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In vitro PROPAGATION OF *Arnica montana* L.: AN ENDANGERED HERBAL SPECIES OF GREAT IMPORTANCE TO MEDICINE

Agnieszka Surmacz-Magdziak, Danuta Sugier
University of Life Sciences in Lublin

Abstract. *Arnica montana* L., a valuable medicinal plant, has been used in the pharmaceutical and cosmetic industry for many years. Traditional methods of reproduction of this protected species are hardly efficient; hence, the method of *in vitro* culture provides the possibility of increasing the multiplication coefficient, which in turn would facilitate introduction of arnica into crop production. The aim of the study was to examine the effect of cytokinins and their concentration on multiplication of shoots and the effect of different concentrations of auxin on rooting of multiplied shoots of *Arnica montana*. The plants of mountain arnica were propagated from shoot tips placed on the MS agar (0.7%) medium supplemented with NAA (0.1 mg $\cdot l^{-1}$). One of the four cytokinins, i.e. BAP, KIN, 2iP and Z, was applied at the concentrations of 0.5, 1.0, 1.5 and 2.0 mg $\cdot l^{-1}$. After 5 weeks of the *in vitro* culture, the shoots obtained were transferred to the MS agar medium supplemented with 0, 0.05, 0.1 and 0.2 mg $\cdot l^{-1}$ NAA for rooting. The greatest number of the explants regenerating shoots and the shoots number were found in the MS medium supplemented with 0.5 mg$\cdot l^{-1}$ BAP. KIN at the concentrations of 1.5 and 2.0 mg$\cdot l^{-1}$ exerted the most substantial impact on the shoot length. No change in shoot length and weight was found in the case of Z, and a bigger number of shoots was obtained at its concentrations ranging 1.0 to 2.0 mg$\cdot l^{-1}$. The shoots of the mountain arnica were characterised by high rhizogenic capacity. The biggest numbers of rooted shoots were found in the medium containing 0.1 mg$\cdot l^{-1}$ NAA. Plants with the greatest number and weight of shoots were obtained in the same medium, while the longest roots were found at 0.05 mg$\cdot l^{-1}$ NAA.

Key words: micropropagation, mountain arnica, growth regulators

INTRODUCTION

Mountain arnica *Arnica montana* L. (Asteraceae) is a valuable medicinal plant [Merfort and Wendisch 1992, Willuhn 1998, Reider et al. 2001, Bilia et al. 2006]. Pharma-

Corresponding author – Adres do korespondencji: Agnieszka Surmacz-Magdziak, Institute of Plant Genetics, Breeding and Biotechnology, University of Life Sciences in Lublin, Akademicka 15 Str., 20-950 Lublin, Poland, e-mail: agnieszka.surmacz@up.lublin.pl

ceutical raw material comprises flower heads – *Arnicae anthodium*, which have been used in therapeutics, herbal and cosmetic industry for many years [Kisiel 1995, Jaroniewski 1996, Kowalczyk 2007a, 2007b]. Preparations made from mountain arnica flowers are widely used for topical treatment of post-trauma effects and inflammatory diseases [Braga et al. 2006, Weremczuk-Jeżyna et al. 2006]. When applied externally, *Arnica montana* flower heads exhibit antibacterial, antifungal, anti-inflammatory and analgesic effects; they also reduce pain, swelling and discoloration resulting from bruises, and possess potent antioxidant and antiradical abilities [Gawlik-Dziki et al. 2011]. Alcoholic and oily extracts are used in gels, creams and ointments or as arnica oil [Wagner and Merfort 2007]. *Arnica montana* can reduce the symptoms of hand osteoarthritis [Widrig et al. 2007] and is widely used both in traditional and homeopathic medicine [Brinkhaus et al. 2006].

Arnica montana L. is endemic to Europe. It is found on mountain meadows of Central Europe, France, the Pyrenees, the Balkans, southern Scandinavia, and more rarely in lowlands in Latvia and Belarus [Meusel and Jäger 1992]. In Poland, the species occurs rather infrequently in the Sudety Mountains, the East Carpathian Mountains, the Suwalszczyzna region, the Mazury region and the Białowieża Primeval Forest [Wojewoda and Cyunel 1963, Kozłowski et al. 2001, Zając and Zając 2001, Forycka et al. 2004, Forycka and Buchwald 2008]. *Arnica montana* is a rare species under strict protection in several European countries, therefore collecting it in its natural state is prohibited [Ellenberger 1998, Baillie et al. 2004, Mirek and Zarzycki 2006]. It is obtained from special plantations for medicinal purposes. Due to great difficulties in establishing and maintaining plantations, the crop area of *Arnica montana* is very small [Buła 1993a, 1993b, Buła 1995, Kozłowski et al. 1999, Weremczuk-Jeżyna and Wysokińska 2000, Sugier 2007].

Despite the fact that *Arnica montana* is a medicinal plant most widely used in clinics either alone or in combination with other herbs, agriculture and *in vitro* techniques are only partially documented [Lê 1998, Kozak 2004, Stefanache et al. 2010, Petrova et al. 2011].

The aim of the study was to determine the type of cytokinin and its concentration applied for multiplication of shoots and to determine the concentration of auxin (in this case NAA) necessary for rooting of multiplied shoots of *Arnica montana*. On the one hand, knowledge about micropropagation of arnica can help to protect this rare species; and on the other hand, it can be used for production of the plant for pharmaceutical purposes in field conditions.

MATERIALS AND METHODS

Plant material. The initial plant material comprised seeds of *Arnica montana* L. provided by the Department of Industrial and Medicinal Plants of the University of Life Sciences in Lublin. The seeds were disinfected with 0.2% $HgCl_2$ for 3 minutes, and then rinsed three times (for 15 minutes each time) with sterile water. The disinfected seeds were placed on Petri dishes loaded with the Murashige and Skoog (MS) [1962] agar (Agar-agar, Sigma) medium (0.7%) supplemented with *gibberellic* acid – GA_3

(2 mg · l^{-1}) and kinetin – KIN (0.01 mg · l^{-1}). The seeds were kept in darkness for about 2 weeks and exposed to light of about 25 µmol · s^{-1}· m^{-2} after germination. The top parts of shoots (about 0.5 cm) were collected from 5-week seedlings; these parts included terminal buds with two leaves. Shoot tips collected from the seedlings were placed in 0.5 l jars containing the MS agar (0.7%) medium supplemented with 0.1 mg ·l^{-1} of 1-naphthaleneacetic acid (NAA) and one of the four cytokinins, i.e. 6-benzylaminopurine (BAP), kinetin (KIN), N6- 2-isopentenyladenine (2iP) and zeatin (Z) at the concentrations of 0.5, 1.0, 1.5 and 2.0 mg ·l^{-1}. Each combination included 30 explants. The stage of organogenesis induction and regeneration of leaf rosettes (reduced shoot with a few leaves) lasted 5 weeks. The cultures were maintained in a phytotron at 25°C ± 2°C, at constant exposition to light provided by fluorescent lamps (25 µmol · s^{-1}· m^{-2}.). Afterwards, explants that regenerated shoots (shoot cluster), those that survived but failed to regenerate shoots and the explants that died out without regenerating shoots were counted. The number of shoots per one explant, their average fresh weight and average length of the shoot were determined. Structures longer than 0.5 cm were assumed to be shoots. The percentage of shoots with changed morphology was also calculated.

Rooting. The shoots were transferred one by one to the MS agar medium supplemented with 0.05, 0.1 and 0.2 mg ·l^{-1} of 1-naphthaleneacetic acid (NAA) for rooting. The medium in the control did not contain auxin. Ten repetitions were performed (ten 0.5 l jars with 3 explants each) for each combination. After 4 weeks, the percentage of rooted shoots as well as the number, length and fresh weight of roots were determined.

Statistical analyses. Significant differences between mean values of morphological features of mountain arnica were analysed using the ANOVA method and Tukey's post hoc tests. The mean values of the morphological features of *Arnica montana* plantlets were subjected to the Principal Component Analysis (PCA). Before analyses, the data were standardized and log transformed. The Statistica and MVSP programs were used.

RESULTS AND DISCUSSION

Some of the experimental explants started organogenesis within about two weeks after they had been placed on the induction medium. At this stage of the experiment, some of the explants failed to react to the media used and some of them started to turn brown, which indicated their death. After 5 weeks of culture growth, it was observed that the best medium for multiplication of shoots of *Arnica montana* was the MS medium enriched with 0.1 mg·l^{-1} NAA and the medium supplemented with 0.5 mg·l^{-1} BAP. In such conditions, 86.1% explants propagated and regenerated shoots (tab. 1). The mean number of shoots (i.e. 7) obtained in these conditions was statistically higher than at a higher BAP concentration and application of KIN, 2iP and Z (fig. 1, phot. 1). Similar results were reported by Conchou et al. [1992], who obtained 7.7 shoots from one explant at a 1 mg·l^{-1} BAP concentration. Yet, Stefanache et al. [2010] obtained 3–4 shoots at the 1 mg·l^{-1} and 2 mg·l^{-1} concentrations of BAP. Weremczuk-Jeżyna and Wysokińska [2000] found that the optimum concentration of BAP for multiplication of *Arnica montana* shoots was 0.25 µM·l^{-1} (0.06 mg ·l^{-1}). The above-mentioned researchers obtained

6.5 shoots from one explant. Studies carried out by other researchers showed that cytokinin was beneficial for multiplication of shoots of *Salvia sclarea*, *Salvia splendens* [Skała and Wysokińska 2001] and *Tibouchina urvilleana* [Kozak and Wnuk 2005].

Table 1 The effect of the type of cytokinin (BAP – 6-benzylaminopurine, KIN – kinetin, 2iP – N6- 2-isopentenyloadenine, Z – zeatin) and its concentration on the frequency (%) of *Arnica montana* L. explants: forming shoots, living but not forming shoots, died-out and shoots with changed morphology

Tabela 1. Wpływ rodzaju cytokininy (BAP – 6-benzynoaminopuryna, KIN – kinetyna, 2iP – N6-2-isopentenyloadenina, Z – zeatyna) oraz jej stężenia na częstotliwość (%) eksplantatów *Arnica montana* L. tworzących pędy, żywych, ale nietworzących pędów, zamarłych oraz pędów o zmienionej morfologii

Cytokinin – Cytokinina		% of explants – % eksplantatów			% of morphologically changed shoots % pędów o zmienionej morfologii
Type Rodzaj	concentration stężenie $mg \cdot l^{-1}$	forming shoots tworzące pędy	living but not forming shoots żywe, ale nietworzące pędy	died-out zamarłe	
BAP	0.5	86.1	7.7	6.2	0
	1.0	72.3	16.9	10.8	0
	1.5	63.4	24.5	12.1	25.0
	2.0	59.2	29.5	11.3	32.0
KIN	0.5	53.1	30.6	16.3	0
	1.0	55.2	29.7	15.1	3.0
	1.5	70.0	15.8	14.2	2.8
	2.0	65.1	21.8	13.1	3.1
2iP	0.5	36.8	49.0	14.2	0
	1.0	36.7	50.1	13.2	0
	1.5	42.0	48.8	9.2	2.5*
	2.0	40.0	48.7	11.3	2.5*
Z	0.5	48.0	41.8	10.2	0
	1.0	66.3	20.5	13.2	2.6
	1.5	53.1	36.7	10.2	0
	2.0	60.0	28.6	11.3	0
Total mean Średnia ogólna		56.7	31.3	12.0	9.2

* chlorosis – chloroza

The experiment conducted showed that BAP at the concentration of 0.5 $mg \cdot l^{-1}$ promoted elongation and had a beneficial effect on the morphology of multiplied shoots with green leaves (phot. 1), whose average length was 2.4 cm (fig. 2). An increase in the BAP concentration caused a ca. 60% decrease in the percentage of explants capable of regenerating shoots (tab. 1) and the number of shoots per explant dropped to 2 (fig. 1). In their study on propagation of *Centaurium erythraea,* Piątczak and Wysokińska [2003] found that an increase in the BAP concentration was accompanied by a drop in the number of explants capable of regenerating shoots and in the number of shoots.

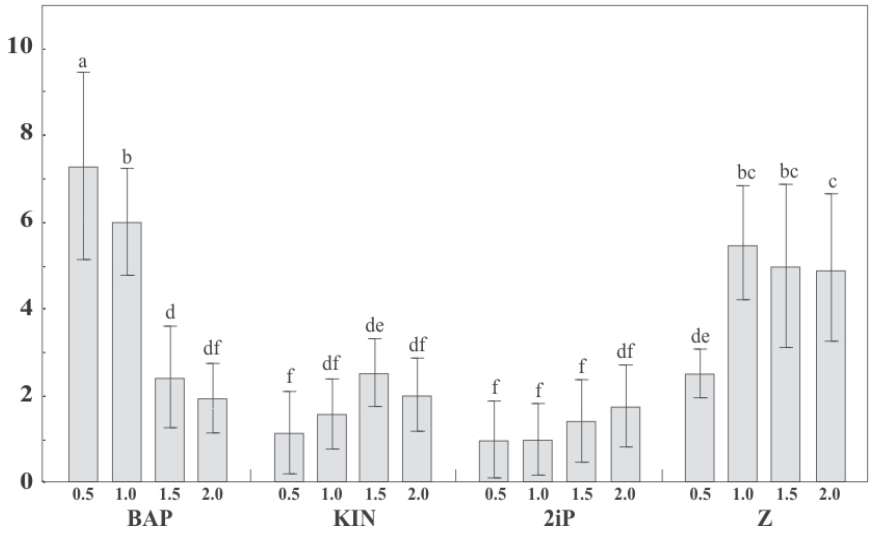

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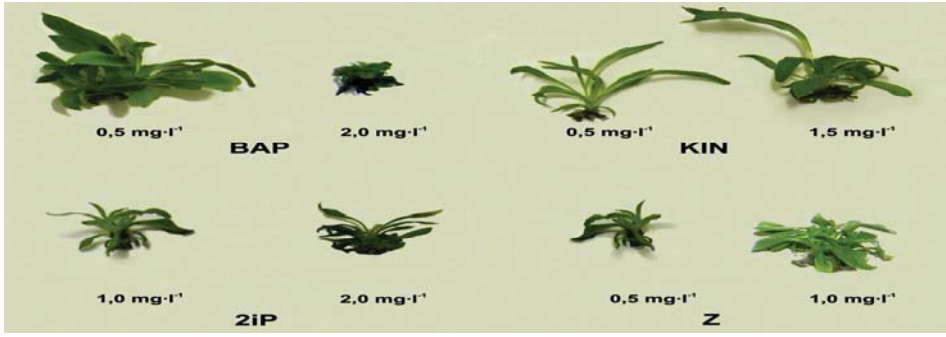
Fig. 1. The effect of the type of cytokinin (BAP – 6-benzylaminopurine, KIN – kinetin, 2iP –N6-2-isopentenyloadenine, Z – zeatin) and its concentration on the number of shoots per explant of *Arnica montana* L.

Rys. 1. Wpływ rodzaju cytokininy (BAP – 6-benzynoaminopuryna, KIN – kinetyna, 2iP – N6- 2-isopentenyloadenina, Z – zeatyna) oraz jej stężenia na liczbę pędów na eksplantat *Arnica montana* L.



Phot. 1. Shoot regeneration from shoot tips of *Arnica montana* L. on MS medium supplemented with NAA and one of the four cytokinins (BAP, KIN, 2iP, Z), after 5 weeks of *in vitro* culture

Fot. 1. Regeneracja pędów z wierzchołków pędów *Arnica montana* L. na pożywce MS z dodatkiem NAA oraz jedną z czterech cytokinin (BAP, KIN, 2iP, Z), po 5 tygodniach kultury *in vitro*

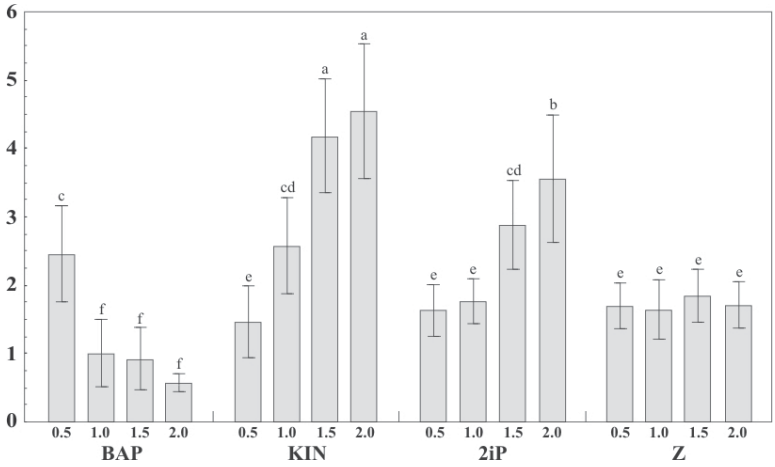


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Fig. 2. The effect of the type of cytokinin (BAP – 6-benzylaminopurine, KIN – kinetin, 2iP – N6- 2-isopentenyloadenine, Z – zeatin) and its concentration on the length of shoots (cm) of *Arnica montana* L.

Rys. 2. Wpływ rodzaju cytokininy (BAP – 6-benzynoaminopuryna, KIN – kinetyna, 2iP – N6- 2-isopentenyloadenina, Z – zeatyna) oraz jej stężenia na długość pędów (cm) *Arnica montana* L.

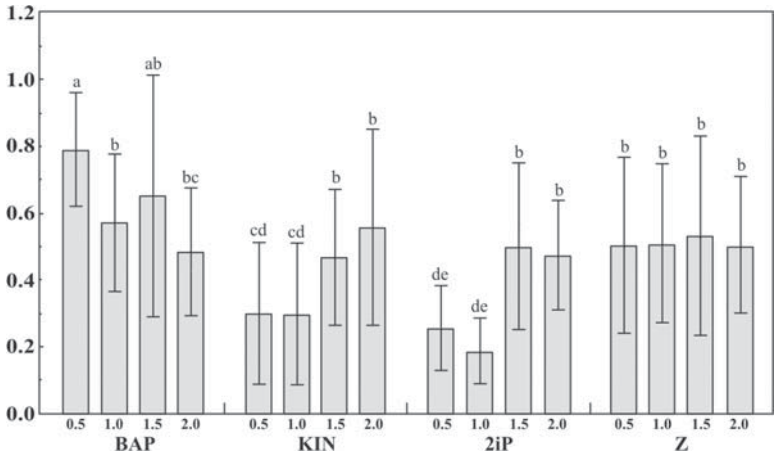

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Fig. 3. The effect of the type of cytokinin (BAP – 6-benzylaminopurine, KIN – kinetin, 2iP – N6- 2-isopentenyloadenine, Z – zeatin) and its concentration on the fresh weight of shoots (g) of *Arnica montana* L.

Rys. 3. Wpływ rodzaju cytokininy (BAP – 6-benzynoaminopuryna, KIN – kinetyna, 2iP – N6- 2-isopentenyloadenina, Z – zeatyna) oraz jej stężenia na świeżą masę pędów (g) *Arnica montana* L.

In the experiment conducted, the mean shoot fresh weight was 0.79 g at the 0.5 mg·l^{-1} concentration of BAP and it was statistically higher than the fresh weight of shoots obtained at the BAP concentrations of 1.0 mg·l^{-1} and 2.0 mg·l^{-1}. It was also higher than the fresh weight of shoots obtained after application of KIN, 2iP and Z (fig. 3). At high concentrations of BAP, the shoots were short (fig. 2), light green and creased (table 1). In their experiment conducted on *Anthemis nobilis*, Echeverrigaray et al. [2000] showed that the percentage of shoots with changed morphology differed significantly depending on the concentrations of BAP and NAA. At a high concentration of BAP alone (18 µM·l^{-1}), as many as 96% explants died and the number of shoots per explant was 1.5.

In the experiment conducted with application of 1.5 mg·l^{-1} KIN, 70% of the explants formed shoots (table 1) and the mean number of shoots regenerated by one explant was 2.5 (fig. 1, phot. 1). According to Malarz et al. [1993], it is possible to obtain as many as 22 shoots within 8 weeks of the *in vitro* culture at the 2.5 mg·l^{-1} concentration of KIN. Petrova et al. [2011] obtained 6.9 shoots with a mean length of 2.4 cm from one explant at the 1 mg·l^{-1} concentration of KIN. In the experiment performed, an increase in the kinetin concentration (up to 2.0 mg·l^{-1}) caused a slight decrease in the percentage of explants capable of regenerating shoots (tab. 1) and in the number of shoots (fig. 1). Studies conducted by Makowczyńska and Andrzejewska-Golec [2009] showed that the increase in kinetin concentration in the medium was accompanied by a lower multiplication coefficient in *Plantago maritima* buds and shoots and it depended on the type of the explant. Rout [2005] reported that a higher concentration of kinetin in the MS medium increased the number of *Clitoria ternatea* explants that regenerated shoots. This experiment showed that the MS medium supplemented with kinetin had a beneficial effect on elongation of the shoots obtained, whose mean length increased from 1.5 cm (in the presence of 0.5 mg·l^{-1} KIN) to 4.5 cm (in the presence of 2.0 mg·l^{-1} KIN). At 1.5 mg·l^{-1} and 2.0 mg·l^{-1} KIN, the shoots were the longest (fig. 2, phot. 1). An increase in the shoot length accompanying increased levels of KIN was also observed in experiments on micropropagation of such plants as *Salvia sclarea* and *Salvia splendens* [Skała and Wysokińska 2001].

At the higher kinetin concentration (2.0 mg·l^{-1}), the shoot fresh weight increased and reached 0.56 g (fig. 3). Slight changes in the morphology of the plants were observed at kinetin concentrations ranging from 1 to 2.0 mg·l^{-1}; no changes were visible at the lowest kinetin concentration in the medium (tab. 1).

The MS medium supplemented with 2iP had a small effect on micropropagation of *Arnica montana*; on average 49.1% of the explants failed to regenerate any shoots, and 12% of explants died. A similar number of shoots per explants (from 0.7 to 1.7) was regenerated at all the 2iP concentrations used (fig. 1, phot. 1). Butiuc-Keul and Deliu [2001] obtained 3.2 shoots from one explant of *Arnica montana* at 2iP concentration of 5.0 µM·l^{-1} (1 mg·l^{-1}). Studies carried out by other authors showed that 2iP had a negative effect on formation of shoots in *Tibouchina urvilleana* [Kozak and Wnuk 2005]. Our experiment showed that the shoots were longer at higher concentrations of 2iP (fig. 2), but they showed symptoms of chlorosis (tab. 1).

The experiment showed that the optimum concentration of zeatin in the MS medium necessary for multiplication of *Arnica montana* shoots was 1 mg·l^{-1} (phot. 1). During the 5 week of the *in vitro* culture, a mean of 5.6 shoots were obtained from one explant

(fig. 1) with a mean length of 1.7 cm (fig. 2). The number of shoots obtained at the Z concentrations of 1, 1.5 and 2,0 mg·l^{-1} was significantly higher than the number of shoots obtained in the presence of Z applied at the concentration of 0.5 mg·l^{-1} (fig. 1). At 1 mg·l^{-1} Z, 66.3% of the explants regenerated shoots and only 2.6% of the shoots were deformed with thick and fragile leaves (tab. 1). Petrova et al. [2011] obtained 8.2 shoots per explants with a mean length of 2.4 cm at 1 mg·l^{-1} Z. According to Weremczuk-Jeżyna and Wysokińska [2000], zeatin may be used in concentrations ranging from 0.1 to 5.5 mg·l^{-1}; if the concentration of zeatin falls below 0.1 mg·l^{-1}, it inhibits formation of shoots without changing their length and morphology. In our experiment, when the concentration of zeatin increased to 2.0 mg·l^{-1}, the percentage and number of explants capable of regenerating shoots decreased only slightly, i.e. to about 60% (table 1) and 4.8 shoots/explant, respectively (fig. 1). When 0.5 mg·l^{-1}zeatin was added, it inhibited regeneration of shoots, decreasing their number to 2.4 in one explant (fig. 1), whereas the percentage of explants capable of regenerating shoots decreased to about 48% (tab. 1). The shoot length was not affected by the concentration of this cytokinin in the medium; therefore, it did not change significantly (fig. 2).

The PCA analysis results point to variability of three morphological features of *Arnica montana* plants (fig. 4). The high eigenvalues for axis I and II of the PCA diagram suggest the presence of two main environmental gradients. The concentration of points suggests their strong morphological similarity and the vectors presented indicate a gra-

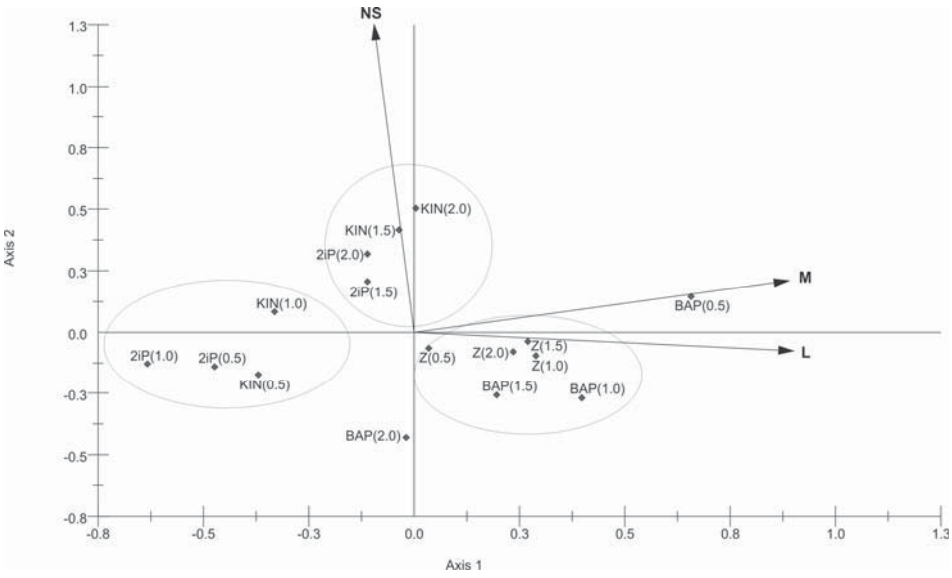


Fig. 4. Results of the PCA analysis on the basis of mean value of morphological features of *Arnica montana* L. plants; NS – number of shoots per explant, L – length of shoots (cm), M – fresh weight of shoots (g)

Rys. 4. Rezultaty analizy PCA na podstawie średnich wartości cech morfologicznych roślin *Arnica montana* L.; NS – liczba pędów na eksplantat, L – długość pędów (cm), M – świeża masa pędów (g)

dient of particular morphological features. On the right-hand side of the PCA analysis diagram, there are plants grown in the presence of BAP (1.0), BAP (1.5), Z (0.5), Z (1.0), Z (1.5), Z (2.0) correlated with the number of shoots per explant and shoot weight gradients (fig. 4). On the left-hand side of the PCA analysis diagram, there are plants grown in the presence of 2iP (0.5), 2iP (1.0), KIN (0.5), KIN (1.0). They are characterized by the lowest number of shoots and weight. Plants grown in the presence of KIN (1.5), KIN (2.0), 2iP (1.5), 2iP (2.0) and situated along the length of the shoot vector have the longest shoots and medium numbers of shoots per explant and weight (fig. 4). Peripherally, on the ordination space there are plants grown in the presence of BAP (0.5), which have the highest fresh weight and the greatest number of shoots per explants; the plants grown in the presence of BAP (2.0) have the shortest shoots and medium numbers of shoots per explant and weight (fig. 4).

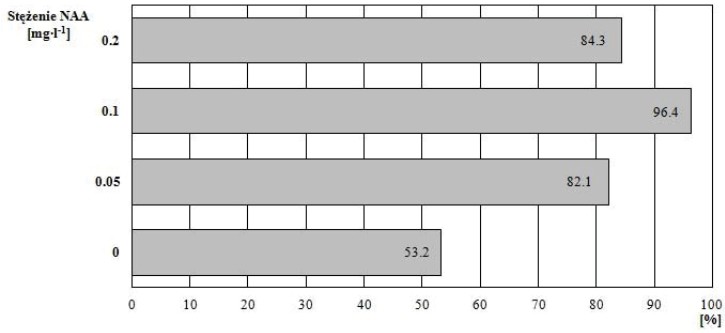
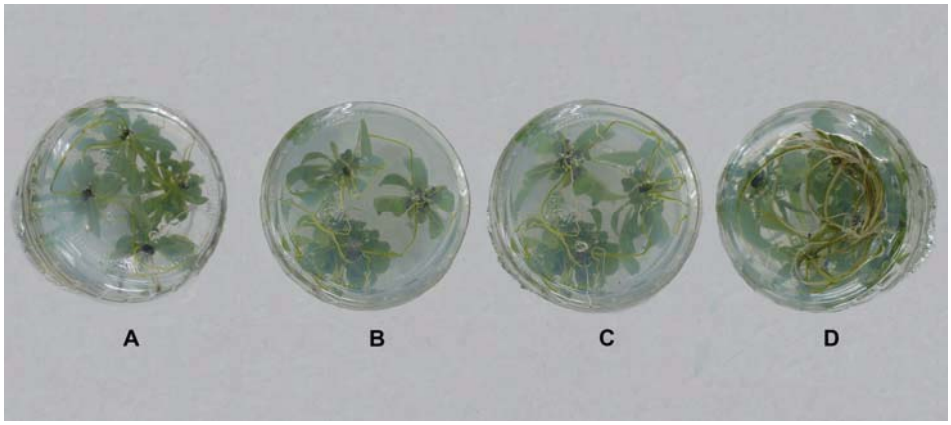


Fig. 5. The effect of NAA concentration on the percent (%) of rooted *Arnica montana* L. shoots (after 4 weeks of *in vitro* culture)

Rys. 5. Wpływ stężenia auksyny NAA na % ukorzenionych pędów *Arnica montana* L. (po 4 tygodniach kultury *in vitro*)

The experiment showed that roots appeared on shoots grown on all kinds of the media, but application of auxin had a positive effect on rhizogenesis (fig. 5, phot. 2). It was also found that the shoots of *Arnica montana* obtained rooted best on the MS agar medium supplemented with 0.1 mg·l^{-1} NAA (phot. 2). In these conditions, about 96.4% of shoots formed roots after 4 weeks of the *in vitro* culture (fig. 5). *Arnica montana* had the largest number of roots (about 5.0) when NAA was used at concentrations of 0.1 and 0.2 mg ·l^{-1} (fig. 6A). The smallest number of roots was regenerated by shoots grown on a medium without auxin (1.2) (fig. 6A). Studies carried out by other authors [Makowczyńska and Andrzejewska-Golec 2009] have also shown that NAA added at lower concentrations accelerates the process of rooting and increases the percentage of rooted shoots of *Plantago maritima*.

A significant effect of the concentration of auxin on the root length was also observed. NAA applied at the concentration of 0.05 mg ·l^{-1} had the most beneficial effect on root elongation. It was also observed that both lack and excess (0.2 mg ·l^{-1}) of auxin



Phot. 2. The effect of different concentrations of NAA on rooting of shoots of *Arnica montana* L., after 4 weeks of *in vitro* culture*:* A – control, B – 0.05 mg·l^{-1} NAA, C – 0.2 mg·l^{-1} NAA, D – 0.1 mg·l^{-1} NAA

Fot. 2. Wpływ zróżnicowanego stężenia NAA na ukorzenianie pędów *Arnica montana* L., po 4 tygodniach kultury *in vitro*: A – kontrola, B – 0,05 mg·l^{-1} NAA, C – 0,2 mg·l^{-1} NAA, D – 0,1 mg·l^{-1} NAA

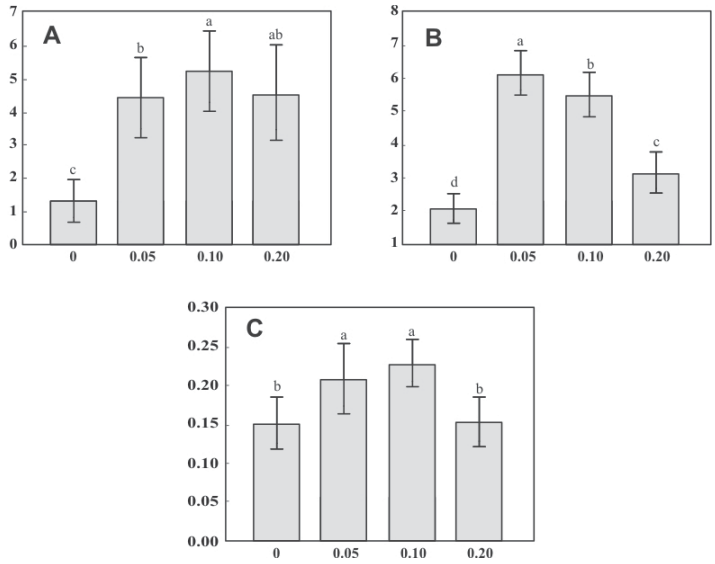


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Fig. 6. The effect of NAA concentration on the number of roots (A), length of roots (cm) (B), and fresh weight of roots (g) (C) in *Arnica montana* L.

Rys. 6. Wpływ stężenia auksyny NAA na liczbę korzeni (A), długość korzeni (cm) (B) oraz świeżą masę korzeni (g) (C) *Arnica montana* L.

inhibited root elongation (fig. 6B). These findings are in agreement with the results of research conducted by Świstowska and Kozak [2005], who found that NAA applied at the highest concentrations used in the experiment seriously inhibited the elongation of roots of *Nematanthus hyb.* 'Tropicana'.

NAA stimulated the growth of root fresh weight in the test plants. Roots formed at 0.05 mg $\cdot l^{-1}$ and 0.1 mg $\cdot l^{-1}$ NAA were characterized by higher fresh weight, whereas those obtained on the medium without NAA and at its maximum concentration exhibited the lowest fresh weight (about 0.15 g) (fig. 6C).

Additionally, regeneration of the callus tissue at the base of the shoots was observed. Callus was formed upon application of all the NAA concentrations. According to other authors [Świstowska and Kozak 2005], NAA has a significant influence of rooting of shoots and on the morphological features of the aboveground parts of *Nematanthus hyb.* 'Tropicana' in sterile cultures. The research has shown that NAA causes a significant increase in root fresh weight, stimulating the development of callus at the same time. In his research on micropropagation of *Prunus tenella* 'Firehill', Alderson et al. [1987] found that NAA caused excessive cell division, which resulted in appearance of thick roots covered with callus.

CONCLUSION

1. The mountain arnica population examined exhibited phenotypic variation in terms of capability micropropagation via direct organogenesis of shoot tips.

2. The number of the shoots obtained as well as their length and fresh weight depended on the type of cytokinin applied to the medium. The greatest number of the explants forming shoots and the shoots number were observed in the MS medium supplemented with 0.5 mg$\cdot l^{-1}$BAP. KIN applied at the concentrations of 1.5 and 2.0 mg$\cdot l^{-1}$ had the greatest impact on shoot length. Increasing levels of Z in the medium did not induce changes in the shoot length and weight, although a bigger number of shoots was obtained at the concentrations ranging from 1.0 to 2.0 mg$\cdot l^{-1}$.

3. The mountain arnica shoots obtained were characterised by high rhizogenic capacity. Rooting of the shoots depended on the concentration of auxin in the rooting media. The medium containing 0.1 mg$\cdot l^{-1}$NAA was most efficient, as 96.1% of the shoots produced roots. Plants with the greatest number and weight of roots were obtained on this medium; the longest roots were produced at 0.05 mg$\cdot l^{-1}$NAA.

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ROZMNAŻANIE *Arnica montana* L. METODĄ *in vitro*: ISTOTNEGO W MEDYCYNIE I ZAGROŻONEGO GATUNKU ZIELARSKIEGO

Streszczenie. *Arnica montana* L. jest cenną rośliną leczniczą wykorzystywaną od wiele lat w przemyśle farmaceutycznym i kosmetycznym. Tradycyjne metody rozmnażania w przypadku tego chronionego gatunku są mało wydajne, stąd zastosowanie metody kultur *in vitro* stwarza możliwość zwiększenia współczynnika mnożenia, co ułatwiłoby wprowadzenie arniki do uprawy polowej. Celem badań była ocena wpływu cytokinin i ich

stężeń do mnożenia pędów oraz zróżnicowanego stężenia auksyny do ukorzeniania namnożonych pędów *Arnica montana* L. Rośliny arniki górskiej rozmnażano z wierzchołków pędów, które umieszczano na agarowym (0,7%) podłożu MS z dodatkiem NAA (0,1 mg $\cdot l^{-1}$) oraz jedną z czterech cytokinin, tj. BAP, KIN, 2iP oraz Z w stężeniach: 0,5; 1,0; 1,5; 2,0 mg $\cdot l^{-1}$. Po 5 tygodniach kultury *in vitro* otrzymane pędy w celu ukorzenienia przenoszono na agarowe podłoże MS z dodatkiem NAA w ilości 0; 0,05; 0,1; 0,2 mg $\cdot l^{-1}$. Najwięcej eksplantatów tworzących pędy oraz ich największą liczbę stwierdzono na pożywce MS z dodatkiem 0,5 mg$\cdot l^{-1}$ BAP. Na długość wytwarzanych pędów największy wpływ miała KIN w stężeniu 1,5 i 2,0 mg$\cdot l^{-1}$. W przypadku Z nie stwierdzono zmiany długości i masy pędów, zaś większą liczbę pędów obserwowano w stężeniach od 1,0 do 2,0 mg$\cdot l^{-1}$. Otrzymane pędy charakteryzowały się dużą zdolnością do ryzogenezy. Najwięcej pędów ukorzeniło się na pożywce zawierającej 0,1 mg$\cdot l^{-1}$ NAA. Na tej pożywce otrzymano również rośliny o większej liczbie i masie korzeni, natomiast najdłuższe korzenie stwierdzono przy stężeniu 0,05 mg$\cdot l^{-1}$ NAA.

Słowa kluczowe: mikrorozmnażanie, arnika górska, regulatory wzrostu

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