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IN VITRO PROPAGATION OF *CATALPA OVATA* G. Don AND *CATALPA BIGNONIOIDES* Walt.

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Shoot cultures of *Catalpa ovata* and *C. bignonioides* were started from epicotyls of aseptically germinated seeds and maintained on Murashige and Skoog (1962) based medium. Optimal BA concentration for shoot multiplication was 0.5 mg^{-1} for *C. ovata* and 1.0 mgl^{-1} for *C. bignonioides*. IBA had little effect on shoot multiplication and 0.1 mgl^{-1} was considered optimal for both species. Shoots of *C. bignonioides* were more then twice longer than shoots of *C. ovata* on all BA concentrations exceeding 0.1 mgl^{-1}. Shoots 1.5-2.0 cm long could be easily rooted, media supplemented with 0.5 mgl^{-1} IBA was considered optimal. Rooted plantlets were adapted in glasshouse after planting in mixtures of peat, sand and organic manure.

Key words: *in vitro,* propagation, shoot cultures, *Catalpa ovata* G. Don., *Catalpa bignonioides* Walt.

Ključne reči: *in vitro,* razmnožavanje, kulture izdanaka, *Catalpa ovata* G. Don., *Catalpa bignonioides* Walt.

INTRODUCTION

Genus *Catalpa* of the Bignoniaceae family contains about 10 species of trees which are planted as ornamental plants or in timber plantations (B a i l e y , 1949). *C. bignonioides* Walt. is native to North America and *C. ovata* Don, to East Asia (China, Japan). They can be distinguished by color of flowers; *C. ovata* has yellow flowers striped orange and spotted dark violet whilst *C. bignonioides* has white flowers with two yellow stripes inside and spotted purplish-brown. Both species are characterized by very large, hart shaped leaves. Decorative appearance of these specimens is enhanced by fruits which are large, up to 40 cm long pods. Leaves, flowers and fruits are known to contain pharmacologically active substances like catalpol and catalposide which have diuretic properties. Bruised leaves of *C. bignonioides* emit an unpleasant odor.

In this paper we present results on the investigation of *in vitro* methods suitable for clonal propagation of *C. ovata* and *C. bignonioides*. *In vitro* propagation of *C. bignonioides* has been elaborated by W y s o k i n s k a and S w i a t e k , 1989.

MATERIAL AND METHODS

Seeds of both species were collected from trees growing in Botanical garden, Belgrade. Seeds were surface steriliezd for 20 min in 10% solution of commercial bleach (4-5% NaOCl) to which few drops of liquid detergent have been added. Seeds were throghly washed in autoclaved water and than germinated on moist filter paper in small petri dishes, 5 seeds per plate. After first signs of germination, seeds were transferred to agar solidified medium, individually in 10 x 100 test tubes. This medium was either hormone-free- or supplemented with 1.0 mgl^{-1} BA and 0.2 mgl^{-1} IBA. After proper germination, epicotyls were excised and first true subculture was performed.

Medium used in all experiments comprised M u r a s h i g e and S k o o g (1962) inorganic salts, vitamins and inositol. It was modified to contain 0.4 mgl^{-1} vitamin B_1, 2.0% sucrose and 0.62% agar. Media was autoclaved for 20 min on 114-115°C, Ph was adjusted to 5.5-5.7 prior to autoclaving. Culture vessels used for shoot multiplication were 100 ml wide neck Erlenmeyer flasks containing 40 ml of medium or 150 ml transfusion bottles containing 50 ml medium. Both types of culture vessels were closed by cotton wool plugs. Conditions in the growth room were 16/8 hours light to darkness, irradiance 5.0-7.2 (10.) Wm^{-2} provided by cool white fluorescent lamps. Temperature in the growth room was adjusted to 25 ± 2°C.

Multiplication index – parameter used to evaluate propagation rate corresponds to the number of new propagula (shoots explants) produced by a single shoot explant during subculture lasting for 4 weeks, which can be used for further subculturing.

Rooting was performed with shoots 1.5-2.0 cm long. Rooting results were scored after 4 weeks. Planting substrate was prepared from mixture of peat, sand and „Beo-humus" (organic manure). Adaptation and further cultivation of rooted plantlets were performed in glasshouse. Transferred plantlets were frequently sprayed with 0.3% Venturin to prevent damage by fungi.

All experiment were repeated at least twice with no less than 24 replicates per treatment.

RESULTS

Experiments were started in spring 1989. Surface sterilization of seeds was successful and the number of seeds found to be contaminated on sterile filter paper was practically nil. Some inborn bacterial contamination were found later specially in *C. ovata*. Upon transfer to agar solidified medium germination was fast and first true subculture which included excision of epiocotyl was performed after three weeks. On hormone free medium epicotyls were long but terminal buds often perished from necrosis. On medium with 1.0 mgl^{-1} BA and 0.2 mgl^{-1} IBA epicotyls were short but healthy, and therefore they were used for establishment of shoot cultures.

From second subculture epicotyl explants of both species were transferred to medium supplemented with 0.5 mgl^{-1} BA and 0.1 mgl^{-1} IBA, which was previously in our laboratory found to be suitable for maintenance of shoot cultures of a number of dicotyledonous tree species including; apple, quince, mulberry, carob, various *Prunus* species and other. Preliminary results showed marked differences in the growth of two *Catalpa* species. Whilst growth of *C. ovata* was satisfactory, shoot multiplication of *C. bignoinioides* was low and shoot length unusally high. To investigate the optimal BA concentration for maintenance of shoot culture stocks of both species, experiments were performed in which BA concentration was varied (0-2.0 mgl^{-1}) and IBA concentration was at constant concentration 0.1 mgl^{-1} (Fig. 1).

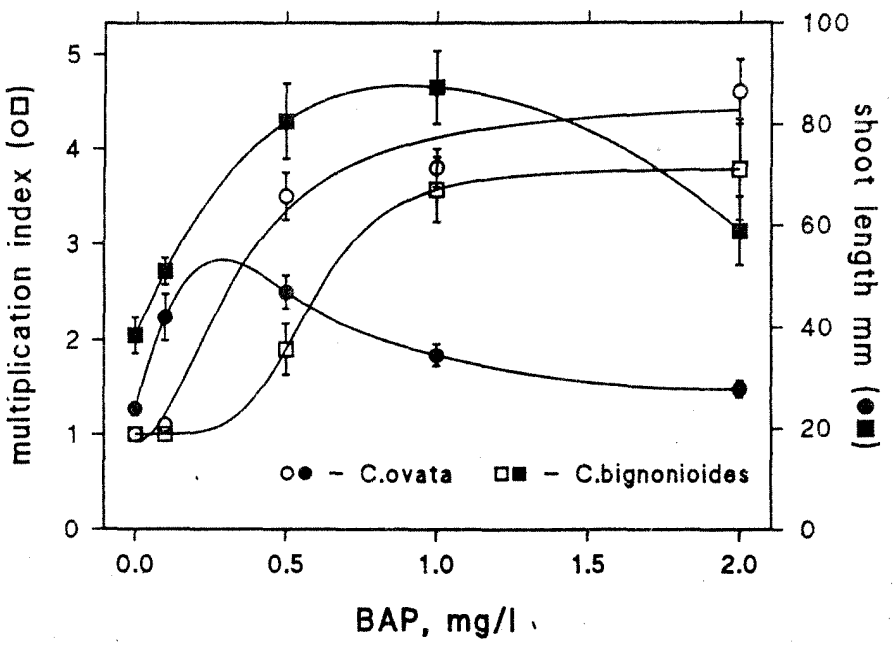

Fig. 1. – Effect of 0-2.0 mgl^{-1} BA on shoot multiplication and shoot elongation in *Catalpa ovata* and *C. bignonioides*.

According to Fig. 1, shoot multiplication of *C. ovata* increased faster with increase of BA concentration than in *C. bignonioides*. In *C. ovata* plateau for shoot multiplication was reached at 0.5 mgl^{-1} BA. Thus at 0.5 mgl^{-1} BA shoot multiplication of *C. ovata* was nearly twice higher than in *C. bignonioides*. The maximum values for shoot length in *C. ovata* is somewhere between 0.1 and 0.5 mgl^{-1} BA and for *C. bignonioides* at 1.0 mgl^{-1}. In the range 0.5-2.0 mgl^{-1} BA shots of *C. bignonioides* were twice longer than shoots of *C. ovata* which was an obvious and striking difference between the two *Catalpa* species (Fig. 2). Considering both shoot multiplication and shoot length, the optimum BA concentration for maintenance of shoot cultures was 0.5 mgl^{-1} for *C. ovata* (as initially provided) and 1.0 mgl^{-1} for *C. bignonioides*. In general *C. bignonioides* at all BA concentration exhibited unusually high shoot elongation not only in relation to *C. ovata* but also in relation to other dicotyledonous tree species.

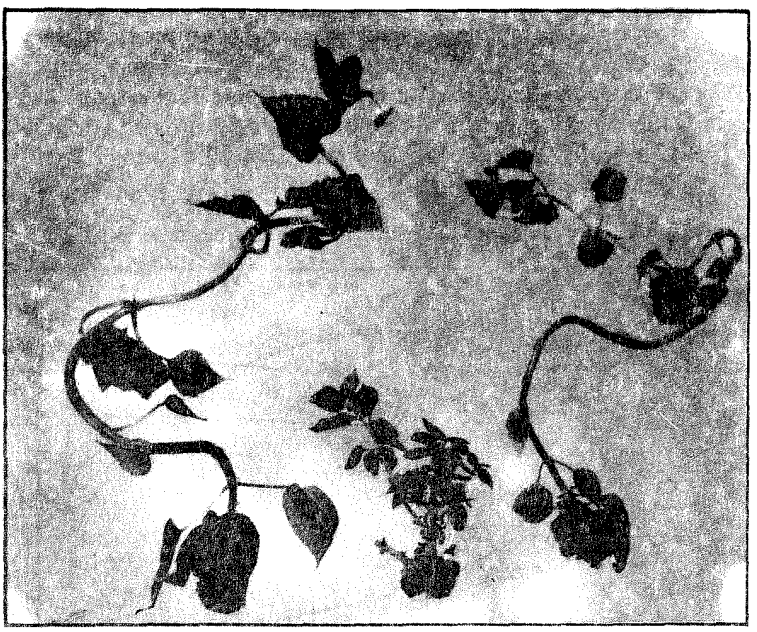

Fig. 2. – Shoot cultures of *Catalpa ovata* (center) and *C. bignonioides* (left and right) after 4 weeks on medium containing 0.5 mgl^{-1} BA and 0.1 mgl^{-1} IBA. Compare difference in shoot length.

Since preliminary results showed that IBA in 0-1.0 mgl^{-1} had little effect on shoot multiplication except that it stimulated callusing, and that 0.1 mgl^{-1} IBA already gave good results in the maintenance of shoot culture stocks, effect of IBA concentration on shoot multiplication was not further investigated.

Single isolated roots of both species could be easily rooted on hormone-free or media containing auxins. The effect of 0-1.0 mgl^{-1} IBA on the number of roots per

rooted shoot and length of longest root is presented in Fig. 3. It is evident that for both species results were nearly identical. Root length decreased with increase of IBA in the medium whilst the number of roots per rooted shoot increased.

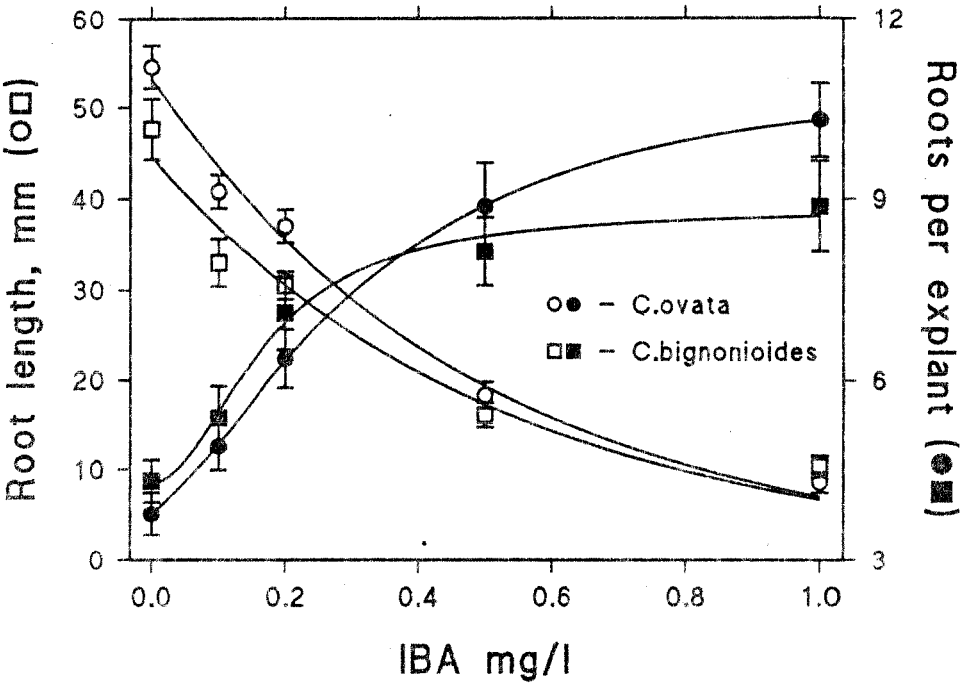

Fig. 3. – Effect of 0-1.0 mgl^{-1} IBA on the average number of roots per rooted culture and root length.

Rooting could also be achieved in a two-stage rooting procedure in which shoots were first placed on media supplemented with 2.0 mgl^{-1} IBA and then tansferred to hormone free medium after 1, 2, 4 or 7 days. The number of roots per rooted shoot was very high, Fig. 4. In *C. ovata* it increased with increased duration of the first stage of rooting but in *C. bignonioides* it was constant in all treatments. Also root length in *C. ovata* decreased with prolonged duration of auxin treatment whilst in *C. bignonioides* values were erratic and roots in general were short.

Adaptation of plantlets after transition to *ex vitro* conditions was affected by the rooting method. In plants rooted in continuous contact with medium supplemented with IBA, success in adaptation was 62-80% for *C. ovata* and 86-100% for *C. bignonioides*. Adaptation of plantlets rooted in the two-stage procedure was less successful, 10-66% for both species. Further growth of transferred plantlets in the glasshouse was fast.

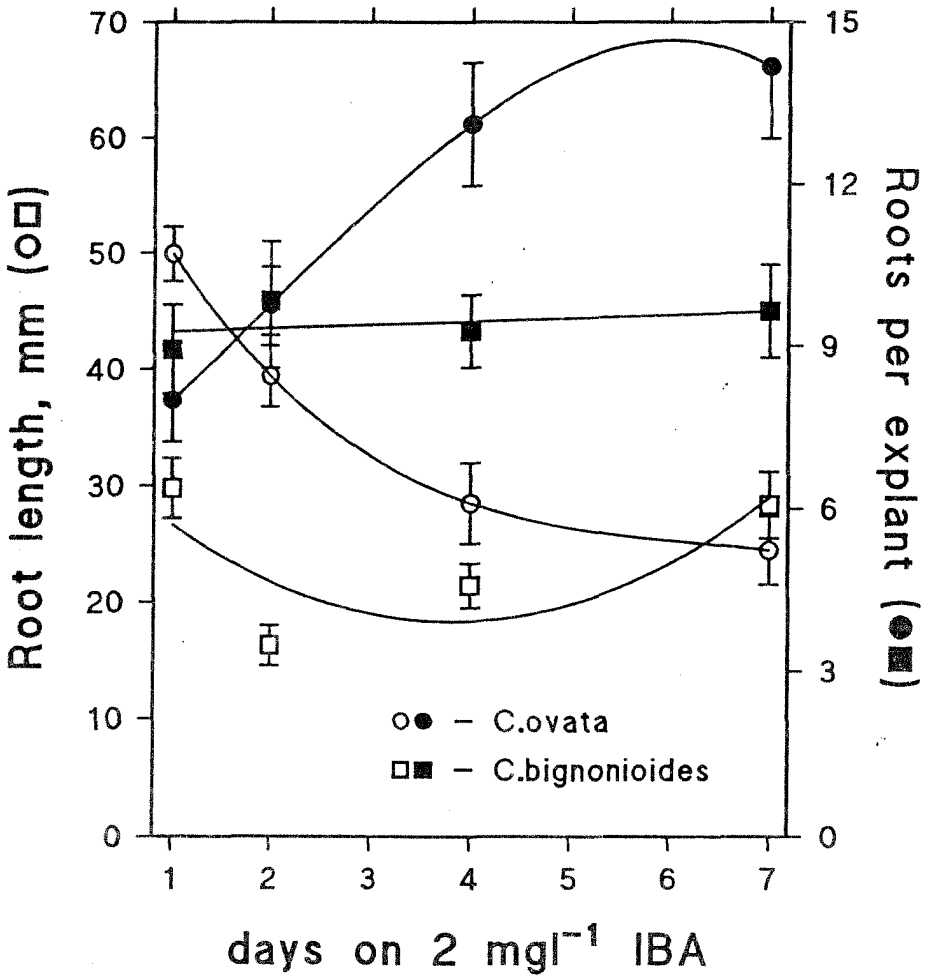

Fig. 4. – Effect of duration of the auxin treatment (1, 2, 4 and 7 days on 2.0 mgl^{-1} IBA) followed by transfer to hormone-free medium on the average number of roots per rooted culture and root length.

DISCUSSION

The technique of *in vitro* propagation of dicotyledonous tree species from epicotyls of aseptically germinated seeds has proved in our laboratory to be a simple and reliable method for fast estblishment of *in vitro* cultures. This method has been specially useful when clonal propagation was not the primary goal of investigation and when preliminary culture requirements for shoot cultures of certain species where

sought. In this way conditions were determined for maintaining shoot cultures of *Ceratonia siliqua* (Vinterhalter et al., 1992; Vinterhalter & Vinterhalter, 1992) and *Morus alba* (Vinterhalter & Grubišić, 1990). Although the use of seedling epicotyls can not be considered as true clonal propagation (Krikorian, 1982) it can be used in species propagated by open pollinated seeds. Wysokinska and Swiatek (1989) presented a multiplication scheme for *C. bignonioides* in which shoot cultures were established by differentiation of callus originating from hypocotyls of two week old seedlings. Reasons for which this propagation scheme is based on the use of callus aren ot clear. We however propose the use of epicotyls and a significant shortcut in the *in vitro* propagation scheme for both *Catalpa* species.

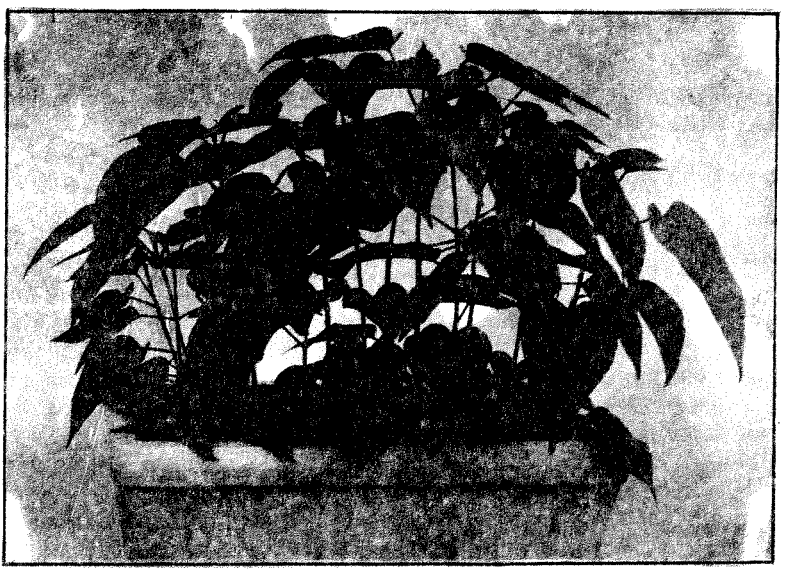

Fig. 5. – Plantlets of *C. bignonioides* after successive adaptation.

Hormonal balances which we used for shoot multiplication and rooting of *Catalpa ovata* are well known to give good results with many dicotyledonous species including almost all top fruit species (apples, pears, quinces, plums, peaches, cherries etc.). *C. bignonioides* required higher cytokinin concentration (1.0 mgl^{-1} BA) for satisfactory shoot multiplication. Wysokinska and Swiatek (1989) recommend even 2.0 mgl^{-1} BA as optimal for shoot multiplication which according to our findings already induced some vitrification and fasciation.

Our results in general support the findings of Wysokinska and Swiatek (1989) and show that other *Catalpa* species like *C. ovata* can also be propagated by *in vitro* methods.

REFERENCES

Bailey, L.H. (1949): Manual of cultivated plants. – The MacMillan Company, New York.

Krikorian, A.D. (1982): Cloning higher plants from aseptically cultured tissues and cells. – Biol. Rev. *57:* 151-218.

Murashige, T., Skoog, F. (1962): A revised medium for rapid growth and bioassays with tobacco tissue cultures. – Physiol. Plant. *15:* 473-497.

Vinterhalter, D., Grubišić, D. (1990): Brzo razmnožavanje belog duda (*Morus alba* L.) metodom kulture *in vitro.* IX Simp. Yug. Soc. Pl. Phys. Knjiga abstrakta, pp. 67.

Vinterhalter, D., Grubišić, D., Bojović-Cvetić, D., Budimir, S. (1992): Lenticel hypertrophy in shoot cultures of *Ceratonia siliqua* L. – Plant Cell Tissue and Organ Culutre, *31:* 111-114.

Vinterhalter, D., Vinterhalter, B. (1992): Factors affecting *in vitro* propagation of Carob (*Ceratonia siliqua* L.). – Arch. Biol. Sci. *44:* 177-186.

Wysokinska, H., Swiatek, L. (1989): Micropropagation of *Catalpa bignonioides* Walt. through tissue cultures. – Acta Soc. Bot. Poloniae, *58:* 185-198.

Rezime

MILICA MANOJLOVIĆ, BRANKA VINTERHALTER, DRAGAN VINTERHALTER

IN VITRO RAZMNOŽAVANJE *CATALPA OVATA* G. DON I *CAPALTA BIGNONIOIDES* WALT.

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Kulture izdanaka *Catalpa ovata* i *C. bignonioides* pokrenute iz epikotila aseptično isklijalog semena gajene su na podlozi po Murashige i Skog-u (1962). Optimalna koncentracija BA za multiplikaciju izdanaka bila je 0.5 mg^{-1} za vrstu *C. ovata* i 1.0 mgl^{-1} za *C. bignonioides.* IBA je vrlo malo uticala na multiplikaciju pa je koncentracija 0.1 mgl^{-1} smatrana optimalnom za obe vrste. Izdanci vrste *C. bignonioides* bili su više nego dva puta duži nego izdanci vrste *C. ovata* na svim podlogama koje su sadržavale više od 0.1 mgl^{-1} BA. Izdanci dužine 1.5-2.0 cm su se lako ožiljavali, a podloga sa 0.5 mgl^{-1} IBA je bila optimalna. Adaptacija ožiljenih biljaka nakon presađivanja u smesu treseta, peska i organskog đubriva bila je uspešna a biljke su dalje gajene u staklari.