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THE EFFECT OF AUXINS: IAA, IBA AND NAA ON ROOTING OF *Hebe buchananii* (HOOK) AND *Hebe canterburiensis* (J.B.ARMSTR.) 'PROSTRATA' *IN VITRO*

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Abstract. The effect of auxins: IAA, IBA and NAA in concentrations of 1.0, 2.5 and 5.0 mg·dm^{-3} on rooting of *Hebe buchananii* and *Hebe canterburiensis* 'Prostrata' *in vitro* was examined. Shoots used in the experiment were excised from aseptically grown shoots on 1/2 MS media. Auxins used in the experiment were showed to have a positive effect on rooting of shoots of *Hebe buchananii* and *Hebe canterburiensis* 'Prostrata' *in vitro*. The biggest number of rooted microshoots and the longest roots of *Hebe buchananii* formed in presence of IBA. On the media supplemented with IAA in concentrations of 2.5 and 5.0 mg·dm^{-3}, IBA in concentration of 5 mg·dm^{-3} and NAA in concentration of 1 mg·dm^{-3} 100% of rooted shoots of *Hebe canterburiensis* 'Prostrata' were obtained. The biggest number of roots occurred on media with 2.5 and 5.0 mg IAA·dm^{-3}. NAA added to the media caused callusing of the shoots base.

Key words: *Hebe*, auxins, rooting, *in vitro*

INTRODUCTION

Genus *Hebe* includes about 140 species of evergreen shrubs which belong to the *Plantaginaceae* family. They are found in nature in the Southern Hemisphere. Most of them are endemits from New Zealand, the other ones come from Australia, South America and New Guinea [Armstrong et al. 2005; Marosz 2006]. Hebe are valuable garden plants which have a very nice, compact growth, decorative leaves and attractive flowers [Cervelli 2001]. They may become an interesting diversification of garden flower-beds and moors. Many species and varieties may be cultivated also as pot plants [Kristensten 1989].

Hebe plants are propagated mainly generatively through seeds sowed in cold hotbeds or vegetatively with use of softwood cuttings rooted in warmed substrates or layers. These methods are easy but not too effective.

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Cultivation of these plants in climate conditions of Poland is difficult because of high termal demands. Obtaining a large number of healthy and even plant material as well as having production independent of weather conditions is possible thanks to propagation *in vitro*. There is no data available on micropropagation of *Hebe*.

Rooting of plants grown in vitro is a very important moment of micropropagation. The main growth regulator used at this stage is auxin, which stimulates forming roots [Jankiewicz 1997]. The most often used auxins are: (IAA), (IBA) and (NAA).

The aim of presented work was to estimate the best auxin and it's concentration for rooting of *Hebe buchananii* and *Hebe canterburiensis* 'Prostrata' *in vitro*.

MATERIAL AND METHODS

The experimental object were 15–20 mm shoots of *Hebe buchananii* (HOOK) and *Hebe canterburiensis* (J.B. ARMSTR.) 'Prostrata' excised from aseptically grown shoot clusters. Explants were placed into 200 ml Erlenmayer flasks filled with 1/2 Murashige and Skoog medium [1962] supplemented with indole-3-acetic acid (IAA), indole-3--butyric acid (IBA) or 1-naphthaleneacetic acid (NAA) in concentrations of 1.0, 2.5 or 5 mg $\cdot dm^{-3}$. As a control the medium without growth regulators was used. Medium pH was adjusted to 5.8 with KOH or NaOH prior to the addition of 0.6% of agar and subsequently autoclaved for 20 min at 120ºC. The cultures were incubated in a culture room at a temperature of 22ºC during the day and 20ºC at night and 16-h photoperiod with irradiance of 35 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Each combination included 20 shoots (5 shoots per flask) in two series. After four weeks the experiment was finished and a plant material was analysed. During experiment data collected were percentage of rooting, number of roots per shoot, root length and weight, percentage of shoots that callused and callus weight.

The results obtained in the experiment were evaluated statistically with the use of analyses of variance and Tukey t-test at 5% level of significance.

RESULTS

Auxins used in the experiment had a positive effect on in vitro rooting of *Hebe buchananii* and *Hebe canterburiensis* 'Prostrata' shoots.

Rhizogenesis of *Hebe buchananii* was best promoted by IBA, at which 83.3% of explants formed roots in comparison to the control media (30.0%) (tab. 1).

The biggest number of roots was observed on the media supplemented with 5 mg IBA$\cdot dm^{-3}$ (6.1 per shoot). Good results were also obtained in with NAA in concentration of 1 mg$\cdot dm^{-3}$ (4.4 per shoot). The least roots formed on the media without growth regulators (1.5 per shoot). All used in the experiment auxins caused elongation of formed roots. Roots of the highest fresh weight were obtained on media supplemented with NAA in concentration of 1 mg$\cdot dm^{-3}$ (10.9 mg) and IBA in concentration of 5 mg$\cdot dm^{-3}$ (10.4 mg). Roots of the smallest fresh weight formed on the control media (0.8 mg).

Table 1. Influence of auxins on rizogenesis of *Hebe buchananii in vitro*
Tabela 1. Wpływ auksyn na ukorzenianie *Hebe buchananii in vitro*

Auxin Auksyna (A)	K	IAA			IBA			NAA		
Concentration Stężenie, mg·dm^{-3} (B)	0	1	2,5	5	1	2,5	5	1	2,5	5
Number of rooted microshoots Liczba ukorzenionych eksplantatów, %	30	70	60	65	70	95	85	70	75	75
	$NIR_A = 22.41$									
Number of roots per shoot Liczba korzeni na jednym pędzie	1.5	2.0	3.1	3.3	3.9	3.9	6.1	4.4	3.8	3.5
	$NIR_A = 1.09$; $NIR_{A\times B} = 2.45$									
Length of roots Długość korzeni, mm	6.2	14.9	16.0	15.8	15.8	18.0	22.9	15.2	14.3	12.7
	$NIR_A = 3.83$									
Fresh weight of roots Świeża masa korzeni, mg	0.8	3.4	3.5	4.2	4.3	8.3	10.4	10.9	9.0	7.2
	$NIR_A = 2.23$; $NIR_{A\times B} = 5.0$									
Number of callused shoots Liczba eksplantatów z kalusem, %	0	0	0	0	0	0	0	20	90	60
	$NIR_A = 15.9$; $NIR_B = 12.5$; $NIR_{A\times B} = 35.60$									
Fresh weight of callus Świeża masa kalusa, mg	0	0	0	0	0	0	0	5.7	29.5	21.4
	$NIR_A = 5.21$; $NIR_B = 4.09$; $NIR_{A\times B} = 11.66$									

K – Control – Kontrola

NAA added to the media caused callusing of shoots base. In presence of this auxin in concentration of 2.5 mg·dm^{-3} 90% of explants formed callus of a mean fresh weight 29.5 mg. A high fresh weight characterised also callus formed on the medium supplemented with 5 mg NAA·dm^{-3} (21.4 mg).

Rooting of *Hebe canterburiensis* 'Prostrata' explants was observed on all used in the experiment combinations. In presence of IAA in concentrations of 2.5 and 5 mg·dm^{-3}, IBA in concentration of 5 mg·dm^{-3} and NAA in concentration of 1 mg·dm^{-3} 100% of shoots formed roots (table 2).

The biggest number of roots was observed in presence of IAA in concentration of 5 mg·dm^{-3} (6.5 per shoot). Significantly less roots were obtained on media without auxin (2.9 per shoot) and on media supplemented with IAA in concentration of 1 mg·dm^{-3} (3.5 per shoot) and NAA in concentration of 2.5 mg·dm^{-3} (3.9 per shoot). Roots of the highest fresh weight formed in combinations with IBA in concetrations of 1 and 5 mg·dm^{-3} and in presence of NAA in all concentrations. Roots of the lowest fresh weight formed on the control media (6.1 mg) and on media supplemented with IAA in all concentrations (suitably 8.3, 9.5 and 10.2 mg). Auxins used in the experiment did not influence root length.

Table 2. Influence of auxins on rizogenesis of *Hebe canterburiensis* 'Prostrata' *in vitro*
Tabela 2. Wpływ auksyn na ukorzenianie *Hebe canterburiensis* 'Prostrata' *in vitro*

Auxin Auksyna (A)	K	IAA			IBA			NAA		
Concentration Stężenie, mg·dm^{-3} (B)	0	1	2,5	5	1	2,5	5	1	2,5	5
Number of rooted microshoots Liczba ukorzenionych eksplantatów, %	95	90	100	100	90	95	100	100	85	75
Number of roots per shoot Liczba korzeni na jednym pędzie	2.9	3.5	5.9	6.5	5.1	5.7	5.7	5.1	4.6	3.9
	$NIR_A = 1.01$; $NIR_{A\times B} = 2.27$									
Length of roots Długość korzeni, mm	15.8	16.6	16.5	18.0	10.0	16.7	18.9	17.0	13.7	10.8
Fresh weight of roots Świeża masa korzeni, mg	6.1	8.3	9.5	10.2	10.0	12.6	18.9	17.0	13.7	10.8
	$NIR_A = 3.74$; $NIR_{A\times B} = 8.37$									
Number of callused shoots Liczba eksplantatów z kalusem, %	0	0	0	0	0	0	0	0	45	35
	$NIR_A = 17.7$; $NIR_{A\times B} = 39.59$									
Fresh weight of callus Świeża masa kalusa, mg	0	0	0	0	0	0	0	0	15.4	10.5
	$NIR_A = 4.06$; $NIR_B = 3.19$; $NIR_{A\times B} = 9.09$									

K – Control – Kontrola

Similarly as with *Hebe buchananii* the presence of NAA in media caused callusing of shoots. In combinations with NAA in concentration of 2.5 and 5 mg·dm^{-3} suitably 45 and 35% of shoots formed callus.

DISCUSSION

Results obtained in conducted experiment confirm stimulative effect of auxins on adventitious shoots formation.

The most rooted microshoots of *Hebe buchananii* were observed on media supplemented with IBA in higher concentrations. In presence of this auxin there was the highest number of roots and they characterised with the biggest length. In case of *Hebe canterburiensis* 'Prostrata' the highest percentage of rooted shoots and the highest number of roots were obtained on media supplemented with IAA in higher concentrations.

In earlier works Dąbski [2002] examining rooting in vitro microshoots of *Columnea hirta* showed that the increase of concentration of IBA and IAA in the media caused increase of number of roots. Indole-3-butyric acid (IBA) was used in rooting of shoots of *Bixia orellana* [De Paiva Neto et al. 2003], *Digital lanata* [Cacho et al. 1991], *Picrorhiza kurroa* [Wawrosch et al. 2003], *Bacopa* [Tiwari et al. 1998] and *Columnea* [Dąbski 2002, Świstowska and Kozak 2004]. While indole-3-acetic acid (IAA) was used in rooting of microshoots of *Ocimum gratissimum* [Gopi et al. 2006] and *Nemathanthus hybridus* [Świstowska and Kozak 2005].

Roots of *Hebe buchananii* of the biggest length formed while supplementing media with IBA. Similar action was observed in case of *Hebe canterburiensis* 'Prostrata' although analyses of viariance did not confirm significance of differences. Stimulative effect of IBA on roots length was also observed by Wawrosch et al. [2003].

Adding NAA to the media caused forming callus at the base of shoots. The higher was concentration of that auxin the smaller was number, length and fresh weight of roots. Callusing shoots in the presence of NAA was also observed in tissue culture of *Columnea hirta* [Świstowska and Kozak 2004].

CONCLUSIONS

1. Presence of auxins positively influences rhizogenesis of *Hebe buchananii* and *Hebe canterburiensis* 'Prostrata' *in vitro*.

2. The most effective auxin in rooting of *Hebe buchananii* is indole-3-butyric acid.

3. Rooting of *Hebe canterburiensis* 'Prostrata' is in the highest degree stimulated by indole-3-acetic acid in concentration of 5 $mg \cdot dm^{-3}$.

4. Presence of 1-naphthaleneacetic acid in the media causes callusing of shoots.

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WPŁYW AUKSYN: IAA, IBA I NAA NA UKORZENIANIE *Hebe buchananii* (HOOK) I *Hebe canterburiensis* (J.B. ARMSTR.) 'PROSTRATA' *IN VITRO*

Streszczenie. Badano wpływ auksyn: IAA, IBA i NAA w stężeniach 1; 2,5 i 5 mg·dm^{-3} na ukorzenianie *Hebe buchananii* i *Hebe canterburiensis* 'Prostrata' *in vitro*. Pędy wykorzystane do doświadczenia uzyskano z ustabilizowanych kultur prowadzonych na zestalonej pożywce ½ MS. Stwierdzono, że zastosowane auksyny mają korzystny wpływ na ukorzenianie pędów *Hebe buchananii* i *Hebe canterburiensis* 'Prostrata' *in vitro*. Najwięcej ukorzenionych mirkosadzonek i najwięcej długich korzeni *Hebe buchananii* uzyskano w obecności IBA. Na pożywce zawierające IAA w stężeniu 2,5 i 5 mg·dm^{-3}, IBA w stężeniu 5 mg·dm^{-3} oraz NAA w stężeniu 1 mg·dm^{-3} stwierdzono 100% ukorzenionych sadzonek *Hebe canterburiensis* 'Prostrata'. Największą liczbę korzeni uzyskano na pożywkach uzupełnionych 2,5 i 5 mg IAA·dm^{-3}. Dodanie do pożywki NAA powodowało kalusowanie podstawy pędu.

Słowa kluczowe: *Hebe*, auksyny, ukorzenianie, *in vitro*

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