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ROOTING OF A TRUMPET CREEPER (*Campsis radicans* (L.) Seem.) MICROSHOOTS IN PRESENCE OF AUXINS

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Abstract. A trumpet creeper (*Campsis radicans*) is an attractive vine propagated vegetatively through cuttings. So far, there is very little available data on propagation of this beautiful species in tissue culture. There was a research conducted in order to estimate the possibility to obtain rooted *Campsis radicans* plants that had been cultivated in tissue culture. The plant material were shoots obtained by multiplication on Murashige and Skoog [1962] (MS) medium which were put in fresh media supplemented with auxins: IAA (indoleacetic acid), IBA (indolebutyric acid) or NAA (naphthaleneacetic acid). The shoots were rooted *in vitro* or transplanted into soil (peat + perlite 1 : 1 w/v). It was noted that *Campsis radicans* is a very difficult plant to root in tissue culture. No rooting was obtained *in vitro*. Use of a stimulating passage with a hormone free medium or the ones containing IAA or IBA in concentrations of 2.5–5 mg·dm^{-3} and rooting shoots directly in soil allowed to obtain 100% of well rooted plants.

Key words: roots, micropropagation, tissue culture, stimulating passage

INTRODUCTION

A trumpet creeper (*Campsis radicans* (L.) Seem.) is an exceptionally attractive vine. Its main decoration are colour, shape and profusion of flowers. Elegant and very distinct combination of red colour of flowers and green leaves makes each trellis or wall, which are covered with trumpet creeper, look original. In the natural stands it is found in the United States, in the area from Pennsylvania to Florida and Texas [Dohse and Elston 2009]. It climbs up to 10–20 meters high. Leaves are opposite, 15–38 cm long, compose of 7–11 leaflets [Dirr 2009]. Flowers are monoecious, large, 6–9 cm long and 3–5 cm in diameter, orange-red, scarlet inside, clustered in corymbs with 4–15 flowers. In natural habitat it blooms from June till September [Bertin 1982, USDA 2004]. In Europe, a trumpet creeper, is regarded as an attractive ornamental vine often planted in private

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gardens. For many years it was cultivated only in botanic gardens and arboreta, but nowadays it is available from many nurseries [Polish Nurserymen Association]. It is used as a cover for fences, arbors, walls or large trellises and as a groundcover. What is interesting, this perennial vine, is considered as a weed in the midwestern and southeastern United States, where it is commonly found [Edwards and Oliver 2004, Beeler et al. 2012]. *Campsis radicans* contains flavonoids that show free radical scavenging activity [Hashem 2007]. In natural habitat it is pollinated by hummingbirds, but in Poland they are substituted by honybees [Kołodziejska-Degórska and Zych 2006]. *Campsis radicans* is known to be generally self-incompatible, although in specific conditions pollination with self pollen may occur [Bertin et al. 1989]. Usually less than 10% of flowers produce mature seeds [Bertin 1982]. In climate conditions of Poland it rarely sets seeds what limits a generative reproduction [Kołodziejska-Degórska and Zych 2006]. It may be propagated also vegetatively by stem cuttings [Ban 2011, USDA 2004] or by rootstocks [Edwards and Oliver 2004].

There is very little information on *in vitro* propagation of *Campsis radicans* [Wei et al. 2007, in Chinese with English abstract] and there are a few articles on micropropagation of plants belonging to the Bignoniaceae family.

In the previous experiments the best method to initiate a tissue culture of *Campsis radicans* was estimated and the conditions for *in vitro* propagation of shoots were also evaluated [Dąbski et al. 2014].

The further step of micropropagation is rooting of microshoots. Auxins are the most important stimulator of roots initiation in tissue culture. Many authors studied the efficiency of these growth regulators on rooting microshoots. Lu [2002] studied the influence of auxins on rooting of *Morus latifolia* 'Poilet' *in vitro*. He noted that the shoots rooted spontaneously in 57% and the addition of auxins to the media increased the rooting percentage and a number of roots. Gabryszewska and Warabieda [1992] studied rooting of *Syringa vulgaris* in tissue culture in presence of auxins IAA, IBA and NAA. All auxins used positively influenced rooting of shoots. The best rhizogenesis was observed when IBA or IAA in concentration of 8 $mg \cdot dm^{-3}$ were added to the media. NAA caused callusing of the shoots bases. Komalavalli and Rao [2000] observed that shoots of *Gymnema sylvestre* formed the most roots on the media containing IBA, while in presence of NAA or IAA shoots formed callus without roots. Microshoots of *Chaenomeles japonica* also formed more and longer roots in presence of IBA in comparison to NAA [Bach et al. 1996]. IBA was used to promote rooting of many ornamental plants. Shoots of *Sesbania sesban* were rooted on the media containing IBA in different concentrations [Jha et al. 2003/4]. The most rooted plants were observed when IBA in concentration of 3 $mg \cdot dm^{-3}$ was added to the medium. However, the IBA in concentration of 2 $mg \cdot dm^{-3}$ was chosen as more successful due to better plants quality and lesser callussing. Ault [2002] obtained the most rooted shoots of *Hymenoxys acaulis* on the MS media with addition of 0.1 mg $IBA \cdot dm^{-3}$ (90%). This auxin used in low concentrations (0.02–0.5 $mg \cdot dm^{-3}$) was also used to root *Prunus armeniaca* [Balla and Vertesy 2001], *Alnus acuminata* [Enrico et al. 2005], *Hagenia abyssinica* [Feyissa et al. 2005] and *Aristolochia indica* [Manjula et al. 1997]. IBA in higher concentrations (1–5 $mg \cdot dm^{-3}$) positively influenced rhizogenesis of *Ficus religiosa* [Desphpande et al. 1998], *Myrtus communis* [Parra and Amo Marco 1996], *Polygonum aubertii* [Dąbski

and Kozak 1998]. NAA is less commonly used to root *in vitro* plants, as it often causes callussing of the shoots bases. Such reaction was observed by Babu et al [2000]. However, Lu [2005] noted that addition of NAA to the medium increased rooting of *Vitis thunbergii*. Similarly in studies of Molinar et al. [1996] NAA in concentration of 1 mg·dm^{-3} was chosen as the best auxin for rooting *Berberis trifoliata*. Martin [2003] reported that NAA used in low concentrations had a positive effect on rooting of *Rotula aquatica*. Shoots of the woody plants, which are usually difficult to propagate in tissue culture, may form roots at high concentrations of auxins. 97% shoots of *Cercis canadensis* formed roots in presence of 10 mg IBA·dm^{-3} [Distabanjong and Geneve 1997]. IBA in concentration of 25 mg·dm^{-3} was used to root shoots of *Acacia catechu in vitro* [Wilhelm 1999] and the same auxin in concentration of 45 mg·dm^{-3} was used to promote rooting of *Photinia fraseri* microshoots [Malagon et al. 1997].

The egzogenous auxins do not always have a positive influence on rooting of microshoots *in vitro*. Rooting of *Forsythia koreana* 'Suwon Gold' was better on the medium without growth regulators than in presence of 0.5 mg IBA·dm^{-3} [Kyung-Ku Shim and Yoo-Mi Ha 1997]. Shoots of *Vaccinium myrtillus* and *Vaccinium pahalae* were put on the media containing IBA or NAA in different concentrations. There were no roots observed in any combination used, although 40% or shoots formed roots later on the medium without growth regulators [Shibli and Smith 1996].

Auxins used in different concentrations and length during *in vitro* rooting may also influence subsequent growth of plants in *ex vitro* conditions. Different types of auxins used in different concentrations influenced accllimatization and surviving of *Viburnum odoratissimum* plants *ex vitro*. Earlier use of IBA increased the number of plants that survived in soil in comparison to NAA [Schoene and Yeager 2005]. Shoots of *Quercus robur* were cultivated in the media with addition of IBA for 7 days and then put in the media without growth regulators, what had a positive influence on rooting, giving 84% of rooted plants in shorter time and with less callus [Puddephat et al. 1999]. Sansberro et al. [1999] in order to root shoots of *Ilex paraguariensis* put them on the media with 1.5 mg IBA·dm^{-3} for 10 days and then transplanted them into the media without growth regulators. The stimulative passage may be also used directly before planting shoots into soil. Palacios et al. [2002] induced rooting of *Lonicera tatarica* shoots on the media containing IBA and after 10–15 days planted them in soil. Bogetti et al. [2001] studied rooting of shoots of *Anacardium occidentale* in presence of auxins IAA, IBA and NAA. They observed differences depending on the period of auxins used (24 hours, 5 days and 30 days). The best results were obtained when IBA in concentration of 20 mg·dm^{-3} was used for 5 days.

The aim of the study was to estimate the influence of auxins on rooting of *Campsis radicans* 'Flamenco' shoots in tissue culture and their subsequent growth in soil.

MATERIAL AND METHODS

The presented study was carried out in the Laboratory of the Department of Ornamental Plants and Landscape Architecture of the University of Life Sciences in Lublin.

Plant material and culture conditions

The plant material for the presented research were shoots of *Campsis radicans* 'Flamenco' 10–15 mm in length with 2 well developed leaves, obtained by multiplication in basal medium during the cultivation conducted by the authors [Dąbski et al. 2014], which consisted of MS salts with addition of 3% (w/v) sucrose, 0.4 mg·dm^{-3} thiamine, 0.5 mg·dm^{-3} pyridoxine, 0.5 mg·dm^{-3} nicotinic acid, 2 mg·dm^{-3} glycine, 100 mg·dm^{-3} myo-inositol and gelled with 6.5 g·dm^{-3} agar. The pH of the medium was adjusted to 5.7, followed by autoclaving at 121°C under steam pressure 1.5 bar for 21 minutes. 40 ml of medium was dispensed in sterilized jars. Each treatment consisted of 5 jars with 5 shoots in each. One flask with 5 shoots was treated as a replication.

Cultures of *Campsis radicans* were placed in a growth chamber under 20 ±2°C and 16-h photoperiod provided with irradiance of 35 μmol·m^{-2}·s^{-1}.

Experimental treatments

Effect of auxins type and concentration on rooting of shoots in vitro. Shoot tips were excised from aseptically grown tissue cultures of *Campsis radicans* and placed in jars containing MS basal medium supplemented with auxins: IAA, IBA and NAA in concentrations of 0.5, 1 and 2 mg·dm^{-3}. The medium without growth regulators was the control. After 4 weeks of cultivation, the root formation and the growth of shoots were evaluated. The following features were taken into consideration during the experiment: length of the main shoot (mm), number of leaves per shoot, length of the longest leave (mm), fresh weight of the main shoot with leaves (mg), number of axillary shoots, length of axillary shoots (mm), fresh weight of axillary shoots (mg), number of roots, length of roots (mm), fresh weight of roots (mg), fresh weight of callus (mg). Some specific issues, such as colour of leaves and callus size, leaf roll, incidence of chlorosis, necrosis or malfomation were also monitored.

The subsequent influence of auxins type and concentration on shoots acclimatization in soil. Shoot tips were excised from aseptically grown tissue cultures of *Campsis radicans* and placed in jars containing MS basal medium supplemented with auxins: IAA, IBA and NAA in concentrations of 2.5, 5 and 10 mg·dm^{-3}. The medium without growth regulators was the control. The shoots were cultivated *in vitro* for 2 weeks and then planted into plastic boxes filled with the mixture of peat : perlite (1 : 1 w/v) for another 4 weeks. The same features were evaluated as in the first experiment.

Experimental design and statistical analysis

A factorial experiment in a complete randomize design was employed in both experiments. The results obtained in the experiments were analyzed statistically with the use of analyses of variance and Tukey t-test at $p = 0.05$ level of significance.

RESULTS AND DISCUSSION

Effect of auxins type and concentration on rooting of shoots in vitro. The growth and development of the main shoot of *Campsis radicans* were affected by the growth regulators added to the media (tab. 1).

Table 1. Growth and branching of *Campsis radicans* shoots depending on the type and concentration of auxins added to the media

Auxin	Concentration ($mg \cdot dm^{-3}$)	Main shoot length (mm)	Number of leaves on the main shoot	Main shoot weight (mg)	Number of axillary shoots per explant	Length of axillary shoots (mm)	Callus weight (mg)
Control	0	12.75_{a}*	5.2_{ab}	32.15_{a}	2_{a}	7.0_{a}	12.51_{d}
IAA	0.5	11.85_{ab}	4.9_{abc}	34.91_{a}	0_{b}	–	13.60_{cd}
	1.0	13.05_{a}	5.8_{ab}	30.71_{a}	2_{a}	7.5_{a}	15.51_{bcd}
	2.0	11.50_{ab}	5.5_{ab}	29.96_{a}	2_{a}	5.0_{a}	20.05_{bcd}
IBA	0.5	13.95_{a}	6.0_{a}	34.93_{a}	2_{a}	8.0_{a}	21.06_{bcd}
	1.0	11.25_{ab}	5.2_{ab}	31.69_{a}	0_{b}	–	22.90_{abc}
	2.0	11.50_{ab}	5.3_{ab}	35.34_{a}	2_{a}	6.0_{a}	26.69_{a}
NAA	0.5	8.30_{bc}	4.7_{bc}	31.87_{a}	2_{a}	5.5_{a}	21.60_{bcd}
	1.0	7.55_{c}	3.7_{c}	36.07_{a}	0_{b}	–	25.26_{ab}
	2.0	7.45_{c}	4.3_{bc}	36.66_{a}	0_{b}	–	32.15_{a}

* means followed by the same letter do not differ significantly at $\alpha = 0.05$

The highest plants were obtained on the control media (12.75 mm), although only the NAA significantly inhibited the length of the main shoot (from 7.45 at the concentration of 2 $mg \cdot dm^{-3}$ to 8.30 at the concentration of 0.5 $mg \cdot dm^{-3}$). NAA added to the media inhibited also the number of emerging leaves.

The axillary shoots were formed incidentally.

It was observed that plants did not form roots in tissue culture while the callus was noted in all combinations. The callus of the biggest weight was formed when NAA or IBA in the highest concentration were added to the media (32.15 and 26.69 mg respectively) in comparison to the results obtained on the media with the smallest concentrations of these auxins (21.60 and 21.06 mg respectively), on the media supplemented with IAA in all concentrations used and on the control media (tab. 1).

On the basis of the visual observations it was noted that plants cultivated in presence of NAA characterized with a very bad quality, with signs of hyperhydricity, no matter the concentration of the growth regulator.

The plants of the Bignoniaceae family are quite difficult to root in tissue culture. Wei et al. [2007] obtained rooting of *Campsis radicans* on the media supplemented with NAA in concentration of 0.5 $mg \cdot dm^{-3}$. In case of *Handroanthus impetiginosus* it was possible to obtain 43% of rooting on the 1/2 WPM media and 25% on the MSG media,

without auxins [Larraburu et al. 2012]. Spontaneous adventitious rooting of approximately 25% was observed on the hormone free medium in case of *Tabebuia donnell-smithii* [Gonzalez-Rodriguez et al. 2010]. However, MS medium without growth regulators failed to induce root formation in *Oroxylum indicum* [Dalal and Rai 2004], although in the research of Talari and Swamy [2013] *in vitro* rooting was observed in all concentrations of IAA, IBA and NAA and the maximum response was observed at 1–2 mg NAA·dm^{-3}. Gokhale and Bansal [2009] also studied micropropagation of *Oroxylum indicum*. They observed that IBA responded best for all parameters of rooting. *Tecomella undulata*, belonging to the Bignoniaceae family, formed roots on the MS medium supplemented with 0.3 mg·dm^{-3} IBA [Danya et al. 2012]. The results show that the growth regulators requirements for root induction are variable even in the same family.

The subsequent effect of auxins type and concentration on shoots acclimatization in soil. The auxins used in the experiment had an effect on growth and development of *Campsis radicans* shoots in soil (tab. 2).

Table 2. Growth and branching of *Campsis radicans* shoots in soil depending on the type and concentration of auxins added to the media in tissue culture

Auxin	Concentration (mg·dm^{-3})	Main shoot length (mm)	Number of leaves on the main shoot	Main shoot weight (mg)	Survival of plants (%)	Length of roots (mm)	Roots weight (mg)
Control	0	11.53_{a}*	4.2_{a}	37.7_{a}	100	11.2_{a}	7.36_{a}
IAA	2.5	11.25_{ab}	2.2_{a}	18.7_{a}	100	11.2_{a}	4.86_{a}
	5.0	10.20_{ab}	3.2_{a}	30.8_{a}	100	9.6_{ab}	3.00_{a}
	10.0	9.15_{ab}	3.2_{a}	28.3_{a}	100	8.6_{ab}	6.41_{a}
IBA	2.5	8.43_{abc}	2.6_{a}	30.1_{a}	100	8.2_{ab}	5.05_{a}
	5.0	7.00_{bcd}	2.0_{a}	29.1_{a}	100	10.5_{ab}	7.00_{a}
	10.0	7.22_{bcd}	2.0_{a}	26.8_{a}	80	6.2_{ab}	2.89_{a}
NAA	2.5	5.27_{cd}	2.0_{a}	23.2_{a}	20	2.0_{c}	4.00_{a}
	5.0	4.33_{d}	2.0_{a}	27.4_{a}	0	–	–
	10.0	3.14_{d}	2.0_{a}	35.3_{a}	0	–	–

* means followed by the same letter do not differ significantly at α = 0.05
** no result means that the plant did not form the organ

The highest plants were obtained on the control media (11.53 mm). Plants of the similar height were noted on the media supplemented with IAA in all concentrations (from 9.15 to 11.25 mm) and IBA in concentration of 2.5 mg·dm^{-3} (8.43 mm).

The smallest shoots were formed when NAA was added to the media, no matter the concentration (from 3.14 to 5.27 mm). Shoots cultivated on the control media formed the most leaves, especially in comparison to ones cultivated on the media supplemented with IBA in concentrations of 5 and 10 mg·dm^{-3} and NAA in all concentrations (2.0 in all combinations), although the difference was not significant statistically. 4% of plants

formed axillary shoots, which were observed on the plants that had been cultivated on the media supplemented with 5 mg IAA·dm^{-3} or 2.5 mg NAA·dm^{-3}.

Rooting of the shoots was influenced by the auxins added to the media in tissue culture (tab. 2). 100% of plants survived the acclimatization and grew successively in soil when the shoots had been cultivated on the control medium or on the media supplemented with IAA in all combinations of IBA in concentration of 2.5 or 5 mg·dm^{-3}. The highest concentration of IBA, 10 mg·dm^{-3}, inhibited the number of plants that well adapted to the new conditions. The poorest rooting was observed when plants had been cultivated in presence of NAA, which used in concentrations of 5 or 10 mg·dm^{-3} completely inhibited rooting of shoots.

The longest roots were observed on the control media, when no growth regulators had been added to the media (11.2 mm), and on the medium supplemented with IAA in concentration of 2.5 mg·dm^{-3} (11.2 mm). Similar results, statistically, were observed when plants had been cultivated in presence of IAA in concentrations of 5 and 10 mg·dm^{-3} (9.6 and 8.6 mm respectively) and IBA in all concentrations used (from 6.2 to 8.2 mm). Significantly shorter roots were formed when 2.5 mg NAA·dm^{-3} had been present in the medium (2.0 mm).

According to Arnold et al. [1995] auxins in high concentration or long treatments may inhibit rooting of plants *in vitro*. In order to prevent this the growth regulators may be used for shorter time prior to planting the shoots in soil. This method was used in the presented experiment. A 100% rooting and acclimatization was obtained when IBA, IAA or auxin-free medium were applied before planting shoots in soil. The correlation between the type of auxin used during rooting and plants adaptation to non-sterile conditions was observed by other authors. Wojtania and Gabryszewska [2000] studied rooting of *Coccoloba uvifera in vitro*. They observed that auxins had no influence on rooting *in vitro* but affected the subsequent growth and rooting of plants in a greenhouse. Higher percentage of rooted plants and better acclimatization was noted when plants had been growing for 4 weeks in the media with addition of IBA in concentration of 0.1 mg·dm^{-3} or without growth regulators. Palacios et al. [2002] used the stimulating passage directly before planting shoots in soil. They induced rooting of *Lonicera tatarica* shoots on the media containing IBA and after 10–15 days planted them in soil. Bogetti et al. [2001] studied rooting of shoots of *Anacardium occidentale* in presence of auxins IAA, IBA and NAA. They observed differences depending on the period of auxins used (24 hours, 5 days and 30 days). The best results were obtained when IBA in concentration of 20 mg·dm^{-3} was used for 5 days. NAA, on the other hand, was the best auxin to root *Ixora coccinea*. It caused forming callus but it had no influence on acclimatization of the plants in soil [Lakshmanan et al. 1997].

CONCLUSIONS

Campsis radicans is a difficult to root plant in tissue culture conditions. Use of a stimulating passage with hormone-free medium or media containing IAA or IBA in low concentrations (2.5–5 mg·dm^{-3}) and rooting shoots directly in soil allows to obtain 100% of established plants.

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UKORZENIANIE MILINU AMERYKAŃSKIEGO (*Campsis radicans* (L.) Seem.) W OBECNOŚCI AUKSYN

Streszczenie. Milin amerykański (*Campsis radicans*) to piękny krzew pnący, który rozmnażany jest wegetatywnie za pomocą sadzonek. Niewiele jest informacji dotyczących mikrorozmnażania tej pięknej rośliny. Przeprowadzono doświadczenia, których celem było uzyskanie ukorzenionych roślin milinu pochodzących z kultur tkankowych. Materiałem roślinnym były pędy uzyskane w drodze rozmnażania na pożywce Murashige i Skooga (MS), które wyłożono na pożywki uzupełnione auksynami: IAA, IBA i NAA. Pędy ukorzeniano *in vitro* lub sadzono w podłożu (torf + perlit 1 : 1 w/v). Zauważono, że *Campsis radicans* jest rośliną trudną do ukorzenienia w kulturach tkankowych. Nie stwierdzono żadnych korzeni *in vitro*. Zastosowanie pasażu stymulacyjnego na pożywce niezawierającej regulatorów wzrostu lub uzupełnionej IAA lub IBA w stężeniach 2,5–5 $mg \cdot dm^{-3}$ i ukorzenianie pędów bezpośrednio w podłożu pozwoliło na uzyskanie 100% ukorzenionych roślin.

Słowa kluczowe: korzenie, mikrorozmnażanie, kultury tkankowe, pasaż stymulacyjny

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