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THE INFLUENCE OF THE SALT COMPOSITION OF BASAL MEDIUM AND GROWTH REGULATORS ON *IN VITRO* GROWTH AND DEVELOPMENT OF *Tibouchina urvilleana* (DC.) Cogn.

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Abstract. *Tibouchina urvilleana* is a very popular small tree or shrub known for its beautiful velvety foliage and violet-blue flowers. It can be used in the mild climate in any landscape or as an indoor container plant in cooler regions. The aim of the present study was to determine the effect of mineral salt composition and growth regulators (BA and IAA) on the growth and development of *Tibouchina urvilleana* shoots. Shoot tips, obtained from aseptically grown shoots, were cultured on Murashige and Skoog (MS) (full and half strength), Nitsch and Nitsch (NN) or Lloyd and McCown (WPM) media supplemented with BA at 2.5 mg·dm^{-3} and IAA at 0.1 mg·dm^{-3}. Also, the effects of BA (1.0, 2.5, 5.0 mg·dm^{-3}) and IAA (0.1 or 0.5 mg·dm^{-3}) applied alone or in combination were investigated. Explants cultured on medium without growth substances were used as a control. The study results showed that the application of full-strength Murashige and Skoog salt mixture has a more beneficial effect on the growth of the main shoot and the induction of axillary shoots of *Tibouchina urvilleana* compared to ½ MS, WPM and NN salt mixtures. The number of axillary shoots increases with the increasing concentration of BA (from 1 to 5 mg·dm^{-3}), but they are characterized by a smaller length. The combined application of BA and IAA does not have a significant effect on the induction of axillary shoots. The addition of IAA at a concentration of 0.5 mg·dm^{-3} to the media containing BA (1 to 5 mg·dm^{-3}) has a beneficial effect on elongation growth of axillary shoots.

Key words: *Tibouchina urvilleana*, mineral salt mixture, cytokinin, auxin, branching, micropropagation

INTRODUCTION

Tibouchina urvilleana (Melastomataceae) is native of Brazil. It is a small ornamental tree or shrub that can be found across the world, in particular in regions where no

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ground frost occurs. This plant owes its exotic appearance to its original shoots, velvety leaves and large (5–8 cm in diameter) violet-blue flowers. *Tibouchina* is propagated from tip cuttings in a greenhouse, but this method is not very efficient. As a result of the rapid progress in and application of state-of-the-art *in vitro* technologies, more and more species are propagated using this method. There are reports on micropropagation of this plant in the available literature: Kozak and Wnuk [2005], Poospooragi [2005], He et al. [2009]. Mineral medium is an important element determining the growth and development of explants in *in vitro* cultures. Its task is to feed explants and to create appropriate physical conditions for their development. There are many types of medium with a strictly defined mineral salt composition, e.g.: salt mixtures formulated by Murashige and Skoog [1962], Gamborg et al. [1968], Nitsch and Nitsch [1969], Lloyd and McCown [1980]. Growth regulators are of very great importance in plant micropropagation. Kozak and Wnuk [2005] found that MS medium with the addition of benzyladenine (BA) at 5 $mg \cdot dm^{-3}$ was the most suitable for the induction of axillary shoots from tips and nodes of *Tibouchina urvilleana*. Poospooragi [2005] reports that shoot multiplication and elongation was optimal in half strength MS medium supplemented with 6 μM (1.4 $mg \cdot dm^{-3}$) BA. He et al. [2009] obtained shoots from the callus induced from leaf explants cultured on MS medium with 15.9 μM (3.5 $mg \cdot dm^{-3}$) thidiazuron (TDZ).

The aim of the present study was to determine the effect of mineral salt composition and growth regulators (BA alone and combined with IAA) on the growth and development of *Tibouchina urvilleana* shoots.

MATERIALS AND METHODS

Shoot tips of 15–20 mm in length, having at least 2 fully developed leaves, were taken from aseptically grown shoots of *Tibouchina urvilleana* (DC.) Cogn. In the first experiment, the effect of the type of basal salts mixture was tested. In this experiment, explants were cultured on media containing mineral salts according to: Murashige and Skoog (MS) (full and half strength) [1962], Nitsch and Nitsch (NN) [1969], Lloyd and McCown (WPM) [1980]. Every medium contained: thiamine – 0.4 $mg \cdot dm^{-3}$, pyridoxine – 0.5 $mg \cdot dm^{-3}$, nicotinic acid – 0.5 $mg \cdot dm^{-3}$, glycine – 2 $mg \cdot dm^{-3}$, myo-inositol – 100 $mg \cdot dm^{-3}$, sucrose – 30 $g \cdot dm^{-3}$, benzyladenine (BA) at 2.5 $mg \cdot dm^{-3}$, and indoleacetic-acid (IAA) at 0.1 $mg \cdot dm^{-3}$. The second experiment was conducted to test medium containing full strength MS mineral salts, organic compounds as in the first experiment, and BA: 1.0, 2.5, 5.0 $mg \cdot dm^{-3}$ alone or in combination with IAA at a concentration of 0.1 or 0.5 $mg \cdot dm^{-3}$ as well IAA at 0.1 and 0.5 $mg \cdot dm^{-3}$ used alone. Explants cultured on medium without growth substances were used as a control. All the media were solidified with Agar-Agar (Sigma) – 6.5 $g \cdot dm^{-3}$. The pH of the media was adjusted to 5.7 before autoclaving.

The cultures were maintained at 22°C ± 2°C, under 16-h photoperiod at photosynthetic photon flux of 35 $\mu mol\ m^{-2}\ s^{-1}$. There were four replications per treatment, each consisting of 5 explants/Erlenmeyer flask. The experiment was repeated twice.

The following characters were evaluated after 6 weeks: length and fresh weight of the main shoot, number of leaves and nodes on the main shoot, number of axillary shoots and their length, number of leaves on axillary shoots, fresh weight of axillary shoots/explant. The results of the experiments were analyzed statistically using a standard statistical procedure with one factorial design, while the Tukey test was used to estimate the differences between the means at the 5% level of significance.

RESULTS AND DISCUSSIONS

Effect of the mineral salt mixture on the growth and development of *Tibouchina urvilleana*

The analysis of variance showed that the type of mineral salt mixture had a significant effect on the growth of the main shoot (tab. 1). The shoot tips growing on MS medium containing BA 2.5 mg·dm^{-3} and IAA 0.1 mg·dm^{-3} showed the best elongation growth (53.5 mm) (fig. 1). They also produced the highest number of nodes and leaves (respectively: 4.3 and 8.7) and reached the highest fresh weight (336.2 mg). The poorest elongation growth of the main stem was observed on WPM medium (45.5 mm). The shoots growing on NN medium were characterized by the lowest fresh weight as well as the lowest number of nodes and leaves (respectively: 229.0 mg, 3.8, 7.7).

Table 1. Influence of mineral salt composition with addition of BA 2.5 mg·dm^{-3} and IAA 0.1 mg·dm^{-3} on the growth of main shoot of *Tibouchina urvilleana* (DC.) Cogn., after 4 weeks of *in vitro* culture

Tabela 1. Wpływ zestawu soli mineralnych z dodatkiem BA 2.5 mg·dm^{-3} i IAA 0.1 mg·dm^{-3} na wzrost pędu głównego *Tibouchina urvilleana* (DC.) Cogn., po 4 tygodniach kultury *in vitro*

Composition of mineral salts Zestaw soli mineralnych	Length of main shoot Długość pędu głównego (mm)	Number of nodes Liczba węzłów	Number of leaves Liczba liści	Fresh weight of main shoot Świeża masa pędu głównego (mg)
MS	53.5 a*	4.3 a	8.7 a	336.2 a
½ MS	49.3 b	4.1 ab	8.2 ab	307.0 ab
NN	48.7 c	3.8 b	7.7 b	229.0 b
WPM	45.5 c	3.9 ab	7.9 ab	253.8 b
Mean – Średnia	49.3	4.0	8.1	281.5

*Means followed by the same letter are not significantly different at α = 0.05, Mean of 40 cultures per treatment

Średnie oznaczone tą samą literą nie różnią się istotnie przy α = 0,05; Średnia z 40 kultur w traktowaniu

The statistical analysis demonstrated the significant effect of the mineral salt mixture on the number and growth of axillary shoots (tab. 2). The explants cultured on medium containing the full strength MS salt mixture produced the highest number of

Fig. 1. Shoots of *Tibouchina urvilleana* obtained on different salt composition of basal medium containig BA at 2.5 mg·dm^{-3} and IAA at 0.1 mg·dm^{-3}, after 4 weeks of culture *in vitro*

Fot. 1. Pędy *Tibouchina urvilleana* uzyskane na pożywkach o różnych zestawach soli mineralnych zawierających BA 2.5 mg·dm^{-3} i IAA 0.1 mg·dm^{-3}, po 4 tygodniach kultury *in vitro*

Table 2. Influence of mineral salt composition with addition of BA 2.5 mg·dm^{-3} and IAA 0.1 mg·dm^{-3} on the induction and growth of axillary shoots of *Tibouchina urvilleana* (DC.) Cogn., after 4 weeks of *in vitro* culture

Tabela 2. Wpływ zestawu soli mineralnych z dodatkiem BA 2.5 mg·dm^{-3} i IAA 0.1 mg·dm^{-3} na indukcję i wzrost pędów kątowych *Tibouchina urvilleana* (DC.) Cogn., po 4 tygodniach kultury *in vitro*

Composition of mineral salts Zestaw soli mineralnych	Number of axillary shoots/explant Liczba pędów kątowych/ eksplantat	Length of axillary shoots Długość pędów kątowych (mm)	Number of leaves Liczba liści	Fresh weight of axillary shoots/explant Świeża masa pędów kątowych/ eksplantat (mg)
MS	4.1 a*	5.8 b	2.4 a	57.1 a
½ MS	3.3 ab	5.8 b	2.4 a	56.4 ab
NN	2.8 b	5.1 b	2.4 a	43.5 b
WPM	2.7 b	6.6 a	2.6 a	55.5 b
Mean – Średnia	3.2	5.8	2.5	53.1

*See explanation table 1, objaśnienia tabela 1

shoots (4.1), whereas the poorest shoot induction was observed on WPM and NN media (2.7 and 2.8).

The longest axillary shoots were obtained on WPM medium (6.6 mm), while the poorest elongation growth of shoots was recorded on NN medium (5.1 mm). On MS and ½ MS media, axillary shoots reached the same length (5.8 mm). The number of

leaves on axillary shoots was similar on all media under study (2.4–2.6). The highest fresh weight of axillary shoots was recorded on MS medium (57.1 mg), whereas the shoots induced on NN medium had the lowest weight (43.5 mg).

The best elongation growth and multiplication of *Tibouchina urvilleana* shoots was obtained on solid medium containing Murashige and Skoog (MS) mineral salts [1962]. MS medium is one of the most frequently used in plant micropropagation, which is confirmed by numerous reports. It was used as a culture medium for the multiplication of *Lagerstroemia reginae* [Sumana and Kaveriappa, 2000], *Callistemon rigidus* [Jiang et al. 2005], *Tibouchina urvilleana* [Kozak and Wnuk, 2005; Poospooragi, 2005; He et al. 2009], *Ficus anastasia* [Al. Malki et al. 2010], *Ilex glabra* [Sun et al. 2010]. Mineral composition is a major components of plant tissue culture media. The supply of macronutrients such as nitrogen, phosphorus and sulphur and micronutrients is important for optimal morphogenesis [Ramage and Williams 2002]. The best growth of *Tibouchina urvilleana* shoots on MS medium can be caused by high contents of ammonium and potassium nitrate which is 2 times higher than in NN medium. The main difference between WPM and MS is the relatively small amount of sodium and chloride ions in WPM. Further, WPM prescribes only 75% of the ammonium and nitrate ions and 60% of the potassium recommended in MS [Kyte 1990].

In the present study, *Tibouchina* shoots were short and branched poorly on Nitsch and Nitsch medium [1969]. In turn, Furmanowa et al. [1994] recommend NN medium for the multiplication of *Catharanthus roseus* shoots. This salt mixture was also used by Al-Juboory [1996] for the propagation of *Gardenia jasminoides* in *in vitro* cultures, Kukreja [1999] for the micropropagation of *Melissa officinalis,* as well as by Kozak et al. [2007] for the initiation of *Kohleria amabilis* cultures.

Effect of BA and IAA on the growth and development of *Tibouchina urvilleana*

On the basis of analysis of variance, it was found that the presence of benzyladenine at a concentration range of 1.0–5.0 mg·dm^{-3} had an inhibitory effect on main shoot elongation (respectively: 42.2 mm and 34.8 mm). This effect was significant in comparison with growth of main shoot on control medium but no significant influence was found on media containing different levels of BA (tab. 3). In the treatments not containing BA, but supplemented with IAA (0.1 and 0.5 mg·dm^{-3}), as well as in the control treatment, the shoots reached a significantly longer length (61.5–68.4 mm). The number of nodes in particular treatments was similar and ranged from 3.4 to 4.4. The growth substances contained in the medium were found to have a significant influence on the number of leaves. The control medium and the presence of IAA in the medium at an amount of 0.1 or 0.5 mg·dm^{-3} had the beneficial effect on this character. Main stem fresh weight was the highest in the treatments containing the highest concentration of BA applied alone (269.4 mg).

It was found on the basis of statistical analysis that the concentration of benzyladenine had an impact on the formation of axillary shoots (tab. 4). The number of axillary shoots increased with the increasing BA concentration (from 3.4 to 5.1). The combined application of BA and IAA did not have a significant effect on the number of axillary shoots. The regeneration of axillary shoots was poor on the media supple-

mented with IAA, but without the addition of BA, and it was 0.2 and 0.5 shoot/explant. In the control treatment, not containing any growth regulators, shoot induction was entirely inhibited (fig. 2).

Table 3. Influence of BA and IAA concentration on the growth of main shoot of *Tibouchina urvilleana* (DC.) Cogn., after 4 weeks of *in vitro* culture

Tabela 3. Wpływ stężenia BA i IAA na wzrost pędu głównego *Tibouchina urvilleana* (DC.) Cogn., po 4 tygodniach kultury *in vitro*

Growth regulators Regulator wzrostu ($mg \cdot dm^{-3}$)		Length of main shoot Długość pędu głównego (mm)	Number of nodes Liczba węzłów	Number of leaves Liczba liści	Fresh weight of main shoot Świeża masa pędu głównego (mg)
BA	IAA				
0	0	61.7 ab*	4.4 a	8.8 a	223.0 ab
	0.1	61.5 ab	4.3 a	8.5 ab	218.8 ab
	0.5	68.4 a	4.1 a	8.2 abc	175.5 b
1.0	0	42.2 cd	3.9 a	7.8 abc	194.0 ab
	0.1	41.3 cd	3.5 a	6.9 bc	203.9 ab
	0.5	57.7 b	3.9 a	7.7 abc	184.9 b
2.5	0	36.8 cd	3.9 a	7.3 abc	223.5 ab
	0.1	42.2 cd	3.4 a	6.7 c	221.5 ab
	0.5	46.7 c	3.6 a	7.2 abc	172,7 b
5.0	0	34.8 d	4.0 a	7.9 abc	269.4 a
	0.1	35.8 d	3.4 a	6.7 c	159.3 b
	0.5	42.9 cd	4.0 a	8.0abc	214.8 ab

*See explanation table 1, objaśnienia tabela 1

The concentration of BA had no positive effect on the length of axillary shoots. The elongation growth of the shoots was inhibited with the increase in BA concentration from 1 to 5 $mg \cdot dm^{-3}$ (respectively: 8.3–5.9 mm), but the differences were not significant. We observed a beneficial effect of the combined application of BA and IAA, in particular at a concentration of 0.5 $mg \cdot dm^{-3}$, on the length of axillary shoots. The shortest shoots were recorded on the media containing only auxin (0.5–4.9 mm). The number of leaves in the treatments containing BA or BA and IAA was similar and did not differ significantly (2.9–3.4). However, the number of leaves was the lowest (0.5–2.1) in the treatments containing only auxin. In analysing the fresh weight of axillary shoots, the medium containing BA at 5 $mg \cdot dm^{-3}$ was found to have the most beneficial effect on this character.

The present study found a beneficial effect of BA at a concentration of 1–5 $mg \cdot dm^{-3}$ on the number of axillary shoots of *Tibouchina urvilleana*. The role of BA, synthetic cytokinin with high biological activity, as an effective cytokinin in shoot multiplication has been established in many plants. Poospooragi [2005] recommend BA at 6 µM as the best for the multiplication of *Tibouchina urvilleana*. Kozak and Wnuk [2005] the biggest number of axillary shoots this species achieved from shoot tips on medium contain-

ing BA at 5 mg·dm^{-3}. The medium containing 1.5 mg·dm^{-3} BA proved to be the best medium for *in vitro* shoot formation of *Lagerstroemia reginae* [Sumana and Kaveriappa, 2000], *Rosa indica* [Shabbir et al. 2009], whereas Lu [2002] observed that the mean number of *Morus latifolia* shoots increased to 4–6 when the BA concentration was increased to 0.5–1 mg·dm^{-3}. Ebrahim and Ibrahim [2000] used MS medium supplemented with BA 5 mg·dm^{-3} for the initiation of an aseptic culture of *Maranta leuconeura*. Axillary buds of *Ficus religiosa* [Deshpande et al. 1998] and of *Simmondsia chinensis* [Bashir et al. 2007] sprouted at a high concentration of BA (5 mg·dm^{-3}).

Table 4. Influence of BA and IAA concentration on the induction and growth of axillary shoots of *Tibouchina urvilleana* (DC.) Cogn., after 4 weeks of *in vitro* culture

Tabela 4. Wpływ stężenia BA i IAA na indukcję i wzrost pędów kątowych *Tibouchina urvilleana* (DC.) Cogn., po 4 tygodniach kultury *in vitro*

Growth regulators Regulator wzrostu (mg·dm^{-3})		Number of axillary shoots/explant Liczba pędów kątowych/ eksplantat	Length of axillary shoots Długość pędów kątowych (mm)	Number of leaves Liczba liści	Fresh weight of axillary shoots/explant Świeża masa pędów kątowych/ eksplantat (mg)
BA	IAA				
0	0	0 e*	-	-	-
	0.1	0.2 e	0.5 d	0.5 b	0.5 e
	0.5	0.5 e	4.9 c	2.1 a	4.1 e
1.0	0	3.4 c	8.3 abc	3.4 a	56.8 bc
	0.1	2.5 d	6.5 bc	3.1 a	26.9 de
	0.5	3.7 bc	12.1 a	3.4 a	39.3 cd
2.5	0	4.4 ab	6.6 bc	2.9 a	53.7 bc
	0.1	4.5 a	7.6 abc	3.2 a	73.9 ab
	0.5	3.6 c	9.5 ab	3.4 a	68.5 ab
5.0	0	5.1 a	5.9 bc	3.1 a	97.5 a
	0.1	4.9 a	5.7 c	3.1 a	57.9 bc
	0.5	4.5 a	6.7 bc	3.4 a	67.9 abc

*See explanation table 1, objaśnienia tabela 1

The addition of IAA at a concentration of 0.1 or 0.5 mg·dm^{-3} to the medium containing BA (1–5 mg·dm^{-3}) did not exert a significant effect on the multiplication of *Tibouchina urvilleana* shoots. This is in agreement with the report of Shahzad et al. [2011] who observed that the addition of auxins to the optimal concentration of BA did not further improve the regeneration capacity of *Veronica anagallis-aquatica* explants. Yalcin-Mendi et al. [2009] compared the media containing BA 1 mg·dm^{-3} and BA 1 mg·dm^{-3} combined with IAA 0.5–1 mg·dm^{-3} and found that increasing auxin concentration decreased the regeneration ratio of *Gazania rigens*. Several reports are also available about the positive effect of using different concentrations of IAA and BA for multiple shoot regeneration. There is a good evidence that auxin can regulate cytokinin biosynthesis. Onagro and Leyser [2007] suggest that apically derived auxin affects

Fig. 2. Growth and development of *Tibouchina urvilleana* shoots on MS medium containing growth regulators, after 4 weeks of culture *in vitro*

Fot. 2. Wzrost i rozwój pędów *Tibouchina urvilleana* na pożywce MS z dodatkiem regulatorów wzrostu, po 4 tygodniach kultury *in vitro*

cytokinin synthesis both the node and in the root, and that the level of cytokinin from these sources correlates with bud activity. The medium containing BA 1 mg·dm^{-3} and IAA 0.1 mg·dm^{-3} was the best for induction of *Chrysanthemum morifolium* shoots [Karim et al. 2003]. Explants of *Lagerstroemia speciosa* produced multiple shoots when cultured on medium with BA 1.5 mg·dm^{-3} and IAA 0.1 mg·dm^{-3} [Zobayed, 2000]. Shekafandeh [2007] found the highest shoot proliferation of *Myrtus communis* on medium containing BA 2 mg·dm^{-3} and IAA 0.2 mg·dm^{-3} or 0.2 NAA mg·dm^{-3}.

CONCLUSIONS

1. The application of full-strength Murashige and Skoog salt mixture has a more beneficial effect on the growth of the main shoot and the induction of axillary shoots of *Tibouchina urvilleana* compared to ½ MS, WPM and NN salt mixtures.

2. The number of axillary shoots slightly increases with the increasing concentration of BA (from 1 to 5 mg·dm^{-3}), but they are characterized by a smaller length.

3. The combined application of BA and IAA does not have a significant effect on the induction of axillary shoots.

4. The addition of IAA at a concentration of 0.5 mg·dm^{-3} to the media containing BA (1 to 5 mg·dm^{-3}) has a beneficial effect on elongation growth of axillary shoots.

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WPŁYW ZESTAWU SOLI MINERALNYCH I REGULATORÓW WZROSTU NA WZROST I ROZWÓJ *Tibouchina urvilleana* (DC.) Cogn. *IN VITRO*

Streszczenie. *Tibouchina urvilleana* jest bardzo popularnym niskim drzewem lub krzewem o pięknym welwetowym ulistnieniu i fioletowoniebieskich kwiatach. Może być uprawiana w gruncie w klimacie łagodnym lub w pomieszczeniu w pojemnikach w chłodnych rejonach. Celem badań było określenie wpływu zestawu soli mineralnych oraz regulatorów wzrostu (BA, IAA) na wzrost i rozwój pędów *Tibouchina urvilleana*. Wierzchołkowe fragmenty pędów, uzyskane z kultur aseptycznych, kultywowano na pożywce Murashige i Skooga (MS) (pełny zestaw i ½ zestawu soli), Nitsch i Nitsch (NN) lub Lloyd i McCown (WPM) zawierającej BA w stężeniu 2,5 mg·dm^{-3} i IAA w ilości 0,1 mg·dm^{-3}. Zbadano również wpływ BA (1,0; 2,5; 5,0 mg·dm^{-3}) i IAA (0,1 lub 0,5 mg·dm^{-3}) zastosowanych pojedynczo oraz w kombinacji. Eksplantaty wyłożone na pożywkę bez regulatorów wzrostu stanowiły kontrolę. Wyniki badań wykazały, że zastosowanie pełnego zestawu soli mineralnych wg Murashige i Skooga, korzystniej wpływa na wzrost pędu głównego i indukcję pędów kątowych *Tibouchina urvilleana*, w porównaniu z zestawem soli ½ MS, WPM i NN. Wraz ze wzrostem stężenia BA (od 1 do 5 mg·dm^{-3}) rośnie liczba pędów kątowych, lecz charakteryzują się one mniejszą długością. Łączne zastosowanie BA i IAA nie ma istotnego wpływu na indukcję pędów kątowych. Dodatek IAA w stężeniu 0,5 mg·dm^{-3} do pożywek zawierających BA (1–5 mg·dm^{-3}) wpływa korzystnie na wzrost wydłużeniowy pędów kątowych.

Słowa kluczowe: *Tibouchina urvilleana*, cytokinina, auksyna, rozkrzewianie pędów, mikrorozmnażanie

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