in vitro* w fizjologii roślinnej" PAN w Krakowie, 65–74.

Litwińczuk W., Wadas M., 2008. Auxin-depended development and habituation of highbush blueberry (*Vaccinium* × *covilleanum* But. *et* Pl.) 'Herbert' *in vitro* shoot cultures. Scientia Hort., 119, 41–48

Marcotrigiano M., Stimart D. P., 1983. *In vitro* organogenesis and shoot proliferation of *Paulownia tomentosa* Steud. (Empress Tree). Plant Science Letters, 31 (2–3), 303–310.

Murashige T., Skoog F., 1962. A revised medium for the rapid growth and bioassays with tobacco tissue cultures. Physiologia Plant., 15, 473–496.

Ozaslan M., Can C., Aytekin T., 2005. Effect of explant source on *in vitro* propagation of *Paulownia tomentosa* Steud. Biotechnology & Biolechnological Equipment, 19, 20–26.

Rajbahak S., Sah S.K., 2010. Micropropagation of *Paulownia tomentosa* through *in vitro* culture technique. St. Xavier's J. Sci., 2(1), 15–20.

Rao C. D., Goh C., Kumar P.P., 1996. High frequency adventitious shoot regeneration from excised leaves of *Paulownia spp.* cultured *in vitro*. Plants Cell Reports, 16, 204–209.

Rout G.R., Reddy G.M., Das P., 2001. Studies on *in vitro* clonal propagation of *Paulownia tomentosa* Steud. and evaluation of genetic fidelity through RAPD marker. Silvae Genetica, 50 (5–6), 208–212.

Taha L.S., Soad Ibrahim M.M., Farahat M.M., 2008. A micropropagation of *Paulownia kowakamii* through *in vitro* culture technique. Austral. J. Basis and Appl. Sci., 2(3), 594–600.

ROZWÓJ PĘDOWYCH KULTUR *in vitro* PAULOWNI PUSZYSTEJ (*Paulownia tomentosa* Steud.) POD WPŁYWEM RÓŻNYCH CUKRÓW

Streszczenie. W pracy badano wpływ wybranych cukrów na wzrost i rozwój pędowych kultur *in vitro* paulowni puszystej (*Paulownia tomentosa*) – wartościowego gatunku drzewa o wielostronnym zastosowaniu. Przetestowano, a następnie określono przydatność kilku cukrów, głównie sacharozy (10–50 g dm^{-3}) oraz glukozy i fruktozy (15,8 i 30 g dm^{-3}). Cukry były składnikiem pożywki MS z dodatkiem BA (1 mg dm^{-3}), NAA (0,1 mg dm^{-3}) i GA_3 (0,1 mg dm^{-3}). Sacharoza w rosnącym stężeniu (20–40 g dm^{-3}) nie oddziaływała wyraźnie na proliferację pędów kątowych, podczas gdy ograniczała istotnie rozwój pędów przybyszowych i występowanie szklistości. Glukoza (15,8 g dm^{-3}) stymulowała zarówno proliferację, jak i elongację pędów kątowych. Sacharoza (10 g dm^{-3}) i fruktoza (15,8 g dm^{-3}) sprzyjały rozwojowi pędów przybyszowych i witryfikacji kultur. Pożywki z dodatkiem sacharozy (30–40 g dm^{-3}) lub glukozy (15,8 g dm^{-3}) mogą być polecane do mikrorozmnażania roślin, podczas gdy sacharoza (50 g dm^{-3}) i fruktoza (15,8 g dm^{-3}) odpowiednio do kolekcjonowania kultur *in vitro* i hodowli twórczej z wykorzystaniem technik opartych na pędach przybyszowych.

Słowa kluczowe: mikrorozmnażanie, pędy kątowe, pędy przybyszowe, sacharoza, glukoza, fruktoza

Accepted for print – Zaakceptowano do druku: 24.11.2011