Micropropagation of *Catalpa bignonioides* Walt. through tissue cultures

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Abstract

The conditions used in the micropropagation of *Catalpa bignonioides* Walt. (*Bignoniaceae*) from callus tissue are described. The multiplication of shoots was best on Schenk and Hildebrandt (SH) solid medium supplemented with 0.5 mg dm^{-3} indolil-3-acetic acid (IAA) and 2.0 mg dm^{-3} benzylaminopurine (BAP). Under these conditions, 11 shoots per culture could be developed within 4 weeks. To develop roots, the shoots were then transferred to basal SH medium without growth regulators. Rooted plantlets were obtained within 2-8 weeks. Regenerated plants were fully capable of further development in soil. Generally, ca 10 plants could be obtained from a fragment of callus within 10-12 weeks.

Key words: *Catalpa bignonioides* Walt., *Bignoniaceae*, micropropagation, tissue cultures, rate of regeneration

INTRODUCTION

Catalpa bignonioides (*Bignoniaceae*) is a woody plant growing wild the south-eastern U.S.A. The fruits, flowers, bark and leaves of the genus *Catalpa* are rich in many kinds of pharmacologically active compounds such as naphthoquinones (Inouye et al. 1975), flavonoids (Harborne 1967, Okuda

et al. 1975) and phenolic acids (Nakaoki and Morita 1955). The presence of iridoid glycosides, mainly catalpol and catalposide has also been found (Bobbitt et al. 1961, Kimura et al. 1963). The latter two have diuretic properties (Suzuki 1968).

We have previously obtained (Wysokińska and Świątek in press) callus and suspension cultures of *Catalpa bignonioides*. A relationship was then found between the synthesis of iridoid compounds and organogenesis: catalpol and catalposide appear only in buds and shoots obtained from callus tissue. In the present paper, the conditions of propagation of *C. bignonioides* by tissue culture to quickly obtain material rich in biologically active compounds have been described. The conditions for micropropagation of plants from the genus *Catalpa* had not been worked out until now.

MATERIAL AND METHODS

INITIAL MATERIAL: CAULOGENIC CALLUSES

Callus tissues were initiated from the hypocotyl of two-week-old *Catalpa bignonioides* seedlings cultivated in sterile culture, as described earlier (Wysokińska and Świątek, in press). For induction of buds and shoots the calluses were cultivated at 4-week intervals on agar Gamborg et al. (1968) medium (B5) containing 0.5 mg dm^{-3} indolil-3-acetic acid (IAA) and 2.0 mg dm^{-3} benzylaminopurine (BAP).

THE EFFECT OF MINERAL MEDIUM AND DURATION OF CULTURE ON THE FORMATION OF SHOOTS

Pieces of caulogenic tissue (each fragment contained one shoot or macroscopically visible bud) were then passed onto one of three mineral media: Schenk and Hildebrandt (1972) (SH), Linsmaier and Skoog (1965) (LS) and Gamborg et al. (1968) (B5), supplemented with growth regulators: IAA 0.5 mg dm^{-3} and BAP 2.0 mg dm^{-3} . The mean fresh weight of an inoculum was: 0.120 g. The inoculates were placed in Erlenmeyer (300 cm^3) flasks, each containing 50 cm^3 of liquid or solid (Difco Bacto Agar 8.0 g dm^{-3}) medium. Flasks with liquid cultures were incubated on a rotary shaker at 100 rpm for 4 weeks and those with agar cultures — for 10 weeks. The dry and fresh weights as well as the length and mean shoot number of these cultures were determined: on solid medium — every 2 weeks, on liquid — after 4 weeks.

THE INFLUENCE OF CYTOKININS ON SHOOT FORMATION

Agar SH media were supplemented with BAP, zeatin — trans-6-(4 hydroxy-3-methylbutyl-2-enyl) aminopurine, kinetin — 6-furfurylamino-purine or 2iP — 6-(γ , γ -dimethylallylamino)-purine in the concentrations indicated in Table 2. These compounds were used with IAA at a concentration of 0.5 mg dm^{-3} . The time of incubation was 4 weeks.

THE ROOTING OF SHOOTS

Shoots (longer than 5 mm) were isolated from callus tissue and transferred to agar SH medium devoid of growth regulators. After 2, 4, 6, 8 and 12 weeks the percentage of shoots forming roots was estimated, as well as the fresh and dry weights of the obtained plantlets.

After 8 weeks, the plantlets ($> 4 \text{ cm}$) were transferred to pots with a specially prepared sterilized mixture of soil, sand and peat (4:4:3 v/v). During the first week of growth the plants were covered with beakers to avoid excessive transpiration.

All the experiments were carried out at $26 \pm 2^\circ\text{C}$ and under continuous fluorescent light (2.4 W m^{-2}).

RESULTS

SHOOT MULTIPLICATION

The calluses forming buds and shoots were obtained from the hypocotyl of *C. bignonioides* seedlings on B5 medium with IAA (0.5 mg dm^{-3}) and BAP (2.0 mg dm^{-3}) (Fig. 2). The same growth substances were used in experiments carried out to compare the multiplication rate (time and number of shoots produced per culture) on various agar media: B5, LS and SH (Fig. 1). Formed shoots from 6 to 9 per culture on LS and SH media respectively, were observed after 2 weeks. However, the highest number of shoots (an average of 9-11 depending on the medium) was always observed after 4 weeks. Longer incubation caused gradual withering of the shoots, and as a consequence — a decrease of their total number. The ageing process was most rapid in cultures on B5 medium where the decrease of shoot number already occurred after 6 weeks. On LS and SH media the shoots grew well for a longer time and the decrease of their number was observed only after 8 or 10 weeks (Fig. 1). Comparison of the results presented on Fig. 1 shows that the highest number of shoots was developed on SH medium (11 per culture) (Fig. 3), while the smallest — on B5 medium (9 per culture). The time of shoot formation was

also the shortest on SH medium. Figure 4 shows the lengths of shoots obtained on various media. The greatest number of group A shoots (0.5-1 cm long) was noticed on B5 medium. Group C shoots (3.1-6 cm) on this medium were the longest and appeared after only 6 weeks. The greatest number of C group shoots was seen on SH and LS media, but they appeared earlier on SH medium. Group D shoots (6.1-10 cm) were observed on LS and SH media, but they were more numerous on SH medium. However, group E shoots (over 10 cm), were noticed only on SH medium.

Fig. 1. The effect of mineral media (B5 — Gamborg et al. 1968; LS — Linsmaier and Skoog 1965; SH — Schenk and Hildebrandt 1972) on the growth of cultures and the formation of shoots *Catalpa bignonioides*. The results represent mean values of 3 series of determinations. Each series was comprised of 5 cultures. Time of growth was 10 weeks, on B5 medium — 6 weeks

Fig. 2. Shoots developed in *Catalpa bignonioides* callus culture on B5 medium with IAA 0.5 mg dm^{-3} and BAP 2.0 mg dm^{-3} , after 4 weeks of growth

Fig. 3. Regeneration of shoots after 6 weeks on solid SH medium with IAA 0.5 mg dm^{-3} and BAP 2.0 mg dm^{-3}

Fig. 4. The length of *Catalpa bignonioides* shoots on various media. Shoot length: 0.5-1 cm (A); 1.1-3 cm (B); 3.1-6 cm (C); 6.1-10 cm (D); over 10 cm (E). Abbreviations as in Fig. 1

Table 1 presents the formation of shoots in liquid media. In liquid culture, rooted shoots capable of further development were obtained only on B5 medium (Fig. 5). Their maximum length was 6 cm. However, in liquid SH and LS media, mainly rosettes of small and very turgid brownish-green or brownish-red leaves were formed. All these structures withered after transferring to agar medium or formed undifferentiated callus tissue.

Table 1

The effect of liquid media on the growth and regeneration of shoots from callus cultures of *Catalpa bignonioides*

Type of medium	Fresh weight per culture (g)	Dry weight per culture (g)	No. of shoots per culture	% Of shoots > 1 cm
LS	2.91	0.295	8.0	15
SH	3.19	0.405	15.4	10
B5	2.28	0.244	13.5	15

Culture conditions: the media were supplemented with IAA 0.5 mg dm^{-3} and BAP 2.0 mg dm^{-3} ; growth 4 weeks. The results represent mean values of 3 determinations. Each determination was comprised of 5 cultures.

The number of shoots per culture, as well as their length depend on the type of cytokinin in the medium (Table 2). The highest number of shoots was obtained on solid medium with BAP or zeatin. The activity of both the cytokinins evaluated on the basis of the effects caused by their equivalent concentrations was similar. On the other hand, increasing the BAP concentration to 2.0 and 5.0 mg dm^{-3} resulted in a marked increase of the number of regenerated shoots (Table 2). Therefore, the use of this synthetic cytokinin seems more advantageous (also because of the price and availability on the market). The other two cytokinins 2iP and kinetin, induced a much smaller number of shoots. Although the elongation of shoots was most pronounced in the presence of 2iP at all the applied concentrations and in the presence of a low concentration of zeatin, this study demonstrated that under these conditions the number of shoots was low (2-4 per culture). Therefore, the application of such conditions for the propagation of *C. bignonioides* does not seem to be very advisable.

Cytokinins affected not only number of shoots and their structure but also the fresh and dry weight in *C. bignonioides* cultures. The highest dry weight was obtained in the material with zeatin, and the lowest — with BAP (Table 2). The increase of cytokinin concentration caused the increase of dry weight which, however, could be observed only to a concentration limit; above this limit a marked decrease in the dry weight of shoots (ca 25%) could be observed e.g. in the presence of zeatin and BAP at concentrations of 1.0 mg dm^{-3} and 5.0 mg dm^{-3} , respectively (Table 2).

Fig. 5. Shoots developed in callus tissue on liquid B5 medium with IAA 0.5 mg dm^{-3} and BAP 2.0 mg dm^{-3} , after 4 weeks of culture

Table 2

The effect of various cytokinins on the formation, growth and rooting of *Catalpa bignonioides* shoots regenerated from callus cultures

Type and concentration of cytokinin (mg dm^{-3}) ^a		Fresh weight per culture (g)	Dry weight per culture (%)	No. of shoots per culture	% Of shoots > 1 cm	Rooting (%)
Z	0.01	0.45	16.10	1.8	61	80
	0.1	0.55	17.25	3.5	51	40
	1.0	1.04	13.05	7.8	31	0
2iP	0.01	0.47	12.55	1.8	64	67
	0.1	0.39	14.30	2.6	50	79
	1.0	0.41	15.15	3.8	50	70
KIN	2.0	0.50	14.07	3.4	56	30
	0.1	0.26	14.02	2.9	31	50
	1.0	0.16	14.12	2.0	35	10
BAP	2.0	0.30	14.82	3.0	37	0
	5.0	0.51	13.50	3.6	33	0
	0.1	0.48	14.00	3.0	37	50
BAP	1.0	0.98	11.90	7.0	40	0
	2.0	1.20	11.80	11.0	21	0
	5.0	1.06	8.90	13.0	8	0

Culture conditions: agar SH medium with IAA 0.5 mg dm^{-3} . Time of growth: 4 weeks. The data represent mean values of 3 successive experiments. ^aAbbreviations: Z — zeatin; 2iP — N⁶-(2-isopentenyl)adenine; KIN — kinetin; BAP — 6-benzylaminopurine

SHOOT ROOTING AND PLANTLET DEVELOPMENT

To develop roots, the shoots were then transferred to the SH agar medium without growth regulators. Under these conditions ca 80% shoots produced roots within 8 weeks of culture (Fig. 6). The roots were abundant, branched about 15 cm long. It was found that the time of rooting was related to the size of the initial shoot (Table 3). Shoots 1.5 cm long started to form roots after 8 days of culture, i.e., twice as quickly as 0.5 cm long shoots. Many shoots also rooted in the presence IAA 0.5 mg dm^{-3} and low cytokinin concentration (Table 2). Thus the possibility of multiplication and rooting of *C. bignonioides* shoots at the same time, without passing through the rooting stage, should be considered. Unfortunately, the formation of roots occurred under conditions providing a low rate of regeneration of shoots.

The plantlets which reached a height of 4 cm or more (Fig. 7) and showed well-developed root and leaf systems were then grown in pots with specially

Table 3

The effect of shoot length on the time of rooting (n = 15)

The initial size of excised shoot (cm)	Rooting of plantlets (%) after days of culture*				
	8	16	40	48	55
0.5	0	40	47	47	47
1.0	13	47	53	60	67
1.5	67	67	80	87	87

Culture conditions: agar SH medium without growth regulators. *Observations of rooting were performed every day. In the table only the days on which changes were noticed are noted.

Fig. 6. The rooting and growth of *Catalpa bignonioides* shoots on SH agar medium without growth regulators. For rooting, shoots longer than 5 mm were taken. The results represent mean values of 3 series of determinations. Each series was comprised of 10 cultures

prepared soil. Figure 8 shows *C. bignonioides* after about 6 months of growing in the soil.

From the presented results it can be seen that the total duration of the regeneration cycle (shoot production, rooting) of *C. bignonioides* by tissue culture was 10-12 weeks and about 10 plants were obtained from a single fragment (ca 120 mg) of callus tissue.

Fig. 7. Rhizogenesis of regenerated shoots after 8 weeks cultivation on SH medium without growth substances

Fig. 8. *C. bignonioides* plant 6 months after transferring it into a soil

DISCUSSION

The presented method of micropropagation of *Catalpa bignonioides* comprises:

1. development of caulogenic callus,
2. obtaining a high rate of regeneration of shoots,
3. root formation in in vitro conditions.

Calluses of *C. bignonioides* obtained according to the method described earlier (Wysokińska and Świątek in press) formed shoots on agar B5, SH and LS media with IAA (0.5 mg dm^{-3}) and BAP (2.0 mg dm^{-3}). The number of shoots and the rate of their formation were the highest on SH medium. The shoots formed on this medium were healthy (ageing symptoms were observed only in the 10th week of culture) and longer than shoots from LS or B5 media.

The rate of regeneration of shoots is markedly affected by cytokinins in the culture medium. BAP seems to be the most suitable for micropropagation of *C. bignonioides*. The highest number of shoots was obtained at a BAP concentration of 5.0 mg dm^{-3} . However, taking into consideration the poor structure (shoots were stunted with small or chlorotic leaves) and relatively low dry weight contents, a lower concentration of this growth regulator, viz. 2.0 mg dm^{-3} should be taken as optimum. BAP was most effective in the induction of shoots of other plants too, e.g. *Salix matsudana* $\times$ *alba* NZ 1002 and *Trifolium repens* (Bhojwani 1980, 1981). The second cytokinin useful in the regeneration of *C. bignonioides* shoots was zeatin. In comparison with BAP at equivalent concentrations its activity was seen to be greater, since it provided a somewhat higher rate of propagation. However, the difference was not large. Moreover economic reasons favour BAP. The other two cytokinins, kinetin and 2iP were much less active than BAP. In the case of 2iP which is a natural cytokinin, the reason may be its inactivation by enzymatic systems present in the tissue (Norton and Norton 1985).

More shoots were formed in liquid cultures of *C. bignonioides* on B5 and SH media than in agar culture. However, these structures after transferring to medium for rooting either did not develop at all (from SH and LS media), or showed weak development (from B5 medium). This can probably explain the process of tissue vitrification in liquid cultures. It may cause among others, physiological or structural changes in the meristem (Vieitez et al. 1985). The results of our study are in agreement with previous findings in which liquid or semisolid (0.4% agar) media, gave better conditions for the propagation of shoots and the main factor limiting the application of these media was the vitrification process (Debergh et al. 1981, Chun et al. 1986, Repčáková et al. 1986).

An important problem in micropropagation of plants is to obtain favourable conditions for rooting of the shoots developed in vitro. *C. bignonioides*

shoots rooted readily in the absence of auxin or other phytohormones. This result indicates that cells of this plant probably contain a relatively high level of endogenous auxin. On the other hand, rooting this species may also occur in the presence of low cytokinin concentrations. The stimulating effect of low concentrations of exogenous cytokinin on root formation has previously been described by Nemeth (1979).

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Mikrorozmnażanie Catalpa bignonioides Walt. metodą kultury tkankowej

Streszczenie

Opisano warunki mikrorozmnażania *Catalpa bignonioides* Walt. (*Bignoniaceae*) z tkanki kalusowej. Rozmnażanie pędów najlepiej przebiega na agarowej pożywce Schenka i Hildebrandta (SH) uzupełnionej IAA $0,5 \text{ mg dm}^{-3}$ i BAP $2,0 \text{ mg dm}^{-3}$. W tych warunkach podczas 4-tygodniowego okresu wzrostu tworzyło się przeciętnie 11 pędów w jednej kulturze. Pędy te po przeniesieniu na podłoże SH bez regulatorów wzrostu formowały korzenie między 2-8 tygodniem hodowli. Ukorzenione roślinki wykazywały pełną zdolność do dalszego rozwoju w glebie. Stosując tę metodę, z fragmentu kalusa można otrzymać w ciągu 10-12 tygodni ok. 10 roślin.