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THE EFFECT OF GROWTH RETARDANTS APPLIED *IN VITRO* ON THE ACCLIMATIZATION AND GROWTH OF *Tibouchina urvilleana* cogn. *IN VIVO*

Danuta Kozak

Agricultural University in Lublin

Abstract. Shoot tips of *Tibouchina urvilleana* Cogn. were cultured 4 weeks *in vitro* in modified Murashige and Skoog (MS) [1962] medium supplemented with growth retardants: paclobutrazol – 0.1, 0.5, 1.0, 5.0 mg·dm^{-3}, flurprimidol – 0.1, 1.0, 5.0 mg·dm^{-3}, chlorocholine chloride (CCC) – 5.0, 50.0, 250.0 mg·dm^{-3}. Explants cultured on the medium without growth substances were used as a control. Rooted microcuttings were transferred to the greenhouse and transplanted into a mixture of 1 peat : 1 perlite, where they were grown for 5 weeks. Plants were then cultivated in a peat substrate during another 5 weeks. Acclimatization of rooted shoots in the greenhouse was affective in 92.5–100%. The survival of plants was lowest when microcuttings were previously cultured on medium with flurprimidol at 5.0 mg·dm^{-3}. Cultivation of *Tibouchina urvilleana* shoots *in vitro* on media with various growth retardants had a significant effect on the further growth of plants *ex vitro*. Paclobutrazol and flurprimidol at 5.0 mg·dm^{-3} inhibited very strong growth of plants after 10 weeks of growth *ex vitro*.

Key words: *Tibouchina urvilleana,* growth retardants, *in vitro*, acclimatization, *in vivo*

INTRODUCTION

Growth retardants are known to be effective in improving survival of plantlets after their transfer to the soil [Oliphant 1990, Ziv 1992, Eliasson et al. 1994]. Their beneficial influence is associated with reduction of wilting of plants [Smith et al. 1990, Novello et al. 1992, Roberts et al. 1992, Roberts and Matthews 1995]. There are also several reports on long-term effects of growth retardants on the growth and development of plants obtained *in vitro* from media with these substances [Ziv 1992, Larsen and Lieth 1993, Ruter 1994, Kozak and Grodek 2005].

The objective of this study was to determine the acclimatization and growth of *Tibouchina urvilleana* plants *ex vitro* which had been previously cultured *in vitro* on the media with the growth retardants (paclobutrazol, flurprimidol, CCC).

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

MATERIAL AND METHODS

Shoot tips taken from aseptically grown shoots were cultivated on the modified Murashige and Skoog (MS) [1962] medium solidified with Agar-Agar – 6.5 g·dm^{-3}, and supplemented with growth regulators. The following growth retardants were used: paclobutrazol – 0.1, 0.5, 1.0, 5.0 mg·dm^{-3}, flurprimidol – 0.1, 1.0, 5.0 mg·dm^{-3}; and chlorocholine chloride (CCC) – 5.0, 50.0, 250.0 mg·dm^{-3}. Explants cultured on the medium without growth substances were used as a control. The ph medium was adjusted to 5.8. The cultures were maintained at 22°C and light intensity of 35 µM m^{-2} s^{-1} and 16-h photoperiod.

After four weeks the plants were transferred to the greenhouse and transplanted into 8 cm pots containing a mixture of 1 peat : 1 perlite (by volume) and placed for further growth and hardening on the bench under polyethylene sheeting, where they were grown for 5 weeks. Plants were then planted to a peat substrate and cultivated during another 5 weeks. The pots were supplied once a week with a nutrient solution (Insol U 0.1%). After 5 weeks of growing *ex vitro* survival rate was estimated, and after 5 and 10 weeks of cultivation in the soil the following data were collected: height of plants, number of leaves/main and axillary shoot, number of axillary shoot and their length.

There were 20 plants per treatment. The plants were grown *ex vitro* in a greenhouse maintained about 25/20°C (day/night). The experiment was repeated twice. 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

Acclimatization of rooted shoots in the new environment in the greenhouse was affective in 92.5–100%. The survival of plants was lowest when microcuttings were previously cultured on medium with flurprimidol at 5.0 mg·dm^{-3}. According to many sources the growth retardants increase the ability of plants to overcome of acclimatization phase. Eliasson et al. [1994] noted that survival of *Prunus serotina* 4 weeks after transfer to the soil was significantly improved by paclobutrazol. Similarly, survival of plantlets of *Philodendron* that had been treated with paclobutrazol during proliferation was much better (100%), compared with control (78%) [Ziv 1992]. Roberts and Matthews [1995] reported that *Chrysanthemum* 'Pennile Reel' treated with paclobutrazol in rooting medium had greater resistance to desiccation. Gribaudo et al. [2001] found that 1.0 mg·dm^{-3} paclobutrazol used during the last stage of micropropagation of *Vitis vinifera* 'Nebbiolo' was less effective during acclimatization than relative humidity.

The present study indicated that pretreatment of *Tibouchina urvilleana* shoots *in vitro* by various growth retardants had a strong effect on the further growth of shoots *ex vitro* (tab. 1). After 5 weeks of growth *ex vitro* plants with the shortest stems were received when paclobutrazol or flurprimidol was applied at 5.0 mg·dm^{-3}. In this treatments the plant's height was 9.4 mm and 15.1 mm, and they characterized thick stems, very short internodes and small leaves. Similar tendency was observed by Kozak and Grodek [2005] in investigations of consequent effect of growth retardants on the growth

Table 1. Effect of various growth retardants in the medium on the further growth of *Tibouchina urvilleana* plants in the greenhouse
Tabela 1. Wpływ obecności w pożywce retardantów wzrostu na dalszy wzrost roślin *Tibouchina urvilleana* w szklarni

Growth retardant Retardant wzostu	Concentration Stężenie $mg \cdot dm^{-3}$	Rate of survival %	Height of plants Wysokość roślin cm		Number of leaves/main shoot Liczba liści/pęd główny	
			after 5 weeks po 5 tygodniach	after 10 weeks po 10 tygodniach	after 5 weeks po 5 tygodniach	after 10 weeks po 10 tygodniach
Control – Kontrola	0.0	100.0	11.2 ab	18.3 a	8.2 abc	10.4 ab
Paclobutrazol Paklobutrazol	0.1	97.5	9.0 abc	17.8 a	8.8 abc	11.1 a
	0.5	100.0	7.8 c	12.7 b	7.9 a-d	8.7 abc
	1.0	97.5	4.5 d	8.4 c	8.0 abc	8.1 bcd
	5.0	97.5	2.9 d	3.6 d	5.4 d	7.0 d
Flurprimidol Fluropirimidol	0.1	100.0	9.5 abc	16.9 a	10.0 a	11.3 a
	1.0	95.0	7.9 c	11.5 bc	7.3 bcd	7.8 cd
	5.0	92.5	1.7 d	2.4 d	6.6 cd	7.8 cd
CCC	5.0	100.0	11.9 a	19.3 a	10.4 a	10.8 a
	50.0	100.0	11.5 a	17.7 a	9.3 ab	10.2 abc
	250.0	100.0	8.5 bc	11.8 b	9.8 ab	10.8 a
Mean – Średnia		98.2	7.9	12.8	8.3	9.5

*Values in vertical 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. Effect of various growth retardants in the medium on the formation and growth of axillary shoots in plants of *Tibouchina urvilleana* cultivated in the greenhouse

Tabela 2. Wpływ obecności w pożywce retardantów wzrostu na formowanie i wzrost pędów kątowych u roślin *Tibouchina urvilleana* uprawianych w szklarni

Growth retardant Retardant wzostu	Concentration Stężenie $mg \cdot dm^{-3}$	Number of axillary shoots/shoot Liczba pędów kątowych/pęd		Length of axillary shoots Długość pędów kątowych mm		Number of leaves/axillary shoot Liczba liści/pęd kątowy	
		after 5 weeks po 5 tygodniach	after 10 weeks po 10 tygodniach	after 5 weeks po 5 tygodniach	after 10 weeks po 10 tygodniach	after 5 weeks po 5 tygodniach	after 10 weeks po 10 tygodniach
Control – Kontrola	0.0	4.6 a	5.3 a	9.3 c	45.4 a	2.4 abc	3.8 ab
Paclobutrazol Paklobutrazol	0.1	2.0 abc	3.4 ab	4.3 e	5.2 de	1.1 c	1.4 c
	0.5	1.5 bc	2.7 b	2.5 e	3.2 e	1.2 c	1.5 c
	1.0	2.4 abc	2.8 ab	9.2 c	11.7 bc	2.0 bc	2.6 abc
	5.0	0.0c	0.0 c	-	-	-	-
Flurprimidol Fluropirimidol	0.1	1.5 bc	1.6 bc	38.0 a	42.2 a	4.5 a	4.5 a
	1.0	1.7 bc	3.2 ab	11.5 bc	13.7 bc	3.5 ab	3.6 ab
	5.0	0.0 c	3.0 ab	-	1.0 e	-	1.5 abc
CCC	5.0	3.0 ab	3.2 ab	8.9 cd	9.2 cd	2.4 abc	2.5 abc
	50.0	1.0 c	2.8 ab	5.0 de	5.6 de	2.0 bc	2.2 bc
	250.0	2.0 abc	2.5 bc	15.5 b	16.5 b	2.5 abc	2.6 abc
Mean – Średnia		1.8	2.8	11.6	15.4	2.4	2.6

*Values in vertical 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

in vitro of *Tibouchina urvilleana* shoots. Eliasson et al. [1994] included paclobutrazol (0.15–0.60 mg·dm^{-3}) to *in vitro* rooting of *Pyrus serotina* and found its significant effect on reduction of shoot height. Henderson et al. [1994] carrying out studies on *Rosa* 'Red Cascade' and *Anigozanthos bicolor* noticed that plants of both species had smaller, darker green leaves when grown with paclobutrazol 4.0 mg·dm^{-3}, but at 1.0 mg·dm^{-3} of paclobutrazol the growth was promoted. On the media with CCC 5.0 and 50.0 mg·dm^{-3} the height of *Tibouchina urvilleana* plants was greater than on the control medium. Also, the favorable effect of paclobutrazol at 0.1 mg·dm^{-3} on the growth of plants was observed. Data of height of plants after 10 weeks of growth *ex vitro* showed similar dwarfing effect of growth retardants. Plants derived from medium with paclobutrazol or flurprimidol at 5.0 mg·dm^{-3} still characterized strong reduction of growth. Ziv [1992] observed that growth retardants used in tissue culture of *Gladiolus* influenced plant height after 5–6 weeks in the greenhouse.

Significant differences were noted in the number of leaves between examined growth retardants pretreatments. The maximal number of leaves was attained in the plants obtained from media with CCC (9.3–10.4), after 5 weeks of growing *ex vitro* and from media containing 0.1 mg·dm^{-3} paclobutrazol or flurprimidol, when plants were cultivated 10 weeks (11.1 and 11.3, respectively). Plants treated with flurprimidol or paclobutrazol at 5.0 mg·dm^{-3} formed 5.4–6.6 very small leaves. *Tibouchina urvilleana* seems to be a plant sensitive to growth retardant application and requires low doses of flurprimidol or paclobutrazol.

The number of axillary shoots emerging from the main shoot depended both on kind of growth retardant and its concentration (tab. 2). The plants derived from control medium, after 5 weeks of growth in the soil, produced the most number of axillary shoots (4.6). Also, the media containing CCC 5.0 mg·dm^{-3} or paclobutrazol 5.0 mg·dm^{-3} were effective in induction of new shoots. On the media with paclobutrazol 5.0 mg·dm^{-3} axillary shoots were not formed. Ziv and Ariel [1991] and Ziv et al. [1994] observed favorable effect of growth retardants on *in vitro* bud proliferation of *Philodendron* and *Nerine*. Axillary shoots derived from medium containing CCC 250.0 mg·dm^{-3} characterized the highest length. After 10 weeks of growth of shoots *ex vitro* formation and growth of axillary shoots were the best when plants were obtained from control medium (5.3 and 45.4 mm respectively).

REFERENCES

Eliasson M. K., Beyl C. A., Barker P. A., 1994. *In vitro* responses and acclimatization of *Prunus serotina* with paclobutrazol. J. Plant Growth Regulation 13, 137–142.

Gribaudo I., Novello V., Restagno M., 2001. Improved control of water loss from micropropagated grapevines (*Vitis vinifera* cv. Nebbiolo). Vitis 40, 3, 137–140.

Henderson K. P., Taji A. M., Williams R. R., 1994. Effect of reduced humidity and paclobutrazol on acclimatization of tissue-culture plants. Inter. Plant Propag. Soc. Comb. Proc. 43, 97–102.

Kozak D., Grodek J., 2005. The consequent effect of growth retardants on the growth and development of *Tibouchina urvilleana* Cogn. shoots *in vitro*. Acta Sci. Pol. Hortorum Cultus 4 (2), 123–128.

Larsen R. U., Lieth J. H., 1993. Shoot elongation retardation owing to daminozide in chrysanthemum: I. Modeling single applications. Scientia Hort. 1–2, 109–125.

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

Novello V., Roberts A. V., 1992. Effects of paclobutrazol and reduced humidity on stomatal conductance of micropropagated grapevines. Acta Hort. 319, 65–70.

Oliphant J., 1990. The use of paclobutrazol in the rooting medium of micropropagated plants. Comb. Proc. Int. Plant Prop. Soc. 40, 358–360.

Roberts A.V., Matthews D., 1995. The preparation *in vitro* of chrysanthemum for transplantation to soil. 5. The 2S, 3S enantiomer of paclobutrazol improves resistance to desiccation. Plant Cell Tissue and Organ Culture 40, 2, 191–193

Roberts A. V., Walker S., Horan I., Smith E. F., Mottley J., 1992. The effect of growth retardants, humidity and lighting at stage III on stage IV of micropropagation in chrysanthemum and rose. Acta Hort. 319, 153–158.

Ruter J. M., 1994. Growth and landscape establishment of *Pyracantha* and *Juniperus* after application of paclobutrazol. HortScience 29, 11, 1318–1320.

Smith E. F., Gribaudo I., Roberts A. V., Mottley J., 1992. Paclobutrazol and reduced humidity improve resistance of micropropagated grapevine to wilting. HortScience 27, 111–113.

Ziv M., 1992. The use of growth retardants for the regulation and acclimatization of *in vitro* plants. Proceed. of the 14 Intern. Conf. on Plant Growth Subst., Amsterdam, 21–26 July, 199, 809–817.

Ziv M., Ariel T., 1991. Bud proliferation and plant regeneration in liquid-cultured philodendron treated with ancymidol and paclobutrazol. J. Plant Growth Regulation 10, 53–57.

Ziv M., Kahany S., Lilien-Kipnis H., 1994. Scaled-up proliferation and regeneration of *Nerine* in liquid cultures. Part I. The induction and maintenance of proliferating meristematic clusters by paclobutrazol in bioreactors. Plant Cell Tissue and Organ Culture 39, 109–115.

WPŁYW RETARDANTÓW WZROSTU ZASTOSOWANYCH *IN VITRO* NA AKLIMATYZACJĘ I WZROST *Tibouchina urvilleana* Cogn. W WARUNKACH *IN VIVO*

Streszczenie. Wierzchołki pędów *Tibouchina urvilleana* Cogn. prowadzono 4 tygodnie *in vitro* na zmodyfikowanej pożywce Murashige i Skooga (MS) [1962] z dodatkiem retardantów wzrostu: paklobutrazol – 0,1; 0,5; 1,0; 5,0 mg·dm^{-3}, fluropirimidol – 0,1; 1,0; 5,0 mg·dm^{-3}, chlorek chlorocholiny (CCC) – 5,0; 50,0; 250,0 mg·dm^{-3}. Kontrolę stanowiła pożywka nie zawierająca retardantów wzrostu. Ukorzenione pędy przenoszono do szklarni i sadzono do mieszaniny torfu i perlitu (1:1), gdzie rosły przez 5 tygodni. Następnie rośliny przesadzono do substratu torfowego i uprawiano przez kolejne 5 tygodni. Rośliny przyjęły się w 92,5–100,0%. Najniższy procent przyjęć wykazywały rośliny pochodzące z pożywki zawierającej 5,0 mg·dm^{-3} fluropirimidolu. Wzrost *in vitro* pędów *Tibouchina urvilleana* na pożywce zawierającej retardanty wzrostu miał istotny wpływ na dalszy wzrost roślin w podłożu. Paklobutrazol i fluropirimidol w stężeniu 5,0 mg·dm^{-3} bardzo silnie hamowały wzrost roślin *ex vitro* po 10 tygodniach.

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

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