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THE INFLUENCE OF TYPE AND ORIENTATION OF EXPLANTS ON *in vitro* GROWTH AND DEVELOPMENT OF *Cosmos atrosanguineus* (Hook.) Voss

Danuta Kozak, Elżbieta Pogroszewska, Mariusz Szmagara
University of Life Sciences in Lublin

Abstract. *Cosmos atrosanguineus* is a tuberous, tender perennial with velvety, dahlia-like, brownish-red flowers that have a chocolate aroma. It is sterile, so no viable seeds are produced. This species is particularly suitable for a border plant in any perennial garden. A study was undertaken to determine whether shoot apex and leaf removal as well as explant orientation had an influence on axillary shoot induction. Explants were prepared from shoots taken from aseptically grown shoot cluster and were cultured for 6 weeks on Murashige and Skoog medium containing BA (1 mg·dm^{-3}). The study results showed that the removal of the apex from the non-defoliated shoot tip improved axillary branching. The removal of developed leaves from the shoot tip with the apex removed caused a reduction in axillary shoot production. The defoliated shoot tip inserted vertically in the medium in an inverted position regenerated the highest number of axillary shoots characterized by the highest fresh weight. The defoliated shoot tip after removal of the apex, placed vertically with the base down, produced axillary shoots characterized by the greatest elongation growth and the highest percentage of shoots that reached a length of more than 1 cm.

Key words: branching, defoliation, decapitation, explant orientation vertical or horizontal

INTRODUCTION

Cosmos atrosanguineus (Hook.) Voss (Asteraceae family) is a herbaceous perennial plant growing to 40–60 cm tall, with fleshy tuberous roots. The flowers are produced in a capitulum 3–4.5 cm in diameter, dark red to maroon-dark brown, and they have the scent of chocolate. This plant is sterile and does not produce seeds, so it has to be propagated by division of the tubers. This method of propagation is very slow, so studies are undertaken to propagate *Cosmos in vitro* in order to obtain a high propagation rate and to produce healthy and pathogen-free plants. Hosoki et al. [2003] report about

Corresponding author – Adres do korespondencji: Danuta Kozak, Institute of Ornamental Plants and Landscape Architecture, University of Life Sciences in Lublin, ul. Leszczyńskiego 58, 20-068 Lublin, Poland, e-mail: danuta.kozak@up.lublin.pl

the possibility of micropropagation of *Cosmos atrosanguineus* by subculturing stem sections with nodes or by dividing axillary shoots. The success of regeneration depends significantly on the type of explant chosen. Shoot tip explants were found to be suitable for *in vitro* propagation of many plants from Asteraceae family: *Achillea millefolium* [Turker et al. 2009], *Arnica montana* [Surmacz-Magdziak and Sugier 2012], *Aster ericoides* [Salazar et al. 2005], *Chrysanthemum morifolium* [Waseem et al. 2009], *Zinnia angustifolia*, *Z. elegans*, *Z. haageana* [Anantasaran and Kanchanapoom 2008].Using of nodes has been reported for: *Artemisia vulgaris* [Sujatha and Kumari 2008], *Calendula arvensis* [Leal et al. 2009], *Centaurea tchihatcheffii* [Ozel et al. 2006], *Chrysanthemum* × *grandiflorum* [Nencheva 2010], *Ch. morifolium* [Waseem et al. 2011], *Cosmos atrosanguineus* [Hosoki et al. 2003], *Echinops spinosissimus* [Murch et al. 2003], *Gynura procumbens* [Chan et al. 2009]. This explants were positioned vertically with normal polarity. There are reports about the positive effect of apex removal [Miller and Drew 1990, Voyiatzi et al. 1995, Pumisutapon et al. 2000, Ngamau 2001, Mohamed-Yasseen 2002] or defoliation [Orlikowska et al. 2000] on shoot branching. Also the way explants are placed on the culture medium is very important in micropropagation. An increase in shoot numbers was observed when shoots were placed on the medium horizontally [Orlikowska et al. 2000, Debnath 2005, Rajeswari and Palival 2008] or vertically in an inverted position with the shoot tip down [Ziv et al. 1970, Seabrook et al. 1976, Kozak 1991, Orlikowska et al. 2000].

The aim of the present study was to evaluate the regeneration ability of different types of *Cosmos atrosanguineus* explants, placed in different orientations.

MATERIALS AND METHODS

In vitro shoot cultures of *Cosmos atrosanguineus* (Hook.) Voss were established by culturing shoot tips and axillary buds collected from plants growing in a greenhouse. They were disinfected in sodium hypochlorite containing 0.5% of active chlorine for 30 minutes and rinsed 3 times in sterilized water. The explants were cultured on basic Murashige and Skoog [1962] (MS) medium containing mineral salts and 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 $g \cdot dm^{-3}$, and Agar-Agar (Lab-Agar™ Biocorp) – 6.5 $g \cdot dm^{-3}$, and supplemented with benzyladenine (BA) at 1 $mg \cdot dm^{-3}$. After several months of multiplication, shoot tips of 2 cm in length and the nodal parts of shoots of 1 cm in length were dissected from the shoot clusters and used for preparing explants. 7 types of explants were used in the experiment: shoot tips with leaves, defoliated shoot tips (except for the youngest leaf at the top), decapitated shoot tips, shoot tips after removal of the shoot apex and leaves, nodes with leaves, and defoliated nodes. The explants were placed on the medium in different orientations: vertically with the shoot tip up, vertically with the shoot tip down, horizontally.

The preliminary experiment showed that among from 4 concentrations of BA (0.2, 1.0, 2.5, 5.0 $mg \cdot dm^{-3}$) the best for multiplication of shoots is MS medium supplemented with BA 1 $mg \cdot dm^{-3}$, so this treatment was used in experiment. The pH of the medium was adjusted to 5.7 before autoclaving. There were four replications per treatment, each

consisting of 5 explants / Erlenmeyer flask. The experiment was repeated twice. The cultures were maintained at 22°C, with light intensity of 35 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and 16-h photoperiod.

The following characters were evaluated after 6 weeks: number of axillary shoots and their maximum and average length, fresh weight of axillary shoots / explant. The study also evaluated the percentage of shoots forming axillary shoots and the percentage of shoots in three length classes (< 5 mm, 5–10 mm, > 10 mm). The results of the experiment were analyzed statistically using a standard statistical procedure with one factorial design and the Tukey test was used to estimate the differences between the means at a 5% level of significance.

RESULTS AND DISCUSSIONS

The type of explant and its orientation on the culture medium showed a high influence on the regeneration and growth of axillary shoots of *Cosmos atrosanguineus*.

When analysing the percentage of explants regenerating axillary shoots, the poorest result (65% and 80%, respectively) was observed in the case of defoliated shoot tips and defoliated, decapitated shoot tips at the vertical orientation with the base down. In the other treatment combinations, the regeneration rate was high, amounting to 90–100% (tab. 1).

Shoot apex removal proved to have a large effect on shoot branching in the case of foliated shoots; they produced 2.1 more axillary shoots compared to the explants with the shoot apex (tab. 1, fig. 1). Shoot apex removal was not shown to have any effect in relation to defoliated explants.

Defoliation had a significant adverse effect in the case of decapitated explants placed vertically with the base down. In this treatment combination, 2.1 fewer shoots per 1 explant were obtained compared to the treatment when leaf removal was not applied (fig. 1, 2). Defoliation had no significant influence on shoot tips with the apex that were placed vertically or nodes that were placed vertically or horizontally.

Analysing the influence of explant orientation on the number of axillary shoots, their highest number (12.3) was found in the case of defoliated shoots placed in an inverted position in relation to the natural orientation (tab. 1, fig. 4). At the vertical orientation with the shoot tip up, this explant type regenerated 3.8 shoots, while at the horizontal orientation – 4.2 shoots. There were no significant differences in the number of shoots obtained from other types of explants at the horizontal or vertical orientation with the shoot tip up.

The experimental results showed a dependence of elongation growth of regenerating axillary shoots on the presence of the shoot apex or leaves on the explants and their orientation on the culture medium. Axillary shoots from shoot tips with leaves and from defoliated, decapitated shoot tips showed the highest maximum length (respectively: 21.3 and 23.2 mm) (tab. 2). Apical and nodal shoot segments placed vertically or horizontally produced shoots that were characterized by a lower maximum length. The horizontal orientation of explants inhibited elongation growth of the shoots regenerated from them (tab. 2, fig. 3, 5, 6).

Table 1. The effect of shoot tip and leaf removal and explant orientation on axillary shoot induction of *Cosmos atrosanguineus* (Hook.) Voss, after 6 weeks of *in vitro* culture

Tabela 1. Wpływ usunięcia wierzchołka wzrostu i liści oraz sposobu ułożenia eksplantatów na indukcję pędów kątowych *Cosmos atrosanguineus* (Hook.) Voss, po 6 tygodniach kultury *in vitro*

Type of explant Rodzaj eksplantatu	Explant orientation Ułożenie eksplantatu	% explants forming axillary shoots % eksplantatów tworzących pędy kątowe	Number of axillary shoots/explant Liczba pędów kątowych z 1 eksplantatu
Shoot tip, non-defoliated Wierzchołek pędu ulistniony	vertically, base down pionowo	90	3.3 c
Shoot tip, non-defoliated,apex removed Wierzchołek pędu ulistniony przycięty	vertically, base down pionowo	95	5.4 b
Shoot tip, defoliated, Wierzchołek pędu bez liści	vertically, base down pionowo	65	3.8 c
Shoot tip, defoliated, apex removed Wierzchołek pędu bez liści przycięty	vertically, base down pionowo	80	3.3 c
Shoot tip, non-defoliated Wierzchołek pędu ulistniony	horizontally poziomo	95	3.7 c
Shoot tip, defoliated Wierzchołek pędu bez liści	horizontally poziomo	100	4.2 c
Shoot tip, defoliated Wierzchołek pędu bez liści	vertically, base up pionowo, odwrócony	100	12.3 a
Node with leaves Węzeł ulistniony	vertically, base down pionowo	100	3.5 c
Node with leaves Węzeł ulistniony	horizontally poziomo	95	3.8 c
Node defoliated Węzeł bez liści	vertically, base down pionowo	90	4.1 c
Node defoliated Węzeł bez liści	horizontally poziomo	100	4.2 c

*Means followed by the same letter are not significantly different at $\alpha = 0.05$ – Średnie oznaczone tą samą literą nie różnią się istotnie przy $\alpha = 0{,}05$

The average length of axillary shoots, similarly to the maximum length, was the highest in the case of shoots regenerated from defoliated, decapitated shoot tips (15.0 mm) (tab. 2, fig. 2). Shoots from the nodes with leaves placed vertically with the base down also reached a significant average length (10.5 mm). In the other treatment combinations, the average shoot length ranged 6.6–9.4 mm (tab. 2).

When analysing the structure of shoot length in particular treatments, the lowest number of short shoots (< 5 mm) was observed in the case of shoots regenerated from the following: nondefoliated nodes (4.3%), decapitated shoot tips with leaves (5.4%), and defoliated, decapitated shoot tips (5.9%), at the vertical orientation with the base

Fig. 1. Shoot clusters of *Cosmos atrosanguineus* obtained from foliated shoot tip (from left) and from foliated shoot tip after shoot apex removal (from right), placed vertically, after 6 weeks of culture *in vitro*

Ryc. 1. Zespoły pędów *Cosmos atrosanguineus* uzyskane z ulistnionego wierzchołka pędu (z lewej) i z przyciętego, ulistnionego wierzchołka pędu (z prawej), ułożonych pionowo, po 6 tygodniach kultury *in vitro*

Fig. 2. Shoot clusters of *Cosmos atrosanguineus* obtained from defoliated shoot tip (from left) and from defoliated shoot tip after shoot apex removal (from right), placed vertically, after 6 weeks of culture *in vitro*

Ryc. 2. Zespoły pędów *Cosmos atrosanguineus* uzyskane z pozbawionego liści wierzchołka pędu (z lewej) i z przyciętego, pozbawionego liści wierzchołka pędu (z prawej), ułożonych pionowo, po 6 tygodniach kultury *in vitro*

down. Defoliated shoot tips placed vertically with the shoot tip up produced the largest number of short shoots (43.4%). The highest percentage of shoots that reached a length of more than 10 mm was observed in the case of foliated, decapitated shoot tips (35.3%) and foliated nodes (34.8%). The lowest number of long shoots was found in the treatment with defoliated nodes placed horizontally (4.8%). The percentage of shoots with a length of 5–10 mm ranged from 34.2% in the treatment with foliated shoot tips placed horizontally to 64.9% in the combination with foliated shoot tips with the shoot apex removed, placed vertically.

Fig. 3. Shoot clusters of *Cosmos atrosanguineus* obtained from foliated shoot tip (from left) and from foliated shoot tip after shoot apex removal (from right), placed horizontally, after 6 weeks of culture *in vitro*

Ryc. 3. Zespoły pędów *Cosmos atrosanguineus* uzyskane z ulistnionego wierzchołka pędu lewej) i z przyciętego, ulistnionego wierzchołka pędu (z prawej) ułożonego poziomo po tygodniach kultury *in vitro*

The type of explant and its orientation were shown to have a significant effect on the fresh weight of axillary shoots obtained from 1 explant. Shoots regenerated from defoliated shoot tips placed in an inverted position in relation to the natural orientation were characterized by the highest fresh weight (411.8 mg). It differed significantly from the fresh weight in the other treatments, which ranged from 66.3 mg to 179.8 mg.

The shoot tip and nodal segment are explants that are used most frequently in micropropagation. In the present study, 3.3 shoots were obtained from the foliated shoot tip and 3.5 shoots from the node of *Cosmos atrosaguineus* placed vertically with the base down on medium MS + BA (1 mg·dm^{-3}). Hosoki et al. [2003] report that the optimal multiplication rate (3.7) for *Cosmos atrosaguineus* was achieved from nodes or by dividing axillary shoots on medium containing BA (0.2 mg·dm^{-3}).

Cosmos atrosaguineus shoots exhibit strong apical dominance. Many authors report that apical dominance reduces significantly shoot branching [Bressan et al. 1982, Voyiatzi et al. 1995, Kucharska et al. 2000]. In the present experiment, the removal of the shoot apex from the non-defoliated shoot tip increased the number of axillary shoots up to 5.4. Tipped shoot explants of *Rosa* 'Dr Verhage' significantly improved shoot branching (3.4 shoots/explant) [Voyiatzi et al. 1995]. Kucharska et al. [2000] found that

Table 2. The effect of shoot tip and leaf removal and explant orientation on axillary shoot growth of *Cosmos atrosanguineus* (Hook.) Voss, after 6 weeks of *in vitro* culture

Tabela 2. Wpływ usunięcia wierzchołka wzrostu i liści oraz sposobu ułożenia eksplantatów na wzrost pędów kątowych *Cosmos atrosanguineus* (Hook.) Voss, po 6 tygodniach kultury *in vitro*

Type of explant Rodzaj eksplantatu	Explant orientation Ułożenie eksplantatu	Maximal length of shoots Maksymalna długość pędów (mm)	Average length of shoots Średnia długość pędów (mm)	% of shoots % pędów			Fresh weight of shoots/ explant (mg) Świeża masa pędów/ eksplantatu (mg)
				< 5 mm	5–10 mm	>10 mm	
Shoot tip, non-defoliated Wierzchołek pędu ulistniony	vertically, base down pionowo	16.0 bc	7.4 cd	24.6	53.9	21.5	79.0 b
Shoot tip, non-defoliated, apex removed Wierzchołek pędu ulistniony przycięty	vertically, base down pionowo	21.3 a	9.4 bc	5.4	64.9	29.7	179.8 b
Shoot tip, defoliated, Wierzchołek pędu bez liści	vertically, base down pionowo	13.2 c	9.2 bc	43.4	44.0	22.6	104.7 b
Shoot tip, defoliated,apex removed Wierzchołek pędu bez liści przycięty	vertically, base down pionowo	23.2 a	15.0 a	5.9	58.8	35.3	104.3 b
Shoot tip, non-defoliated Wierzchołek pędu ulistniony	horizontally poziomo	13.6 c	7.1 cd	42.5	34.2	23.3	95.9 b
Shoot tip, defoliated Wierzchołek pędu bez liści	horizontally poziomo	9.6 d	5.8 d	36.3	53.8	10.0	54.5 b
Shoot tip, defoliated Wierzchołek pędu bez liści	vertically, base up pionowo, odwrócony	14.3 c	7.8 cd	30.6	46.7	22.7	411.8 a
Node with leaves Węzeł ulistniony	vertically, base down pionowo	17.7 b	10.5 b	4.3	60.9	34.8	131.0 b
Node with leaves Węzeł ulistniony	horizontally poziomo	15.8 bc	8.0 bcd	27.4	52.1	20.5	66.3 b
Node defoliated Węzeł bez liści	vertically, base down pionowo	13.4 c	7.7 cd	41.3	45.3	13.3	67.6 b
Node defoliated Węzeł bez liści	horizontally poziomo	8.1 d	6.6 d	26.2	69.0	4.8	79.8 b

*Means followed by the same letter are not significantly different at $\alpha = 0.05$ – Średnie oznaczone tą samą literą nie różnią się istotnie przy $\alpha = 0,05$

shoot apex removal stimulated strongly branching in *in vitro* cultures of *Rosa manetti*. Miller and Drew [1990] found that in *Carica papaya* the removal of the apex promoted the growth of axillary shoots. Apex removal in *Hylocereus undatus* explants [Mohamed-Yasseen 2002] and seedling decapitation in *Zantedeschia aethiopica* 'Green Goddess' also resulted in an increased number of axillary shoots [Ngamu 2001]. Orlikowska et al. [2000] observed that the removal of the shoot apex in *Codiaeum variegatum* slightly improved axillary branching (from 1.5 to 2.4). Berrios and Economou [1992] had opposite observations; they report that the decapitated shoot tip of *Gardenia* explants produced fewer shoots than the shoot apex and nodes. Greater branching was observed by the removal of the shoot apex in *in vivo* cultures of the following: *Simmondsia chinensis* [Ravetta and Palzkill 1992], *Verbascum thapsus* [Naber and Aarssen 1998], *Lythrum salicaria* [Venecz and Aarssen 1998].

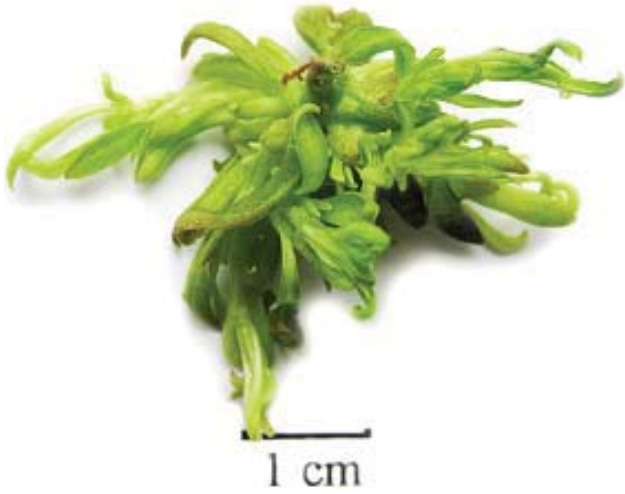

Fig. 4. Shoot cluster of *Cosmos atrosanguineus* obtained from defoliated shoot tip, placed vertically with shoot tip down, after 6 weeks of culture *in vitro*

Ryc. 4. Zespół pędów *Cosmos atrosanguineus* uzyskany z pozbawionego liści wierzchołka pędu ułożonego pionowo wierzchołkiem do dołu po 6 tygodniach kultury *in vitro*

The results of the presented study showed that leaf removal in the *Cosmos atrosaguineus* shoot tip with the apex removed caused poorer regeneration of axillary shoots. Berrios and Economou [1992] had similar observations in their study of *Gardenia*. On the other hand, Orlikowska et al. [2000] found that defoliated shoots produced more axillary shoots in the case of *Codiaeum variegatum* 'Excellent' in comparison to non-defoliated ones (respectively: 4.4 and 2.5). Kada et al. [1991] obtained 3 shoots per explant from the defoliated shoot tip of *Cistus* × *purpureus*.

The present study indicates that defoliated shoot tips of *Cosmos atrosaguineus* have to be inverted to obtain high regeneration of axillary shoots. Such a response of explants to the apolar orientation is frequently found in bulbous plants [Ziv et al. 1970, Seabrook

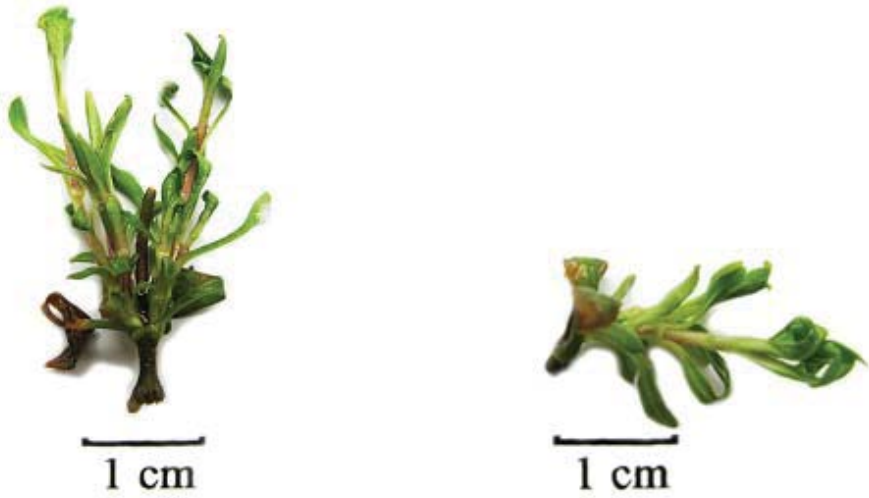

Fig. 5. Shoot clusters of *Cosmos atrosanguineus* obtained from foliated node placed vertically (from left) and from foliated node, placed horizontally (from right), after 6 weeks of culture *in vitro*

Ryc. 5. Zespoły pędów *Cosmos atrosanguineus* uzyskane z ulistnionego węzła ułożonego pionowo (z lewej) i z ulistnionego węzła ułożonego poziomo (z prawej) po 6 tygodniach kultury *in vitro*

Fig. 6. Shoot clusters of *Cosmos atrosanguineus* obtained from defoliated node placed vertically (from left) and from defoliated node, placed horizontally (from right), after 6 weeks of culture *in vitro*

Ryc. 6. Zespoły pędów *Cosmos atrosanguineus* uzyskane z pozbawionego liści węzła ułożonego pionowo (z lewej) i z pozbawionego liści węzła ułożonego poziomo (z prawej) po 6 tygodniach kultury *in vitro*

et al. 1976, Kozak 1991]. Orlikowska et al. [2000] also observed a beneficial effect of the inversion of defoliated shoots of *Codiaeum variegatum* on the number of shoots. This polarity effect of the tissues may be related to polar transport of auxins that exists in such tissues [Seabrook et al. 1976]. But Saini and Jaiwal [2002] report that epicotyl segments of *Vigna mungo* placed vertically in the medium in an inverted position did not regenerate shoots but developed callus at both ends. Epicotyl explants of *Citrus sinensis* × *Poncirus trifoliata* placed in a vertical upright position produced more shoots than those incubated in an inverted position [Garcia-Luis et al. 2006].

The horizontal orientation of non-defoliated and defoliated shoot tips and nodes of *Cosmos atrosaguineus* slightly increased the number of regenerated shoots (from 0.1 to 0.4). There are many reports about the beneficial effect of the horizontal orientation of explants on the regeneration ability of explants: *Myrtus communis* [Nobre 1994], *Codiaeum variegatum* [Orlikowska et al. 2000] *Vaccinium vitis-idaea* [Debnath 2005], *Albizia odoratissima* [Rajeswari and Paliwal 2008]. The increased axillary shoot proliferation by placing the explants in horizontal position could be attributed to greater uptake of the medium constituents due to increased contact with the medium [Mackay and Kitto 1988, Vieitez et al. 1993]. Contrary to that, no shoot tip proliferation was observed from the horizontally placed shoot tip of *Casuarina cunninghamiana* [Shen et al. 2010]. Similar trends were found in the case of the horizontal orientation of the hypocotyl and epicotyl of *Vigna subterranea*. Kone et al. [2009] observed a drastic decrease in the frequency of shoot induction and in the number of shoots / explant.

The shoot tip of *Cosmos atrosaguineus* after removal of the shoot apex and developed leaves, placed vertically with the base down, produced axillary shoots characterized by the highest elongation growth and the highest percentage of shoots that reached a length > 1 cm. The positive effect of defoliation on the number of *Codieum variegatum* shoots of more than 1 cm in length was observed by Orlikowska et al. [2000]. In the present study, a lower percentage of shoots of more than 1 cm was observed at the horizontal orientation of explants. Debnath [2005] also reported that changing the orientation of *Vaccinium vitis-idea* explants reduced elongation growth of axillary shoots.

CONCLUSIONS

1. Shoot apex and leaf removal as well as explant orientation were shown to have an influence on the growth and development of *Cosmos atrosaguineus*.

2. Apex removal from the non-defoliated shoot tip improves axillary branching.

3. Leaf removal from the shoot tip with the apex removed causes a reduction in axillary shoot production.

4. The defoliated shoot tip inserted vertically in the medium in an inverted position regenerated the highest number of axillary shoots characterized by the highest fresh weight.

5. The defoliated and decapitated shoot tip, placed vertically with the base down, produced axillary shoots characterized by the greatest elongation growth and the highest percentage of shoots that reached a length of more than 1 cm.

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WPŁYW RODZAJU I SPOSOBU UŁOŻENIA EKSPLANTATU NA WZROST I ROZWÓJ ONĘTKA KRWISTOCZERWONEGO *Cosmos atrosanguineus* (Hook.) Voss *in vitro*

Streszczenie. *Cosmos atrosanguineus* jest bulwiastą byliną wrażliwą na mróz. Posiada welwetowe, podobne do dalii, brązowo-czerwone kwiaty o zapachu czekolady. Jest rośliną sterylną (nie wytwarza nasion). Ma zastosowanie głównie jako roślina obwódkowa do ogrodów bylinowych. Podjęto badania, których celem było określenie wpływu usunięcia wierzchołka pędu i liści oraz sposobu ułożenia eksplantatów na indukcję pędów kątowych. Eksplantaty pobrano z zespołów pędów uzyskanych w kulturach sterylnych i pro-

wadzono na pożywce Murashige i Skooga uzupełnionej BA 1 mg·dm^{-3}. Wyniki badań wykazały, że usunięcie wierzchołka wzrostu z ulistnionych fragmentów pędów stymulowało ich rozkrzewianie. Usunięcie liści z przyciętych wierzchołkowych fragmentów pędów powodowało zahamowanie wytwarzania pędów kątowych. Największą liczbę pędów kątowych charakteryzujących się największą świeżą masą uzyskano z pozbawionych liści wierzchołków pędów ułożonych w pozycji odwrotnej do naturalnej. Przycięte i pozbawione liści wierzchołkowe fragmenty pędów ułożone w pozycji naturalnej wytwarzały pędy charakteryzujące się najsilniejszym wzrostem elongacyjnym i najwięcej pędów osiągało długość powyżej 1 cm.

Słowa kluczowe: rozkrzewianie, usuwanie liści, dekapitacja, ułożenie eksplantatów horyzontalne / wertykalne

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