

* Marcelina KRUPA-MAŁKIEWICZ¹, Piotr ŻURAWIK², Paulina PIÓRKOWSKA¹

MICROPROPAGATION OF *CROCOSMIA* × *CROCOSMIIFLORA* ‘LUCIFER’

MIKROROZMNAŻANIE *CROCOSMIA* × *CROCOSMIIFLORA* ‘LUCIFER’

¹ Department of Plant Genetics, Breeding and Biotechnology, West Pomeranian University of Technology, Szczecin

² Department of Horticulture, West Pomeranian University of Technology, Szczecin

Streszczenie: Celem pracy było określenie wpływu zastosowanych roślinnych regulatorów wzrostu na morfogenezę *Crocosmia* × *crocosmiiflora* w kulturach *in vitro*. Materiał badawczy stanowiły nasiona krokosmii odmiany ‘Lucifer’. Nasiona kiełkowały na pożywce MS bez dodatku roślinnych regulatorów wzrostu. Eksplantaty pierwotne do założenia kultury *in vitro* stanowiły 1–2-centymetrowe fragmenty merystemów wierzchołkowych pędów wraz z węzłem i pąkami kątowymi. Rośliny namnażano na pożywce MS uzupełnionej BAP, NAA i 2,4-D. Najlepszą pożywką do namnażania krokosmii była pożywka MS bez dodatku roślinnych regulatorów wzrostu. W celu zainicjowania formowania cebulek namnożone pędy wykładano na pożywkę MS uzupełnioną 2iP, BAP, 2,4-D oraz IBA. Cebulki o największej średnicy obserwowano na pożywce MS bez dodatku regulatorów wzrostu.

Key words: *crocosmia*, *in vitro*, multiplication, plant growth regulators.

Słowa kluczowe: *in vitro*, krokosmia, mikrorozmnażanie, roślinne regulatory wzrostu.

INTRODUCTION

(*Crocosmia* × *crocosmiiflora*) ‘Lucifer’ belongs to the *Iridaceae* family, growing in its natural habitat in south Africa. *Iridaceae* is a large and diverse family with about 65 genera of approximately 2025 species with a wide range of uses, but only a few of these attractive species have been used for developing new horticultural crops (Ascough et al. 2009, Ahouran et al. 2012). The exceptional variety of flower and leaf shape, size and colour make this family an ideal resource for developing new floricultural crops. Among breeders there has been great interest in biotechnological methods, including *in vitro* culture, which can accelerate and intensify the breeding process (Kulpa and Nowak 2011). Crops grown in the field or greenhouse are limited by the natural climatic conditions under which they grow, and control over flowering time is minimal. In most bulbous crops, flowering is genetically programmed to occur at certain times of the year, depending on environmental conditions (Armitage and Laushman 1990). *In vitro* cultures are used to obtain in a short amount of time

* Corresponding author – Adres do korespondencji: dr inż. Marcelina Krupa-Małkiewicz, Department of Plant Genetics, Breeding and Biotechnology, West Pomeranian University of Technology, Szczecin, Poland, e-mail: mkrupa@zut.edu.pl

a large population of progeny genetically identical with the maternal plants. Furthermore, micropropagation can be a useful tool for the clonal propagation and preservation of plants. The effectiveness of micropropagation protocols could also provide a foundation for plant genetic transformation, which could result in the development and introduction of new ornamental varieties into commercial markets (Ahouran et al. 2012).

Knowledge of the optimal conditions favoring differentiation under *in vitro* conditions is an important step in the application of *in vitro* manipulation techniques in higher plants. Successful plant regeneration depends on factors such as the genotype, explant type, age of the donor plants and the medium compositions, especially the plant growth regulators auxins/cytokinins (Boltenkov and Zarembo 2005, Gao et al. 2010).

Hence, the aim of this research was to determine the influence of the concentration of PGRs (Plant Growth Regulators) – auxins and cytokinins, in the medium on the morphogenesis of *Crocoshmia* × *crocoshmiiflora* 'Lucifer' *in vitro*.

MATERIAL AND METHODS

The plant material consisted of *Crocoshmia* × *crocoshmiiflora* 'Lucifer' seeds from collections of Study of Ornamental Plants in the Department of Horticultural West Pomeranian University of Technology in Szczecin were plant material. Before germination, the seeds were disinfected in two steps. The first time – a 70% solution of alcohol was applied for 30 s, in the second step – a 7% solution of NaOCl was used for 10 min. After that seeds were rinsing three times in sterile distilled water for 5 min per rinse. Next, 100 seeds were placed in 100 ml Erlenmayer flasks (5 per flask). Each flask contained 20 ml of MS medium (Murashige and Skoog 1962). Germination was conducted at a temperature 25°C in white light ($40 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) for 16 h. The initiation stage lasted 6 weeks.

The explants initiated for growth were put onto multiplication MS medium. Shoot fragments of the size 1–2 cm, and with an apex or node with lateral meristems were used as primary explants for *in vitro* culture. The multiplication was induced on media containing macro- and microelements according to MS supplemented with 1-naphthaleneacetic acid (NAA) and 6-benzylaminopurine (BAP), BAP and 2,4-dichlorophenoxyacetic acid (2,4-D), 0.5 and 2.3 $\text{mg} \cdot \text{dm}^{-3}$ BAP (Table 1).

Table 1. Mean values of morphological traits *Crocoshmia* × *crocoshmiiflora* 'Lucifer' propagated on MS medium with different concentrations of plant growth regulators ($\text{mg} \cdot \text{dm}^{-3}$)

Tabela 1. Średnie wartości cech morfologicznych *Crocoshmia* × *crocoshmiiflora* 'Lucifer' namnażanych na pożywkach MS o zróżnicowanej zawartości roślinnych regulatorów wzrostu ($\text{mg} \cdot \text{dm}^{-3}$)

Medium ($\text{mg} \cdot \text{dm}^{-3}$) Pożywka ($\text{mg} \cdot \text{dm}^{-3}$)	Plant height (cm) Wysokość roślin (cm)	Roots length (cm) Długość korzeni (cm)	No of leaves Liczba liści
1 BAP + 1 NAA	12.5 c	2.6 c	2.7 a
0.5 BAP + 2 2,4-D	13.6 bc	3.0 c	2.2 a
0.5 BAP	11.1 c	3.1 c	2.6 a
1 BAP	15.4 b	5.2 b	1.9 a
Control – Kontrola	23.7 a	14.6 a	2.1 a
Mean – Średnia	15.3	5.7	2.3
LSD _{0,05} – NIR _{0,05}	2.36	1.92	0.94

*Means in the same column 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).

The media were selected on the basis of literature data (Koh et al. 2007, Ascough et al. 2009, Ahouran et al. 2012). Each jar filled with 30 ml of medium contained 6 explants. Each combination consisted of 10 replications – one jar per replication. Plants placed on MS medium without the addition of growth regulators constituted the control at all stages of the experiment. Every 4 weeks the cultures were transferred onto fresh media.

The multiplied shoots were placed on MS bulb media supplied with: 5.0 mg · dm⁻³ 2-isopentenyladenine (2iP), 0.5 mg · dm⁻³ BAP with 2.0 mg · dm⁻³ 2,4-D, 2.0 mg · dm⁻³ BAP with 1.0 mg · dm⁻³ indole-3-butyric acid (IBA), 0.5 and 2.3 mg · dm⁻³ BAP (Table 2).

Table 2. Mean values of morphological traits *Crocoshmia* × *crocoshmiiflora* rooted on MS medium with different concentrations of plant growth regulators (mg · dm⁻³)

Tabela 2. Średnie wartości cech morfologicznych *Crocoshmia* × *crocoshmiiflora* 'Lucifer' ukorzenianych na pożywkach MS o różnicowanej zawartości roślinnych regulatorów wzrostu (mg · dm⁻³)

Medium (mg · dm ⁻³) Pożywka (mg · dm ⁻³)	Plant height (cm) Wysokość roślin (cm)	Roots length (cm) Długość korzeni (cm)	No of leaves Liczba liści	Diameter of bulbs (cm) Średnica cebulek (cm)
5.0 2iP	16.2 bc	3.9 d	2.5 a	0.69 d
0.5 BAP + 2.0 2,4-D	13.2 d	3.5 d	0.8 c	0.80 b
2 BAP + 1 IBA	17.1 b	6.4 bc	2.3 a	0.63 e
2.3 BAP	15.5 bcd	4.9 cd	1.9 b	0.73 c
0.5 BAP	13.9 cd	7.3 ab	1.6 b	0.60 f
Control – Kontrola	21.1 a	9.1 a	2.5 a	0.84 a
Mean – Średnia	16.2	5.9	2.4	0.72
LSD _{0,05} – NIR _{0,05}	2.39	2.01	0.25	0.02

*Means in the same column 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).

All the media were supplemented with 8 g · dm⁻³ agar and 30 g · dm⁻³ sucrose, pH was adjusted to 5.7, and autoclaved at 121°C (0.1 MPa) during the time required according to the volume of medium in the vessel. All cultures were incubated in a growth room at a temperature of 25°C under 16-h photoperiod from a fluorescent lamp (40 μmol · m⁻² · s⁻¹).

The mean experimental measurement values at the multiplication stage (shoot and root length, and the numbers of multiplied shoots), and at the bulb stage (shoot and root length, the numbers of multiplied shoots and the diameter of bulbs) are presented in Tables 1, 2.

The results were statistically analyzed. The significance of differences at a significance level of $\alpha = 0.05$, was determined by means of variance analysis and Tukey's test. Homogenous groups among the analysed combinations were labeled with successive letters of the alphabet.

RESULT AND DISCUSSION

Nodal culture

Ten days after placing *crocoshmia* seeds on the MS medium, 70% of them had germinated. The other seeds either did not grow or became infected. The height of *Crocoshmia* × *crocoshmiiflora* 'Lucifer' seedlings at the initiation stage ranged from 7.0 to 18.0 cm, while the number of leaves ranged from 4 to 9 per plant. After 4 weeks, explants were placed

on MS medium supplemented with NAA, BAP and 2.4-D (Table 1). The average height of the resultant crocosmia plants was 15.3 cm and the tallest (23.7 cm) were observed on MS without the addition of growth regulators (Table 1). Among seedlings growing on MS media supplied with plant growth regulators, the longest crocosmias were obtained on media MS supplemented with $1.0 \text{ mg} \cdot \text{dm}^{-3}$ BAP. Their length was 15.4 cm (Fig. 1).

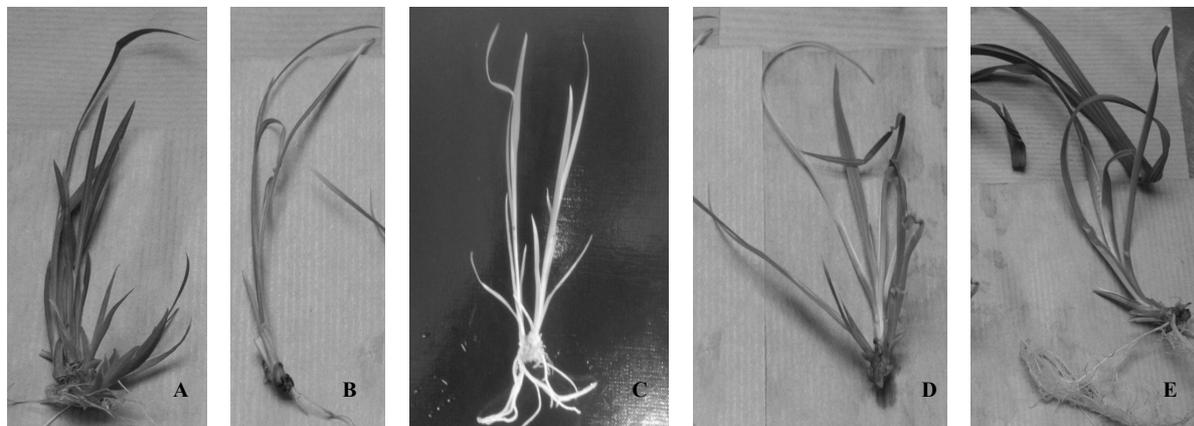


Fig. 1. Crocosmia regenerated on MS media supplemented with $1.0 \text{ mg} \cdot \text{dm}^{-3}$ BAP and $1.0 \text{ mg} \cdot \text{dm}^{-3}$ NAA (A), $0.5 \text{ mg} \cdot \text{dm}^{-3}$ BAP and $2.0 \text{ mg} \cdot \text{dm}^{-3}$ 2,4-D (B), $0.5 \text{ mg} \cdot \text{dm}^{-3}$ BAP (C), $1.0 \text{ mg} \cdot \text{dm}^{-3}$ BAP (D) and on MS media (E)

Rys. 1. Krokoscemia regenerowana na pożywce MS z dodatkiem $1,0 \text{ mg} \cdot \text{dm}^{-3}$ BAP i $1,0 \text{ mg} \cdot \text{dm}^{-3}$ NAA (A), $0,5 \text{ mg} \cdot \text{dm}^{-3}$ BAP i $2,0 \text{ mg} \cdot \text{dm}^{-3}$ 2,4-D (B), $0,5 \text{ mg} \cdot \text{dm}^{-3}$ BAP (C), $1,0 \text{ mg} \cdot \text{dm}^{-3}$ BAP (D) oraz MS bez dodatku roślinnych regulatorów wzrostu (E)

The shortest (11.1 cm), on the other hand, were plantlets obtained on media MS supplemented with $0.5 \text{ mg} \cdot \text{dm}^{-3}$ BAP (Table 1). A similar relationship was observed by Bacchetta et al. (2003) for *Lilium spp.* bred from bulbs. Plants on the MS medium either without the addition of plant growth regulators or the $2.0 \text{ mg} \cdot \text{dm}^{-3}$ BAP-enriched MS medium were characterized by longer shoots than those on MS media with the addition of either auxins (IAA and IBA) or a combination of auxins and cytokinins. Similarly accelerated development of these *Alstroemeria* shoots was observed by Lin et al. (1997), when a combination of TDZ and BAP was used. According to Bacchetta et al. (2003), BAP is the most effective cytokinin for adventitious shoot regeneration from explants of Asiatic genotypes.

In the author's own research, no statistical differences were observed in the number of crocosmia multiplied leaves. The largest number of leaves (2.7) was observed in seedlings growing on MS media supplemented with $1.0 \text{ mg} \cdot \text{dm}^{-3}$ BAP and $1.0 \text{ mg} \cdot \text{dm}^{-3}$ NAA and the lowest (1.9) – on MS media supplemented with $1.0 \text{ mg} \cdot \text{dm}^{-3}$ BAP (Table 1). According to Cousineau and Donnelly (1991), Turk et al. (1994) and Gao et al. (2010), it is necessary to add a combination of auxins/cytokinins to initiate leaf regeneration in bulb plants.

The composition of the medium had no significant influence on the length of crocosmia roots. Seedlings growing on the control medium without the addition of growth regulators were characterized by the longest roots (14.6 cm). Plants from this medium were included in a separate homogeneous group. Crocosmias propagated on media with the addition of auxins/cytokinins were characterized by significantly shorter roots than crocosmias from the

control medium. Their length ranged from 2.6–5.2 cm (Table 1). At the plant breeding stage, morphological features were also observed. On the basis of these observations, it was found that the structure of plants from the control medium was very fragile (Fig. 1), despite the fact that they were the tallest and had the longest roots. Seedlings from MS medium supplemented with $1.0 \text{ mg} \cdot \text{dm}^{-3}$ BAP and $1.0 \text{ mg} \cdot \text{dm}^{-3}$ NAA proved to be the best in this respect. Their leaves were thick, well-formed and strongly branching, whereas seedlings from MS medium supplemented with $0.5 \text{ mg} \cdot \text{dm}^{-3}$ BAP in combination with $2.0 \text{ mg} \cdot \text{dm}^{-3}$ 2.4-D had a strong tendency to develop callus tissue.

Bulb forming

The crocosmia shoots once bred were transferred onto a new medium at the next stage to initiate bulb formation. Explants were placed on MS media supplied with different concentrations and combinations of the growth regulators (Table 2). An MS medium without the addition of plant growth regulators was the control. According to Koh et al. (2007), the best medium for crocosmia bulb formation is MS supplemented with 2.4-D and BAP. The positive influence of BAP on the formation of *Crocus sativus* bulbs is also confirmed by research conducted by Sharma et al. (2008), who received the best effects after using a $3.0 \text{ mg} \cdot \text{dm}^{-3}$ BAP-enriched MS medium.

In the author's own research, crocosmia from MS medium (without the addition of plant growth regulators) formed bulbs where the mean diameter was 0.84 cm. Slightly smaller bulbs in comparison to control group were obtained on MS medium supplemented with $0.5 \text{ mg} \cdot \text{dm}^{-3}$ BAP and $2.0 \text{ mg} \cdot \text{dm}^{-3}$ 2.4-D. The diameter of bulbs obtained was 0.80 cm (Table 2). Despite the fact that crocosmia from this combination of media on average allowed the largest bulbs to be obtained, the resultant plants were characterised by the lowest height (13.2 cm), the shortest roots (3.5 cm) and the lowest number of leaves (0.8). The average height of the plants at the bulb formation stage was 16.2 cm, while the control plants proved to be the tallest (21.1 cm).

The bulb formation process in *in vitro* cultures is the essential stage in the subsequent adaptation of the plants to natural conditions. As food reserves for plants are stored in the bulbs, it was a significant aim of this study to select the most appropriate medium enabling the growth of crocosmia (*Crocoshmia* × *crocoshmiiflora*) bulbs. On the basis of our results, it was found that the addition of BAP to MS medium has a stimulating effect on crocosmia bulb formation. Results similar to those obtained in this study were also recorded by Plessner et al. (1990) for crocuses (*Crocus sativus* L.) and by Bruyn and Ferreira (1992) for gladiolus plants (*Gladiolus dalenii* and *Gladiolus tristis*).

CONCLUSIONS

On the basis of the obtained results, the following conclusions have been drawn:

1. Crocosmia should be propagated *in vitro* on a Murashige and Skoog (1962) medium without growth regulators. Plants propagated under these conditions grow tall and have the longest roots.
2. The optimal media for bulb formation in crocosmia are MS without growth regulators and MS supplemented with $0.5 \text{ mg} \cdot \text{dm}^{-3}$ BAP and $2.0 \text{ mg} \cdot \text{dm}^{-3}$ 2.4-D.

REFERENCES

- Ahouran M., Hosseini R., Zarghami R. 2012. Corms as a source of explants for the successful clonal propagation of *Crocus cancellatus*. J. Crop Sci. Biotech. 15 (1), 47–51.
- Armitage A.M., Laushman J.M. 1990. Planting date, in-ground time affect cut flowers of *Acidanthera*, *Anemone*, *Allium*, *Brodiaea*, and *Crococsmia*. Hort. Sci. 25 (10), 1236–1238.
- Ascough G.D., Erwin J.E., van Staden J. 2009. Micropropagation of iridaceae – a review. Plant Cell. Tiss. Org. Cult. 97, 1–19.
- Bacchetta L., Remotti P.C., Bernardini C., Saccardo F. 2003. Adventitious shoot regeneration from leaf explants and stem nodes of *Lilium*. Plant Cell. Tiss. Org. Cult. 74, 37–44.
- Boltenkov E.V., Zarembo E.V. 2005. *In vitro* regeneration and callogenesis in tissue culture of floral organs of the genus *Iris* (Iridaceae). Biol. Bull. 32 (2), 138–142.
- Bruyn M.H., Ferreira D.I. 1992. *In vitro* corm production of *Gladiolus dalenii* and *Gladiolus tristis*. Plant Cell. Tiss. Org. Cult. 31 (2), 123–128.
- Cousineau J.C., Donnelly J.D. 1991. Adventitious shoot regeneration from leaf explants of tissue cultured and greenhouse-grown raspberry. Plant Cell. Tiss. Org. Cult. 27, 249–255.
- Gao X., Yang D., Cao D., Ao M., Sui X., Wang Q., Kimatu J.N., Wang L. 2010. *In vitro* micropropagation of *Freesia hybrida* and the assessment of genetic and epigenetic stability in regenerated plantlets. J. Plant Grow. Regul. 29, 257–267.
- Koh J.C., Lee E.J., Park H.G. 2007. Effect of several plant growth regulators on callus and corm formation of *Crococsmia* × *crococsmiflora* *in vitro*. Flower Res. J. 12, 238–244.
- Kulpa D., Nowak N. 2011. *In vitro* flowering of *Petunia* × *atkinsiana* D. Don. Folia Hort. 23 (2), 125–129.
- Lin S.H., De Jeu M.J., Jacobsen E. 1997. Direct shoot regeneration from excised leaf explants of *in vitro* grown seedlings of *Alstroemeria* L. Plant Cell. Rep. 16, 770–774.
- Murashige T., Skoog F. 1962. A revised medium, for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant, 15, 473–479.
- Plessner O., Ziv M., Negbi M. 1990. *In vitro* corm production in the saffron crocus (*Crocus sativus* L.). Plant Cell. Tiss. Org. Cult. 20, 89–94.
- Sharma K.D., Tathour R., Sharma R., Goel S., Sharma T.R., Singh B.M. 2008. *In vitro* cormlet development in *Crocus sativus*. Biol. Plant. 52 (4), 709–712.
- Turk B.A., Harry J.S., Zimmerman R.H. 1994. Adventitious shoot regeneration from *in vitro* cultured leaves of *Rubus* genotypes. Plant Cell. Tiss. Org. Cult. 38, 11–17.

Abstract. The aim of this research was to determine the influence of the concentration of PGRs– auxins and cytokinins in the medium on morphogenesis of *Crococsmia* × *crococsmiflora* ‘Lucifer’ *in vitro*. Plant material were seeds of *crococsmia* ‘Lucifer’. The germination was conducted at MS medium without any growth regulators. Shoot fragments of size 1–2 cm, with an apex or node with lateral meristems were used as primary explants for *in vitro* culture. The multiplication was induced on the media according to MS supplemented with combinations of BAP, NAA and 2.4-D. *Crococsmia* multiplication *in vitro* should be conducted on MS media without growth regulators. Proliferated shoots were placed on the MS bulb forming media supplemented with combinations of 2iP, BAP, 2.4-D and IBA. Maximum diameter of bulbs in comparison to the control, was obtained on MS medium supplemented with 0.5 mg · dm⁻³ BAP and 2.0 mg · dm⁻³ 2.4-D.