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THE INFLUENCE OF THE MINERAL COMPOSITION OF THE MEDIUM ON *IN VITRO* PROPAGATION OF *KOHLERIA AMABILIS* (PLANCH. ET LINDEN) FRITSCH SHOOTS

Danuta Kozak, Jerzy Hetman, Małgorzata Witek

Institute of Ornamental Plants and Landscape Architecture, Agricultural University, Lublin
dkozak@autograf.pl

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S u m m a r y

The aim of the study was to test the influence of the mine ral salt composition of the medium on *in vitro* multiplication and growth of kohleria shoots. Shoot tips were cultured on Murashi ge and Skoog (MS) (full and half strength), Gamborg et al. (B_5), Nitsch and Nitsch (NN) or Lloyd and McCown (WPM) media supplemented with BA 1 mg·dm ^{3}. The influence of passa ge time on the induction and growth of shoots on the medium con taining half strength of MS mineral salts and BA 1 mg·dm ^{3}, GA_3 1 mg·dm ^{3} was also studied. The explants used in the experiments were obtained from aseptically grown shoot clusters. A signifi cant influence of the medium type and the time of propagation on the number, length and weight of axillary shoots was observed. The medium containing full or half strength of mineral salts ac cording to MS was the best. The propagation of kohleria shoots should take place at 4 week passages.

Key words: *Kohleria amabilis*, *in vitro*, mineral salt composition, time of passage

INTRODUCTION

In horticultural production, the traditional methods of propagation of ornamental plants are more and more frequently replaced by the *in vitro* culture method. This technology allows healthy and homogenous material to be obtained for sale in a short time. It is of special significance in the case of valuable plant species, but which are little known on the floriculture market. In available literature, there are reports on micropropagation of many plants from the family Gesneriaceae (Start and Cumming, 1976; Cook, 1977; Bilkey and McCown, 1979; Vlahos, 1989, Dąbski and Kozak, 1996, 1997; Kozak and Dąbski, 1995a, b; Maghami, 2003; Araujo et al., 2004; Zhang, 2004). But data on *in vitro* propagation of *Kohleria amabilis* (Planch. et Linden) Fritsch are lacking.

An appropriately selected medium has an essential influence on the morphogenesis and the achievement of desired effects in tissue cultures. The effect of growth regulators on explants growing *in vitro* is possible thanks to supplying plants with essential nutrients, notably mineral salts. There are many types of growth media with a strictly defined mineral composition, e.g. basal salt mixtures according to Murashige and Skoog (1962), Gamborg et al. (1968), Nitsch and Nitsch (1969), Lloyd and McCown (1980). The multiplication stage is composed of passages the time of duration of which is adapted to the respective plant species and variety in order to obtain the highest possible multiplication ratio. During the duration of the passage, the chemical composition of the medium changes and adversely affects plant quality.

The aim of this study was to test the influence of the mineral composition and the time of passage on the induction and growth of kohleria shoots.

MATERIAL AND METHODS

Shoot tips and nodal sections of shoots taken from kohleria plants growing in a greenhouse were used as explants in the experiments. They were sterilized in sodium hypochlorite solution containing 0.75% of active chlorine for 30 min. and rinsed three times in sterile water. The explants were placed on modified Nitsch and Nitsch (NN) (1969) medium supplemented with kinetin (1mg·dm^{-3}) and GA_3 (1 mg·dm^{-3}). Regenerating shoots were multiplied in 4-week passages on medium containing $^1/_2$ macro- and micronutrients according to Murashige and Skoog (MS) (1962), NaFeEDTA 40,3 mg·dm^{-3} and organic compounds: 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, 30 g·dm^{-3} sucrose, BA 1 mg·dm^{-3}, GA_3 1 mg·dm^{-3}. After

several passages of multiplication, shoot tips of 6–10 mm in length, with 2 pairs of well developed leaves, were dissected from the shoot clusters and used in the experiments.

In the first experiment, the explants were cultured on the media containing mineral salts according to: Murashige and Skoog (MS) (1962) (full- and half strength), Gamborg et al. (B_5) (1968), Nitsch and Nitsch (NN) (1969), Lloyd and McCown (WPM) (1980). Every medium contained iron and organic compounds as at the initial stage and BA 1 mg·dm^{-3}. In the second experiment, medium contained half strength of MS mineral salts, iron, organic compounds as at the initial stage and growth regulators: BA (1 mg·dm^{-3}), GA_3 (1 mg·dm^{-3}). All the media were solidified with 6.5 g·dm^{-3} Agar-Agar (Sigma). The pH of the media was adjusted to 5.7 before autoclaving.

Seven shoots were incubated per 250 ml Erlenmeyer flask. Twenty one shoots were used for each combination. Each flask with 7 explants was a replicate. The experiment was repeated twice.

The cultures were maintained at 22°C with a photon flux density of 35 µM·m^{-2}·s^{-1} and a 16-h photoperiod. The first experiment lasted 4 weeks. In the second experiment, subculturing was performed after 3, 4, 5 and 6 weeks. The number of axillary shoots from 1 explant, their length and fresh weight, and fresh weight of shoot clusters were determined. The results of the experiment were analyzed statistically using a standard statistical procedure with one factorial design and the Tukey test was used to estimate the differences between the means at a 5% level of significance.

RESULTS AND DISCUSSION

Analysis of the study results showed a significant influence of the medium type on the number of shoots formed from an explant. The largest number of shoots was obtained on $^1/_2$ MS medium and standard MS medium, whereas the lowest number on NN and B_5 medium (Tab. 1). WPM medium had a smaller effect on the trait studied.

It was found that the shoots growing on NN and B_5 medium formed the significantly longest axillary shoots. The significantly shorter shoots were obtained on WPM and $^1/_2$ MS medium. In turn, MS medium had a slightly weaker stimulating influence on shoot growth than WPM and $^1/_2$ MS medium.

Among the medium types studied, B_5 showed the significantly highest influence on the fresh weight of axillary shoots. Shoots with the significantly lowest fresh weight were obtained on NN and WPM medium. The favourable effect of $^1/_2$ MS and MS medium on the abovementioned trait was also observed.

A significant influence of the medium type on the fresh weight of the shoot cluster was demonstrated. Shoot clusters obtained on $^1/_2$ MS and MS medium were characterised by the highest weight, whereas the lowest weight was noted on WPM medium. NN and B_5 medium indirectly influenced the trait studied.

A significant influence of the time of passage on the growth and development of kohleria shoots was also noted. The largest number of axillary shoots was obtained during the passage lasting 6 weeks (Tab. 2). These shoots were also characterised by the largest length and the highest fresh weight compared to the shoots origi-

Table 1
The influence of the medium kind on the differentiation of shoots of *Kohleria amabilis* (Planch. et Linden) Fritsch *in vitro.*

Kind of the medium	Number of axillary shoots/1 explant	Length of axillary shoots (mm)	Fresh weight of axillary shoots (mg)	Fresh weight of shoot cluster (mg)
$^1/_2$ MS	16.1a*	4.0c	386.5ab	872.1a
MS	15.3ab	4.5bc	355.2ab	741.4ab
NN	9.6c	5.5a	236.8b	634.1b
B_5	10.6c	5.0ab	473.2a	652.7ab
WPM	12.3bc	3.9c	280.5b	599.3b
Means	12.8	4.6	346.4	699.9

* Means followed by the same letter are not significantly different at α 0.05

Table 2
The influence of the propagation time on the differentiation of shoots *Kohleria amabilis* (Planch. et Linden) Fritsch *in vitro.*

Time of the passage (weeks)	Number of axillary shoots/1 explant	Length of axillary shoots (mm)	Fresh weight of axillary shoots (mg)	Fresh weight of shoot cluster (mg)
3	7.2c*	3.9b	39.8c	269.9c
4	11.3bc	4.7ab	115.8bc	549.9b
5	13.9ab	5.1ab	149.9b	628.5b
6	18.3a	5.6a	337.6a	1091.8a
Means	12.7	4.8	160.8	635.0

* See explanation table 1

nating from the 3-week passage. Over the same period, a shoot cluster with the significantly highest fresh weigh was obtained. At the same time, it was observed that the quality of the new shoots worsened as the passage was prolonged. They became excessively elongated and had smaller leaves. Apart from that, a large part of the leaves browned. The shoots after 4 weeks of the duration of *in vitro* culture showed the best quality.

The chemical composition of the medium is one of the factors determining the success of *in vitro* cultures (Kępczyńska and Kępczyński, 1998). In this study, it was shown that the mineral composition has a significant influence on the induction and growth of kohleria shoots. Based on the results, ½ MS and MS media were singled out as the ones which have the most favourable effect on regenerating kohleria shoots. Smolik and Rzepka-Plevneš (1996) recognised both media as optimal in the micropropagation of *Saintpaulia ionantha*. But for *Sinningia speciosa*, the number of shoots and leaves and the mean shoot length were better on ½ MS medium (Araujo et al., 2004). MS medium is commonly used in the *in vitro* multiplication of different plant species from the family Gesneriaceae: *Achimenes* (Vlahos, 1989), *Aeschynanthus* (Dąbski and Kozak, 1996), *Episcia* (Bilkey and McCown, 1979), *Hypocyrta* and *Columnea* (Kozak and Dąbski, 1995a,b), *Saintpaulia ionantha* (Start and Cumming, 1976; Maghami, 2003; Zhang, 2004) and *Sinningia* (Londe et al., 2004). In the experiment conducted, the other media showed a lower value. Geier (1988) used NN medium (Nitsch and Nitsch, 1969) to initiate the hybrid cultures (*Kohleria amabilis* x *K. bogoten-*

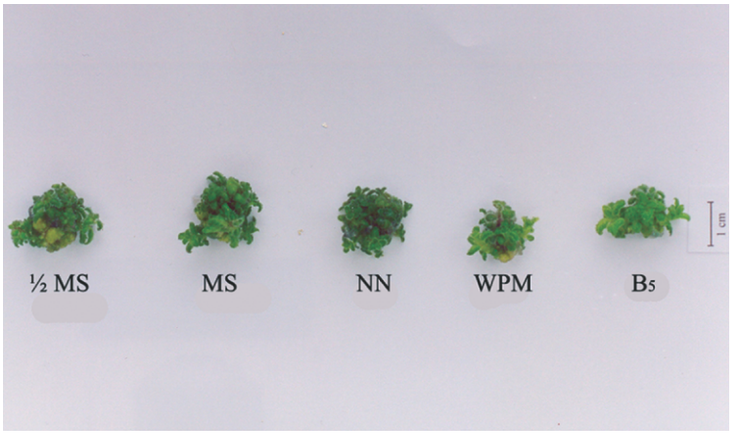

Fig. 1. Cluster of shoots of *Kohleria amabilis* obtained on the diffrent media after 4 weeks of culture in vitro.

sis) x K. *eriantha*, however, in own studies the poorest growing plants were obtained on it. B_5 medium had a very favourable influence on the fresh weight and elongation growth of kohleria shoots, but it had a weak effect on their number. For *Sinningia allagophylla*, B_5 medium was used containing a concentration of mineral salts half lower than the standard medium according to Gamborg et al. (1968)(Gomes and Kirszenzaft Shepherd, 2002). But WPM medium inhibited the elongation growth and increase of fresh weight of kohleria shoots.

The duration of the passage during the multiplication of kohleria had a significant influence on the traits studied. During the 6-week passage, the highest multiplication ratio was obtained, but the quality of the new shoots was unsatisfactory. A similar time of passage was applied during the multiplication of other plants from the family Gesneriaceae (Kozak and Dąbski, 1995a,b; Dąbski and Kozak, 1996). et al. (2004) also multiplied *Hedera helix* 'Dark Pittsburgh' and 'Kolibri' at 6-week intervals. Based on the results and observations, 4-week passages were chosen for the multiplication of kohleria. The following were also multiplied at such intervals: *Gerbera* (Soczek and Hempel, 1989), *Chaenomeles japonica* (Bach et al., 1996), *Rosa* (Kucharska et al., 2000; Kucharska and Orlikowska, 2005).

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Wpływ zestawu soli mineralnych na namnażanie kohlerii /*Kohleria amabilis* (Planch. et Linden) Fritsch/ *in vitro*

Streszczenie

Celem badań było określenie wpływu zestawu soli mineralnych na namnażanie i wzrost pędów *Kohleria amabilis* (Planch. et Linden) Fritsch. Wierzchołkowe fragmenty pędów wykładano na pożywki zawierające zestawy soli mineralnych wg: Murashige i Skoog (MS) (pełny zestaw lub ½), Gamborg i in. (B_5), Nitsch i Nitsch (NN) lub Lloyd i McCown (WPM). Pożywki uzupełniono BA 1 mg·dm^{-3}. Zbadano również wpływ długości trwania pasażu na pożywce zawierającej ½ MS oraz BA 1 mg·dm^{-3} i GA_3 1 mg·dm^{-3} na indukcję i wzrost pędów. Eksplantaty wykorzystane do doświadczeń pochodziły z ustabilizowanych kultur *in vitro*. Wykazano istotny wpływ rodzaju pożywki i długości trwania pasażu na liczbę, wzrost i świeżą masę nowych pędów. Najlepsze były zestawy soli mineralnych ½ MS i MS. Namnażanie pędów kohlerii powinno odbywać się w odstępach 4-tygodniowych.