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THE INFLUENCE OF EXPLANTS TYPE AND ORIENTATION ON GROWTH AND DEVELOPMENT OF *Mandevilla sanderi* (Hemsl.) Woodson *in vitro*

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ABSTRACT

Mandevilla sanderi is an important commercial ornamental pot plant. Traditional vegetative propagation is limited due to the low rate, therefore there is a need to develop an alternative, more efficient method. There is an interest in development of micropropagation technology for the species, as it allows to obtain a lot of offsprings in a relatively short time. The aim of the present work was to estimate an influence of explants type and position on regeneration of *Mandevilla sanderi* in tissue culture. Four different types of explants (leafy shoot tips, decapitated leafy shoot tips, defoliated shoot tips, decapitated and defoliated shoot tips) were used in the experiment, which were placed on the media vertically, while defoliated shoot tips were placed horizontally or vertically upside down. The explants were cultivated on a Murashige and Skoog (MS) medium supplemented with 1 mg·dm^{-3} benzyladenine (BA) and 0.5 mg·dm^{-3} indole-3-butyric acid (IBA). It was noted that both explants orientation and positioning, influenced the multiplication rate. Defoliated shoot tips placed horizontally were characterized by higher multiplication rate (6.8) in comparison to upside down vertical positioning (3.2). It was also observed that removal of shoot apex improved axillary branching, while defoliation of shoots placed in a normal position reduced multiplication rate.

Key words: regeneration, multiplication rate, explant defoliation, explant decapitation

INTRODUCTION

Mandevilla sanderi (Hemsl.) Woodson (syn. *Dipladenia sanderi*) is an attractive woody climber native to Brazil. It belongs to the Apocynaceae family. It has gained an increasing popularity over the last decade, due to its pretty, funnel-shaped, pink, white or red flowers blooming from late spring till autumn. Mandevilla may be grown outdoors in warm, tropical climates, but usually it is cultivated indoors as a houseplant or as a pot plant for decoration of balconies and terraces. The species may be propagated by stem cuttings in late spring or early summer, however the method is inefficient. In order to establish an effective method for production of healthy plants and to obtain a high propagation rate an *in vitro* studies were undertaken by some researches. It was proven that the success of regeneration depended significantly on the type of explant chosen and the way of its placement on a culture medium. Wojtania et al. [2006] tested 2 types of *Mandevilla sanderi* explants: apical and basal shoot segments obtained in tissue culture. The mul-

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tiplication rate was 2.5 shoots per apical explant and 3.1 shoot per basal one. Yonghong et al. [2004] compared regeneration ability of 3 types of explants of that species: terminal buds, mature stem segments and immature stem segments and noted that terminal buds had been the best source of explants. Nodal segments excised from seedlings were used for *Mandevilla velutina* [Biondo et al. 2007] and *Mandevilla guanabarica* [Cordeiro et al. 2014], while Biondo et al. [2004] used nodes from mature plants of *in vitro Mandevilla illustris.* Other explants were also used in micropropagation of mandevilla: young leaves, stem segments, root explants for *Mandevilla velutina* [Handro et al. 1988] or shoots obtained from *in vitro* plantlets for *Mandevilla moricandiana* [Cordeiro et al. 2012]. Several authors observed a positive effect of horizontal positioning of explants on their regenerative ability [Orlikowska et al. 2000, Debnath 2005, Rajeswari and Palival 2008] or a vertical upside down one [Ziv et al. 1970, Seabrook et al. 1976, Kozak 1991, Orlikowska et al. 2000]. The increase in shoot branching was also observed after removal of apex [Miller and Drew 1990, Voyiatzi et al. 1995, Pumisutapon et al. 2009, Ngamau 2001, Mohamed-Yasseen 2002] or shoots defoliation [Orlikowska et al. 2000].

The aim of the present study was to evaluate the regeneration ability of different types of *Mandevilla sanderi* (Hemsl.) Woodson explants, placed on the medium in different orientations.

MATERIAL AND METHODS

In vitro shoot cultures of *Mandevilla sanderi* (Hemsl.) Woodson were initiated from the shoot tips and axillary buds collected from plants cultivated in a greenhouse. They were surface disinfected by immersion in sodium hypochlorite containing 0.5% of active chlorine for 30 minutes, then rinsed 3 times in distilled sterile water. The explants were cultured on basic Murashige and Skoog [1962] medium (MS) containing mineral salts and thiamine – 0.4 mg·dm^{-3}, pyridoxine – 0.5 mg·dm^{-3}, nicotinic acid – 0.5 mg·dm^{-3}, glycine – 2 mg·dm^{-3}, myo-inositol – 100 mg·dm^{-3}, sucrose – 30 g·dm^{-3}. The medium was supplemented with benzyladenine (BA) at 1 mg·dm^{-3} and indole-3-butyric acid (IBA) 0.5 mg·dm^{-3} and solidified with agar (Lab-Agar™ Biocorp) – 6.5 g·dm^{-3}. The pH of the medium was adjusted to 5.7 before autoclaving. After several months of multiplication, shoot tips, 15–20 mm in length, with 2 nodes were isolated and used for an experiment. Following types of explants were prepared: shoot tips, decapitated shoot tips, defoliated shoot tips (except for the youngest leaf at the top), shoot tips after removal of the shoot apex and leaves (decapitated and defoliated). They were placed on the medium vertically with the shoot tip up. In case of defoliated shoot tips, horizontal and vertical position with the shoot tip down was also used. The cultures were maintained at 22 ±2°C under 16-h photoperiod and light intensity of 35 µmol·m^{-2}·s^{-1}. There were four replications per treatment, each consisting of 5 explants cultivated in Erlenmeyer flask of 200 ml capacity. The experiment was repeated twice.

Following features were evaluated in the experiment after 8 weeks of cultivation: length and fresh weight of main shoots, number and size of leaves and number of nodes per main shoot; percentage of shoots with axillary shoots, number, length and fresh weight of axillary shoots, number of leaves and nodes on axillary shoots, percentage of rooted shoots, number, length and fresh weight of roots; percentage of shoots with callus, size and fresh weight of callus. A multiplication rate (number of micro-cuttings per explant obtained by division of main and axillary shoots that could be used for further cultivation) was also counted per each treatment. The results of the experiment were analyzed statistically with the use of a one-factorial analysis of variance and the Tukey test was used to estimate the significant differences between the means at the 5% level of significance.

RESULTS

On the basis of the obtained results it was noted that the type of explant and its positioning on the media had an influence on growth and development of the main shoot (Tab. 1).

Analyzing the effect of decapitation or defoliation of the shoot tip on growth of the main shoot it was noted that shoots grown from shoot tips, defoliated or leafy (32.1 and 30.1 mm respectively), were significantly longer in comparison to decapitated ones

Table 1. Influence of the type and orientation of explant on growth of *Mandevilla sanderi* main shoot after 8 weeks of cultivation

Explant type	Explant orientation	Main shoot length (mm)	Main shoot fresh weight (mg)	Number of nodes	Number of leaves	Length of leaves (mm)	Leaf width (mm)
Leafy shoot tip	vertically, base down	30.1 a	167.1 a	5.9 a	7.9 ab	21.5 a	9.6 a
Leafy shoot tip, apex removed	vertically, base down	20.0 b	145.4 a	4.1b	6.8 b	20.7 a	10.0 a
Shoot tip, defoliated	vertically, base down	32.1 a	247.7 a	6.4 a	9.2 a	19.0 a	9.9 a
Shoot tip, defoliated, apex removed	vertically, base down	20.0 b	144.4 a	3.8 b	6.3 b	19.8 a	9.8 a
Shoot tip, defoliated	horizontally	28.6 ab	154.7 a	6.1 a	8.2 a	19.8 a	8.4 a
Shoot tip, defoliated	vertically, base up	25.4 ab	137.8 a	5.9 a	10.1 a	9.0 b	4.7 b

Means in each column followed by the same letter are not significantly different at α = 0.05

Table. 2. Influence of the type and orientation of explants on branching of *Mandevilla sanderi* shoots after 8 weeks cultivation in tissue culture

Explant type	Explant orientation	Shoots with axillary shoots (%)	Number of axillary shoots per shoot	Length of axillary shoots (mm)	Fresh weight of axillary shoots (mg)	Number of nodes on axillary shoot	Number of leaves on axillary shoots
Leafy shoot tip	vertically, base down	10 b	1.0 b	24.5 a	50.1 b	3.0 cd	4.5 bc
Leafy shoot tip, apex removed	vertically, base down	60 a	1.0 b	11.0 b	83.0 ab	2.4 d	3.3 c
Shoot tip, defoliated	vertically, base down	47 ab	1.0 b	22.4 a	156.3 a	5.2 b	7.6 a
Shoot tip, defoliated, apex removed	vertically, base down	75 a	1.3 a	14.4 ab	118.7 ab	4.2 b–d	6.8 ab
Shoot tip, defoliated	horizontally	55 a	1.5 a	20.6 ab	102.4 ab	4.4 bc	6.7 ab
Shoot tip, defoliated	vertically, base up	5 b	1.0 b	18.0 ab	134.5 ab	7.4 a	9.0 a

Means in each column followed by the same letter are not significantly different at α = 0.05

(20.0 mm). It was also observed that removing leaves from shoot tips cultivated in a normal position, slightly stimulated elongation (by 2 mm).

Comparing the effect of orientation of defoliated shoot tips, no significant differences were stated regarding the shoots length in case of vertical, horizontal or upside down positioning (32.1, 28.6, 25.4 mm respectively). However, on the basis of the obtained results it was noted that shoots placed horizontally or upside down were slightly shorter.

It was observed that neither decapitation or defoliation of explants nor their orientation had a significant effect on fresh weight of the main shoot. However, on the basis of the obtained data, it might be stated that the highest fresh weight was noted in case of shoot tips with the leaves removed and placed vertically on the medium (247.7 mg), while the smallest one was observed when shoot tips had been defoliated and placed on the media vertically with the base up (137.8 mg).

Statistical analysis proved that decapitation of explants significantly decreased the number of nodes on the main shoots, both in case of leafy and defoliated ones (4.1 and 3.8 respectively), in comparison to shoot tips with the apex (from 5.9 to 6.4). Similar results were obtained in regards to the number of leaves. It was observed that leaves differed in their morphological features. Those formed on the main shoot grown from a defoliated shoot tip orientated vertically with the base up, were significantly shorter and narrower (9.0 and 4.7 mm respectively) in comparison to other treatments, in which leaves were from 19.0 to 21.5 mm long and from 8.4 to 10.0 mm wide.

The type of explant and its positioning on the media influenced branching of the main shoot (Tab. 2). Analyzing a regeneration capacity of the studied explants, it was observed that the most shoots with axillary ones appeared when defoliated shoot tips with the apex removed were used as explants (75%). Statistically less shoots branched when leafy or defoliated shoot tips placed vertically with the base up or down were used (5 and 10%, respectively). The axillary shoots formed on the lowest node at the shoot base, which is presented in Photo 1. It was also observed that defoliated shoot tips with the apex removed or not, placed on the media vertically base down or horizontally formed more axillary shoots (1.3 and 1.5 pcs per shoot respectively) in comparison to the remaining treatments (1.0 pcs per shoot). The obtained axillary shoots differed in length. Those formed on leafy or defoliated shoot tips orientated vertically with the base down were longer (24.5 and 22.4 mm respectively) than the ones noted on leafy shoot tips with the apex removed, placed on the media in the same position (11.0 mm). Axillary shoots that were formed on defoliated shoot tips placed vertically with the base down were characterized by the highest fresh weight as well (156.3 mg). Axillary shoots of significantly lower fresh weight were noted when leafy shoot tips orientated vertically with the base down were used as explants (50.1 mg).

Analyzing the number of nodes and leaves formed on axillary shoots it was noted that defoliation of explants increased their amount. The highest number of nodes was obtained when defoliated shoot tips were placed on the media vertically with the base up (7.4), in comparison to other treatments. Vertical positioning of the leafy, decapitated shoot tips caused that shoots formed the least nodes and leaves (2.4 and 3.3, respectively).

On the basis of the statistical analysis it was proved that the type of explant and its positioning on the media influenced rooting of *Mandevilla sanderi* shoots in tissue culture (Tab. 3). It was noted that when leafy shoot tips placed vertically were used as explants, the most shoots formed roots (85%). Significantly less rooted shoots were observed when defoliated shoot tips were placed vertically in a normal position (30%) or upside down (15%) or additionally had the apex removed (15%). The studied explants formed from 1 to 3.5 roots. The highest number of roots was observed when defoliated shoot tips were placed on the media upside down (3.5 pcs), in comparison to leafy shoot tips in a normal position (1.0 pcs) or defoliated shoot tips orientated horizontally (1.0 pcs).

Regarding the length and fresh weight of the obtained roots, the differences between the means were not proven. However, on the basis of the obtained results, it was noted that roots formed on defoliated shoot tips placed upside down and defoliated shoot tips with the apex removed weighted less than those obtained in other combinations (3.4 and 5.0 mg, respectively in comparison to 18.6–32.7 mg).

A callus tissue was observed in all the treatments. It was proved that statistically less shoots formed callus when defoliated shoot tips positioned vertically

Photo 1. Leafy shoot tip orientated vertically after 8 weeks of cultivation in tissue culture

Photo 2. Leafy shoot tip with the apex removed orientated vertically after 8 weeks of cultivation *in vitro*

Photo 3. Defoliated shoot tip orientated vertically after 8 weeks of cultivation in tissue culture

Photo 4. Defoliated shoot tip with the apex removed orientated vertically after 8 weeks *in vitro*

Photo 5. Defoliated shoot tip orientated horizontally after 8 weeks of cultivation in tissue culture

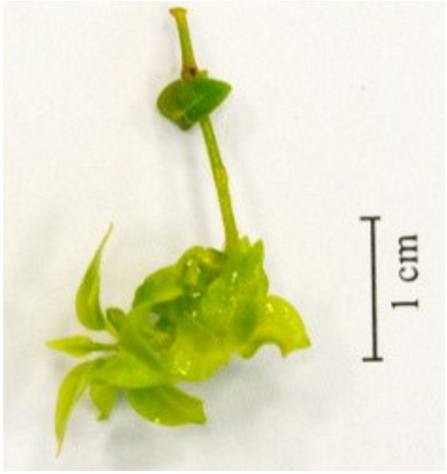

Photo 6. Defoliated shoot tip orientated upside down after 8 weeks of cultivation in tissue culture

Table 3. Influence of the type and orientation of explants on rooting of *Mandevilla sanderi* shoots after 8 weeks cultivation in tissue culture

Explant type	Explant orientation	Rooted shoots (%)	Number of roots	Roots length (mm)	Fresh weight of roots (mg)	Shoots with callus (%)	Callus volume (cm^3)	Callus fresh weight (mg)
Leafy shoot tip	vertically, base down	85 a	1.0 b	18.8 a	19.4 a	100 a	2.19 ab	574.9 b
Leafy shoot tip, apex removed	vertically, base down	65 ab	3.0 ab	20.4 a	30.5 a	90 a	1.26 b	382.2 b
Shoot tip, defoliated	vertically, base down	30 b	3.0 ab	24.1 a	18.6 a	60 ab	1.95 ab	520.4 b
Shoot tip, defoliated, apex removed	vertically, base down	15 b	1.8 ab	20.0 a	5.0 a	95 a	2.32 ab	596.9 b
Shoot tip, defoliated	horizontally	65 ab	1.0 b	19.8 a	32.7 a	100 a	3.23 a	1041.7 a
Shoot tip, defoliated	vertically, base up	15 b	3.5 a	19.7 a	3.4 a	35 b	3.60 a	1504.3 a

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

Table 4. Influence of the type and orientation of *Mandevilla sanderi* explant on the multiplication rate after 8 weeks of *in vitro* cultivation

Type of explant	Leafy shoot tip	Leafy shoot tip, apex removed	Shoot tip, defoliated	Shoot tip, defoliated, apex removed	Shoot tip, defoliated	Shoot tip, defoliated
Explant orientation	vertically, base down	vertically, base down	vertically, base down	vertically, base down	horizontally	vertically, base up
Multiplication rate	5.4 ab	3.9 bc	5.2 ab	2.7 c	6.8 a	4.2 bc

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

with the base up were used as explants. The obtained callus tissue differed in volume. Significantly bigger callus tissue was observed when defoliated shoot tips placed horizontally or upside down were used as explants (3.23 and 3.60 cm^3, respectively) in comparison to callus formed on decapitated, leafy shoot tips placed in a normal position on the media (1.26 cm^3). They characterized by higher fresh weight as well (1041.7 and 1504.3 mg, respectively), in comparison to other treatments.

Photos 1–6 show growth and development of *Mandevilla sanderi* shoots after 8 weeks of cultivation in tissue culture depending on the type of explant and its positioning on the culture media.

Mandevilla shoot tips explants showed a good elongation growth and some of them formed axillary shoots. In order to obtain new progeny plants, the main shoot and axillary shoots might be divided into nodal sections and used for further multiplication. Analyzing the number of the *Mandevilla sanderi*,

secondary explants obtained on shoots orientated in a natural position, depending on the explant type, higher number was noted in case of leafy shoot tips (5.4 pcs) and defoliated leafy shoot tips (5.2 pcs) (Tab. 4). Less secondary explants was obtained when the apex was removed, both for leafy and defoliated shoot tips (3.9 and 2.7, respectively). Horizontal orientation of defoliated shoots was very advantageous for the number of produced micro-shoots, as it allowed to obtained the highest multiplication rate (6.8).

DISCUSSION

In case of many plants species, an apical dominance or apical control occurs, therefore shoot apex grows predominantly and growth of axillary buds is retarded. Decapitation of the shoot tip might break this phenomenon and allow the buds to grow out [Shimizu-Sato et al. 2009]. In the presented study, the apex removal and horizontal position improved the axillary branching of *Mandevilla sanderi* shoots. Orlikowska et al. [2000] reported that in the case of *Codiaeum variegatum,* shoot tip removal increased a number of axillary shoots from 1.5 to 2.4. The influence of the apex removal was even more advantageous for Co*smos atrosanguineus* (3.3–5.4 axillary shoots obtained) [Kozak et al. 2013]. The same effect was observed in tissue culture of many ornamental plants, such as *Rosa* 'Dr Verhage' [Voyiatzi et al. 1995], *Rosa manetti* [Kucharska et al. 2000], *Zantedeschia aethiopica* [Ngamu 2001].

The influence of leaves removal on branching of *Mandevilla sanderi* in tissue culture was not observed in the present experiment. Similar observations were found in the case of *Cosmos atrosanguineus* [Kozak et al. 2013], but defoliated shoots of *Codiaeum variegatum* produced significantly more axillary shoots (3.9) in comparison to non-defoliated ones (1.5) [Orlikowska et al. 2000]. Renau-Morata et al. [2005] in studies on *Cedrus atlantica* and *C. libani* also observed bud sprouting from defoliated micro-cuttings. Defoliated stems with nodal buds were used to initiate the tissue culture of *Syngonium podophyllum* [Kalimuthu and Prabakaran 2014].

Comparing the orientation of explants, it was observed that among 4 studied explant types of *Mandevilla sanderi* that were placed vertically with the base down, the best axillary shoot induction was noticed from defoliated shoot tips with the apex removed (75% branched shoots with 1.3 axillary shoots). The upside down position of shoots was significantly less advantageous for production of axillary shoots (5% branching and 1.5 axillary shoot). Good results were also obtained in case of shoot tips placed in a horizontal position (55% of branched shoots with 1.5 axillary shoots). Beneficial effect of the horizontal orientation of explants on their regeneration ability was reported by Orlikowska et al. [2000] for *Codiaeum variegatum*, Nobre [1994] for *Myrtus communis*, Rajeswari and Paliwal [2008] for *Albizia odoratissima*, and Saini et al. [2010] for *Citrus jambhiri.* Favorable effects of inverted explant position were reported for several plants, such as *Cosmos atrosanguineus* [Kozak et al. 2013], *Lens culinaris* [Bermejo et al. 2012] or *Codiaeum variegatum* [Orlikowska et al. 2000].

All studied explants of *Mandevilla sanderi* produced callus tissue at the base. Callus regeneration was also observed by Wojtania et al. [2006] in *Dipladenia sanderi* tissue culture, Saini and Jaiwal [2002] in tissue culture of *Vigna mungo* and by Faroq et al. [2002] for *Annona squamosa*.

Presented results are a part of the research that aims at increasing the multiplication rate of *Mandevilla sanderi* in tissue culture. The obtained results indicate that the appropriate choice of explant type and its positioning on the media might significantly enhance or reduce the coefficient of *in vitro* propagation of the species. In case of *Mandevilla sanderi,* it seems that the best way to obtain progeny plants is division of shoots into nodal defoliated and decapitated explants placed on the media vertically with the base up, as it allows to obtain the largest number of secondary explants for further multiplication. However, a further research should be undertaken to develop a more efficient micropropagation technology for the species.

CONCLUSIONS

1. Type of explant as well as its positioning on the media have an influence on growth and branching of *Mandevilla sanderi in vitro.*

2. Apex removal improves the axillary branching of *Mandevilla sanderi.*

3. Defoliation of shoots placed in a normal position reduces the multiplication rate.

4. The use of defoliated shoot tips orientated horizontally allows to obtain the highest multiplication rate.

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REFERENCES

Biondo, R., Soares, A.M., Bertoni, B.W., Franca, S.C., Pereira, A.M. S. (2004). Direct organogenesis of *Mandevilla illustris* (Vell.) Woodson and effect of its aquaeous extract on the enzymatic and toxic activities of *Crotalus durissus* terrificus snake venom. Plant Cell Rep., 22, 549–552.

Biondo, R., Souza, A.V., Bertoni, B.W., Soares, A.M., Franca, S.C., Pereira, A.M.S. (2007). Micropropagation, seed propagation and germplasm bank of *Mandevilla velutina* (Mart.) Woodson. Sci. Agric. (Piracicaba, Braz.), 64(3), 263–268.

Bermejo, C., Espósito, M.A., Cravero, V., Anido, F.L., Cointry, E. (2012). *In vitro* plant regeneration from cotyledonary nodes of recombinant inbred lines of lentil. Sci. Hortic., 134, 13–19.

Cordeiro, S.Z., Simas, N.K., Henriques, A.B., Lage, C.L.S., Sato, A. (2012). Micropropagation of *Mandevilla moricandiana* (A.D.C.) Woodson. In Vitro Cell. Dev. Biol. Plant, 48, 620–626.

Cordeiro, S.Z., Simas, N.K., Henriques, A.B., Sato, A. (2014). Micropropagation and callogenesis in *Mandevilla guanabarica* (Apocynaceae), an endemic plant from Brazil. Crop Breed. Appl. Biotechnol., 14(2). http://dx.doi.org/10.1590/1984-70332014v14n2a19

Debnath, S.C. (2005). Micropropagation of lingonberry: influence of genotype, explant orientation, and overcoming TDZ-induced inhibition of shoot elongation using zeatin. HortScience, 40(1), 185–188.

Handro, W., Floh, E.I.S., Ferreira, C.M., Guerra, M.P. (1988). Tissue, cell culture and micropropagation of *Mandevilla velutina*, a natural source of bradykinin antagonist. Plant Cell Rep., 7, 564–566.

Kalimuthu, K., Prabakaran, R. (2014). *In vitro* Micropropagation of *Syngonium podophyllum*. Int. J. Pure App. Biosci. 2(4), 88–92.

Kozak, D. (1991). Shoots regeneration from various parts of *Narcissus* cv. Carlton through tissue culture. Prace Inst. Sad. Kwiac., B Rośl. Ozdobne, 16, 41–48.

Kozak, D., Pogroszewska, E., Szmagara, M. (2013). The influence of type and orientation of explants on *in vitro* growth and development of *Cosmos atrosanguineus* (Hook.) Voss. Acta Sci. Pol. Hortorum Cultus, 12(1), 41–53.

Kucharska, D., Golis, M., Podwyszyńska, M., Wiśniewska-Grzeszkiewicz, H., Orlikowska, T. (2000). Propagation of *Rosa manetti* rootstock *in vitro*. Zesz. Nauk. Inst. Sad. Kwiac., 7, 365–374.

Miller, R.M., Drew, R.A. (1990). Effect of explant type on proliferation of *Carica papaya* L. *in vitro*. Plant Cell. Tiss. Organ Cult. 21, 39–44.

Mohamed-Yasseen, Y. (2002). Micropropagation of pitaya (*Hylocereus undatus* Britton et Rose). In Vitro Cell. Dev. Biol. Plant., 38(5), 427–429.

Murashige, T., Skoog, F. (1962). A revised medium for rapid growth and bio-assays with tobacco tissue cultures. Physiol. Plant. 15, 473–479.

Ngamau, K. (2001). Development of an *in vitro* culture procedure using seeds from *Zantedeschia aethiopica* 'Green Goddess' as explant. Gartenbauwissenschaft, 66(3), 133–139.

Nobre, J. (1994). *In vitro* shoot proliferation of *Myrtus communis* L. from field-grown plants. Sci. Hortic. 58(3), 253–258.

Orlikowska, T., Sabała, I., Kucharska, D. (2000). The effect of leaf and shoot tip removal and explant orientation on axillary shoot proliferation of *Codiaeum variegatum* Blume var. *pictum* Muell. Arg. cv. Excellent. Sci. Hortic., 85, 103–111.

Pumisutapon, P., Visser, R.G.F., De Klerk, G.-J. (2009). Apical dominance in *Alstroemeria* cultured *in vitro*. Acta Hortic. 829, 145–148.

Rajeswari, V., Paliwal, K. (2008). *In vitro* adventitious shoot regeneration from seedling explant of *Albizia odoratissima* L.f. (Benth.). In Vitro Cell. Dev. Biol. Plant. 44(2), 78–83.

Renau-Morata, B., Ollero, J., Arrilaga, I., Segura, J. (2005). Factors influencing axillary shoot proliferation and adventitious budding in cedar. Tree Physiol., 25(4), 477–486.

Saini, R., Jaiwal, P.K. (2002). Age, position in mother seedling, orientation, and polarity of the epicotyl segments

of blackgram (*Vigna mungo* L. Hepper) determines its morphogenic response. Plant Sci., 163(1), 101–109.

Saini, H.K., Gill, M.S., Gill, M.I.S. (2010). Direct shoot organogenesis and plant regeneration in rough lemon (*Citrus jambhiri* Lush.). Indian J. Biotechnol., 9, 419–423.

Seabrook, J.E.A., Cumming, B.G., Dionne, L.A. (1976). The *in vitro* induction of adventitious shoot and root apices on *Narcissus* (daffodil and narcissus) cultivar tissue. Can. J. Bot., 54, 814–819.

Shimizu-Sato, S., Tanaka, M., Mori, H. (2009). Auxin–cytokinin interactions in the control of shoot branching. Plant Mol. Biol., 69, 429–435.

Voyiatzi, Ch., Voyiatzis, D.G., Tsiakmaki, V. (1995). *In vitro* shoot proliferation rates of the rose cv. (hybrid tea) 'Dr. Verhage', as affected by apical dominance regulating substances. Sci. Hortic. 61, 241–249.

Wojtania, A., Gabryszewska, E., Woźniak, P. (2006). Zastosowanie kultur *in vitro* w rozmnażaniu *Dipladenia sanderi* Hemsl. Zesz. Probl. Postęp. Nauk Rol., 510, 717–722.

Yonghong, L., Daxing, Z., Lijuan, X. (2004). Studies of the micropropagation of *Mandevilla sanderi.* For. Sci. Technol., 29(5), 49–51.

Ziv, M., Halevy, A.H., Shilo, R., 1970. Organ and plantlets regeneration of *Gladiolus* through tissue culture. Ann. Bot., 34, 671–675.