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Micropropagation of *Stryphnolobium japonicum*

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Abstract. Observations of Japanese pagoda trees indicate that they undergo a full cycle of vegetative and generative development without self-renovation. The aim of this study was to obtain a successive media protocol propagation of Japanese pagoda tree by *in vitro* cultures. The effects of growth regulators were studied with reference to primary regeneration, shoot multiplication and rooting. As explants source were used the part of shoot of 90-year-old tree. Explants were placed on MS (Murashige and Skoog 1962) basal medium with the addition of 6-benzylaminopurine – BAP (1.0–2.0 mg·dm^{-3}), thidiazuron – TDZ (0.1–0.3 mg·dm^{-3}) and indole-3-acetic acid – IAA (0.5–1.0 mg·dm^{-3}). BAP was the growth regulator which significantly increased shoot regeneration on initial explants. TDZ in turn, inhibited the formation of adventitious shoots and caused the explants which had been placed on the medium to die. The multiplication of the Japanese pagoda tree by *in vitro* cultures should be conducted on MS media with the addition of 0.5 mg·dm^{-3} BAP, and they should be rooted on media with the addition of 0.3 mg·dm^{-3} indole-3-butyric acid – IBA. It seems, that devising an efficient method of Japanese pagoda tree micropropagation is realistic.

Key words: *in vitro* cultures, growth regulators, old tree, Japanese pagoda tree

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Introduction

The Japanese pagoda tree [*Stryphnolobium japonicum* (L.) Schott = *Sophora japonica* L.], also known as the scholar tree, is a tree occurring naturally in China and on the Korean Peninsula. It was brought to Poland in the first half of the 19th century. The tree's late and abundant blooming (end of July–August) and exotic-looking fruit pods are its main ornamental qualities. The plants have low habitat demands, and a confirmed high tolerance to drought and salinity (the so-called xerism), and to air pollution. Therefore, the tree is frequently planted in cities in Southern and Western Europe – in parks, green areas and along streets with high traffic intensity (Meyer 1978). The Japanese pagoda tree is little known in Poland, mostly single, old specimens can be encountered in parks (Szymanowski 1959, Seneta and Dolatowski 2008) and in dendrological collections (Nowak 1999). The Japanese pagoda tree is frost tender. Under Poland's climatic conditions – seedlings and young trees can be damaged by frost. The Japanese pagoda tree is proposed to be selected for cultivation mostly in western parts of Poland (Bojarczuk et al. 1980), but old trees can be found in south-eastern parts of the country e.g. in Przeworsk, Łańcut, Zwierzyniec and Kraków (Frazik-Adamczyk 2003). Observations of trees in Szczecin and Kraków (Frazik-Adamczyk 2003, Kubus 2004) indicate that they undergo a full cycle of vegetative and generative development, without self-renovation – seeds usually do not ripen and are not capable of germinating. The low number of cultivated Japanese pagoda trees can be explained by the problems in their propagation.

The aim of this study was to develop a method of micropropagation of Japanese pagoda tree [*Stryphnolobium japonicum* (L.) Schott = *Sophora japonica* L.].

Material and methods

Initial explants were taken from young, unlignified shoots derived from approximate 90-year-old Japanese pagoda tree specimen (Fig. 1). The shoots for the studies were sampled at the end of May, before flowering.

Prior to the isolation fragments of shoots were soaked for 20 min. in water with the addition of a detergent (Ludwik washing-up liquid), then immersed in 70% ethanol solution for 30 s. After the preliminary disinfection, the explants were disinfected with

Fig. 1. Micropropagation of *Stryphnolobium japonicum* (L.)Schott (=*Sophora japonica* L.) a. 90-years tree b. young, unlignified shoots, c. initiation stage, d. proliferation stage

0.5% $HgCl_2$ for 10 minutes and rinsed in sterile deionised water. Next, under sterile conditions, the disinfected explants were soaked in an ascorbic acid solution (100 mg·dm^{-3}) for 15 min and finally placed on the initiation medium, 20 explants per each medium. Single-node 1.0 to 2.0 cm long explantates, were placed on MS medium (Murashige and Skoog, 1962) enriched in the following cytokinins: 6-benzylaminopurine (BAP) at a concentration of 1.0 and 2.0 mg·dm^{-3} and thidiazuron (TDZ) at a concentration of 0.1 and 0.2 mg·dm^{-3} and auxin – indole-3-acetic acid (IAA), in the quantity of 0.5, 1.0 and 2.0 mg·dm^{-3} (Table 1). Plants placed on MS medium without the addition of growth regulators constituted control at all stages of the experiment. The initiation stage lasted 6 weeks.

The explants initiated for growth were put onto multiplication MS media supplemented with BAP at concentrations ranging from 0.5 to 5.0 mg·dm^{-3} (Table 2).

The multiplied shoots were placed on MS rooting media with the following auxins added: IAA and IBA (indole-3-butyric acid) at a concentration from 0.1 to 0.3 mg·dm^{-3} (Table 3).

All the media were cantained with 8 g·dm^{-3} agar and 30 g·dm^{-3} sucrose, and their pH was adjusted to 5.7 by adding 0.1 M of a NaOH and HCl. The media were heated and 15 ml were poured into 100 ml Erlenmeyer flasks and next, they were autoclaved after adding the growth regulators at a temperature of 121°C for 20 minutes.

The cultures were mainteined in a growth room at a temperature of 24 ±1°C under 16-h photoperiod from fluorescent lamp (photosynthetic photon flux density 40 μmol·m^{-2}·s^{-1}).

The mean values of measurements at proliferation (shoot length, numbers of: leaves, internodes, axillary shoots, shoot weight) and rooting stage (plant height, number of leaves, root length, root number) obtained in the experiments are presented in tables.

The results were statistically analyzed. The significance of differences was determined by means of variance analysis and Tukey's test, at the level of significance of $\alpha = 0.05$.

Table 1. Primary regeneration of initial explants of *Stryphnolobium japonicum* (L.) Schott (=*Sophora japonica* L.) *in vitro*

Plant growth regulators [mg·dm^{-3}]			Regeneration (%)	Callus
IAA	BAP	TDZ		
	Control – MS		0	–
0.5	–	–	15	–
1.0	–	–	10	–
2.0	–	–	20	–
1.0	1.0	–	55	+++
–	1.0	–	50	+
–	2.0	–	40	++
–	–	0.1	10	–
–	–	0.2	15	–
	Mean		23.89	

Table 2. Multiplication of shoots of *Stryphnolobium japonicum* (L.)Schott (=*Sophora japonica* L.) on media with BAP at different concentrations

Containing of BAP [mg·dm^{-3}]	Shoot number/explant	Shoot length [cm]
0.5	3.07 ab	1.32 b
1.0	4.15 a	2.31 b
2.0	2.14 b	1.42 b
3.0	1.08 c	1.37 b
4.0	1.06 c	1.51 b
5.0	1.10 c	1.42 b

Table 3. Rooting of shoots of *Stryphnolobium japonicum* (L.) Schott (=*Sophora japonica* L.) on media containing auxins at different concentrations

Auxin [mg·dm^{-3}]		Shoot length [cm]	Root number	Root length [cm]	Percent of rooted plants [%]
IAA	IBA				
0.1		3.21 ab	0.00 c	–	0
0.2		4.25 a	0.00 c	–	0
0.3		3.20 ab	1.85 b	1.56 a	100
	0.1	2.20 bc	0.00 c	–	0
	0.2	3.20 ab	0.00 c	–	0
	0.3	1.94 c	3.12 a	1.52 a	92

Results and discussion

As it is emphasised by Iturriaga et al. (1994) the initiation and development of *in vitro* cultures of plants from the family *Fabaceae* (=*Leguminosae*) are difficult. This author used only young, 3- or 4-month old *Sophora tomiro* seedlings for initiation. Also Jordan et al. (2001) initially used seedling fragments as explants while initiating *in vitro* cultures of plants belonging to this species. In our own research fragments of young shoots taken from an adult plant were used for initiation. On the basis of the results obtained it has been concluded that the initiation of *in vitro* cultures is possible provided that the mineral composition of the medium is appropriate. BAP turned out to be the growth regulator which significantly increased the initiation frequency (Table 1). TDZ in turn, independent of its concentration, inhibited the formation of axillary shoots and resulted in explants dying out. The cytokinins BAP and TDZ are frequently used at initial stage in micropropagation of woody plants. Their positive effect on the formation of adventitious shoots was found in the research by Meyer (1998), Salajowa et al. (1999) and Kaya and Gocke (1997).

The explants placed on the control medium did not begin to grow *in vitro* cultures and died. The shoots which initiated growth were characterized by proper leaf development and green colour. The method of disinfection applied in our study was effective. Only 15% of the explants placed on the medium were contaminated.

A negative effect of cytokinins on the multiplication of the Japanese pagoda tree was found (Table 2). Despite the proper light green colour, the shoots placed on the media complemented with cytokinins did not multiply and did not elongate. It may have been caused by too high a concentration of cytokinins used in the experiment (from 1.0 to 5.0). Although cytokinins are usually recommended for tree plant multiplication, it is difficult to determine their appropriate content in the medium. While multiplying *Sophora toromiro* Jordan et al. (2001) used a medium with the addition of 0.5 to 2.5 mg·dm^{-3} TDZ, and Shu et al. (2003) only 0.5 mg·dm^{-3} BAP combined with 0.5 mg·dm^{-3} kinetin and 0.1 mg·dm^{-3} NAA. In our experiment, the proper shoot development was observed only on the media with the lowest concentration of cytokinin (0.5 mg·dm^{-3} BAP).

Rooting is a critical phase of tree micropropagation process especially in the case of cultures derived from explants taken from old tree specimens. At the final stage of the experiment single-node fragments of the Japanese pagoda tree were put on three kinds of mineral composition media according to Murashige and Skoog (1962) with the addition of 0.1, 0.2 and 0.3 mg·dm^{-3} IBA to enable their rooting (Table 3). The microseedlings did not root well, however, the formation of a root system was observed only on the medium complemented with the highest auxin concentrations used – 0.3 mg·dm^{-3}. Zhao et. al (2003) obtained the best results of rooting *Sophora flavescens* on MS media with 0.1 mg·dm^{-3} NAA.

The results presented here are only preliminary, and their the need for further investigations. Han et al. (1993) emphasise that regenerative capabilities in the case of arboreal plants are closely related to the genotype. While multiplying the black locust (*Robinia pseudoacacia*) Davis and Keathley (1987) collected primary explants from five adult, 20–30-year-old tree, out of which only explants taken from 2 of the trees started growing *in vitro* cultures.

It seems, however, that devising an efficient method of Japanese pagoda tree micropropagation is realistic, which would contribute to popularizing this attractive tree recommended for urban green areas in our country.

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