Proc. Indian Acad. Sci. (Plant Sci.), Vol. 93, No. 6, November 1984, pp. 655–660

Micropropagation of *Salix babylonica* through *in vitro* shoot proliferation

K K DHIR, RAJIV ANGRISH and MONIKA BAJAJ
Department of Botany, Panjab University, Chandigarh 160014, India

MS received 22 July 1983; revised 15 September 1984

Abstract. Shoots originating from nodal stem explants of *Salix babylonica* showed maximum proliferation through axillary branching on a modified Murashige-Skoog medium supplemented with 1 mg/l of benzylaminopurine. Incorporation of naphthaleneacetic acid or gibberellic acid did not enhance shoot proliferation in the presence of benzylaminopurine. Shoots were grown to a suitable length on a medium with low concentration of benzylaminopurine. Subsequently they were excised and allowed to root on a basal medium, with or without a short pre-treatment with naphthaleneacetic acid. Rooted plantlets were transferred to pots and maintained under high humidity conditions to prevent their desiccation. The high humidity conditions were gradually withdrawn and the plantlets established as independently growing saplings.

Keywords. *Salix babylonica*; micropropagation; *in vitro* shoot proliferation.

1. Introduction

In the past few years micropropagation of woody plants through *in vitro* shoot proliferation has gained increasing attention. Generally shoot apices are cultured on a cytokinin supplemented medium. This results in reduced apical dominance along the shoot axis and encourages the growth of axillary buds into lateral branches. This method is direct as it avoids the callus/suspension stages that often are morphogenetically recalcitrant and associated with mutations (Murashige 1974; Bonga 1976; Abbott 1978; Anderson 1980).

Willows are of considerable value in horticulture and forestry. This paper describes the micropropagation of *Salix babylonica* Roxb, the weeping willow, through shoot proliferation *in vitro*.

2. Material and methods

2.1 *Plant material*

A 15-year old *Salix babylonica* tree was used as the source of explants. Current year's branches, 20–40 cm long, were excised from the tree round the year except during bud burst and active shoot growth in March and April when new branches were developing. The branches were made into stem explants (2·5 cm) with one generative axillary bud each. The leaf was excised from the stem explant leaving only a portion of the petiole attached to it. Cut ends of each explant were plugged with molten wax. The explants were then washed with a solution of 0·05% neutral laboratory detergent and finally surface-sterilized with 0·2% $HgCl_2$ for about 10 min. Explants were washed 4–5 times with sterile distilled water to remove traces of $HgCl_2$. Fresh cuts were made at the

plugged ends of the stem explants and the resultant explants (*ca.* 1 cm) with one generative bud each were planted on variously modified media. The generative buds sprouted after 7–10 days and developed into a male catkin. After 20–25 days the catkin senesced and abscissed. However, a vegetative shoot developed from the extra-axillary position of each reproductive bud (figure 1). Such shoots were utilised in proliferation.

2.2 *Culture medium*

Murashige-Skoog (1962) medium modified to contain half the concentration of its major salts but full minor salts and vitamins and 3% sucrose was used as the basal medium (BM) (Angrish and Nanda 1982). To obtain enhanced shoot proliferation BM was further supplemented with two cytokinins, *i.e.* kinetin (KN) and 6-benzylamino-purine (BA), alone and in combination with α-naphthaleneacetic acid (NAA) or gibberellic acid-3 (GA) as detailed elsewhere in the text. Media were gelled with 0·9% agar and pH was adjusted at 5·8 before autoclaving at 1·1 kg/cm^2 for 17 min.

2.3 *Cultural conditions*

Cultures were maintained in an air-conditioned room (27 ± 2°C). Twelve hours of light and 12 hr of dark cycles of white light from two 4′ cool-white fluorescent lamps maintained at a distance of 30 cm from the surface of the culture formed the source of illumination.

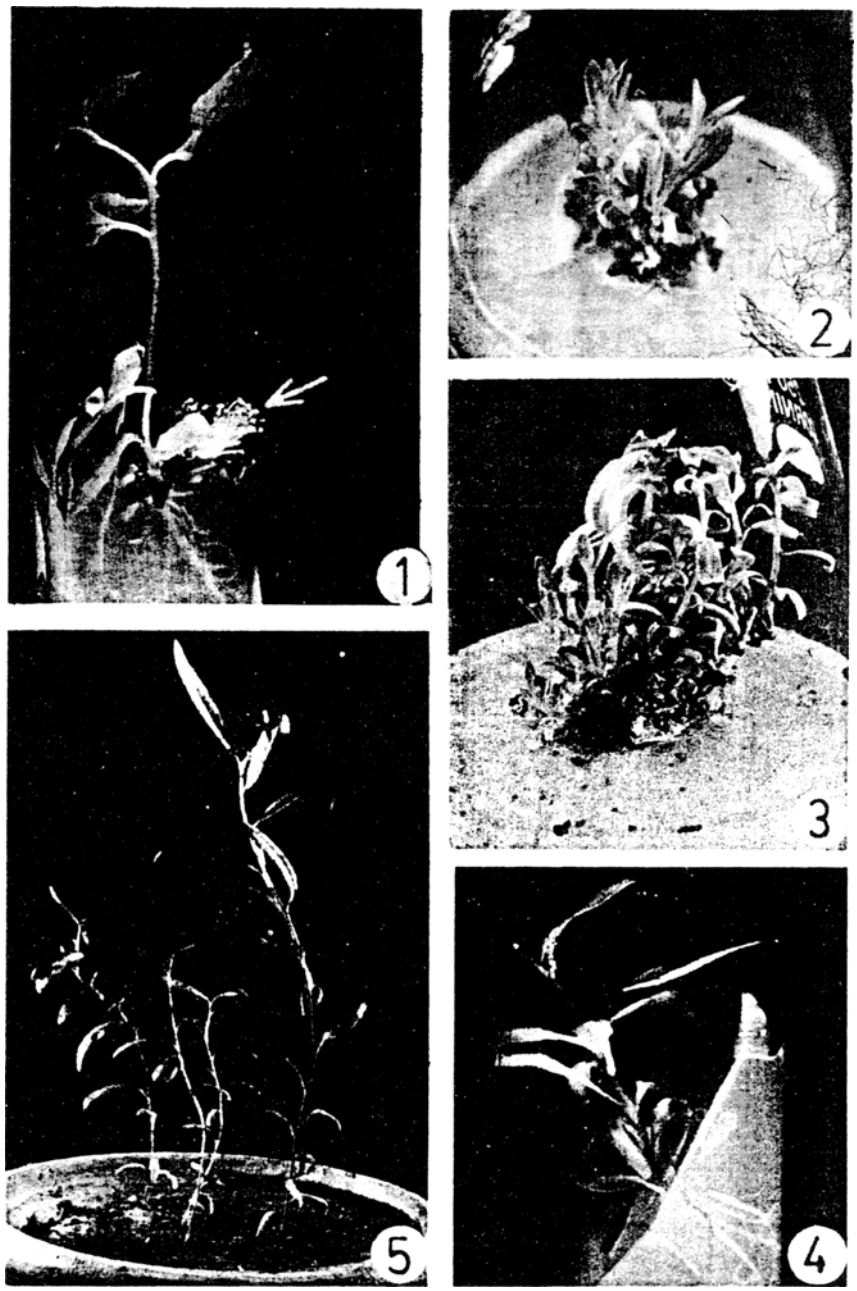
3. Results

3.1 *Shoot proliferation in vitro*

Explants (20 replicates per treatment) were cultured on the BM supplemented with different concentrations (0, 0·5, 1, 2·5, 5 mg/l) of KN and BA in 15·2 × 2·5 cm culture tubes, each containing 25 ml of the medium. After 50 days the explants along with the developing mass of shoots were sub-cultured in 100 ml flasks with 50 ml of the culture medium each to meet the increasing requirement of nutrition and space. Final observations on the number of shoots developing from each explant were recorded after another 50 days, *i.e.* 100 days after the commencement of experimentation.

On BM one or sometimes two shoots developed from the extra-axillary position of the node of each explant and grew to a considerable extent (figure 1). However, on KN and BA supplemented media the development of such extra-axillary shoots was accompanied by simultaneous development of lateral shoots in the axils of their leaves. Some laterals again showed axillary branching. Such a pattern of growth resulted in a mass of shoots in each culture vessel (figure 2). Of the two cytokinins tried 1 mg/l BA (BM-BA medium) caused proliferation of maximum number of shoots (table 1).

In an attempt to further enhance shoot proliferation, BM-BA medium was supplemented with different concentrations of NAA and GA. Experimental protocol was the same as described for cytokinins alone. It is seen (table 2) that the number of shoots produced on media supplemented with these regulators was less as compared to BM-BA alone. The latter was thus established to be optimal for maximum shoot proliferation. Individual shoots when excised and sub-cultured on BM-BA medium again developed into shoot masses through axillary shoot proliferation. As the lag period for the



Figures 1–5. 1. A nodal stem explant cultured on BM after 30 days. Note a male catkin (arrow) that has developed from the axillary reproductive bud. A fairly long shoot of extra-axillary origin is also seen. 2. A mass of shoots developed on BM + 1 mg/l BA (BM-BA) medium after 100 days. 3. Fairly long shoots developed on BM + 0·1 mg/l BA medium 25 days after transfer from BM-BA medium. 4. A 15 day old plantlet produced after the rooting of an excised shoot on BM. 5. About 50 day old saplings established in soil under natural conditions.

Table 1. Number of shoots developed on the BM with different concentrations of KN and BA after 100 days.

Concentration (mg/l)	KN	BA
0	1·5 ±0·2	—
0·5	3·1 ±0·4	9 ±1
1	5·5 ±0·6	15·2±2
2·5	4·75±0·5	4·4±0·7
5	3·3 ±0·5	1·1±0·2

± Standard error.

Table 2. Number of shoots developed on BM-BA medium supplemented with different concentrations of NAA and GA after 100 days.

Concentration (mg/l)	NAA	GA
0 (Control)	14·75±1·8	—
0·5	10·5 ±0·85	12·35±1·13
1	10·6 ±1·25	10·3 ±0·85
2·5	6·35±0·84	5·4 ±0·57

± Standard error.

regeneration of shoots from the stem explants was eliminated in this case, an average of 23 ± 2 shoots per vessel were produced in 100 days.

3.2 *Procurement of shoots for rooting treatment*

It was found that 2–3 cm long shoots were convenient for rooting treatment. However the majority of shoots produced on BM-BA medium were 0·5–1 cm long and were therefore too small for excision and subsequent handling. To obtain shoots of suitable length, individual shoot masses produced on BM-BA medium after 100 days were transferred to a medium with a low (0·1 mg/l) concentration of BA. The length of the shoots was recorded after 25 days. It is seen (table 3) that while no significant increase in shoot length occurred on the BM-BA medium, on BM + 0·1 mg/l BA medium shoots grew to an average length of about 3 cm (figure 3). Such shoots could be conveniently excised and handled for subsequent rooting operations.

3.3 *Rooting of shoots*

About 2–3 cm long shoots (20 replicates) were excised from the *in vitro* raised shoots and planted directly on the BM or after an instant (about 50 sec) dip of the basal part of excised shoot in sterile solution of 10 mg/l NAA. It was found that in both the cases about 75% of the shoots rooted within 10 days and formed plantlets (figure 4). However roots developing from the untreated shoots were thin and weakly growing as compared to

Table 3. Extension growth of shoots produced on BM-BA medium upon transfer to BM + 0·1 mg/l BA medium after 25 days.

Medium	Shoot length (cm)
BM-BA (Control)	0·83 ± 1·06
BM + 0·1 mg/l BA	3·54 ± 0·37

± Standard error.

those developing from the NAA-treated ones which were thick and vigorously growing. Subsequent growth of plantlets was also better in the latter case.

3.4 *Transfer and acclimatization of plantlets in soil*

A fine mixture of sand and leaf compost (3 : 1) filled in earthenware pots (9 cm dia.) was used as the potting substrate. The pots along with the soil were autoclaved to kill any soil-borne infection. Rooted plantlets were gradually pulled out of the agar medium and immersed in water. Agar particles sticking to the root system were removed using a fine brush. The plantlets were allowed to remain in water for about 1 hr to completely leach out sucrose and other organic substances of the medium from their surface. It was observed that if such a practice was not followed a high proportion of plants is infected with fungus in the pots.

Plantlets were then planted in pots. Each pot was covered with a polythene bag to maintain high humidity around the plants. Pots were suitably watered from time to time. Knop's (Knop 1834) nutrient solution (5 ml) was added to each pot at weekly intervals. After about a week the plants started initiating new leaves. During the 2nd week the polythene bags were removed for 3–4 hr daily to expose the plants to conditions of natural humidity. After about 4 weeks when the plants had attained a height of 7–10 cm, these were transferred to bigger (22 cm dia.) pots and were maintained under natural conditions of day length and temperature. Plants were kept covered by polythene sheet for a major part of the day for another week. Thereafter they could withstand natural conditions and developed into 20–25 cm long saplings within 3 months (figure 5).

4. Discussion

The present report shows the important role of BA in the multiplication of shoots of *Salix babylonica* through axillary shoot proliferation. Vieitez and Vieitez (1980) also found that BA induced more intense development of axillary shoots in *Castanea sativa* than KN and zeatin. In the present case supplementation of BM-BA medium with NAA or GA reduced the number of shoots. Similarly there are other reports where regulators like auxins or gibberellins do not elicit a synergistic effect with cytokinins in supporting shoot multiplication (Lane 1978, 1979; Vieitez and Vieitez 1980). The optimal concentration of BA that supported maximum shoot multiplication in the present case is different from the one causing their rapid extension growth. Thus for the harvest of maximum number of shoots of desirable length, their transfer to a medium with low

concentration of cytokinin is recommended. The practice of transfer of excised shoots to a rooting (auxin containing) medium generally followed for the rooting of shoots (Anderson 1980) was not necessary in the present case and only short pre-treatment of auxin is recommended. Papov *et al* (1976) also found that excised shoot tips of cherry rooted upon transfer to a BM after pre-treatment with an auxin. Such a behaviour of some species appears to be due to their easy-to-root nature. The main difficulty encountered in the establishment of plantlets is the rapid desiccation of plantlets due to the abrupt shift to low humidity conditions in the field. This can be avoided by suitably covering the plants with polythene. It is our considered opinion that other willow and popular species can be similarly propagated.

Acknowledgement

The authors are thankful to the late Prof. K K Nanda for critical suggestions.

References

Abbott A J 1978 Practice and promise of micropropagation of woody species; *Acta Hortic.* **79** 113–127

Anderson W C 1980 Mass propagation by tissue culture: principles and practice. in: *Nursery production of fruit plants through tissue culture—applications and feasibility* USDA Science and Education Administration, Agricultural Research Results, Northeastern Series, No 11, December 1980 pp. 1–10

Angrish R and Nanda K K 1982 Dormancy and flowering process in reproductive buds of *Salix babylonica* cultured in vitro; *Z. Pflanzenophysiol.* **106** 263–269

Bonga J M 1976 Applications of tissue culture in forestry. in: *Applied and fundamental aspects of plant cell tissue and organ culture* (ed) J Reinert and Y P S Bajaj (Berlin: Springer Verlag) pp. 93–108

Knop W 1834 Bereitung einer koezentrierter Nahrstofflosung fur Pflanzen; *Mitt. Landev. Varstn.* **30** 292–294

Lane D W 1978 Regeneration of apple plants from shoot meristem tips; *Plant Sci. Lett.* **13** 281–285

Lane D W 1979 Regeneration of pear plants from shoot meristem tips; *Plant Sci. Lett.* **16** 337–342

Murashige T 1974 Plant propagation through tissue culture; *Annu. Rev. Plant Physiol.* **25** 135–166

Murashige T and Skoog F 1962 A revised medium for rapid growth and bioassays with tobacco cultures; *Physiol. Plant.* **15** 473–497

Popov Yu G, Vysotskii V K and Truscheckkin V G 1976 Culture of isolated cherry stem apices; *Fiziol. Rast.* **23** 513–518

Vieitez A and Vieitez L E 1980 Plantlet formation from embryonic shoots of chestnut grown in vitro; *Physiol. Plant.* **50** 127–130