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MICROPROPAGATION OF *Allium neapolitanum* Cirillo

Magdalena Stelmaszczuk, Danuta Kozak
University of Life Sciences in Lublin

Abstract. *Allium neapolitanum* is a valuable species of snow-white flowers, which is suitable for cultivation in flowerbeds, rock gardens as well as in containers. Whole buds of *Allium neapolitanum* were excised from bulbs in the beginning of October and then they were cultured on Murashige and Skoog (MS) medium containing 2 mg BA·dm^{-3} and 0.1 mg NAA·dm^{-3} for shoot initiation. After several passages on the same medium for shoot multiplication, bases of shoots were placed for 2 subcultures on MS medium supplemented with BA or 2-iP in concentration of 2 or 5 mg·dm^{-3} separately or in combination with NAA in concentration of 0.1 or 1 mg·dm^{-3} to obtain multiplication. MS medium without growth regulators was used as a control. The best results were obtained on the medium supplemented with 5 mg BA·dm^{-3} and 0.1 mg NAA·dm^{-3}. On average 5.7 shoots regenerated from 1 shoot base during 12 weeks. Three types of auxins, IAA, IBA and NAA in concentration of 0.5 mg·dm^{-3}, were used for rooting. It was observed that NAA enhanced root formation but reduced roots length. The best quality rooted shoots were obtained on medium supplemented with 0.5 mg·dm^{-3} IAA. The survival rate of the plantlets under *ex vitro* condition was 70% after 4 weeks.

Key words: auxin, cytokinin, axillary shoot induction, rooting, acclimatization

ABBREVIATIONS

BA – 6-benzylaminopurine,
2-iP – 6-γ,γ-(dimethylallylamino)-purine,
IAA – indole-3-acetic acid,
IBA – indole-3-butyric acid,
NAA – naphthalene-acetic acid,
MS – Murashige and Skoog medium.

Corresponding author: Danuta Kozak, Institute of Ornamental Plants and Landscape Architecture, University of Life Sciences in Lublin, 58 Leszczyńskiego Str. 20-068 Lublin, Poland, tel. (+48) 81 533-82-41 e-mail: danuta.kozak.@up.lublin.pl

INTRODUCTION

Genus *Allium* contains around 500–700 species [De Hertogh and Le Nard 1993]. There are edible, medicinal and ornamental species among them. In Poland, there are around 20 ornamental species in cultivation and their assortment increases continuously. *A. neapolitanum* is a bulbous ornamental perennial with round umbels consisting of many snow-white, sweet scented flowers. It blooms in April. Leaves are strap-shaped, greeny-greyish, which dry up during blooming. It reaches 25–30 cm of height. The species looks beautifully in flowerbeds, rock gardens and containers. It may be grown anywhere in full sun and in well drained soil.

The propagation rate of many species of *Allium* in the field cultivation is very slow and it takes many years to produce a new variety so that *in vitro* propagation technique has been studied by numerous researchers [Dunstan and Short 1977, Evenor et al. 1997 a,b, Xu et al. 2005, Cho et al. 2007, Mehta et al. 2013]. The first works on the subject of *in vitro* propagation of *Allium* were carried out in the 70s and they related to *Allium sativum* [Havranek and Novak 1973, Abo El-Nil 1977], *Allium cepa* [Dunstan and Short 1977, George et al. 1987] and *Allium porrum* [Debergh and Standaert-de Metsenaere 1976].

More publications on *in vitro* culture of alliums appeared in later years. The studied species were: *Allium ampeloprasum* [Seabrook 1994, Ziv and Lilien-Kipnis 1997], *Allium ascalonicum* [Hidayat 2005, Cho et al. 2007], *Allium cepa* [Mohamed-Yasseen and Splittstoesser 1992, Quinta-Sierra et al. 2005], *Allium sativum* [Xu et al. 2005, Kim et al. 2006] and of ornamental alliums the research was done on *Allium aflatunense* [Evenor et al. 1997 a,b, Subotić et al. 2006] and *Allium giganteum* [Inagaki et al. 1992, 1994, Šušek et al. 2002].

It was shown that explants containing meristematic tissue is the most suitable for multiple shoot regeneration of *Allium tuberosum* [Pandey et al. 1992], *A. sativum* [Ziv et al. 1983, Mohamed-Yasseen et al. 1994], *A. ascalonicum* [Mohamed-Yasseen et al. 1994] and *A. ampeloprasum* [Ziv et al. 1983, Gantait et al. 2009]. Several authors reported that higher cytokinin (BA 1.8–6 mg·dm^{-3}) and the lower auxin (NAA 0.2–1 mg·dm^{-3}) level promoted direct multiple shoot induction from shoot tips in *A. ampeloprasum* [Ziv et al. 1983], *A. sativum* [Mohamed-Yasseen et al. 1994]. A medium supplemented with cytokinin (BA 0.5 mg·dm^{-3} or kinetin 2.5 mg·dm^{-3}) only without any addition of auxins, was found to be the most efficient for initiation of multiple shoots of *A. tuberosum* [Pandey et al. 1992] and *A. ampeloprasum* [Gantait et al. 2009]. No reports on the *in vitro* propagation of *A. neapolitanum* have been published so far. In the present study the *in vitro* propagation method of *Allium neapolitanum* Cirillo based on shoot cultures is described.

MATERIAL AND METHODS

Initiation of culture. *Allium neapolitanum* bulbs of 5 cm in circumference obtained from the M. Thoolen B.V. Velserbroek – Holland were used in the experiment. After digging out the bulbs were soaked in 0.4% Topsin M 500 SC (Nippon Soda Tokyo)

solution for 60 minutes and then kept in a storage room with air circulation at the temperature of 20–22°C. In the beginning of October outward scales were stripped out and bulbs were thoroughly washed in water with addition of detergent, disinfected with 0.4% Topsin solution for 60 minutes, dipped in 70% ethyl alcohol for a few seconds and then in a sodium hypochlorite solution (NaOCl) containing 2% of active chlorine for 30 minutes. After disinfection, bulb fragments were rinsed with sterile distilled water. Whole buds (shoot tips with 3 leaf primordia) of 2–3 mm length, and 2 mm of base width were excised. Before being put on the media, they were dipped in sodium hypochlorite solution containing 1% of active chlorine for 15 minutes and rinsed with sterile distilled water.

Isolated explants were placed in test tubes of 25 × 100 mm dimensions on Murashige and Skoog [1962] medium containing mineral salts, thiamine – 0.4 $mg \cdot dm^{-3}$, pyridoxine – 0.5 $mg \cdot dm^{-3}$, nicotinic acid – 0.5 $mg \cdot dm^{-3}$, glycine – 2 $mg \cdot dm^{-3}$, myo-inositol – 100 $mg \cdot dm^{-3}$, sucrose – 30 $g \cdot dm^{-3}$, and Agar-Agar (Lab-Agar^{TM} Biocorp) – 6.5 $g \cdot dm^{-3}$ and growth regulators: BA in concentration of 2 $mg \cdot dm^{-3}$ and NAA in concentration of 0.1 $mg \cdot dm^{-3}$. The pH of the medium was adjusted to 5.7 before autoclaving. Sprouting shoots were further multiplied on the same medium in Erlenmeyer flasks to produce plant stock. Cultures were subcultured every 6 weeks. The cultures were maintained at 22°C, with light intensity of 35 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and in 16-h photoperiod.

Effect of BA, 2-iP and NAA on shoot multiplication. Individual shoots isolated from shoot clumps were trimmed off and shoots bases were cultured on MS medium supplemented with BA or 2-iP in the concentration of 2 or 5 $mg \cdot dm^{-3}$ separately or in combination with NAA in concentration of 0.1 or 1 $mg \cdot dm^{-3}$. MS medium without growth regulators was a control. Shoots obtained after six weeks of culture were cut off, roots were removed and bases of shoots were transferred onto the same medium for another 6 weeks. Each treatment consisted of 35 explants, with 7 replicates. Cultures were kept in Erlenmeyer flasks of 250 ml capacity. The experiment was repeated twice.

Rooting of in vitro propagated shoots and growth of plantlets *ex vitro*. To induce rooting, shoots were excised from the multiple shoot clusters proliferated on the MS medium supplemented with 2.0 mg $BA \cdot dm^{-3}$ and 0.1 mg $NAA \cdot dm^{-3}$. Individual shoots of 60–65 mm length were transferred to MS medium containing 0.5 $mg \cdot dm^{-3}$ of IAA, IBA or NAA. MS medium without growth regulators was used as a control.

The cultures were kept in Erlenmeyer flasks of 250 ml capacity. Each combination consisted of 5 explants (1 flask) with 4 repetitions.

In order to acclimatize the *in vitro* propagated plantlets, 20 shoots rooted on MS medium supplemented with 0.5 $mg \cdot dm^{-3}$ of IAA, were transferred to plastic boxes filled with peat and perlite (1:1 v/v). Boxes were covered with plastic film. The experiment was repeated twice.

Data collection and statistical analysis. In the experiment concerning effect of BA, 2-iP and NAA on shoot multiplication the following values were measured after the first (6 weeks) and second (12 weeks) subculture: length of the main shoot, number and length of axillary shoots, number and length of roots. Data collected after 4 weeks of rooting included: number and length of roots, length of the main shoot and number of regenerating shoots. During acclimatization stage of plants the following features were evaluated after 4 weeks: survival rate (%), plants height, width of bulb thickening, num-

ber of axillary shoots, number and length of roots. The results of the experiments were analyzed statistically with the use of a standard statistical procedure with one factorial design and the Tukey test was used to estimate the significant differences between the means at a 5% level of significance.

RESULTS AND DISCUSSION

Effect of BA, 2-iP and NAA on shoot multiplication. The growth regulators used in the experiment inhibited elongation of the main shoot in the first subculture, however the influence was not significant statistically. On the media supplemented with 5 mg BA·dm^{-3} and 0.1 mg NAA·dm^{-3} main shoots were the shortest (55.0 mm). The control medium promoted elongation of the main shoot to the highest extent (85 mm). Similar tendencies could be observed in the second subculture and in the case of axillary shoots length. No significant differences between axillary shoots length in studied treatments were observed (tab. 1).

Table 1. ffect of BA and NAA on the main and axillary shoots growth and development of *Allium neapolitanum*

Growth regulators in subculture media (mg·dm^{-3})	First subculture			Second subculture		
	length of main shoot (mm)	number of axillary shoots	length of axillary shoots (mm)	length of main shoot (mm)	number of axillary shoots	length of axillary shoots (mm)
0	85.0a*	1.9b	44.5a	116.0 a	2.5 c	40.0a
BA 2	63.0a	2.2ab	33.4a	58.0 c	4.2 b	29.2a
BA 2+NAA 0.1	60.0a	3.1a	29.3a	62.0 bc	4.8 a	28.2a
BA 2+NAA 1	74.0a	2.9a	27.9a	69.0 b	3.5 bc	25.7a
BA 5	61.0a	3.2a	23.5a	61.0 bc	4.6 ab	21.2a
BA 5+NAA 0.1	55.0a	3.5a	21.3a	53.0 c	5.7 a	18.7a
BA 5+NAA 1	59.0a	3.1a	23.8a	55.0 c	4.5ab	20.2a
Mean	65.0	2.8	29.1	68.0	4.2	26.2

*Values in vertical columns followed by the same letter do not differ significantly at P = 0.05

The growth regulators used in the experiment significantly affected the number of regenerating shoots in both subcultures. The smallest number of shoots was obtained on the control medium (1.9 and 2.5 appropriately). The significant effect of BA concentration was noted, however, it was observed that the higher concentration of BA (from 2 to 5 mg·dm^{-3}) the bigger number of shoots (2.2 and 3.2 appropriately). Looking at the effect of addition of NAA in concentration of 0.1 and 1 mg·dm^{-3} to the media the slight increase in number of shoots at NAA used in lower concentration. Similar tendencies were observed in the second subculture. The most axillary shoots in both subcultures

were obtained on the media with addition of 5 mg BA·dm^{-3} and 0.1 mg NAA·dm^{-3} (2.5 and 5.7 appropriately) (tab. 1, fig. 1).

Table 2. Effect of BA and NAA on the regeneration and growth of *Allium neapolitanum* roots

Growth regulators in subculture media (mg·dm^{-3})	First subculture		Second subculture	
	number of roots	length of roots (mm)	number of roots	length of roots (mm)
0	10.3a*	18.9a	11.2 b	31.4 a
BA 2	4.8b	7.5b	7.1 c	7.6 bc
BA 2+NAA 0.1	5.5b	4.2b	1.8 f	3.9 c
BA 2+NAA 1	8.8a	5.5b	12.4 a	6.2 bc
BA 5	4.0b	5.8b	4.9 d	8.8 b
BA 5+NAA 0.1	3.7b	7.5b	3.7 e	5.1 bc
BA 5+NAA 1	3.5b	6.6b	4.3 de	7.4 bc
Mean	5.8	8.0	6.5	10.0

*See explanation Table 1

The significant influence of BA and NAA on number and elongation of roots could be observed (tab. 2). The presence of BA separately and BA in combination with NAA in the media significantly influenced rhizogenesis in the first subculture. The most roots were obtained on the control medium (10.3). The addition of BA in concentration of 2 mg·dm^{-3} and NAA in concentration of 1 mg·dm^{-3} to the media inhibited rhizogenesis in the smallest degree (8.8) while the media supplemented with BA in concentration of 5 mg·dm^{-3} and NAA in concentration of 1 mg·dm^{-3} had the most inhibiting effect on roots development (3.5). The most roots were formed on the media containing 2 mg BA·dm^{-3} and 1 mg NAA·dm^{-3} (12.4) and on the control one (11.2) (second subculture).

The elongation of roots was the most promoted on the control medium (18.9 mm after the first subculture and 31.4 mm after the second subculture). The addition of BA or BA in combination with NAA to the media strongly inhibited the elongation of roots (tab. 2).

2-iP added to the media separately or in combination with NAA had no significant effect on main shoot growth (tab. 3). Elongation of axillary shoots was stimulated the most on the media containing 2 mg·dm^{-3} 2-iP and NAA in concentration of 1 mg·dm^{-3} (51.7 mm) or on the media supplemented with 5 mg 2-iP·dm^{-3} and 0.1 mg NAA·dm^{-3} (52.5 mm) in the first subculture and on the media with addition of 2-iP in concentration of 2 mg·dm^{-3} (50.8 mm) in the second subculture.

The significant effect of 2-iP and 2-iP in combination with NAA on the number of axillary shoots regenerating in the first subculture was not noted, however the smallest number of axillary shoots was obtained on the control medium (1.2). In the first subculture the highest multiplication coefficient was observed in the presence of 2-iP in concentration of 5 mg·dm^{-3} (2.0). In the second subculture, the number of axillary shoots

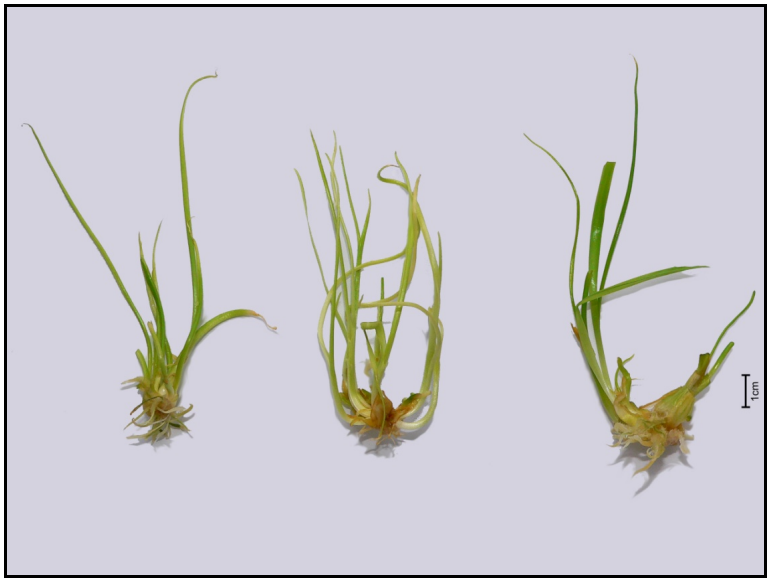

Fig. 1. Shoot clusters of *Allium neapolitanum* obtained after 2 passages on the MS medium supplemented with: BA 2 mg·dm^{-3}, BA 2 mg·dm^{-3} + NAA 0.1 mg·dm^{-3}, BA 2 mg·dm^{-3} + NAA 1 mg·dm^{-3} (left-to-right)

Fig. 2. Shoot clusters of *Allium neapolitanum* obtained after 2 passages on the MS medium supplemented with: 2iP 2 mg·dm^{-3}, 2iP 2 mg·dm^{-3} + NAA 0.1 mg·dm^{-3}, 2iP 2 mg·dm^{-3} + NAA 1 mg·dm^{-3} (left-to-right)

Table 3. Effect of 2-iP and NAA on the of main and axillary shoots growth and development of *Allium neapolitanum*

Growth regulators in subculture media ($mg \cdot dm^{-3}$)	First subculture			Second subculture		
	length of main shoot (mm)	number of axillary shoots	length of axillary shoots (mm)	length of main shoot (mm)	number of axillary shoots	length of axillary shoots (mm)
0	55.0a*	1.2a	32.0bc	51.0a	2.1b	28.2bc
2-iP 2	59.0a	1.6a	48.9ab	55.0a	2.3b	50.8a
2-iP 2+NAA 0.1	55.0a	1.7a	29.8c	65.0a	2.5ab	31.3abc
2-iP 2+NAA 1	50.0a	1.7a	51.7a	64.0a	3.0ab	36.7ab
2-iP 5	47.0a	2.0a	25.5c	42.0a	3.5a	14.5c
2-iP 5+NAA 0.1	55.0a	1.8a	52.5a	45.0a	2.9ab	25.3bc
2-iP 5+NAA 1	45.0a	1.5a	35.0bc	57.0a	2.4b	13.5c
Mean	52.0	1.6	39.3	54.0	2.7	28.6

*See explanation Table 1

ranged from 2.1 on the control medium to 3.5 on the media supplemented with 2-iP in concentration of 5 $mg \cdot dm^{-3}$. The value differed significantly from the number of shoots obtained on the control medium and on the media supplemented with 2-iP in concentration of 2 $mg \cdot dm^{-3}$ and 2-iP 5 $mg \cdot dm^{-3}$ and NAA in concentration of 1 $mg \cdot dm^{-3}$ (tab. 3, fig. 2).

Table 4. Effect of 2-iP and NAA on the regeneration and growth of *Allium neapolitanum* roots

Growth regulators in subculture media ($mg \cdot dm^{-3}$)	First subculture		Second subculture	
	number of roots	length of roots (mm)	number of roots	length of roots (mm)
0	8.7a*	23.4a	9.7b	22.8a
2-iP 2	6.3bc	12.6b	7.4b	15.6b
2-iP 2+NAA 0.1	10.8a	8.9c	10.8a	16.8b
2-iP 2+NAA 1	8.9a	6.4c	13.5a	7.3c
2-iP 5	4.9c	6.7c	7.9b	10.6bc
2-iP 5+NAA 0.1	5.9bc	7.5c	8.7b	11.9bc
2-iP 5+NAA 1	8.1ab	6.9c	10.4ab	8.5c
Mean	7.6	10.3	9.8	13.4

*See explanation Table 1

The significant effect of 2-iP and NAA on number of roots was observed. The most roots in the first subculture were observed in the presence of 2 mg 2-iP·dm^{-3} and 0.1 mg NAA·dm^{-3} (10.8). However, the significant differences in number of roots on the media: 2 mg 2-iP·dm^{-3} and 1 mg NAA·dm^{-3} (8.9), control medium (8.7) and 5 mg 2-iP·dm^{-3} and 1 mg NAA·dm^{-3} (8.1) was not observed. In the second subculture the most roots regenerated on the following media: 2-iP in concentration of 2 mg·dm^{-3} and NAA in concentration of 1 mg·dm^{-3} (13.5), 2-iP in concentration of 2 mg·dm^{-3} and NAA in concentration of 0.1 mg·dm^{-3} (10.8), 2-iP in concentration of 5 mg·dm^{-3} and NAA in concentration of 1 mg·dm^{-3} (10.4).

The roots were significantly longer on the control medium (23.4 and 22.8 mm appropriately). Addition of 2-iP separately or in combination with NAA significantly inhibited elongation of roots.

In the presented study the most efficient induction of axillary shoots occurred on MS medium containing 5 mg BA·dm^{-3} and 0.1 mg NAA·dm^{-3}. The obtained results are in agreement with other reports that BA and NAA promote induction of shoots. Xu et al. [2008] reported that for *Allium chinense* the most effective combination of growth regulators to promote shoots induction were BA in concentration of 1 mg·dm^{-3} with combination of NAA in concentration of 1 mg·dm^{-3}, in presence of which around 17 shoots were produced per cluster during 8 weeks. Nagakubo et al. [1993] obtained the most multiple shoots of *Allium sativum* on the medium containning 2.3 mg·dm^{-3} BA and 0.9 mg·dm^{-3} NAA. Higher cytokinin level (6 mg BA·dm^{-3}) in combination with NAA (1 mg·dm^{-3}) induced multiple axillary shoots growth in *Allium ampeloprasum* [Ziv et al. 1983]. Similarly, addition of BA (0.5–1.2 mg·dm^{-3}) separately to the medium promoted multiple shoot culture in *Allium tuberosum* [Pandey et al. 1992, Haque 1998, 2000].

Results of our experiment with the use of 2-iP separately or in combination with NAA proved less stimulatory effect of this cytokinin on multiple shoot induction in comparison to BA. The positive effect of 2-iP in combination with NAA was observed in *Allium* by many researches. Kim et al. [2006] noted the highest number of *Allium sativum* multiple shoots (7.0) on LS medium with 2-iP in concentration of 5 mg·dm^{-3} and NAA in concentration of 0.2 mg·dm^{-3}. Medium containing 6–8 mg 2-iP·dm^{-3} and 1–2 mg NAA·dm^{-3} was used for induction and multiplication of *Allium porrum* shoots from basal region [Dunstan and Short 1979]. Much lower level of 2-iP (0.5 mg·dm^{-3}) with combination of 0.1 mg NAA·dm^{-3} was recommended for multiplication of *Allium sativum* by Makowska and Kotlińska [2001].

In the present study shoot clumps formed roots during multiplication stages. Presence of cytokinins alone in the medium strongly inhibited this process. It is well known that cytokinins have an inhibitory effect on rhizogenesis [Brault and Maldiney 1999, Werner et al. 2001, Stenlid 2006, George et al. 2008, Kuderová and Hejátko 2009, Wróblewska 2012]. In studies on *Hippeastrum johnsonii* Zakizadeh et al. [2013], used different concentrations of 2-iP (0, 14, 16 and 18 mg·dm^{-3}) and NAA (1, 2, 4 mg·dm^{-3}) for rooting bulblets. A well developed root system was achieved in the MS medium containing 16 mg·dm^{-3} 2-iP and 4 mg·dm^{-3} NAA. Ružić and Vujović [2008] observed rooting in multiplication phase of *Prunus avium* with the use of medium with kinetin 0.2–0.4 mg·dm^{-3} and IBA 1 mg·dm^{-3} (100 % rooted shoots) or 2-iP 0.2 mg·dm^{-3} and IBA 1 mg·dm^{-3} (77.2 % rooted shoots).

Rooting of in vitro propagated shoots and growth of plantlets ex vitro. Among the three auxins used in the experiment, NAA had the most positive effect on roots induction (6.2), while addition of IAA and IBA inhibited rhizogenesis. It was noted that the longest roots formed on the control medium, without addition of auxins (26.3 mm), and the shortest on the medium with addition of NAA in concentration of 0.5 mg·dm^{-3} (13.7 mm) (tab. 5, fig. 3).

The length of the main shoot ranged from 165.8 mm on the medium with addition of 0.5 mg NAA·dm^{-3} to 174.7 mm in the presence of 0.5 mg·dm^{-3} IAA. There was not significant differences observed between treatments used. It was noted, that the addition of IBA in concentration of 0.5 mg·dm^{-3} had the most inhibitory effect on the number of axillary shoots (tab. 5).

In the presented study rhizogenesis occured on all tested media. The most roots were observed on the medium supplemented with NAA in concentration of 0.5 mg·dm^{-3}, but obtained roots were the shortest and stunned. On the medium containing IAA in concentration of 0.5 mg·dm^{-3} the microshoots grew the most intensively and they formed roots of a good quality. Similarly, Gantait et al. [2009] reported that well developed roots of *Allium ampeloprasum* were obtained on the MS medium with 0.5 mg IAA·dm^{-3}. To root shoots of *Allium wallichii* Wawrosch et al. [2001] recommended MS medium supplemented with 2 mg·dm^{-3} IBA. In *Allium sativum*, 1 mg·dm^{-3} IBA proved to be the best for *in vitro* rooting [Mehta et al. 2013]. MS medium without auxins was used in rooting stage of *Allium sativum* [Ma et al. 1994, Haque et al. 1997] and *Allium tuberosum* [Song et al. 2002].

Table 5. Effect of auxins on the growth and development of *Allium neapolitanum* roots and shoots after 4 weeks of *in vitro* culture

Auxin (0.5 mg·dm^{-3})	Number of roots	Length of roots (mm)	Length of main shoot (mm)	Number of axillary shoots
0 (control)	5.0b	26.3a	175.4a*	2.5a
IAA	5.3b	24.3ab	184.7a	2.2a
IBA	5.0b	15.9bc	173.5a	1.4b
NAA	6.2a	13.7c	165.8a	2.1a
Mean	5.4	20.1	174.9	2.1

*See explanation Table 1

Allium neapolitanum plants obtained on the medium supplemented with IAA in concentration of 0.5 mg·dm^{-3} and placed in the mixture of peat and perlite survived in 70% and they reached 218.8 mm height. They formed 2.6 axillary shoots, 6.4 roots of the mean length of 342.6 mm. The width of bulb thickening was 8.5 mm (tab. 5, fig. 4).

Tubić et al. [2011] reported that plants of *Allium schoenoprasum* survived acclimatization very successfully (95%) in pots with a mixture of compost and sand (2:1). Also over 95% of *in vitro* propagated plantlets of *Allium chinense* were successfully acclima-

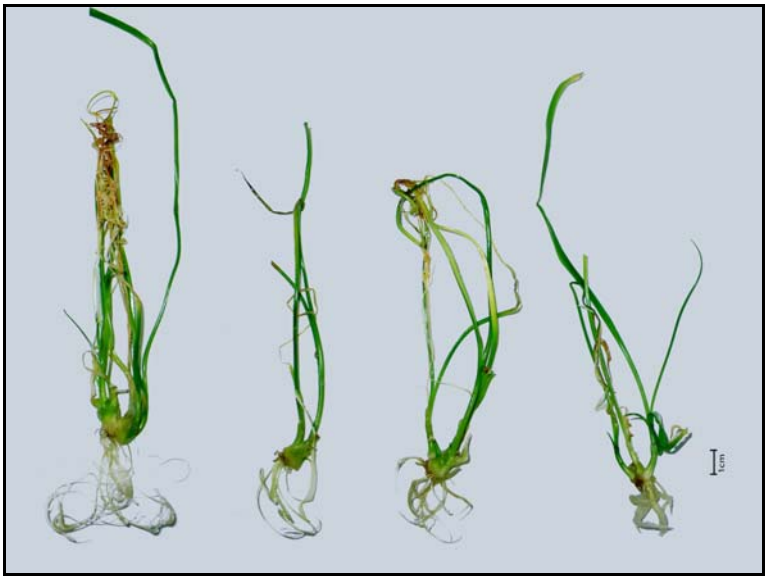

Fig. 3. Microshoots of *Allium neapolitanum* after 4 weeks of rooting on the MS medium supplemented with: MS 0 (control), IAA 0.5 mg·dm^{-3}, IBA 0.5 mg·dm^{-3}, NAA 0.5 mg·dm^{-3} (left-to-right)

Fig. 4. *Allium neapolitanum* plant after 4 weeks of *ex vitro* growth

Table 6. Growth and development of *Allium neapolitanum* plants after 4 weeks of *ex vitro* cultivation

% of survival	Plants height (mm)	Width of bulb thickening (mm)	Number of developing shoots	Number of roots	Roots length (mm)
70.0	218.8	8.5	2.6	6.4	342.6

tized in pots filled with compost [Xu et al. 2008]. Bekheet [2004] noted the highest percentage of *Allium sativum* plants survival when peatmoss and perlite mixture (1:1) were used as the transplanting medium.

CONCLUSIONS

1. The best multiplication of *Allium neapolitanum* shoots were obtained on MS medium supplemented with BA in concentration of 5 $mg \cdot dm^{-3}$ and NAA in concentration of 0.1 $mg \cdot dm^{-3}$. On average 5.7 shoots regenerated from 1 shoot base during 12 weeks.
2. Cytokinins had an inhibitory effect on rhizogenesis of shoot clumps during 2 subsequent subcultures.
3. Among the 3 types of auxins (IAA, IBA NAA, each used in concentration of 0.5 $mg \cdot dm^{-3}$), added to the culture medium for rooting, NAA enhanced root formation but reduced their length. The best quality of rooted shoots was achieved on the medium supplemented with IAA.
4. The survival rate of the plantlets under *ex vitro* conditions in a peat : perlite (1:1 v/v) mixture after 4 weeks was 70%.

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MIKROROZMNAŻANIE *Allium neapolitanum* Cirillo

Streszczenie. *Allium neapolitanum* to cenny gatunek o ozdobnych śnieżnobiałych kwiatach, nadający się do uprawy na rabatach, skalniakach i w pojemnikach. Do badań wykorzystano całe pąki *Allium neapolitanum,* które izolowano z cebul na początku października i wykładano na pożywkę Murashige i Skooga (MS) zawierającą 2 mg BA·dm^{-3} i 0,1 mg NAA·dm^{-3} w celu inicjacji pędów. Po kilku pasażach namnażania na tej samej pożywce pobierano podstawę pędów, które kultywowano w ciągu 2 pasaży na pożywce MS uzupełnionej BA lub 2iP w stężeniu 2 lub 5 mg·dm^{-3} pojedynczo lub łącznie z NAA w stężeniu 0,1 lub 1 mg·dm^{-3}. Kontrolę stanowiła pożywka MS bez regulatorów wzrostu. Najlepsze wyniki namnażania pędów uzyskano na pożywce z dodatkiem 5 mg BA·dm^{-3} i 0,1 mg NAA·dm^{-3}, na której z jednej podstawy pędu otrzymano 5,7 pędów po 12 tygodniach kultury. Do ukorzeniania pędów zastosowano trzy auksyny: IAA, IBA i NAA

w stężeniu 0,5 mg·dm^{-3}. W obecności NAA uzyskano największą liczbę korzeni, lecz były one najkrótsze. Ukorzenione pędy najlepszej jakości otrzymano na pożywce uzupełnionej 0,5 mg·dm^{-3} IAA. Rośliny przenoszone do podłoża do warunków *ex vitro* przyjmowały się w 70%.

Słowa kluczowe: auksyna, cytokinina, indukcja pędów kątowych, ukorzenianie, aklimatyzacja

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