

# MICROPROPAGATION OF *INCARVILLEA DELAVAYI* BUREAU ET FRANCHET (BIGNONIACEAE)

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## Abstract

A simple and efficient protocol for micropropagation of *Incarvillea delavayi* (Bignoniaceae), an ornamental plant from China, was developed in this study. As initial plant material, adventitious shoots were used. Effect of 0, 0.1, 0.5 and 1 mg l<sup>-1</sup> BAP in half-strength MS medium on shoot multiplication was studied. For rooting, 0.1 and 0.3 mg l<sup>-1</sup> NAA were tested. The highest shoot number (8.60 ± 0.78 shoots per explant) and plant height (5.50 ± 0.31 cm) were obtained on medium supplemented with 1 mg l<sup>-1</sup> BAP. The shoots optimally rooted on medium with addition of 0.3 mg l<sup>-1</sup> NAA (8.48 ± 0.51 roots per shoot; root length 1.64 ± 0.18 cm). Well rooted plants were transferred *ex vitro*. Eight weeks after *ex vitro* transfer, 62.5% plants survived. The protocol optimized here, can be used for large-scale propagation of ornamental plant *I. delavayi*.

Keywords: α-naphthalenacetic acid, 6-benzylaminopurine, Bignoniaceae, *Incarvillea delavayi*, micropropagation, ornamental plant

## INTRODUCTION

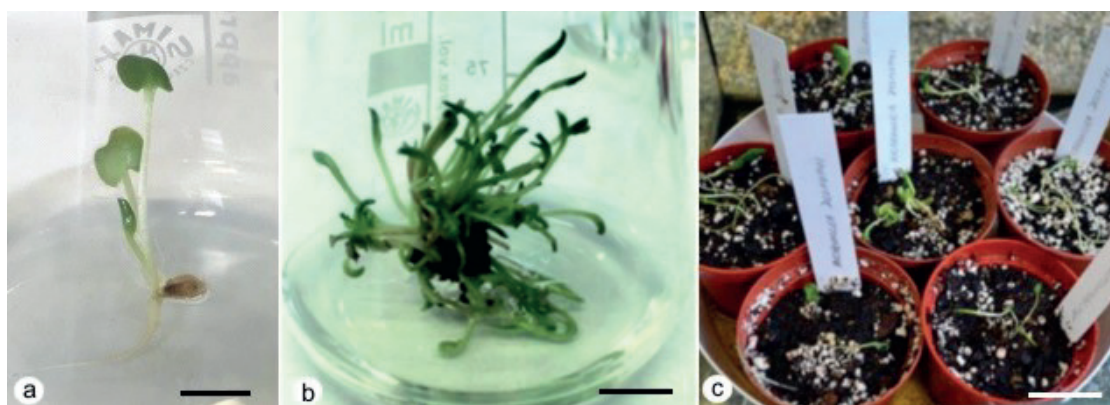
*Incarvillea delavayi* Bureau et Franchet (Bignoniaceae) is a perennial herb originating from Chinese province Yunnan (Chen *et al.*, 2004). The plant bears 2–6 showy pink trumpet-shaped flowers (Cutting, 1921) and it is being used as an ornamental plant in many countries. There are also several ornamental cultivars available. *Incarvillea delavayi* is conventionally propagated from seeds; however due to cross pollination occurring in this plant species (Cutting, 1921), the progeny display various genotypes. Alternatively, the plant can be propagated vegetatively by clamp division. Nevertheless, this process is difficult and not very efficient. *In vitro* propagation provides a rapid and reliable system for production of a large number of

genetically uniform plantlets (Jha and Ghosh, 2005), which is especially important for mass propagation of genotypes with significantly ornamental traits and for already developed cultivars of *I. delavayi*. Thus, the objective of this study was to develop an efficient system for micropropagation of *I. delavayi* via adventitious shoot organogenesis.

## MATERIALS AND METHODS

### Plant Material

As initial plant material, seeds of *Incarvillea delavayi* were used. They were provided by Botanischer Garten St. Gallen, Switzerland, in 2015. Twenty seeds were surfaced sterilized and cultivated as described by Viehmannova *et al.* (2016). After



1: Micropropagation of *Incarvillea delavayi*. a) In vitro germinated seedling (bar = 0.6 cm); b) Adventitious shoots produced on a half-strength MS medium supplemented with 1 mg l<sup>-1</sup> BAP after 30 days of cultivation (bar = 1 cm); c) Plants after ex vitro transfer (bar = 2 cm)

I: Effect of BAP on plant height, number of shoots, number of roots and root length in *Incarvillea delavayi*. The results are expressed as means and S.E. (standard error)

BAP (mg l <sup>-1</sup> )	Plant height (cm) (mean ± S.E.)	Number of shoots per explant (mean ± S.E.)	Number of roots per explant (mean ± S.E.)	Root length (cm) (mean ± S.E.)
0	4.20 ± 0.39 a	2.20 ± 0.20 a	1.21 ± 0.51 a	0.41 ± 0.08 a
0.1	4.81 ± 0.29 ab	3.21 ± 0.25 a	0.00 ± 0.00	0.00 ± 0.00
0.5	5.15 ± 0.32 ab	7.52 ± 0.76 b	0.00 ± 0.00	0.00 ± 0.00
1	5.50 ± 0.31 b	8.60 ± 0.78 b	0.00 ± 0.00	0.00 ± 0.00

Mean values in a column, followed by different letters, were significantly different according to the Tukey's HSD test ( $P < 0.05$ )

10–14 days when the seeds germinated (Fig. 1a), one seedling was randomly chosen. For standardization of the experiment, all initial plant material was multiplied from this single genotype by regular 4-week sub-cultures of adventitious shoots on half-strength MS medium (Murashige and Skoog, 1962) supplemented with 100 mg l<sup>-1</sup> myo-inositol, 30 g l<sup>-1</sup> sucrose, 8 g l<sup>-1</sup> agar (Agar-Agar, dänisch, Carl Roth GmbH & CoKG, Germany) and 0.5 mg l<sup>-1</sup> BAP.

### Shoot Multiplication and Rooting of Shoots

For shoot propagation, shoots ca. 1 cm high were transplanted onto half-strength MS medium supplemented with 0.1, 0.5 and 1 mg l<sup>-1</sup> 6-benzylaminopurine (BAP). As control, medium without cytokinin was used. Cultures were maintained under 16/8 hours light/dark regime at 25/20 °C with 36 µmol m<sup>-2</sup> s<sup>-1</sup> cool white fluorescent light. After 30 days, plant height, number of newly developed shoots, and number and length of roots were evaluated.

Effect of 0.1 or 0.3 mg l<sup>-1</sup> α-naphthalenacetic acid (NAA) was tested on rooting of the shoots. The auxin was added in half-strength concentrated MS medium. Number and length of developed roots were evaluated after 30 days of plant cultivation.

In each treatment, for both shoot multiplication and rooting, twenty explants were used in two replications. The data obtained from the experiment

were statistically evaluated using analysis of variance (ANOVA), and the significantly means were identified using Tukey's HSD test at the 5% level of significance (STATISTICA 12.0 StatSoft, USA).

### Ex Vitro Transfer

Well rooted plants were transferred *ex vitro* according to the protocol of Cepkova *et al.* (2015), using garden substrate (AGRO garden substrate, AGRO CS Ltd., Czech Republic) and perlite (1:1). Eight weeks after *ex vitro* transfer, the survival rate of plants was recorded.

## RESULTS AND DISCUSSION

In the current study, the effect of cytokinin BAP at various concentrations was tested on shoot organogenesis. The highest number of adventitious shoots was produced using 1 mg l<sup>-1</sup> BAP, i.e., the highest concentration of BAP tested. At this concentration, approx. 8.60 new shoots per explant were developed (Tab. I; Fig. 1b). Even though comparable results were achieved using 0.5 mg l<sup>-1</sup> BAP, the shoots on 1 mg l<sup>-1</sup> BAP grew more vigorously than plants on medium with lower concentration of BAP. The number of newly developed shoots decreased with lower BAP concentrations, and the medium lacking cytokinin produced just a minimum of shoots (Tab. I). Moreover, the height of plants increased with higher concentrations

II: Effect of NAA on root induction in *Incarvillea delavayi*. The results are expressed as means and S.E. (standard error)

NAA (mg l <sup>-1</sup> )	Number of roots per explant (mean ± S.E.)	Root length (mean ± S.E.)	Rooting (%)
0	1.21 ± 0.51 a	0.41 ± 0.08 a	30
0.1	4.71 ± 0.51 b	1.16 ± 0.15 b	85
0.3	8.48 ± 0.51 c	1.64 ± 0.18 b	100

Mean values in a column, followed by different letters, were significantly different according to the Tukey's HSD test ( $P < 0.05$ )

of BAP. In the family Bignoniaceae, similar concentrations of BAP were successfully used for axillary bud proliferation and shoot multiplication of medicinally and economically important timber tree *Tecomella undulata* (Chhajjer and Kalia, 2016). On the contrary, in *Zeyheria montana* from the same botanical family, very low concentration, i.e., 0.1 mg l<sup>-1</sup> BAP applied together with 0.5 mg l<sup>-1</sup> gibberellic acid (GA<sub>3</sub>) proved to be optimal for production of new shoots from nodal segments (Cardoso and da Silva, 2013). Likewise, in medicinal tree species *Stereospermum personatum*, 0.1 mg l<sup>-1</sup> BAP produced the maximum of shoots from explant (Shukla *et al.*, 2009). However, for some species of the Bignoniaceae family, other types of cytokinins provided even more satisfactory results, e.g., zeatin in *Tabebuia donnell-smithii* (Gonzalez-Rodriguez *et al.*, 2010), or kinetin in *Campsis radicans* (Dabski *et al.*, 2014).

For rooting of *I. delavayi*, 0.3 mg l<sup>-1</sup> NAA proved to be more efficient (100% rooted plants) than lower

NAA concentration (85% rooted plants). The former treatment provided approx. 8.48 roots per explant. Each root reached approx. 1.64 cm in length. Both number and length of roots decreased with lower concentration of NAA (Tab. II). After rooting, the plants were transferred *ex vitro*. Two months after their cultivation in the greenhouse, 62.5% plants survived (Fig. 1c). No morphological changes in these plants were observed and the plants grew continuously and produced new leaves. Among the species of the Bignoniaceae family, NAA had similar significant effect on rooting in *Tecomella undulate* (Kumari and Singh, 2012), and in combination with indole-3-acetic acid (IAA) in *Oroxylum indicum* (Dalal and Rai, 2004). In the latter study, indole-3-butyric acid (IBA), which is commonly used for rooting in trees from the Bignoniaceae family (Gonzalez-Rodriguez *et al.*, 2010; Shukla *et al.*, 2009; Cardoso and da Silva, 2013), was not optimal for root development in *O. indicum*.

## CONCLUSION

An efficient protocol for mass *in vitro* propagation of an ornamental plant *I. delavayi* was developed in this study. For multiplication of adventitious shoots, half-strength MS medium supplemented with 1 mg l<sup>-1</sup> BAP was optimal and the shoots successfully rooted on half-strength MS medium with addition of 0.3 mg l<sup>-1</sup> NAA. This is the first time that the *in vitro* propagation of *I. delavayi* has been reported.

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