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In vitro rooting of *Crocosmia* × *crocosmiiflora* 'Lucifer'

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ABSTRACT

The aim of this study was to determine the effect of various auxins and their concentrations on *Crocosmia* × *crocosmiiflora* 'Lucifer' rooting in *in vitro* cultures and *in vivo* conditions. The initial research materials were shoots obtained from a stabilized *in vitro* culture. MS media were supplemented with the following auxins: IAA, IBA and NAA at concentrations of 0.5, 1.0, 2.0 and 5.0 mg dm^{-3}. The results of the study showed that hormone applications had a positive effect on the root development of *Crocosmia* microshoots. 100% rooted shoots were observed in each of the applied combinations of the experiment. The highest number of roots and the longest roots were formed in the presence of IBA at a concentration of 1.0 mg dm^{-3}. IAA, IBA and NAA affected plant height and root morphology of *Crocosmia* differently. The addition of higher concentrations of auxins to the medium (2.0 and 5.0 mg dm^{-3}) resulted in the formation of short roots. The applied IBA concentrations determined the percentage of the plants that were adapted to *in vivo* conditions, as well as their vegetative features and the yield of descendant tubers. At the end of the vegetation period, higher IBA concentrations, i.e., 1.0, 2.0 and 5.0 mg dm^{-3}, caused a decrease of up to 50-75% in the obtained plants and also inhibited plant height, as well as the weight and diameter of the tubers formed, compared to the control.

Key words: IAA, IBA, Iridaceae, micropropagation, morphological features, NAA

INTRODUCTION

The Iridaceae family includes over 2025 species with a wide range of uses, i.e. members of *Iris* yield orrisroot (a substance used in the manufacturing of perfumes, soaps or powders). The feathery stigmas of *Crocus sativa* yield saffron, which is used as flavouring and food colouring and as a medicinal ingredient. In natural conditions, these species reproduce vegetatively through tubers or rhizomes, their characteristic feature being sword-like leaves. Due to their high commercial potential, this group of plants is used for further horticultural development. Many plant breeders throughout the world have conducted research on the *Watsonia* species, *Iris*, *Crocosmia* × *crocosmiiflora*, or *Crocosmia aurea* (Niederwieser et al. 2002, Boltenkov and Zarembo 2005, Ascough et al. 2007, Koh et al. 2007, Hannweg et al. 2013, Krupa-Małkiewicz et al. 2013). *Crocosmia* × *crocosmiiflora*, whose natural habitat is in South Africa, are valuable ornamental plants that produce very decorative and attractive flowers and leaves. These plants are commonly cultivated in gardens on flowerbeds or as cut flowers. *Crocosmia* is mainly reproduced by tubers, and more rarely generatively due to the poor ability of seed setting. Thus, to improve the reproduction efficacy of these plants, and thus also their commercialization, *in vitro* cultures are used.

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This is a quick and relatively cheap method which allows for a large number of pathogen-free plants over a short time period, with even growth and without the influence of external environmental conditions. Out of 12 genera from this family, only 40 species are reproduced using the tissue culture method (Ascough et al. 2009).

Plant rooting is one of the most important stages of micropropagation. According to Ascough et al. (2011), Memon (2012) and Hannweg et al. (2013), a medium supplemented with plant growth regulators usually speeds up the rooting process and increases the rooting percentage. The main plant growth regulator used at this stage is auxin, which stimulates the proliferation of adventitious root meristems on the shoots. The growth of root meristems should be initiated by a suitable selection of auxin dose that does not limit concurrent root growth (Koetle et al. 2010, Ascough et al. 2011, Pop et al. 2011). The most often applied auxins include indole-3-acetic acid (IAA), indole-3-butyric acid (IBA) and 1-naphthaleneacetic acid (NAA). A well-developed root system allows easy and quick rooting of microshoots cultured in *in vivo* conditions. Moreover, it decreases the risk of root damage and consequently plant decay (Memon 2012).

The aim of this study was to select the most optimum auxin and its concentration for *Crocosmia* × *crocosmiiflora* 'Lucifer' rooting in *in vitro* cultures and its adaptation to *in vivo* conditions.

MATERIAL AND METHODS

The research material consisted of 15-20 mm shoots of *Crocosmia* × *crocosmiiflora* 'Lucifer' obtained from a sterile stabilized *in vitro* culture. The explants were placed on a medium according to the composition of MS macro- and microelements by Murashige and Skoog (1962). IAA, IBA, and NAA auxins at concentrations of 0.5, 1.0, 2.0, and 5.0 mg dm^{-3} were added to each medium. In the experiment, the MS medium without the addition of plant growth regulators was used as the control. Each combination included 48 shoots (six shoots per flask) in eight series. After six weeks in culture, the number of roots, the root length, weight of roots, plant height and the number of leaves per treatment were recorded.

All cultures were incubated in a growth room at a temperature of 25°C under a 16-h photoperiod with a photosynthetic photon flux density (PPFD) of 40 µmol m^{-2} s^{-1}. All of the media contained 8 g dm^{-3} agar (Biocorp, Poland), 30 g dm^{-3} sucrose and 100 mg dm^{-3} myo-inositol, and the pH was adjusted to 5.7. The media were heated and 30 ml was poured into a 450 ml flask and next they were autoclaved at 121°C (0.1 MPa) during the time required according to the volume of medium in the vessel.

The plants obtained in *in vitro* cultures, in the sites where IBA auxin was applied, were planted separately (according to the concentration of IBA auxin) in pots filled with peat substrates and were left in a mist house for two weeks. Next, they were moved to a heated greenhouse and placed on cultivation tables. In the first 10 days of May, the plants were transplanted from pots into uncovered soil at a spacing of 10 × 15 cm. A dose of 10 g m^{-2} of Azofoska fertilizer was applied as top dressing once during the vegetation season. At the end of the vegetation season, the percentage of plants that survived the adaptation period was established, plant height was measured, and leaf greenness index was determined using a SPAD-502 chlorophyll meter, and newly formed leaves were also counted. After digging up the plants, the tubers were additionally dried in the absence of light and at a temperature of 22-24ºC, and then they were cleaned from encrusted leaves and roots. After cleaning, the weight, diameter and the number of adventitious cormlets of the descendent tubers were established.

The results obtained in *in vitro* cultures were statistically analysed. The significance of differences was determined by means of variance analysis and the Tukey test, at the level of significance of $p < 0.05$. In turn, the results concerning vegetative features and yield after the adaptation period were evaluated based on the mean values due to the high percentage of decayed plants.

RESULTS

The results obtained from this experiment showed a stimulating effect of the applied IBA auxin on the number of roots of *Crocosmia* × *crocosmiiflora* 'Lucifer' rooting in *in vitro* cultures (Fig. 1). The highest number of roots were formed by *Crocosmia* shoots grown on the MS medium supplemented with IBA in comparison to NAA and IAA (48.2% and 59.5%, respectively). From all of the concentrations of auxins applied in the experiment, 1.0 mg dm^{-3} was found to be the most efficient in the stimulation of adventitious roots (Tab. 1). However, a different interaction was observed between the type of auxin used and its concentration. The highest number of roots were formed by *Crocosmia* shoots grown on the MS medium supplemented with 1.0 mg dm^{-3} of

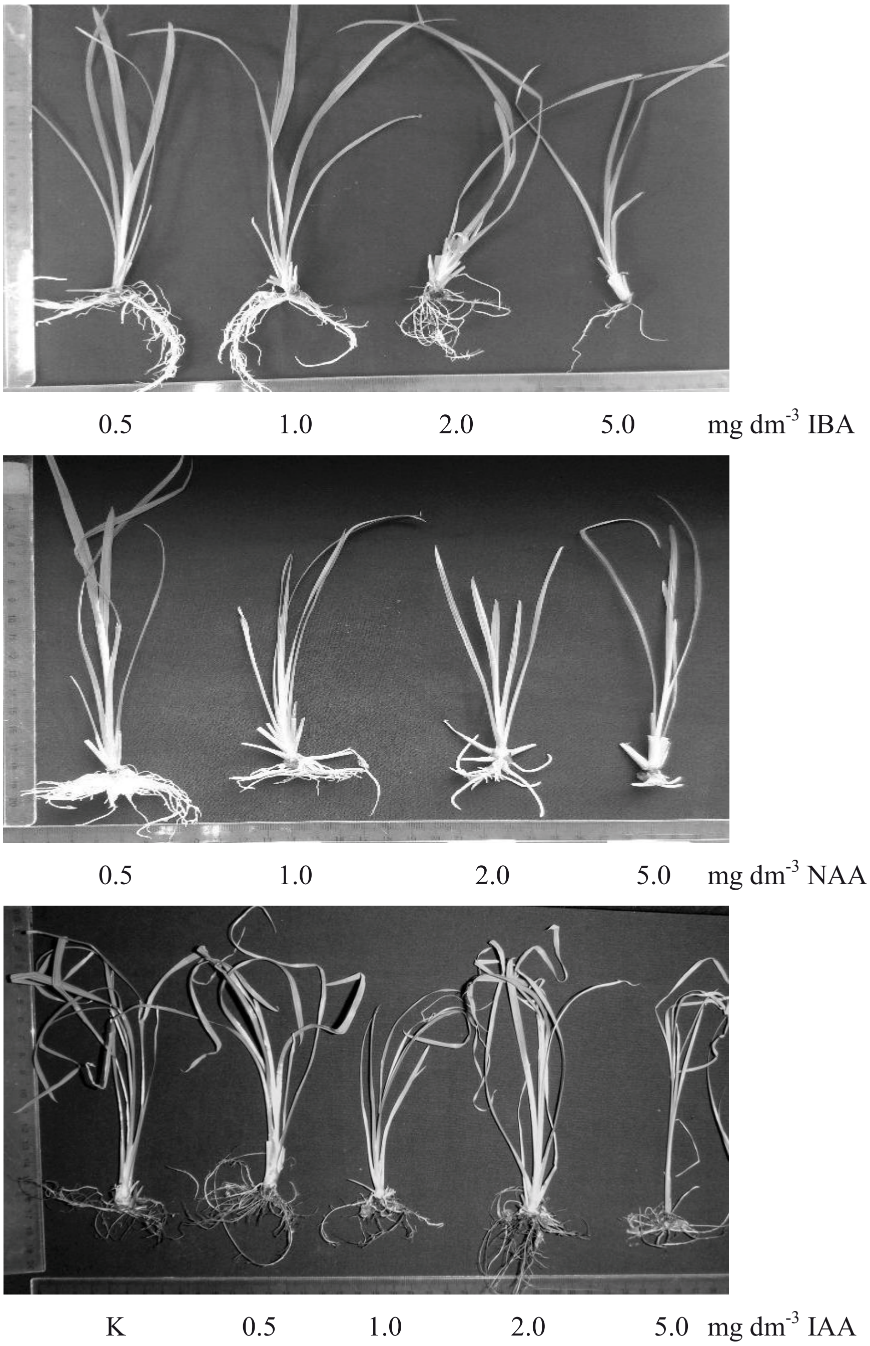

Figure 1. Influence of IBA, NAA and IAA auxins and their concentrations (0.5, 1.0, 2.0, 5.0 mg dm^{-3}) on the rooting of *Crocosmia* × *crocosmiiflora* 'Lucifer' in *in vitro* culture

auxin, the lowest in the control and the MS medium supplemented with 5.0 mg dm^{-3}. *Crocosmia* shoots grown on the MS medium with the addition of 0.5 mg dm^{-3} NAA developed the highest number of roots in comparison with 1.0, 2.0 and 5.0 mg dm^{-3}. In turn, there was no significant effect of IAA concentration on the number of *Crocosmia* roots in this experiment (Tab. 1).

The type of auxin also significantly affected *Crocosmia* root length (Fig. 1, Tab. 1). Based on the obtained results, we concluded that in most of the cases, the rooting of shoots on MS with the addition of IAA and IBA formed longer roots compared to NAA (11.8% and 71.1%, respectively). Based on the obtained results, we concluded that shoots rooted on MS with the addition of auxins, irrespective of their concentrations, formed shorter roots compared to the control. However, the shortest roots were observed in shoots grown on MS supplemented with 5.0 mg dm^{-3} of auxins used in the experiment (up to 67.1% in comparison to control) (Tab. 1). A significant interaction was also observed between

Table 1. The effect of auxins on the number of roots, root length (cm) and weight of roots (g) of *Crocosmia* × *crocosmiiflora* 'Lucifer'

Auxin (A)	Concentration (C) (mg dm^{-3})					Mean
	0	0.5	1.0	2.0	5.0	
			number of roots			
IBA	7.94	9.13	16.19	13.06	6.25	10.51
NAA	7.94	10.69	6.38	5.75	4.69	7.09
IAA	7.94	5.94	6.25	4.75	8.06	6.59
Mean	7.94	8.58	9.60	7.85	6.33	
$LSD_{0.05}$						
Auxin (A)			LSD_A = 1.66			
Concentration (C)			LSD_C = 2.29			
A(C)			$LSD_{A(C)}$= 3.96			
			root length (cm)			
IBA	11.78	9.92	11.06	9.29	7.39	9.89
NAA	11.78	6.44	6.25	2.78	1.66	5.78
IAA	11.78	9.81	10.28	11.31	12.09	11.06
Mean	11.78	8.72	9.20	7.80	7.05	
$LSD_{0.05}$						
Auxin (A)			LSD_A = 1.39			
Concentration (C)			LSD_C = 1.91			
A(C)			$LSD_{A(C)}$ = 3.31			
			weight of roots (g)			
IBA	0.48	0.51	1.19	1.40	1.03	0.92
NAA	0.48	1.00	1.08	0.65	0.45	0.68
IAA	0.48	0.74	0.65	0.61	0.50	0.60
Mean	0.48	0.75	0.89	0.89	0.66	
$LSD_{0.05}$						
Auxin (A)			LSD_A = 0.19			
Concentration (C)			LSD_C = 0.26			
A(C)			$LSD_{A(C)}$ = 0.46			

Least Significant Differences test LSD at $p < 0.05$

the type of auxins used in the experiment and their concentration. Shoots grown on the control medium and MS supplemented with 1.0 mg dm^{-3} IBA developed longer roots than *Crocosmia* grown on MS with 5.0 mg dm^{-3} IBA. However, an increase in NAA concentration resulted in the inhibition of root elongation compared to the control. In addition, there was no significant effect of IAA on the root length irrespective of its concentration.

The addition of IBA to the MS medium stimulated the gain of fresh weight of *Crocosmia* roots (Tab. 1). A slightly lower fresh weight of roots formed on the shoots was observed in the presence of NAA and IAA (35.3% and 53.3%, respectively). Irrespective of the type and concentration of auxins used in this experiment, we observed that the weight of *Crocosmia* roots was higher than in the control. However, an uneven interaction between the auxin concentration and type was observed from the analysis of variance. The increase in IBA concentration (from 1.0 to 5.0 mg dm^{-3}) in the MS medium resulted in a stimulation of the fresh weight of roots in comparison to the control and shoots grown on MS with 0.5 mg dm^{-3} IBA. Different interactions were observed in explants grown on MS with the addition of NAA. The highest gain of fresh weight compared to the control was observed when NAA was used in the concentration of 0.5 and 1.0 mg dm^{-3} and in explants grown on MS with 5.0 mg dm^{-3} NAA. The addition of IAA into the medium, irrespective of their concentration, had no significant effect on the fresh weight of *Crocosmia* roots (Tab. 1).

The types of auxins used in the experiment also significantly affected the aboveground plant features (Tab. 2). On comparing the effect of the type of auxins on the discussed feature, it was observed that the highest plants (29.37 cm) were obtained when using IBA. A slower growth of the *Crocosmia* explants was observed when IAA

Table 2. The effect of auxins on plant height (cm) and the number of leaves of *Crocosmia* × *crocosmiiflora* 'Lucifer'

Auxin (A)	Concentration (C) (mg dm^{-3})					Mean
	0	0.5	1.0	2.0	5.0	
			plant height (cm)			
IBA	21.48	19.28	19.95	29.37	21.87	22.39
NAA	21.48	16.41	13.06	10.88	11.38	14.64
IAA	21.48	16.69	17.34	22.19	23.72	20.28
Mean	21.48	17.46	16.79	20.81	18.99	
$LSD_{0.05}$						
Auxin (A)			$LSD_A = 1.98$			
Concentration (C)			$LSD_C = 2.72$			
A(C)			$LSD_{A(C)} = 4.72$			
			number of leaves			
IBA	6.44	8.81	10.06	9.00	4.38	7.74
NAA	6.44	6.19	5.50	4.38	3.94	5.29
IAA	6.44	5.31	6.63	4.81	5.25	5.49
Mean	6.44	6.77	7.06	6.06	4.52	
$LSD_{0.05}$						
Auxin (A)			$LSD_A = 0.70$			
Concentration (C)			$LSD_C = 0.95$			
A(C)			$LSD_{A(C)} = 1.65$			

Least Significant Differences test LSD at $p < 0.05$

was used. However, the addition of NAA to the MS medium unfavourably affected the examined morphological feature. A significant interaction was also observed between the concentrations of auxins used in the experiment and plant height. On average, the highest were *Crocosmia* shoots grown on the MS medium with 2.0 mg dm^{-3} of auxins used in the experiment compared to the control with 0.5 and 1.0 mg dm^{-3} concentrations. *Crocosmia* grown on the MS medium supplemented with 2.0 mg dm^{-3} IBA concentration was higher than other concentrations used. In turn, *Crocosmia* grown on the MS medium supplemented with NAA was lower compared to the control. Explants from the MS medium supplemented with IAA concentrations of 2.0 and 5.0 mg dm^{-3} formed longer roots compared to the control and explants from the MS supplemented with 0.5 mg dm^{-3} IAA.

The number of leaves formed was significantly affected by the addition of various types and concentrations of auxins (Tab. 2). However, a higher number of leaves per one plant on average (7.74) was observed after the addition of IBA to the MS medium. In turn, the mean number of *Crocosmia* leaves on MS with the addition of NAA and IAA was smaller by as much as 46.3-72.4%, respectively. The plants from the control medium and MS with 0.5 and 1.0 mg dm^{-3} concentrations of auxins used in the experiment developed the highest number of leaves in comparison to the 5.0 mg dm^{-3} concentration, irrespective of the type of auxins. Analysing the effect of IBA on the examined morphological feature, we concluded that the number of leaves was highest when 0.5, 1.0 and 2.0 mg dm^{-3} concentrations were used and the smallest on the medium with the addition of 5.0 mg dm^{-3} IBA. However, a higher number of leaves per one plant were observed when a 0.5 mg dm^{-3} concentration of IAA was applied. Although, the increase in IAA concentration decreased the number of *Crocosmia* leaves. There was no significant effect of IAA concentration on the number of *Crocosma* leaves.

The percentage of plants adapted to the conditions in uncovered soil was 87.5% at the end of the vegetation season at the control site and at the site where the lowest IBA concentration (0.5 mg dm^{-3}) was applied. In the case of higher concentrations, i.e. 1.0, 2.0, and 5.0 mg dm^{-3}, plant survivability was lower by as much as 50.0-75.0% (Tab. 3). Irrespective of the IBA concentration applied, the cultivated plants were characterized by a lower height compared to the control *Crocosmia*. However, the highest differences were noted in the sites where concentrations of 1.0, 2.0, and 5.0 mg dm^{-3} were applied. The auxin applied in the experiment did not affect the number of leaves and leaf greenness index (Tab. 4).

Table 3. The percentage of *Crocosmia* plants of the 'Lucifer' cultivar adapted to open air growing conditions at the end of the growing season

Parameter	IBA (mg dm^{-3})				
	0.0	0.5	1.0	2.0	5.0
Plants (%)	87.5	87.5	12.5	37.5	12.5

Table 4. Vegetative traits of *Crocosmia* plants of the 'Lucifer' cultivar depending on the concentration of the rooting agent (IBA) at the end of the growing season

Parameter	IBA (mg dm^{-3})				
	0.0	0.5	1.0	2.0	5.0
Plant height (cm)	32.1	29.9	25.5	20.2	19.2
Number of leaves (pcs.)	5.5	6.1	5.0	5.0	5.5
Leaf greenness index (SPAD)	47.3	48.7	48.2	47.9	46.8
Diameter of daughter corms (mm)	15.1	15.5	12.8	12.0	9.9
Weight of daughter corms (g)	2.49	2.74	1.78	1.08	0.88
Number of adventitious corms (pcs.)	1.0	1.2	0.0	0.0	0.0

Compared to the control, 0.5 mg dm^{-3} IBA application to plants rooting in *in vitro* cultures affected an insignificant increase of 2.6% in corm diameters on average, and a weight of 10.0% on average. However, further increase in concentration from 1.0 to 5.0 mg dm^{-3} resulted in a considerable decrease in the weight and diameter of the obtained corms at a level from 15.2% to 34.4%, and from 28.5 to 64.7%, respectively. In the sites where 1.0, 2.0, and 5.0 mg dm^{-3} concentrations were applied, adventitious cormlets were not formed at the base of descendent corms. Such corms were only obtained in the control site and in the site where the lowest IBA concentration (0.5 mg dm^{-3}) was applied (Tab. 4). Irrespective of IBA concentration applied in plants rooting in *in vitro* cultures, corms formed in *in vivo* conditions are suitable for planting in the subsequent cultivation cycle.

DISCUSSION

The results obtained in this experiment indicate that the addition of various types and concentrations of auxins to the MS medium affected the structure and root growth of *Crocosmia* × *crocosmiiflora* 'Lucifer' in *in vitro* culture. However, in *Crocosmia* single shoots were rooted on a PGR-free MS medium (Krupa-Małkiewicz et al. 2013). Similar results were obtained by Boltenkov et al. (2005) for *Iris*, Koetle et al. (2010) for *Dierama erectum* Hilliard, and Hannweg et al. (2013) for *Crocosmia aurea*. The occurrence of root development on a PGR-free medium may depend on the presence of endogenous auxins in regenerated cormlets. However, according to many researchers (Koetle et al. 2010, Ascough et al. 2011, Memon 2012, Hannweg et al. 2013), the addition of plant growth regulators usually speeds up the rooting process and increases rooting percentage. Ascough et al. (2011) indicated that NAA at all concentrations significantly inhibited root elongation, with the resultant roots being short and thick. However, the inclusion of IBA showed a positive effect on the stimulation of adventitious roots. A compact root system allows easier and quicker adaptation of plants to *in vivo* conditions without the threat of delicate root system damage.

The process of adventitious root formation is influenced by several internal and external factors, such as the juvenile or mature origin of the microshoots, the rooting potential of the genotype, and the type of auxins and their concentrations. Auxins induce adventitious root meristems on the shoots and root gravity response (Koetle et al. 2010, Memon 2012, Sevik and Guney 2013).

The effects of the auxin group of hormones on rooting and plant development have been reported in a wide range of plant species. Hussain and Khan (2004) studied the effectiveness of IAA and IBA in *Rosa bourboniana* and *Rosa gruss-an-teplitz*; Ascough et al. (2007) analysed the influence of plant hormones on the micropropagation of *Watsonia* species; Hannweg et al. (2013) studied the effective method for the micropropagation of *Crocosmia aurea*. The studies show that, in general, the auxin group of hormones has an effect on rooting. The results obtained from this experiment are in agreement with the results of the above-mentioned authors. Root generation is one of the most important seedling quality indicators. According to the results of this study, the most rooted microshoots of *Crocosmia* × *crocosmiiflora* 'Lucifer' were observed on MS media supplemented with IBA at the concentration of 1.0 mg dm^{-3}. The plants from that media combination were characterized by the longest and the highest number of roots. Moreover, the weight of the roots of *Crocosmia* rooted on the MS medium with IBA addition was 91.6% higher compared to the control. Similar results were described by Koetle et al. (2010) for *Dierama erectum* and Ascough et al. (2009) for *Sisyrinchium laxum*. According to Ahouran et al. (2012), only seedlings that form hairy roots or seedlings with well-shaped taproot systems are able to collect water from the natural environment more easily, and their chance for adaptation to *in vivo* conditions is much higher. The auxins applied in this experiment also affected the structure of *Crocosmia* roots. The roots from the control medium, as well as from the media with lower auxin additions (0.5 and 1.0 mg dm^{-3}), were normal and thin. In turn, higher auxin concentrations (2.0 and 5.0 mg dm^{-3}) inhibited root elongation growth, and they were thick and short. The unfavourable effect of NAA on rooting in *in vitro* cultures of *Sisyrinchium laxum* was also observed by Ascough et al. (2009) and Koetle et al. (2010) in *Dierama erectum*, which according to Koetle et al. (2010) may be due to the stability of NAA in the tissue in its free form and results in scrawny root growth. According to Ludwig-Müller (2000), IBA has a greater ability to promote adventitious root formation as compared with IAA. Moreover, IBA biosynthesis shows that its concentrations in plants may be regulated by plant hormones and various stresses.

The addition of auxins to MS media affected the morphological features of the underground parts of the examined plants. The average height of the plants from the media with the addition of IAA and IBA was at the level of the control. However, the addition of NAA to MS media inhibited the growth of *Crocosmia* plants. In turn, when analysing the effect of selected auxins on the number of *Crocosmia* leaves, we concluded that the value of the discussed morphological features was higher compared to the control only after the application of IBA. In the case of other auxins (IAA and NAA), the number of leaves was at the level of the control. Similar observations were described by Hussain and Khan (2004) in *Rossa gruss-an-teplitz*. As a result, they concluded that hormone applications do not have a positive effect on the number of leaves.

The study conducted on *Crocosmia* adaptation to *in vivo* conditions is the first of its kind and requires replication in subsequent years.

CONCLUSIONS

1. IBA at a concentration of 1.0 mg dm^{-3} was the most efficient at inducing roots in *Crocosmia* × *crocosmiiflora*.
2. The applied auxins (IAA, IBA and NAA) in MS media affected the root structure. Higher auxin concentrations (2.0 and 5.0 mg dm^{-3}) inhibited root elongation growth, and they were thick and short.
3. The IBA auxin applied for *Crocosmia* rooting in *in vitro* cultures at concentrations from 1.0 to 5.0 mg dm^{-3} affected a decrease in the percentage of plants which were adapted to *in vivo* conditions and obtained the yield of descendant corms.

AUTHOR CONTRIBUTIONS

All authors contributed to all aspects of this manuscript, including the development of the ideas, writing, revisions and joint responsibility for the content.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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