

## THE CONSEQUENT EFFECT OF GROWTH RETARDANTS ON THE GROWTH AND DEVELOPMENT OF *Tibouchina urvilleana* Cogn. SHOOTS *IN VITRO*

Danuta Kozak<sup>1</sup>, Jarosław Grodek<sup>2</sup>

<sup>1</sup>Agricultural University in Lublin

<sup>2</sup>Polish Academy of Sciences of Lublin

**Abstract.** The consequent effect of growth retardants: paclobutrazol – 0.1, 0.5, 1, 5 mg l<sup>-1</sup>, flurprimidol – 0.1, 1, 5 mg l<sup>-1</sup>, chlorocholine chloride (CCC) – 5, 50 mg l<sup>-1</sup> on the growth and development of *Tibouchina urvilleana* Cogn. *in vitro* was examined. Paclobutrazol at concentration of 1 or 5 mg l<sup>-1</sup> and flurprimidol at 5 mg l<sup>-1</sup> inhibited the growth of shoots significantly. It was noted that shoots originating from these media had thicker, straighter stems and shorter internodes. The stimulating influence of CCC at 5 mg l<sup>-1</sup> on the elongation of the main shoot was observed. Axillary shoots formation was the best when shoots were derived from the medium supplemented with flurprimidol 1 mg l<sup>-1</sup>. Paclobutrazol at 0.5 and 1 mg l<sup>-1</sup> and flurprimidol at 5 mg l<sup>-1</sup> influenced the formation and fresh weight of roots increase, but flurprimidol was more effective. Growth retardants (except paclobutrazol at 5 mg l<sup>-1</sup>) applied in the earlier passage stimulated elongation of roots.

**Keywords:** *Tibouchina urvilleana*, growth retardants, *in vitro*

### INTRODUCTION

*Tibouchina urvilleana* Cogn. (*Melastomataceae*) is an ornamental shrub or small tree 1–4 m tall. Shoots of *Tibouchina* indicate high growth rate, so there is a need for growth retardants to obtain compact plants for use as interior potted plants [Abdullah et al. 1988, Johansen et al. 1999]. It is well known that growth substances, especially growth retardants, can have long-term effects on the growth and development of plants [Ziv 1992, Larsen and Lieth 1993, Ruter 1994]. The purpose of this experiment was to determine the further growth and development *in vitro* of *Tibouchina urvilleana* shoots which had been previously subjected to the effect of three growth retardants (paclobutrazol, flurprimidol, CCC).

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Corresponding author – Adres do korespondencji: Danuta Kozak, Department of Ornamental Plants, Agricultural University in Lublin, Leszczyńskiego 58, 20-068 Lublin, Poland, e-mail: dkozak@autograf.pl

## MATERIAL AND METHODS

Shoot tips having 2 fully developed leaves were cultivated on the basic Murashige and Skoog (MS) [1962] medium containing: mineral salts and  $\text{NaH}_2\text{PO}_4$  –  $170 \text{ mg l}^{-1}$ , thiamine –  $0.4 \text{ mg l}^{-1}$ , pyridoxine –  $0.5 \text{ mg l}^{-1}$ , nicotinic acid –  $0.5 \text{ mg l}^{-1}$ , glycine –  $2 \text{ mg l}^{-1}$ , myo-inositol –  $100 \text{ mg l}^{-1}$ , sucrose –  $30 \text{ g l}^{-1}$  and Agar-Agar –  $6.5 \text{ g l}^{-1}$ , and supplemented with growth regulators. The following growth retardants were used: paclobutrazol –  $0.1, 0.5, 1, 5 \text{ mg l}^{-1}$ , flurprimidol –  $0.1, 1, 5 \text{ mg l}^{-1}$ ; and chlorocholine chloride (CCC) –  $5, 50 \text{ mg l}^{-1}$ . Explants cultured on the medium without growth substances were used as a control. After four weeks, roots were removed and shoots were transferred to the basic medium without growth substances, where explants were cultured for 4 weeks. The following data were collected: length of main shoot, number of leaves/main and axillary shoot, number of axillary shoot and their length; and the number, length and fresh weight of roots.

There were four replications per treatment, each consisting of 5 explants/Erlemeyer flask. The cultures were maintained at  $22^\circ\text{C}$  and light intensity of  $35 \mu\text{M m}^{-2} \text{ s}^{-1}$  and 16-h photoperiod. Data were analyzed as a one-way completely randomized design, and mean separation by Tukey's at a 5% level of significance.

## RESULTS AND DISCUSSION

The present study indicated that growth retardants applied in the multiplication stage had a consequent effect on the further growth of shoots (tab. 1, phot. 1, 2). Length of main shoots depended both on kind of growth retardant and its concentration. On the media with CCC  $5 \text{ mg l}^{-1}$  the length of shoots was significantly greater than on the control medium. Also, the favorable effect of paclobutrazol at  $0.1 \text{ mg l}^{-1}$  and CCC at  $50 \text{ mg l}^{-1}$  on the growth of shoots was observed. Shoots derived from treatments with paclobutrazol and flurprimidol at higher concentrations ( $1\text{--}5 \text{ mg l}^{-1}$ ) characterized weaker elongation. Flurprimidol at  $5 \text{ mg l}^{-1}$  had the strongest retardation post-effect. Shoots obtained on this media and further cultivated on the medium without growth substances, had thicker stems and very short internodes. No significant differences were noted in the number of leaves between examined growth retardants treatments.

The mean fresh weight of shoots ranged from  $246.51 \text{ mg}$  (on the medium with  $5 \text{ mg l}^{-1}$  paclobutrazol) to  $743.97$  (on the medium with  $0.1 \text{ mg l}^{-1}$  paclobutrazol). It was noted that increasing the concentration of paclobutrazol up to  $5 \text{ mg l}^{-1}$  retarded the increase of fresh weight, about 66.9%. There was no influence of CCC and flurprimidol concentrations on the fresh weight of the main shoot.

As shown in table 2, growth retardants had a significant consequent effect on the number and growth of axillary shoots. On the media with paclobutrazol ( $0.5\text{--}5 \text{ mg l}^{-1}$ ) or with flurprimidol  $1 \text{ mg l}^{-1}$ ,  $1.0\text{--}1.8$  axillary shoots were formed. The control medium and the media containing flurprimidol  $0.1$  and  $5 \text{ mg l}^{-1}$  or CCC  $5$  and  $50 \text{ mg l}^{-1}$  were not effective in induction of new shoots. Axillary shoots derived from medium containing paclobutrazol at concentrations of  $0.5$  or  $1 \text{ mg l}^{-1}$  characterized the highest length and fresh weight.



Phot. 1. Plants of *Tibouchina urvilleana* derived from the MS medium containing different concentrations of paclobutrazol, after 4 weeks *in vitro* on the basic MS medium

Fot. 1. Rośliny *Tibouchina urvilleana* pochodzące z pożywki MS z dodatkiem paklobutrazolu w różnych stężeniach, po 4 tygodniach kultury *in vitro* na pożywce podstawowej MS



Phot. 2. Plants of *Tibouchina urvilleana* derived from the MS medium containing different concentrations of flurprimidol, after 4 weeks *in vitro* on the basic MS medium

Fot. 2. Rośliny *Tibouchina urvilleana* pochodzące z pożywki MS z dodatkiem flurpirimidolu w różnych stężeniach, po 4 tygodniach kultury *in vitro* na pożywce podstawowej MS

Table 1. The consequent effect of the growth retardants on further growth of main shoot after 4 weeks of culture *in vitro* on the basic MS mediumTabela 1. Następczy wpływ retardantów na dalszy wzrost pędu głównego po 4 tygodniach kultury *in vitro* na pożywce podstawowej MS

Growth retardant Retardant wzrostu	Concentration Stężenie (mg l <sup>-1</sup> )	Length of main shoot Długość pędu głównego (mm)	Number of leaves/shoot Liczba liści/pęd	Fresh weight of shoot Świeża masa pędu (mg)
Control – Kontrola	0	68.94 bc <sup>8</sup>	8.9 abc	487.55 b
	0.1	81.92 ab	10.0 ab	743.97 a
Paclobutrazol	0.5	62.61 c	10.5 a	708.62 a
Paklobutrazol	1.0	43.84 d	9.9 ab	575.58 ab
	5.0	33.06 de	7.9 bc	246.51 c
Flurprimidol	0.1	68.37 bc	10.1 a	697.57 ab
Fluopirimidol	1.0	49.89 cd	10.2 a	654.92 ab
	5.0	19.61 e	9.3 ab	645.18 ab
CCC	5.0	89.05 a	9.5 ab	665.5 ab
	50.0	73.89 ab	10.1 a	586.60 ab
Mean – Średnia		59.12	9.6	601.2

\*Values in columns followed by the same letter do not differ significantly at p = 0.05

\*Wartości w kolumnach oznaczone tą samą literą nie różnią się istotnie przy p = 0,05

Table 2. The consequent effect of the growth retardants on the formation and growth of axillary shoot after 4 weeks of culture *in vitro* on the basic MS mediumTabela 2. Następczy wpływ retardantów na tworzenie i wzrost pędów kątowych po 4 tygodniach kultury *in vitro* na pożywce podstawowej MS

Growth retardant Retardant wzrostu	Concentration Stężenie (mg l <sup>-1</sup> )	Number of axillary shoots/shoot Liczba pędów kątowych/pęd	Length of axillary shoots Długość pędów kątowych (mm)	Number of leaves/shoot Liczba liści/pęd	Fresh weight of 1 axillary shoot Świeża masa 1 pędu kąтового (mg)
Control Kontrola	0	0.1 d	2.00 e	2.0 c	1.40 e
	0.1	0.7 bcd	9.50 bcd	2.8 b	10.19 cd
Paclobutrazol	0.5	1.2 ab	14.36 ab	3.9 a	31.72 a
Paklobutrazol	1.0	1.3 ab	17.33 a	4.0 a	32.19 a
	5.0	1.0 bc	6.75 cd	4.1 a	17.47 bc
Flurprimidol	0.1	0.4 cd	11.86 abc	2.7 b	12.96 cd
Fluopirimidol	1.0	1.8 a	11.54 abc	3.5 a	22.93 ab
	5.0	0.3 d	3.50 e	4.0 a	28.45 a
CCC	5.0	0.3 d	4.66 de	2.7 b	5.8 de
	50.0	0.4 cd	3.24 e	2.0 c	4.9 de
Mean – Średnia		0.8	8.47	3.2	16.80

\*Values in columns followed by the same letter do not differ significantly at p = 0.05

\*Wartości w kolumnach oznaczone tą samą literą nie różnią się istotnie przy p = 0,05

The results of the rooting study (tab. 3) indicated that the treatment with 0.5 and 1 mg l<sup>-1</sup> paclobutrazol or 5 mg l<sup>-1</sup> flurprimidol significantly increased the number of roots. Paclobutrazol at 5 mg l<sup>-1</sup> had a harmful post-effect on the rooting of shoots. Growth retardants, except paclobutrazol at 5 mg l<sup>-1</sup>, promoted elongation of roots. All

studied growth retardants had a significant effect on the fresh weight of roots. In all treatments with retardants, the produced roots were heavier compared to the control medium (from 75.44 mg on the medium with 50 mg l<sup>-1</sup> CCC to 361.04 mg on the medium with flurprimidol 5 mg l<sup>-1</sup>).

Table 3. The consequent effect of the growth retardants on the formation and growth of roots after 4 weeks of culture *in vitro* on the basic MS medium

Tabela 3. Następczy wpływ retardantów na regenerację i wzrost korzeni po 4 tygodniach kultury *in vitro* na pożywce podstawowej MS

Growth retardant Retardant wzrostu	Concentration Stężenie (mg l <sup>-1</sup> )	Number of roots Liczba korzeni	Length of roots Długość korzeni (mm)	Fresh weight of roots/explant Świeża masa korzeni/eksplantat (mg)
Control – Kontrola	0	4.5 bc	28.54 cd	80.76 c
	0.1	6.9 bc	41.18 ab	204.72 b
Paclobutrazol	0.5	9.3 ab	44.74 ab	365.37 a
Paklobutrazol	1.0	8.7 ab	39.57 abc	341.08 a
	5.0	3.7 c	26.34 d	169.72 bc
Flurprimidol	0.1	6.3 bc	34.97 bcd	172.36 bc
Fluopirimidol	1.0	7.2 bc	48.48 a	238.75 b
	5.0	11.8 a	34.31 bcd	441.80 a
CCC	5.0	6.1 bc	40.54 ab	163.91 bc
	50.0	6.7 bc	37.19 a-d	156.20 bc
Mean – Średnia		7.1	37.59	233.47

\*Values in columns followed by the same letter do not differ significantly at p = 0.05

\*Wartości w kolumnach oznaczone tą samą literą nie różnią się istotnie przy p = 0,05

The results of the experiment imply that the use of growth retardants in the medium caused a big long-term effect on the shoots of *Tibouchina urvilleana*. Many authors reported that growth retardants have an after-effect on: the size, yield and fruit quality [Ben and Poniedziałek 1994, Oosthuizen et al. 1995], promoting flowering of field-grown *Rhododendron* and *Kalmia* [Gent 1995], and reduction of wilting of *in vitro* grown plants after transfer to the soil [Novello et al. 1992, Roberts et al. 1992, Smith et al. 1992]. Larsen and Lieth [1993] noted that the persistence of the daminozide effect on *Chrysanthemum* shoot elongation was about 34 days. Paclobutrazol influenced plant height for *Pyracantha* after 9 months in the landscape [Ruter 1994]. Ziv [1992] observed a dwarfing effect of growth retardants used in tissue culture of *Gladiolus* even after 5–6 weeks in the greenhouse.

Further research is being undertaken for estimating the consequent influence of growth retardants on the growth of *Tibouchina urvilleana* shoots after transfer to the soil.

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## NASTĘPCZY WPŁYW RETARDANTÓW NA WZROST I ROZWÓJ PĘDÓW *Tibouchina urvilleana* cogn. *IN VITRO*

**Streszczenie.** Badano następczy wpływ następujących retardantów: paklobutrazolu – 0,1; 0,5; 1; 5 mg l<sup>-1</sup>, fluopirimidolu – 0,1; 1; 5 mg l<sup>-1</sup>, chlorku chlorocholiny (CCC) – 5, 50 mg l<sup>-1</sup> na wzrost i rozwój pędów *Tibouchina urvilleana* Cogn. *in vitro*. Paklobutrazol w stężeniu 1 i 5 mg l<sup>-1</sup>, a także fluopirimidol w stężeniu 5 mg l<sup>-1</sup> istotnie hamowały wzrost pędów. Pędy pochodzące z tych pożywek były grubsze oraz sztywniejsze i miały krótsze międzywęzła. Obserwowano stymulujący wpływ CCC w stężeniu 5 mg l<sup>-1</sup> na wzrost elongacyjny pędu głównego. Największą liczbę pędów kątowych wytworzyły pędy inkubowane wcześniej na pożywce zawierającej 1 mg l<sup>-1</sup> fluopirimidolu. Paklobutrazol w stężeniu 0,5 i 1 mg l<sup>-1</sup> oraz fluopirimidol w ilości 5 mg l<sup>-1</sup> wpływały na regenerację i przyrost świeżej masy korzeni. Fluopirimidol wywierał silniejsze działanie. Retardanty (z wyjątkiem paklobutrazolu w stężeniu 5 mg l<sup>-1</sup>) zastosowane we wcześniejszym pasażu stymulowały wzrost elongacyjny korzeni.

**Słowa kluczowe:** *Tibouchina urvilleana*, retardanty wzrostu, *in vitro*

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