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THE INFLUENCE OF GROWTH REGULATORS AND EXPLANT POSITION ON THE GROWTH AND DEVELOPMENT OF *Mandevilla sanderi* (Hemsl.) WOODSON *in vitro*

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ABSTRACT

Mandevilla is a valuable ornamental pot vine. However, due to a low propagation rate, it is difficult to keep up with the demand. Micropropagation would allow to produce lots of plants for the market. The aim of the study was to determine the effect of the growth regulators addition to the media and explants orientation on multiplication of *Mandevilla sanderi*, an exotic, ornamental pot plant. The shoot tips were placed vertically or horizontally on the Murashige and Skoog medium supplemented with benzyladenine (BA) or isopentenyladenine (2iP), at concentrations of 1, 2.5 or 5 mg·dm^{-3} singly or in combination with thidiazuron (TDZ) at concentrations of 0.01, 0.025 or 0.05 mg·dm^{-3}. Maximum multiplication rate was noted on the media supplemented with 2.5 mg·dm^{-3} 2iP + 0.025 mg·dm^{-3} TDZ or 5 mg·dm^{-3} 2iP, when explants were placed horizontally. All the treatments resulted in callus formation. Medium supplemented with the highest concentration of BA combined with TDZ was the most active in callus growth.

Key words: cytokinins, Dipladenia, explant orientation, micropropagation, rocktrumpet

INTRODUCTION

Mandevilla sanderi (Hemsl.) Woodson (syn. *Dipladenia sanderi*) is an exotic flowering climbing plant. As a potted or a container plant, it makes an excellent decoration to the home, balcony, deck or patio. It is native to Brazil and belongs to the *Apocynaceae* family. Flowers are in shades of pink or red, often with a throat in contrasting color and they appear from late spring till autumn. Traditionally, the species is propagated by stem cuttings. In order to establish a method for production of healthy plants and to obtain a high propagation rate an *in vitro* studies were conducted by many researches. According to the literature data, the success of regeneration depends significantly on the plants genotype, type and concentration of growth regulators and the way explants are placed in the culture medium. Wojtania et al. [2006] tested regeneration ability of apical and basal segments of *Dipladenia sanderi* using BA singly or together with thidiazuron (TDZ) or/and 2iP (2-isopentenyl adenine). The best shoot proliferation (2.5–3.1) was obtained on the medium containing BA in concentration of 1 mg·dm^{-3} together with TDZ (0.015 mg·dm^{-3}) or with TDZ and 2iP (each 1.0 mg·dm^{-3}). Yonghong et al. [2004] reported that for induction of axillary buds in this species the medium should contain BA 2-4 mg·dm^{-3} + NAA 0.1 mg·dm^{-3}. For *Mandevilla moricandiana* Cordeiro et al. [2012] suggested medium supplemented with BA alone (1–5 mg·dm^{-3}) or together with IAA

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(1–5 mg·dm^{-3}). For nodal segments of *Mandevilla illustris*, excised from seedlings, all the tested cytokinins: BA (0.44–22.0 μM), 2iP (0.49–24.65 μM), zeatin (0.46–22.85 μM) gave similar results (1–1.53 shoots per node) [Biondo et al. 2004]. BA used in concentration of 0.44 μM was the best in micropropagation of *Mandevilla velutina* [Biondo et al. 2007], however for *Mandevilla guanabarica* TDZ (2.27 μM) or 2iP (0.49 μM) were better [Cordeiro et al. 2014]. Explants of *Mandevilla* are usually positioned vertically on the medium. However, there are a few reports that the regeneration might be better when shoots are placed horizontally [Ponchia and Gardiman 1993, Sudha and Seeni 1996, Liao and Chuang 2014, Silva et al. 2017, Kozak et al. 2019].

The aim of the presented study was to evaluate the regeneration ability of shoot tip explants of *Mandevilla sanderi* (Hemsl.) Woodson, placed vertically or horizontally on the Murashige and Skoog (MS) medium supplemented with BA or 2iP, used singly in concentration of 1, 2.5, 5 mg·dm^{-3} or together with TDZ – 0.01, 0.025, 0.05 mg·dm^{-3}.

MATERIAL AND METHODS

The plant material were *in vitro* shoots of *Mandevilla sanderi* (Hemsl.) Woodson cultured on Murashige and Skoog [1962] (MS) medium solidified with agar (BioMaxima) – 6.5 g·dm^{-3}. The medium was supplemented with benzyladenine (BA) at 1 mg·dm^{-3} and indole-3-butyric acid (IBA) at 0.5 mg·dm^{-3}. For the experiment, shoot tips 15–20 mm in length with 2 nodes were isolated, and after removing the leaves from nodes, they were placed on the MS media supplemented with cytokinins: BA or isopentenyladenine (2iP), used individually in concentration of 1, 2.5, 5 mg·dm^{-3} or in combination with thidiazuron (TDZ) – 0.01, 0.025, 0.05 mg·dm^{-3} in two orientations: vertically or horizontally. The pH of the medium was adjusted to 5.7 before autoclaving. The cultures were maintained at 22°C ±2°C and under light intensity of 35 μmol·m^{-2}s^{-1} with 16h photoperiod. There were three replications per treatment, each consisting of 7 explants cultivated in a flask of 300 ml capacity. The experiment was repeated twice.

The following features were evaluated in the experiment after 8 weeks of cultivation: length (mm) and fresh weight (mg) of main shoots, number of nodes, percentage of shoots with axillary shoots, number, length (mm) and fresh weight (mg) of axillary shoots, percentage of rooted shoots, number, length (mm) and fresh weight (mg) of roots; volume (cm^3) and fresh weight of callus; multiplication rate (number of microcuttings per explant obtained by division of main and axillary shoots).

In the case of horizontally placed explants, the shoot tips died, therefore the features regarding the main shoot are presented only for the vertically placed explants.

The results of the experiment were analyzed statistically using a standard statistical procedure with one- or two-factorial design, while the Tukey test was used to estimate the differences between the means at the 5% level of significance.

RESULTS

Effects of combinations of BA or 2iP with TDZ on growth and development of shoot tips placed vertically

The analysis of variance showed a significant effect of studied cytokinins on the main shoot growth of *Mandevilla sanderi* placed vertically in the media (Tab. 1, Fig. 1). Elongation of shoots was considerably higher in response to single 2iP treatments (35.57–40.15 mm) or in combination with TDZ (30.62–39.00 mm) in comparison to the addition of BA (15.62–22.64 mm) or the control (17.29 mm). Similar results were obtained in the case of fresh weight of the main shoot. The medium supplemented with 2iP 1 mg·dm^{-3} promoted the maximum main shoot fresh weight (313.45 mg). Statistically similar results were obtained on the 2.5 mg·dm^{-3} 2iP + 0.025 mg·dm^{-3} TDZ (261.91 mg) and 5 mg·dm^{-3} 2iP + 0.05 mg·dm^{-3} TDZ (240.58 mg). 2iP used individually or in combination with TDZ was also superior to the control or BA used at concentration of 1 or 2.5 mg·dm^{-3} or at concentration of 5 mg·dm^{-3} in combination with TDZ at concentration of 0.05 mg·dm^{-3} in regards to the number of nodes. The most nodes were obtained on the medium supplemented with 2iP at a concentration of 1 mg·dm^{-3} (6.40), while the least on the control medium (3.62).

Regeneration of axillary shoots depended on the type of cytokinin used (Tab. 1, Fig. 1). There was no

Table 1. The influence of growth regulators on shoots growth of *Mandevilla sanderi* explants positioned vertically after 8 weeks of cultivation

PGRs ($mg \cdot dm^{-3}$)	Main shoot length (mm)	Main shoot fresh weight (mg)	Number of nodes	Explants with axillary shoots (%)	Number of axillary shoots per explant	Length of axillary shoots (mm)	Number of nodes per axillary shoot
Control	17.29 d*	33.26 e	3.62 c	0	–	–	–
BA + TDZ							
1.0 + 0	15.62 d	44.70 de	4.39 bc	46.5	1.00 ab*	5.83 c	2.50 a
2.5 + 0	15.79 d	41.12 de	3.79 c	75.0	1.27 a	9.57 bc	3.41 a
5.0 + 0	22.64 cd	123.08cde	5.73 ab	76.2	1.69 a	11.24 abc	3.27 a
1.0 + 0.01	19.93 d	133.47cde	5.43 ab	61.9	1.00 ab	10.15 bc	3.15 a
2.5 + 0.025	21.06 d	109.25 de	5.44 ab	76.2	1.19 a	12.75 abc	3.50 a
5.0 + 0.05	16.56 d	65.17 de	3.83 c	85.7	1.50 a	16.42 abc	3.86 a
2iP + TDZ							
1.0 + 0	40.15 a	313.45 a	6.40 a	19.0	1.00 ab	27.25 a	4.25 a
2.5 + 0	35.57 ab	224.80 bc	6.14 a	14.3	1.00 ab	15.67 abc	3.67 a
5.0 + 0	39.86 a	226.81 bc	6.19 a	42.9	1.44 a	19.18 ab	3.61 a
1.0 + 0.01	30.62 abc	219.79 bc	6.24 a	9.5	1.00 ab	12.50 abc	4.00 a
2.5 + 0.025	33.14 abc	261.91 ab	5.86 ab	33.3	1.14 ab	16.21 abc	3.43 a
5.0 + 0.05	39.00 a	240.58 ab	6.29 a	57.1	1.50 a	19.09 ab	3.38 a

* Means in each column followed by the same letter are not significantly different at $\alpha = 0.05$

branching observed on the control medium. Percentage of explants with proliferated shoots was higher on the media with BA (except for the result noted on 2iP and TDZ in the highest concentrations used). A combination of BA at 5 $mg \cdot dm^{-3}$ and TDZ at 0.05 $mg \cdot dm^{-3}$ provided the most effective shoot proliferation (85.7%). It was observed that new shoots regenerated mostly from nodes at the base of shoots and sometimes from upper nodes. The most axillary shoots were obtained when explants were cultivated on the medium supplemented with 5 mg of BA (1.69), however the number of axillary shoots in other treatments was statistically similar. The longest axillary shoots were obtained on the medium containing 2iP in concentration of 1 $mg \cdot dm^{-3}$ (27.25 mm), and it was significantly higher than those obtained on the media supplemented with BA used individually in concentration of 1 $mg \cdot dm^{-3}$ (5.83 mm) or 2.5 $mg \cdot dm^{-3}$ (9.57 mm) and in combination with TDZ in the lowest concentration (10.15 mm). Fresh weight of axillary shoots was also the highest in presence of 2iP in concentration of 1 $mg \cdot dm^{-3}$ (186.95 mg). Number of nodes produced on axillary shoots was not significantly different in any of the treatments, however the most nodes were obtained when the explants were cultivated in presence of 2iP in the lowest concentration (4.25 per axillary shoot).

In the case of vertically placed explants, the highest frequency of rooting (95.2 %) was noted on 2.5 $mg \cdot dm^{-3}$ 2iP + 0.025 $mg \cdot dm^{-3}$ TDZ (95.2%) – Table 1. It was observed that shoots rooted better when they were cultivated on the control medium or in presence of 2iP. There were no rooted shoots on the media containing BA in concentration of 2.5 $mg \cdot dm^{-3}$ or 5 $mg \cdot dm^{-3}$ BA + 0.05 $mg \cdot dm^{-3}$ TDZ. On the media supplemented with BA in concentration of 1 $mg \cdot dm^{-3}$ and on the control media, shoots produced significantly less roots (1.0–1.20) than in the remaining treatments (1.50–4.06). The length of roots also depended on the cytokinins used. Roots obtained on the media supplemented with 2.5 $mg \cdot dm^{-3}$ BA + 0.025 $mg \cdot dm^{-3}$ TDZ were significantly longer (32.33 mm) than roots obtained on the media containing 1 $mg \cdot dm^{-3}$ BA (8.50 mm), 5 $mg \cdot dm^{-3}$ BA (13.75 mm) and 2.5 $mg \cdot dm^{-3}$

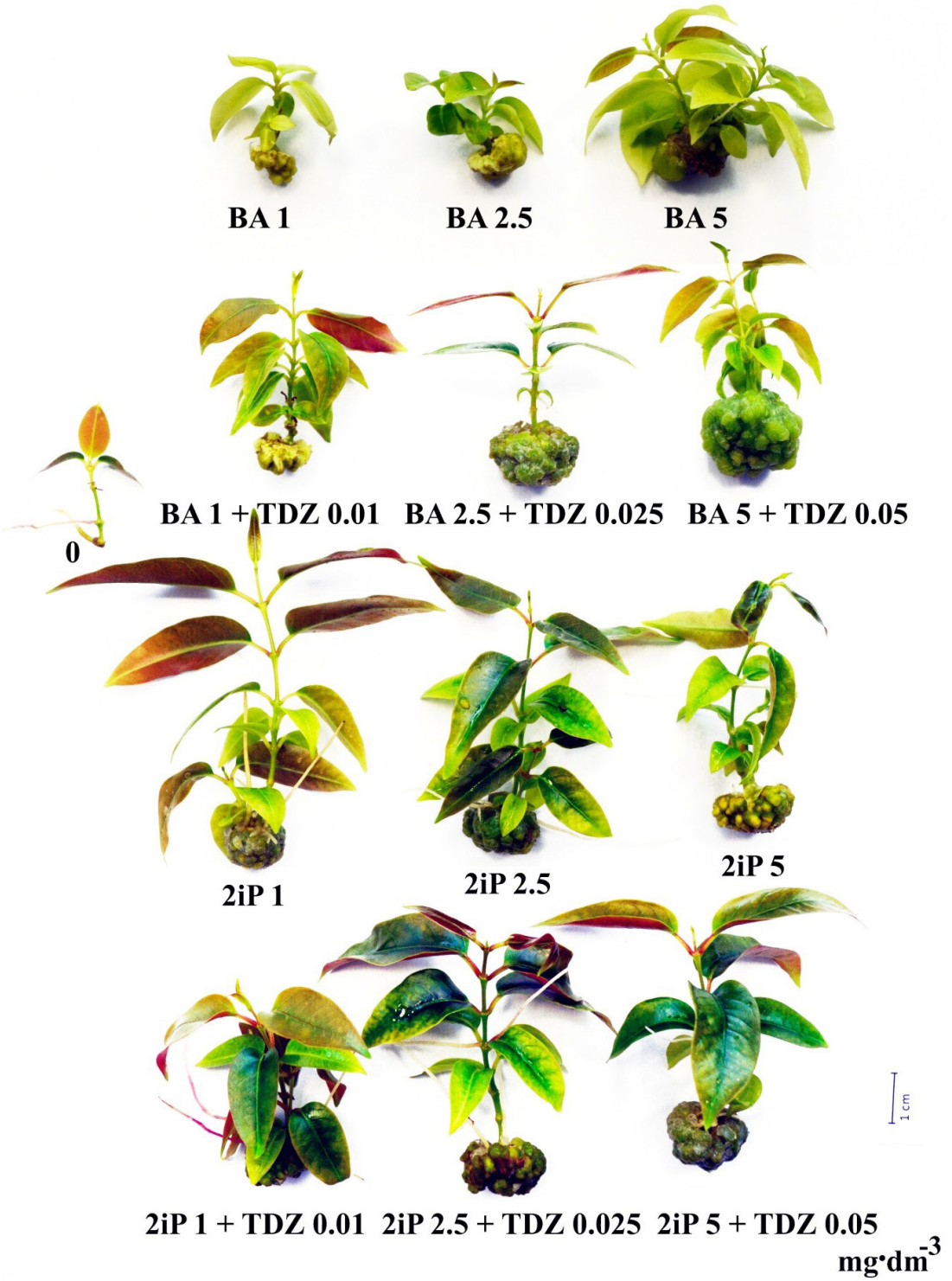

Fig. 1. The influence of growth regulators and vertical orientation of explants on growth and development of *Mandevilla sanderi* after 8 weeks of cultivation

Table 2. The influence of growth regulators on roots and callus growth on *Mandevilla sanderi* explants positioned vertically after 8 weeks of cultivation

Growth regulators concentration ($mg \cdot dm^{-3}$)	Rooted shoots (%)	Number of roots per explant	Length of roots (mm)	Fresh weight of roots (mg)	Callus volume (cm^3)	Callus fresh weight (mg)
Control	47.6	1.20 bc*	24.55 ab	8.92 a	–	–
BA + TDZ						
1.0 + 0	15.4	1.00 bc	8.50 b	1.30 a	0.54 c	169.63 ef
2.5 + 0	0	–	–	–	0.88 c	292.48 c–f
5.0 + 0	9.5	1.50 ab	13.75 b	2.25 a	3.53 ab	832.26abc
1.0 + 0.01	28.6	1.83 ab	18.42 ab	7.50 a	0.83 c	256.26 def
2.5 + 0.025	14.3	1.33 ab	32.33 a	19.50 a	2.23 bc	656.79 b-e
5.0 + 0.05	0	0	–	–	5.17 a	1330.45 a
2iP + TDZ						
1.0 + 0	81.0	3.00 ab	24.18 ab	45.21 a	2.06 bc	622.52 b–e
2.5 + 0	61.9	2.69 ab	14.45 b	14.27 a	3.71 ab	896.83 ab
5.0 + 0	47.6	3.20 ab	14.90 ab	17.36 a	3.47 ab	951.98 ab
1.0 + 0.01	76.2	4.06 a	22.00 ab	40.55 a	2.17 bc	551.24 b–f
2.5 + 0.025	95.2	3.30 a	21.40 ab	31.85 a	3.31 ab	769.57 a–d
5.0 + 0.05	57.1	3.33 a	16.08 ab	17.27 a	4.15 ab	1080.99ab

* Means in each column followed by the same letter are not significantly different at $\alpha = 0.05$

2iP (14.45 mm). There were no statistical differences proved in the case of the fresh weight of roots.

Callus induction at the shoots base was observed in all the treatments with cytokinins (Tab. 2, Fig. 1). Generally, callus was green and lumpy. The highest volume and fresh weight of callus were obtained on the media supplemented with BA and TDZ in the highest concentrations used (5.17 cm^3 and 1330.45 mg, respectively).

Effects of combinations of BA or 2iP with TDZ on growth and development of shoot tips placed horizontally

In the presented experiment, the growth of the main shoots placed on the media horizontally was inhibited. The shoot tips died and the explants produced axillary shoots. Therefore, there is no data on the main shoot growth and development of the explants placed horizontally on the media.

All of the explants produced axillary shoots (100%) – Table 3, Figure 2. The number of axillary shoots per explant depended on the cytokinins added to the media. The media supplemented with 2.5 or 5 BA and 0.025 or 0.05 TDZ or 2iP alone at 5 $mg \cdot dm^{-3}$ promoted the highest number of axillary shoots (3.28, 3.05 and 3.05, respectively). Definitely less axillary shoots per explant were noted on the control media (1.42) and in presence of 2iP in concentration of 1 $mg \cdot dm^{-3}$ alone or in combination with TDZ (1.80 and 1.86). The obtained axillary shoots were the longest on the media containing 2iP in concentration of 1 $mg \cdot dm^{-3}$ (29.8 mm). The stimulatory effect of 2iP at the concentration of 1 $mg \cdot dm^{-3}$ was also observed in regards to the fresh media of the axillary shoots (315.86 mg). It was noted that axillary shoots formed more nodes in presence of 2iP used in concentration of 1 $mg \cdot dm^{-3}$ (4.07) than on the media containing BA in the highest concentration used alone or with TDZ (2.72 and 2.92 respectively).

Rooting of the explants depended on the cytokinins used in the experiment (Tab. 4). The highest frequency of rooting was noted in presence of 2iP in concentration of 1 $mg \cdot dm^{-3}$ alone or in combination with TDZ (100% and 95% respectively). In the remaining treatments 5.0 to 63.2% of explants formed roots. On the media supplemented with 2.5 $mg \cdot dm^{-3}$

Table 3. The influence of growth regulators on axillary shoots growth of *Mandevilla sanderi* explants positioned horizontally after 8 weeks of cultivation

Growth regulators concentration ($mg \cdot dm^{-3}$)	Shoots with axillary shoots (%)	Number of axillary shoots per explant	Length of axillary shoots (mm)	Fresh weight of axillary shoots (mg)	Number of nodes per axillary shoot
Control	100	1.42 c*	8.97 e	51.82 d	3.16 ab
BA + TDZ					
1.0 + 0	100	2.20 abc	14.74 cde	128.16bcd	3.72 ab
2.5 + 0	100	2.65 ab	12.58 de	109.05 cd	3.50 ab
5.0 + 0	100	2.67 ab	8.63 e	126.75 cd	2.72 b
1.0 + 0.01	100	2.25 abc	15.07 cde	143.67bcd	3.42 ab
2.5 + 0.025	100	3.28 a	13.61 cde	195.64 bc	3.19 ab
5.0 + 0.05	100	3.05 a	12.35 de	139.96bcd	2.92 b
2iP + TDZ					
1.0 + 0	100	1.80 bc	29.80 a	315.86 a	4.07 a
2.5 + 0	100	2.21 abc	22.35 b	212.43 b	3.69 ab
5.0 + 0	100	3.05 a	19.56 bc	203.37 b	3.91 ab
1.0 + 0.01	100	1.86 bc	18.17 bcd	178.99 bc	3.49 ab
2.5 + 0.025	100	2.30 abc	19.45 bcd	159.19 bc	3.1 ab
5.0 + 0.05	100	2.05 abc	16.50 bcd	112.41 cd	3.29 ab

* Means in each column followed by the same letter are not significantly different at $\alpha = 0.05$

Table 4. The influence of growth regulators on roots and callus growth on *Mandevilla sanderi* explants positioned horizontally after 8 weeks of cultivation

PGRs ($mg \cdot dm^{-3}$)	Rooted shoots (%)	Number of roots per explant	Length of roots (mm)	Fresh weight of roots (mg)	Callus volume* (cm^3)	Callus fresh weight (mg)
Control	0	–	–	–	–	–
BA + TDZ						
1.0 + 0	15.0	2.00 ab*	13.07 a	6.10 b	1.30 de	413.47 fg
2.5 + 0	0	–	–	–	1.06 de	433.13efg
5.0 + 0	8.3	2.00 ab	9.00 a	3.50 b	6.59 a	1779.02 a
1.0 + 0.01	30.0	1.83 bc	16.28 a	7.93 b	2.12 de	536.15d-g
2.5 + 0.025	35.7	1.20 bc	20.80 a	3.66 b	5.52 ab	1528.63abc
5.0 + 0.05	5.3	1.00 bc	27.00 a	4.10 b	4.83 abc	1594.92ab
2iP + TDZ						
1.0 + 0	100	4.55 a	29.59 a	79.23 a	2.12 de	951.38 c-f
2.5 + 0	63.2	2.83 ab	23.48 a	24.18 ab	1.78 de	889.08 c-f
5.0 + 0	5.0	2.00 ab	15.10 a	10.40 b	2.41 cde	1019.54b-e
1.0 + 0.01	95.0	1.89 b	26.14 a	12.71 b	2.90 bcd	1127.55a-d
2.5 + 0.025	10.0	1.00 bc	30.50 a	15.45 ab	2.63 cd	813.45 def
5.0 + 0.05	0	–	–	–	2.00 de	929.16 c-f

* Means in each column followed by the same letter are not significantly different at $\alpha = 0.05$

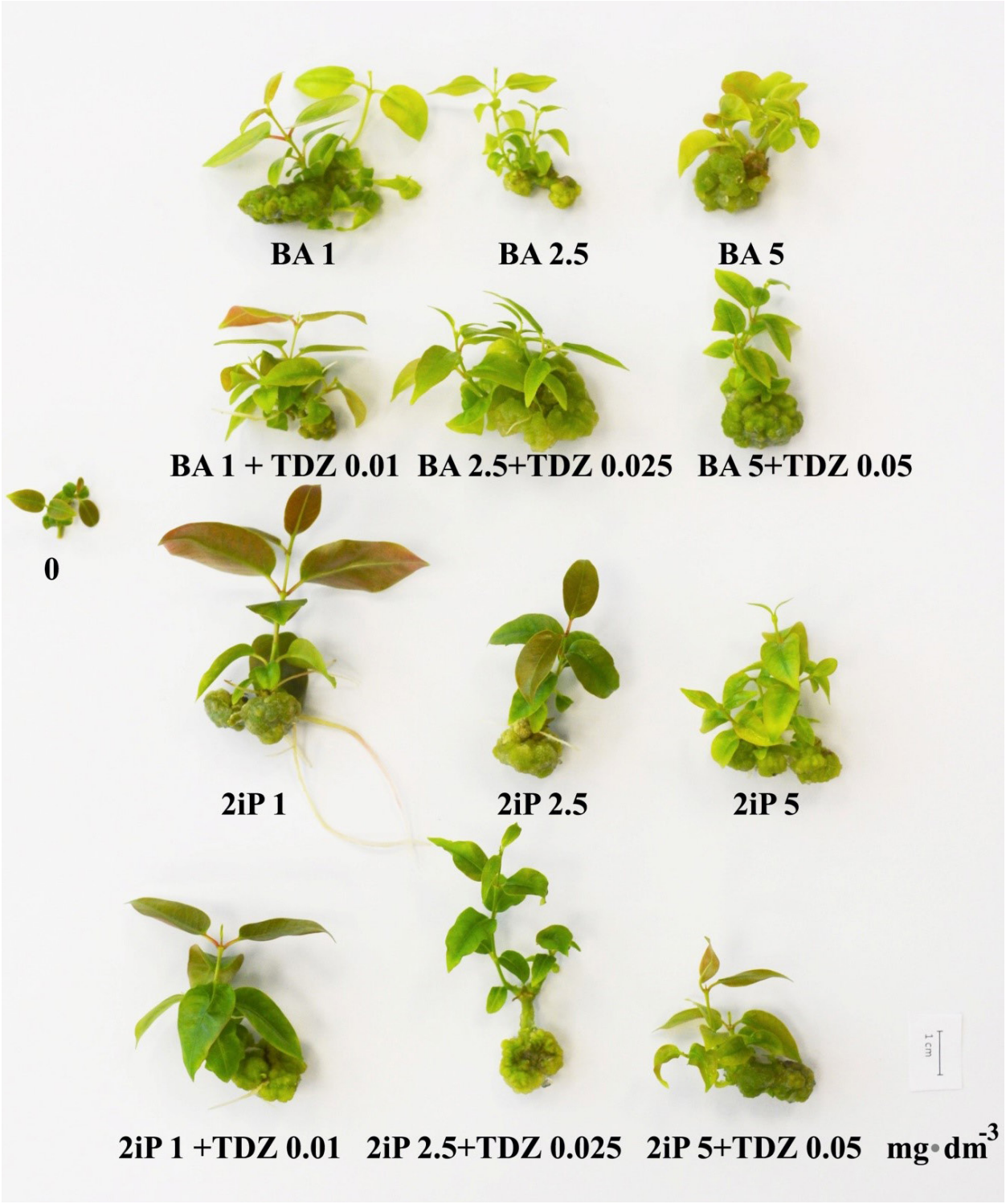

Fig. 2. The influence of growth regulators and horizontal orientation of explants on growth and development of *Mandevilla sanderi* after 8 weeks of cultivation

of BA, 5 mg·dm^{-3} 2iP + 0.05 mg·dm^{-3} TDZ and the control there were no rooted shoots. The highest number of roots per explant was noted in presence of 2iP in concentration of 1 mg·dm^{-3} (4.55) in comparison to 2iP used with TDZ in lower concentrations (1.0–1.89) or BA in combination with TDZ in all concentrations (1.0–1.83). There were no significant differences in the length of roots between studied treatments. Roots formed in presence of 2iP at 1 mg·dm^{-3} characterized with the highest fresh weight (79.23 mg). Roots of similar fresh weight were obtained on the media with 2iP in concentration of 2.5 mg·dm^{-3} alone or in combination with TDZ (24.18 and 15.45 mg respectively).

In the case of explants placed horizontally, callus was in all the treatments except the control (Tab. 4). In the presence of BA at 5 mg·dm^{-3} the highest callus volume was noted (6.59 cm^3), and it did not differ significantly from those on 2.5 mg·dm^{-3} BA + 0.025 mg·dm^{-3} TDZ and 5 mg·dm^{-3} BA + 0.05 mg·dm^{-3} TDZ (5.52 and 4.83 cm^3, respectively). The fresh weight of callus was enhanced by BA 5 mg·dm^{-3} (1779.02 mg), and statistically similar results were obtained when BA was used together with TDZ in two higher concentrations (1528.63 and 1594.92 mg).

The effect of combinations of BA and 2iP with TDZ and orientation of explants on the multiplication rate of Mandevilla sanderii

The calculations done on the basis of the obtained data indicated that *Mandevilla sanderii* multiplication rate, obtained by division of the main and axillary shoots into segments suitable for further multiplication (secondary explants), depended on the orientation of explants and cytokinins used and ranged from 1.76 to 7.30 (Tab. 5). Horizontal orientation promoted production of secondary explants (5.16) in comparison to the vertical position (3.45). Taking into consideration both cytokinins used and the explant position, it was noted that the highest multiplication rate was obtained on horizontally placed explants on the media supplemented with 2iP in concentration of 2.5 mg·dm^{-3} in combination with 0.025 mg·dm^{-3} TDZ (7.30) or 2iP in concentration of 5 mg·dm^{-3} (7.15). Statistically similar results were noted on the media containing 2iP in concentrations of 1 or 2.5 mg·dm^{-3} (6.05 and 6.16) and the same position of explants or BA in concentrations of 5 mg·dm^{-3} alone or in combination with TDZ (5.38 and 5.90 respectively) and vertical explants orientation.

Table 5. The influence of growth regulators and orientation of explants on the multiplication rate of *Mandevilla sanderi* after 8 weeks of cultivation

Growth regulators concentration (mg·dm^{-3})	Explant orientation	
	vertical	horizontal
Control	1.76 g*	2.53 efg
BA + TDZ		
1.0 + 0	1.81 fg	4.45 b–f
2.5 + 0	2.25 efg	4.55 b–e
5.0 + 0	2.90 d–g	4.08 b–g
1.0 + 0.01	2.24 efg	4.40 b–f
2.5 + 0.025	3.09 d–g	6.14 abc
5.0 + 0.05	3.76 b–g	5.31 a–d
2iP + TDZ		
1.0 + 0	4.43 b–f	6.05 abc
2.5 + 0	3.90 b–g	6.16 ab
5.0 + 0	5.38 abc	7.15 a
1.0 + 0.01	3.28 d–g	4.52 b–e
2.5 + 0.025	4.14 b–f	7.30 a
5.0 + 0.05	5.90 abc	4.39 b–f
Mean	3.45 B	5.16 A

* Means followed by the same letter are not significantly different at α = 0.05

DISSCUSION

BA is one of the most commonly applied cytokinins, used for shoot multiplication of many ornamental plants, such as *Hibiscus sabdariffa* [Gomez-Leyva et al. 2008], *Lagerstroemia* [Niranjan et al. 2010], *Ficus anastasia* [Al Malki and Elmeer 2010], *Rosa* sp. [Pawłowska 2011], *Ficus carica* [Mustafa and Taha 2012], *Dracaena sanderiana* [Aslam et al. 2013], whereas 2iP is less popular. In the presented study 2iP was more effective in the improvement of multiplication rate of shoots than BA. It resulted in a stronger growth of *Mandevilla sanderi* shoots, which were then divided into segments. According to other researchers, 2iP promotes elongation of shoots of *Prunus armeniaca* [Murai et al. 1997], *Rosa* 'Bianca' and 'Tora' [Mahmood and Hauser 2015], *Rhododendron tomentosum* [Jesionek et al. 2016]. It was noted that BA significantly reduced the elongation of shoots when compared to 2iP in the studies of Chuenboonngarm et al. [2001] on *Gardenia jasminoides*, Khan et al. [2015] on *Vitis vinifera*, and Mahmood and Hauser [2015] on 'Bianca' and 'El Torro' roses.

Many authors reported that shoot proliferation can be significantly enhanced by the addition of cytokinins in low concentrations. BA at concentration of 0.5–2 mg·dm^{-3} combined with TDZ at concentration of 0.15–0.55 mg·dm^{-3} were used successfully for *Acacia sinuata* [Vengadesan et al. 2002], *Mandevilla sanderi* [Wojtania et al. 2006], *Rubus occidentalis* × *R. idaeus* [Dai et al. 2006], *Hibiscus acetosella* [Sakhanokho 2008], and *Fraxinus americana* [Palla and Pijut 2011]. In the present study the combined use of BA and TDZ did not significantly affect the number of axillary shoots and multiplication rate of *Mandevilla sanderi*. Also, addition of small amount of TDZ to the medium containing 2iP did not stimulate shoot induction. The use of 0.05 mg·dm^{-3} TDZ together with 5 mg·dm^{-3} 2iP, at horizontal explants orientation, reduced multiplication rate significantly (7.15 and 4.39, respectively). Similar to our observations, Thomsone and Gertnere [2003] noted a negative effect of addition of 0.5 mg·dm^{-3} TDZ to the medium together with 15 mg·dm^{-3} 2iP on a number of new shoots of *Rhododendron* 'Nowa Zembla' (4.4 and 1.3, respectively). On the contrary, Jesionek et al. [2016] reported that media supplemented with 2iP 2.26 mg·dm^{-3} and TDZ 0.22 mg·dm^{-3} effectively stimulated microshoot formation of *Rhododendron tomentosum*. Kokotkiewicz et al. [2012] found that the highest number of microshoots of *Cyclopia genistoides* was obtained when two cytokinins, 2iP and TDZ were used.

The orientation of explants plays an important role in their regeneration potential. The horizontal position of the explants was reported to promote shoot formation in many plants, such as *Prunus laurocerasus* [Ponchia and Gardiman 1993], *Rauvolfia micrantha* [Sudha and Seeni 1996], *Citrus jambhiri* [Saini at al. 2010], *Quercus aliena* [Liao and Chuang 2014], and *Aloysia triphylla* [Silva et al. 2017]. According to the present results, a higher multiplication rate was achieved, when explants of *Mandevilla sanderi* were placed horizontally. It was observed that in such explant orientation, 100% explants produced axillary shoots and number of axillary shoots from one explant was two times higher in comparison to the vertical position. The increased axillary shoot proliferation in horizontal position could be connected with greater uptake of the medium constituents due to an increased contact with the medium [Mackay and Kitto 1988, Garcia-Luis et al. 2006]. Kozak et al. [2019] conducted research on the influence of *Mandevilla sanderi* explants positions on their growth and development in tissue culture. The authors found out that both vertical and horizontal positions of explants with shoot tips were advantageous in comparison to other explants treatments. Vertical placement was more responsive for shoot induction and multiplication than horizontal placement in *Celastrus paniculatus* [Nair and Seeni 2001], and *Murraya koengii* [Sharma et al. 2010].

During the current study, 2iP promoted root induction and growth more than BA. Ružić and Vujović [2008] noticed that in many combinations with 2iP, and particularly in those with KIN, rhizogenesis was induced in *Prunus avium*. Soh et al. [1998] suggested that cytokinins have stimulating effect on root formation from callus of *Vigna unguicula.* Shoots of *Eustoma grandiflorum* cultured on the media enriched with BA in concentration of 2 mg·dm^{-3} produced more and longest roots than in a control [Kaviani et al. 2014]. Mastuti et al. [2016] reported that almost all callus *Physalis angulata* induced on a medium with kinetin produced roots.

Development of big amount of callus at shoot bases was noted. All the hormonal treatments resulted in callus formation. Medium supplemented with the

highest concentration of BA combined with TDZ was the most advantageous for callus growth. High values of fresh weight of callus were noted in presence of 1 mg·dm^{-3} 2iP + 0.01mg·dm^{-3} TDZ, when explants were placed horizontally and in the treatment with 5 mg·dm^{-3} 2iP and 0.05 mg·dm^{-3} TDZ, at vertical position. Callus regeneration was also observed by Wojtania et al. [2006] in *Dipladenia sanderi* tissue culture on media containing 0.5–2 mg·dm^{-3} BA used singly or combined with 0.015 mg·dm^{-3} TDZ. In the study on *Vigna radiata* Gulati and Jaiwal [1992] observed that kinetin, Zt or BA in concentration of 5 × 10^{-6} – induced variable amounts of callus at the base of the shoot tip. 2iP in concentration of 15 mg·dm^{-3} used together with TDZ 0.2–0.5 mg·dm^{-3} promoted callus formation of *Vaccinium corymbosum* 'Duke' [Capeletti et al. 2016]. TDZ stimulated callus production in many plants cultured *in vitro*: *Vitis* [Kim and Kim 2002], *Camellia sinensis* [Gonbad et al. 2014], *Quercus aliena* [Liao and Chuang 2014], and *Simmondsia chinensis* [Taha et al. 2016].

CONCLUSIONS

In the presented study it was proved that multiplication rate and quality of shoots cultivated *in vitro* depends on the cytokinins added to the media and the orientation of explants. Horizontal orientation of explants on the medium supplemented with 2iP in concentration of 2.5 mg·dm^{-3} combined with TDZ in concentration of 0.025 mg·dm^{-3} or used alone in concentration of 5 mg·dm^{-3} allows to obtain the highest multiplication rate, what has a practical value for a large scale micropropagation of the species. 2iP is also advantageous in regards to the shoots quality, measured by their length and rhizogenesis.

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