GLASNIK INSTITUTA ZA BOTANIKU I BOTANIČKE BAŠTE UNIVERZITETA U BEOGRADU
BULLETIN DE L'INSTITUT ET DU JARDIN BOTANIQUES DE L'UNIVERSITE DE BEOGRAD
Tom XVIII, 1984, Beograd

UDC 581.144 : 582.738 (497.1)

DRAGOLJUB GRUBIŠIĆ, LJUBINKA ĆULAFIĆ and MIRJANA NEŠKOVIĆ

IN VITRO VEGETATIVE PROPAGATION OF CERCIS SILIQUASTRUM L.

Institute for Biological Research „Siniša Stanković", Beograd
Institute of Botany and Botanical garden, Faculty of Science, Beograd

Grubišić, D., Ćulafić, Lj. and Nešković, M. (1984):: *In vitro vegetative propagation of Cercis siliquastrum L.* – Glasnik Instituta za botaniku i botaničke bašte Univerziteta u Beogradu, Tom XVIII, 77–80.

The ornamnetal tree *Cercis siliquastrum* L. can be vegetatively propagated by *in vitro* culture methods, using apical and lateral buds as initial explants. Bud multiplication and subsequent shoot elongation are optimally induced without changes in the medium, at 10^{-5} M benzylaminopurine (BAP). A 100% rhizogenesis may be obtained by applying naphtalene acetic acid (NAA, 10^{-5} M) while roots elongate in hormone free medium with half–strength mineral solution. A great number of plantlets, ready for the transfer into soil, is obtainable after only three subcultures.

Key words: *Cercis siliquastrum* L., vegetative propagation *in vitro*, BAP, NAA.
Ključne reči: *Cercis siliquastrum* L., vegetativno razmnožavanje *in vitro*, BAP, NAA.

INTRODUCTION

Seed propagation of woody species is not considered to be a convenient method for horticultural practice, since the period necessary for obtaining seedlings may be rather long. Vegetative propagation by spontaneous or hormone–induced rooting of cuttings is a more suitable method, but not readily applicable in certain species. For many herbaceous ornamentals, large scale propagation by *in vitro* technique has already been proven as an efficient and commercially justified method. Therefore, much attention has been paid recently to the extension of those methods to woody plants. Good results have been reported for some *Pinophyta*, such as *Pinus pinaster, P. radiata, P. silvestris, Thuja plicata* etc. (David, 1982), and species of *Acer, Betula, Quercus, Populus* etc. (Brown and Sommer, 1982). *Cercis siliquastrum* L. is an ornamental, thermophyllous species, widespread in mediterranean and submediterranean regions (Šilić, 1973) and also cultivated in parks throughout the country, as a very decorative plant. The seeds

have a rather long period of dormancy (P r o f u m o, 1979), which hampers rapid large–scale production of seedlings. Hence, the purpose of the present work was to study the feasibility of *in vitro* vegetative propagation, which may be of importance in horticultural practice.

MATERIAL AND METHODS

Seeds were harvested in January 1983, from an old tree in the Botanical Garden in Belgrade. They were dipped in 50% H_2SO_4 for 20 min and then sterilized by 4% sodium hypochlorite (20 min) rinsed 3 times in sterile water and sown on moist filter paper, in petri dishes. After 3 days the seed coats and testa were removed and isolated embryos planted in test tubes, containing MS mineral solution (M u r a s h i g e and S k o o g, 1962), 0.7% agar and 3% sucrose. When seedlings were 7–10 days old (2–3 cm long), fragments of cotyledons and hypocotyls were isolated and further cultivated. Apical buds and nodes with lateral buds were also put in culture.

The culture medium was supplemented by (in mg l^{-1}): thiamin 0.4, pyridoxine 0.5, nicotinic acid 0.5 and myo–inositol 100. Benzylaminopurine (BAP), 6–furfuryla minopurine (Kin), naphtalene acetic acid (NAA), dihlorphenoxyacetic acid (2,4–D), gibberellins A_3 and A_7 were added as indicated in the text. The cultures were transferred to fresh media every 4 weeks. They were grown at 25 ± 2°C, in white light of Sylvania Grolux and Tež „Tesla" (4 W m^{-2}), at a daylength of 16 h.

RESULTS

Fragments of cotyledons, hypocotyls and internodes were put initially on media containing auxins (2,4–D, NAA or IAA) and cytokinins (Kin and BAP) in various proportions. Only calluses were produced in these explants. The callus growth was generally poor in all media. Some of them developed roots when cytokinin content was lower. The transfer of calluses to media with high BAP to IAA ratio did not induce the formation of bud initials. After a few passages the calluses turned brown and died.

Apical and lateral buds were cut with adjacent stem tissue and cultivated initially on a medium containing only BAP (5×10^{-5} M). A callus tissue appears on the basal part of primary explants. Lateral buds grow out through the callus and develop into shoots. Approximately 10 buds develop on each explant and they reach a length of 3 or more cm. In later experiments different concentrations of BAP were tested (5×10^{-6}, 10^{-5} and 2×10^{-5} M) and the medium one was found most suitable. At 10^{-5} M BAP satisfactory bud multiplication was induced, while shoot elongation was not seriously impaired. In a series of experiments, each explant out of 20 produced on the average 7.4 buds (S.E. = 0.67). About 33% of these buds were longer than 5 cm, and could immediately be used for rooting. The rest of buds were induced to elongate by GA_3 or GA_7 (1 mg l^{-1}); they reached a length of 10.4 cm and 11.8 cm, respectively, within 3 weeks and also were induced to form roots. Rooting was induced by NAA (10^{-5} M). Rooted plantlets were suitable for transfer into soil and further cultivation in the greenhouse.

DISCUSSION

Occurrence of plant regeneration in calluses grown *in vitro* has been reported in some woody species of the order *Fabales*, as in *Dilbergia sissoo* (M u k h o p a d h y a y

and Mohan Ram, 1981) and *Albizzia lebbek* (Upadhyaya and Chandra, 1983).

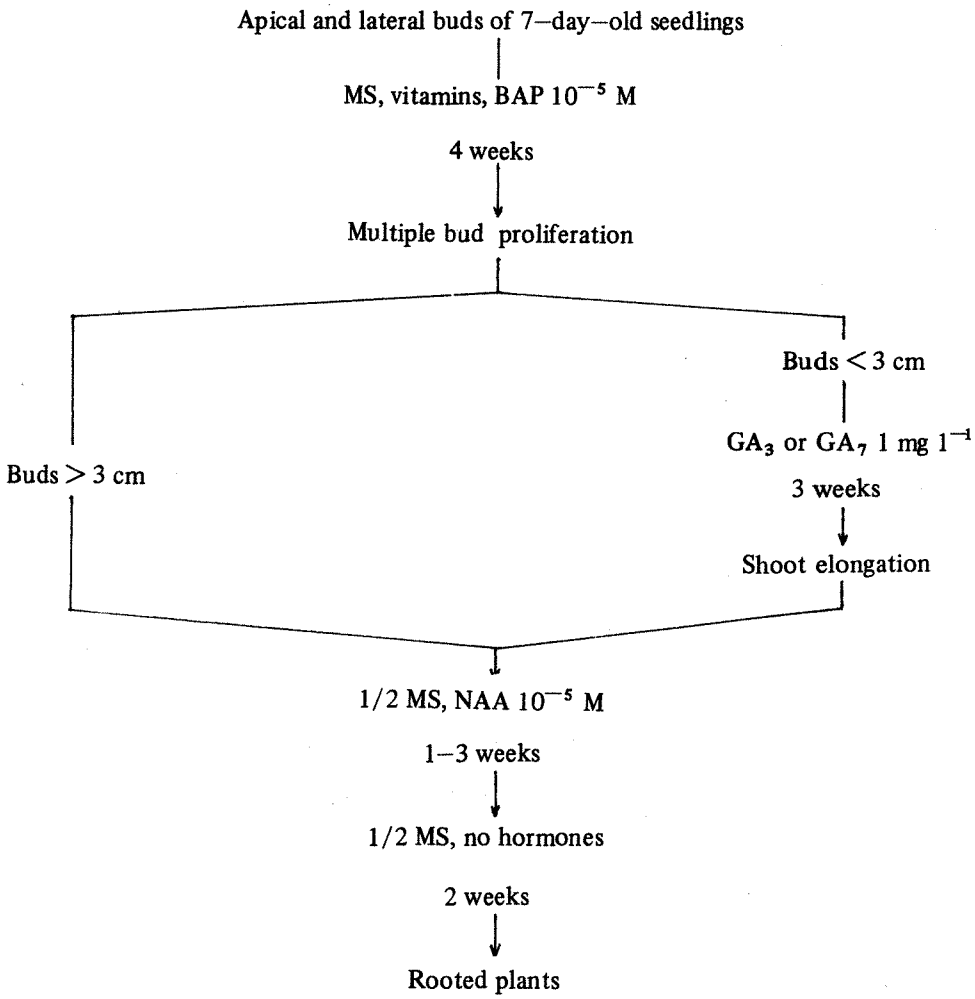

Fig. 1. – Scheme for vegetative propagation of apical and lateral buds.

We obtained callus tissue in cotyledon, hypocotyl and internode explants of *C. siliquastrum*, but organogenic capacity of this tissue was limited to root production only. No buds were formed even in usual bud–inducing media. Upadhyaya and Chandra (1983) demonstrated than in *Albizzia lebbek* each organ explant required a specific combination of hormones, which might explain the failure of bud induction in *C. siliquastrum* tissue.

However, micropropagation of *C. siliquastrum* from apical and lateral buds can be easily obtained. The procedure described here does not require much time, since two

steps, bud multiplication and elongation, can be subsequently induced even without changing the medium. Plant propagation can successfully be obtained by following the procedure displayed in Fig. 1. As can be seen, a period of 3–4 months is sufficiently long to multiply initial explants by a factor of 5, and to produce plantlets ready for transfer into soil.

REFERENCES

Brown, C. L. and Sommer, H. E. (1982): Vegetative propagation of dicotiledonous trees. – In: Tissue culture in forestry. – Bonga J. M. and Durzan D.S. (Eds). M. Nijhoff/Dr. W. Junk Publ., The Hague/Boston/London, pp. 109–150.

David, A. (1982): *In vitro* propagation ot gymnosperms. – In: Tissue culture in forestry. – Bonga J. M. and Durzan D. (Eds). M. Nijhoff/Dr. W. Junk Publ., The Hague/Boston/London. pp. 72–108.

Mukhopadhyay, A. and Mohan Ram, H. Y. (1981): Regeneration of plantlets from excised roots of *Dalbergia sissoo*. – Indian J. Exper. Biol., 19: 1113–15.

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

Profumo, P., Gastaldo, P. and Martinucci, R. (1979): On inhibitory action of endosperm on germination of *Cercis siliquastrum* seeds. – Experientia, 35: 1452–53.

Šilić, Č. (1973): Atlas drveća i grmlja. – Zavod za izdavanje udžbenika, Sarajevo, str. 124.

Upadhyaya, S. and Chandra, N. (1983): Shoot and plantlet formation in organ and callus cultures of *Albizzia lebbek* Benth. – Ann. Bot., 52: 421–424.

Rezime

DRAGOLJUB GRUBIŠIĆ, LJUBINKA ĆULAFIĆ i MIRJANA NEŠKOVIĆ

VEGETATIVNO RAZMNOŽAVANJE CERCIS SILIQUASTRUM L. U KULTURI IN VITRO

Institut za biološka istraživanja „Siniša Stanković", Beograd
Institut za botaniku i botanička bašta,
Prirodno–matematički fakultet, Beograd

Ukrasna drvenasta vrsta *Cercis siliquastrum* L., može se razmnožavati vegetativnim putem metodom kulture *in vitro* iz apikalnih i bočnih pupoljaka. Pri optimalnoj koncentraciji benzilaminopurina (BAP, 10^{-5} M) dolazi do bočnog grananja i umnožavanja pupoljaka, a da pri tom nije inhibirano njihovo izduživanje. Kod izduženih izdanaka indukuje se ožiljavanje (100%) naftilsirćetnom kiselinom (NAA, 10^{-5} M), a prenošenjem na medijum bez auksina omogućava se izduživanje korenova, posle čega se biljke prenose u zemlju.