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THE INFLUENCE OF IBA, IAA AND NAA ON ROOTING OF *CELOSIA ARGENTEA* VAR.*CRISTATA* (L.) KUNTZE *IN VITRO* CULTURE

WPŁYW IBA, IAA I NAA NA UKORZENIANIE *CELOSIA ARGENTEA* VAR.*CRISTATA* (L.) KUNTZE W KULTURACH *IN VITRO*

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Streszczenie. Istotnym etapem rozmnażania wegetatywnego roślin jest ukorzenianie. Dobrze wykształcony system korzeniowy pozwala na łatwiejszą i szybszą adaptację roślin do warunków *ex vitro*. Celem badań było określenie wpływu egzogennych auksyn na ukorzenianie *Celosia argentea* var.*cristata* (L.) Kuntze w kulturach *in vitro* oraz adaptacja otrzymanych roślin do warunków *ex vitro*. Pożywkę ukorzeniajacą MS uzupełniono IBA, IAA i NAA w stężeniach 0.5, 1.0, 1.5 oraz 2.0 mg · dm^{-3}. Na podstawie otrzymanych wyników badań stwierdzono pozytywny wpływ zastosowanych auksyn na formowanie korzeni i ich morfologię. Najdłuższe i najgrubsze korzenie obserwowano u roślin na pożywce z dodatkiem 1.0 mg · dm^{-3} IBA. Największy procent roślin celozji zaadaptowanych do warunków *ex vitro* otrzymano po zastosowaniu IBA i NAA (1.0 mg · dm^{-3}). Wyższe stężenia auksyn (1.5 oraz 2.0 mg · dm^{-3}) miały negatywny wpływ na przeżywalność roślin, która wynosiła 12.5–62.5%.

Key words: *Amaranthaceae*, auxins, cockscomb, *in vitro*, micropropagation, rooting.
Słowa kluczowe: *Amaranthaceae*, auksyny, cockscomb, *in vitro*, mikrorozmnażanie, ukorzenianie.

INTRODUCTION

Celosia argentea var.*cristata* (L.) Kuntze is an ornamental plant which belongs to *Amaranthaceae* family. It is widely grown for ornamental purpose in the tropic and subtropics such as in Malaysia (Bodhipadma et al. 2010; Abu Bakar et al. 2014) and is often used for landscaping and roadside because of the beautiful and very attractive colors of the flowers (Taha and Wafa 2012). What is more, the plant produces some useful chemical compounds such as an antiviral protein, betalain, and anthocyanins. In Chinese medicine, dried leaves, flowers and seeds of celosia are used for hematological and gynecologic disorders (Abu Bakar et al. 2014). Because of the rich values and the high popularity of *Celosia argentea* var.*cristata* (L.) Kuntze, the *in vitro* technique has been applied in order to achieve an efficient mass propagation, rooting, and adaptation to *ex vitro* conditions. The *in vitro* technique allows to increase the efficiency of plant breeding without an environment impact. Moreover, micropropagation could enhance production of adventitious roots, which is an essentials step in vegetative propagation (Monteuuis and Bon 2000; Pop et al. 2011; Abu Bakar et al. 2014).

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The plant hormones, auxins, play the key role in roots formation (De Klerk et al. 1999). Auxins are biosynthesized from tryptophan, indole, or indole glycerol phosphate and its catabolism involving decarboxylation by peroxidases. The presence of amino acid, saccharide, or inositol can change auxin activity, causing its inactivation (Fogaça and Fett--Neto 2005). Correct selection of the type and auxin concentration should initiate the growth of root meristems, without the simultaneous growth of roots. According to many authors (De Klerk et al. 1999; Fogaça and Fett-Neto 2005; Pop et al. 2011) the growth of root meristems should be initiated by suitable selection of auxin dose, not limiting the concurrent root growth. The most commonly used auxins include indole-3-butyric acid (IBA), indole-3-acetic acid (IAA), and 1-naphthaleneacetic acid (NAA).

A well-developed root system reduces the risk of damaging the delicate root structure and their easier adaptation for *ex vitro* conditions. Thus, the objective of the study was selection of the optimal auxin and its concentration for *Celosia argentea* var. *cristata* (L.) Kuntze *in vitro* rooting and *ex vitro* adaptation of the obtained microcuttings.

MATERIAL AND METHODS

The research material consisted of 15–20 mm shoots of *Celosia argentea* var.*cristata* (L.) Kuntze obtained from sterile stabilized *in vitro* culture. Explants were placed on the MS medium (Murashige and Skoog 1962) supplemented with IBA, IAA, and NAA at the subsequent concentrations 0.5, 1.0, 1.5, and 2.0 mg · dm^{-3}. MS without auxin was used as the control. Each combination included 96 shoots (6 shoots per flask) in sixteen series. After four weeks of *in vitro* culture, root and shoot length, number of newly formed shoots and fresh weight (FW g) were determined.

MS media was solidified with 8g · dm^{-3} agar (Biocorp, Poland) and supplemented with 30 g · dm^{-3} sucrose and 100 mg · dm^{-3} myo-inositol. The pH of the media was adjusted to 5.7 prior autoclaving at 121ºC (0.1 MPa) for the time required according to the volume of medium in the vessel. Cultures were incubated in growth room at 25ºC under 16-h photoperiod with a photosynthetic photon flux density (PPFD) of 40 µmol · m^{-2} · s^{-1}.

For acclimatization, the plantlets with well-developed roots were transferred (according to the concentrations of auxins) to the pots filled with soil and vermiculate in 1 : 1 ratio. After 2 weeks of adaptation the percentage of survived was estimated.

The results obtained in *in vitro* cultures were statistically analyzed (Statistica). The experiments were conducted in a completely randomized design. The significance of differences was determined by means of variance analysis and Tukey’s test, at the level of significance of $\alpha = 0.05$. Homogenous groups between analysed combinations were labeled with successive letters of alphabet. In turn, after the adaptation period, the percentage of survived plants was evaluated.

RESULTS AND DISCUSSION

The key step of the vegetative plant reproduction is a well-developed root system. Compact root system allows for easier and faster adaptation of plants to *ex vitro* conditions, without the risk of damaging the delicate root structure (Memon 2012). The obtained results indicate the positive effect of the exogenous auxins (IBA, IAA and NAA) on the adventitious roots formation in *Celosia argentea* var.*cristata* (L.) Kuntze under *in vitro* conditions. The best effect was observed for IBA (1.0 mg · dm^{-3}), which induced root length in range 4.87 cm (Table 1). Microcutting exposed to IAA had intermediate performance displaying less root formation than IBA or NAA treated (Table 1). In our study, it was also observed that the addition of IBA to MS medium had a stimulating effect on the length of shoots, compared to the remaining auxins used in the experiment (Table 2). However, the mean value for shoot length decrease as the IBA concentration increased. As a result, it can be concluded that exogenous auxin applications enhance apical dominance of plant. Explants with the longest shoots were obtained in the case of control plants (4.16 cm). On the contrary, types and concentrations of auxins did not have a significant influence on the number of new shoots on a single plant and fresh weight, which was at the level of the control (3.06 and 1.98 g, respectively) – Table 3, 4.

Similar to the current results IBA was found to be the best among the auxin tested in induction of roots from shoots explants of *Celosia argentea* (Abu Bakar et al. 2014) and *Eucalyptus saligna* and *E. globulus* (Fogaça and Fett-Neto 2005). According to, Nissen and Sutter (1990) the presence of IBA in the medium allows obtaining higher rooting efficiency of microshoots due to relatively higher stability of this auxin. However, IAA is five times more readily photo-oxidized than IBA and is more susceptible to enzymatic degradation.

The role of auxins (IBA, IAA, NAA) in the initiation and regeneration of roots *in vitro* has been described for many plant species. Sevik and Guney (2013) studied the effectiveness of IAA, IBA, NAA and GA_3 in *Melissa officinalis* L.; Hussain and Khan (2004) studied the effectiveness of IAA and IBA in *Rosa bourboniana* and *R. grussan-tepliz*; Hausman (1993) analyzed the influence of auxin level and ethylene production during root formation by poplar shoots. Generally, these studies showed that auxins play a central role in the determination of rooting capacity.

According to many authors (Kozai 1991; Jain and Babbar 2003; Memon 2012; Yaseen et al. 2013) *in vitro* grown plants have low photosynthetic activity because of low irradiance and limited gas exchange. This abnormal physiology of *in vitro* plantlets may lead to slow growth and low survival rate after acclimatization into *ex vitro* condition (Mosaleeyanon et al. 2004; Xiao and Kozai 2006; Memon 2012). Improvement of *in vitro* rooting techniques will facilitate the production of greater number of roots and helps in better survival of plantlets upon transferring them to *ex vitro* conditions. In our study, after two weeks, the plant regeneration and root-induced plantlets were hardened in a heated greenhouse. The percentage of plants that survived the adaptation period is presented in Fig. 1. The percentage of plants adapted to the conditions in uncovered soil was 25% at the end of vegetation season in the control site. Well-rooted *Celosia* plantlets showed higher percentage (75%) of survival upon *ex vitro* transplantation during acclimatization. In case of higher concentrations, i.e., 1.5 and 2.0 mg · dm^{-3}, plants survivability was lower, as much as 12.5–62.5% (Fig. 1, 2).

Table 1. Effect of auxins and their concentrations on roots length [cm] of *Celosia argentea* var.*cristata* (L.) Kuntze
Tabela 1. Wpływ rodzaju i stężenia auksyn na długość korzeni [cm] *Celosia argentea* var.*cristata* (L.) Kuntze

Auxin – Auksyna (I)	Concentration – Stężenie [mg · dm^{-3}] (II)										Mean Średnia	
	0		0.5		1.0		1.5		2.0			
IBA	0.28 ± 0.06	b	4.13 ± 0.54	a	6.04 ± 0.48	a	3.35 ± 0.42	a	5.18 ± 0.56	a	4.87	a
IAA	0.28 ± 0.06	c	5.49 ± 0.70	a	3.46 ± 0.51	ab	3.63 ± 0.41	ab	2.68 ± 0.31	bc	4.18	a
NAA	0.28 ± 0.06	b	3.80 ± 0.65	a	4.83 ± 0.57	a	5.10 ± 0.52	a	3.37 ± 0.31	a	4.55	a
Mean – Śednia	0.28	b	4.47	a	4.78	a	4.02	a	3.74	a		

$LSD_{0.05}$ – $NIR_{0.05}$
Auxin – Auksyna: LSD_{I} – NIR_{I} = 2.23
Concentration – Stężenie: LSD_{II} – NIR_{II} = 2.01
Auxin x concentration
Auksyna x stężenie: $LSD_{I/II}$ – $NIR_{I/II}$ = 2.73

Mean followed by the same letter are not significantly different ($p < 0.05$; least significant differences test – LSD) – Średnie w kolumnach oznaczone tymi samymi literami nie różnią się istotnie ($p < 0.05$; najmniejsza istotna różnica – NIR).

Table 2. Effect of auxins and their concentrations on shoot length [cm] of *Celosia argentea* var.*cristata* (L.) Kuntze
Tabela 2. Wpływ rodzaju i stężenia auksyn na długość pędów [cm] *Celosia argentea* var.*cristata* (L.) Kuntze

Auxin – Auksyna (I)	Concentration – Stężenie [mg · dm^{-3}] (II)										Mean Średnia	
	0		0.5		1.0		1.5		2.0			
IBA	4.16 ± 1.52	a	4.19 ± 1.94	a	4.34 ± 1.58	a	3.18 ± 1.24	b	3.89 ± 1.96	ab	3.95	a
IAA	4.16 ± 1.52	a	2.91 ± 1.10	b	2.76 ± 1.10	b	3.10 ± 1.28	b	2.88 ± 1.39	b	3.16	b
NAA	4.16 ± 1.52	a	3.60 ± 1.74	ab	3.29 ± 2.06	ab	2.78 ± 1.42	bc	2.24 ± 1.03	c	3.21	b
Mean – Średnia	4.16	a	3.56	ab	3.47	b	3.01	b	3.00	b		

$LSD_{0.05}$ – $NIR_{0.05}$
Auxin – Auksyna: LSD_{I} – NIR_{I} = 0.73
Concentration – Stężenie: LSD_{II} – NIR_{II} = 0.66
Auxin x concentration
Auksyna x stężenie: $LSD_{I/II}$ – $NIR_{I/II}$ = 0.90

Mean followed by the same letter are not significantly different ($p < 0.05$; least significant differences test – LSD) – Średnie w kolumnach oznaczone tymi samymi literami nie różnią się istotnie ($p < 0.05$; najmniejsza istotna różnica – NIR).

Table 3. Effect of auxins and their concentrations on number of new shoots of *Celosia argentea* var.*cristata* (L.) Kuntze
Tabela 3. Wpływ rodzaju i stężenia auksyn na liczbę nowych pędów *Celosia argentea* var.*cristata* (L.) Kuntze

Auxin – Auksyna (I)	Concentration – Stężenie [mg · dm^{-3}] (II)										Mean Średnia	
	0		0.5		1.0		1.5		2.0			
IBA	3.06 ± 1.23	b	4.06 ± 0.92	a	3.06 ± 0.84	b	3.25 ± 1.28	b	2.93 ± 1.47	b	3.27	a
IAA	3.06 ± 1.23	a	3.37 ± 1.5	a	3.37 ± 1.36	a	3.62 ± 1.73	a	3.37 ± 1.25	a	3.36	a
NAA	3.06 ± 1.23	a	2.62 ± 1.36	a	2.94 ± 1.17	a	2.75 ± 1.06	a	3.0 ± 1.25	a	2.87	a
Mean – Średnia	3.06	a	3.35	a	3.12	a	3.20	a	3.10	a		

$LSD_{0.05}$ – $NIR_{0.05}$
Auxin – Auksyna
Concentration – Stężenie
Auxin x concentration
Auksyna x stężenie

LSD_I – NIR_I = 0.64
LSD_{II} – NIR_{II} = 0.58
$LSD_{I/II}$ – $NIR_{I/II}$ = 0.78

Mean followed by the same letter are not significantly different ($p < 0.05$; least significant differences test – LSD) – Średnie w kolumnach oznaczone tymi samymi literami nie różnią się istotnie ($p < 0.05$; najmniejsza istotna różnica – NIR).

Table 4. Effect of auxins and their concentrations on fresh weight [g] of *Celosia argentea* var.*cristata* (L.) Kuntze
Tabela 4. Wpływ rodzaju i stężenia auksyn na świeżą masę [g] *Celosia argentea* var.*cristata* (L.) Kuntze

Auxin – Auksyna (I)	Concentration – Stężenie [mg · dm^{-3}] (II)										Mean Średnia	
	0		0.5		1.0		1.5		2.0			
IBA	1.98±1.34	a	1.97±0.94	a	2.29±1.8	a	1.91±0.24	a	3.11±0.33	a	2.25	a
IAA	1.98±1.34	a	1.51±0.13	a	1.65±0.16	a	1.96±0.12	a	2.00±0.16	a	1.82	a
NAA	1.98±1.34	b	2.73±0.28	b	2.58±0.21	b	4.41±0.46	a	1.75±0.15	b	2.69	a
Mean – Średnia	1.98	a	2.07	a	2.17	a	2.76	a	2.29	a		

$LSD_{0.05}$ – $NIR_{0.05}$
Auxin – Auksyna
Concentration – Stężenie
Auxin x concentration
Auksyna x stężenie

LSD_I – NIR_I = 1.08
LSD_{II} – NIR_{II} = 0.98
$LSD_{I/II}$ – $NIR_{I/II}$ = 1.32

Mean followed by the same letter are not significantly different ($p < 0.05$; least significant differences test – LSD) – Średnie w kolumnach oznaczone tymi samymi literami nie różnią się istotnie ($p < 0.05$; najmniejsza istotna różnica – NIR).

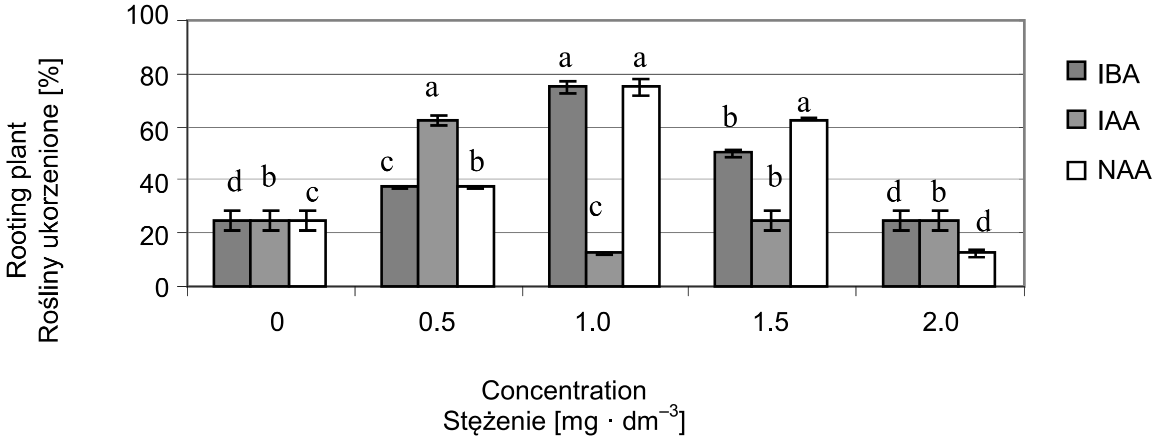

Fig. 1. Percentage of *Celosia argentea* var.*cristata* (L.) Kuntze *a* plants rooting *in vivo*
Ryc. 1. Procent roślin *Celosia argentea* var.*cristata* (L.) Kuntze ukorzenionych *in vivo*

Fig. 2. Influence of auxins and their concentration on rooting of *Celosia argentea* var.*cristata* (L.) Kuntze
Ryc. 2. Wpływ auksyn i ich stężenia na ukorzenianie pędów *Celosia argentea* var.*cristata* (L.) Kuntze

CONCLUSION

The results presented in this study demonstrate the usefulness of different kind of auxins for efficient *in vitro* rooting of *Celosia argentea* var.*cristata* (L.) Kuntze. Rooting of celosia microshoots depend on the type of auxin and its concentration in the medium. In the present study, the most efficient at inducing celosia roots was 1.0 mg · dm^{-3} IBA. IBA, IAA and NAA auxins applied for *Celosia* rooting *ex vitro* at concentrations of 1.5 and 2.0 mg · dm^{-3} affected a decrease in the percentage of plants that survived.

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Abstract. The formation of adventitious roots is an essential step in vegetative propagation. Well-developed root system allows for easier and faster adaptation of plants to *ex vitro* conditions. The aim of this study was to determine the effect of exogenous auxin on *Celosia* *argentea* var.*cristata* (L.) Kuntze rooting under *in vitro* conditions and on *ex vitro* adaptation of those plants. MS rooting media were supplemented with IBA, IAA and NAA at subsequent concentrations 0.5, 1.0, 1.5, and 2.0 mg · dm^{-3}. The results showed, that subsequent hormones had a positive effect on root development and morphology. The longest and thickest roots were formed in the presence of IBA at the concentration of 1.0 mg · dm^{-3}. The highest percent of *ex vitro* survived plants was obtained after application of IBA and NAA (1.0 mg · dm^{-3}). Plants survival *ex vitro* decreased (12.5–62.5%), when culture treated with IBA and NAA (1.5 and 2.0 mg · dm^{-3}).