

COMPARISON OF *Allium aflatunense* B. Fedtsch. 'PURPLE SENSATION' AND *Allium karataviense* Regel. 'IVORY QUEEN' REGENERATIVE CAPABILITIES IN TISSUE CULTURE

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Abstract. *Allium aflatunense* and *Allium karataviense* are valuable bulbous plants grown in a garden. Moreover, *Allium aflatunense* is one of the most decorative species for cut flowers industry. These plants characterize with a low multiplication rate. Immature inflorescence stem sections and leaf bases isolated from '*Allium aflatunense* 'Purple Sensation' and *Allium karataviense* 'Ivory Queen' bulbs in October were used as source of explants for shoot regeneration. The use of a two – step sterilization procedure (2% Cl⁻ – 30 min and 1% Cl⁻ – 15 min) gave much better results than a one – step (2% Cl⁻ – 30 min). Inflorescence explants in inverted orientation were cultured on a Murashige and Skoog [1962] medium containing BA in concentration of 2 mg·dm⁻³ and NAA in concentration of 1 mg·dm⁻³. Leaf bases placed in normal and inverted orientation were incubated on MS medium supplemented with: BA 2 mg·dm⁻³ plus NAA 1 mg·dm⁻³, BA 5 mg·dm⁻³ plus NAA 1 mg·dm⁻³ and TDZ 1 mg·dm⁻³ plus NAA 1 mg·dm⁻³. In subsequent experiments clumps consisting of 2 shoots were cultured on MS medium containing BA 0.5, 1, 2 mg·dm⁻³ with NAA 0.1 mg·dm⁻³ and BA 2 mg·dm⁻³ with IAA 0.1 mg·dm⁻³ for 6 weeks. A multiplication rate during four subcultures on medium with addition of BA 0.5 mg·dm⁻³ and NAA 0.1 mg·dm⁻³ was also examined. Explants from young inflorescences and from immature leaf bases were found effective to form shoots. The orientation of leaf explants did not have a significant effect on the number of regenerating shoots. 5.7 to 7.5 shoots were achieved for *Allium aflatunense* 'Purple Sensation' on studied media during 6 weeks of multiplication and 3.5 to 5.1 shoots for *Allium karataviense* 'Ivory Queen'. Multiplication rate of 31.3 shoots for *Allium aflatunense* 'Purple Sensation' and 26.6 shoots for *Allium karataviense* 'Ivory Queen' was possible to obtain on medium supplemented with BA 0.5 mg·dm⁻³ and NAA 0.1 mg·dm⁻³ after 32 weeks of culture.

Key words: ornamental alliums, inflorescence stem, leaf base, polarity explants, shoot multiplication

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,
TDZ – thidiazuron,
MS – Murashige and Skoog medium,
NaOCl – sodium hypochlorite.

INTRODUCTION

One of the most decorative ornamental alliums is *Allium aflatunense* ‘Purple Sensation’ with pink purple flowers gathered in spherical inflorescences of 10 cm in diameter which grow on 90–150 cm tall stalks. These plants are ideal for containers, mass planting, borders and as cut flowers. They bloom from late spring to early summer. Another valuable ornamental plant is *Allium karataviense* ‘Ivory Queen’. Because of its short stems it is suitable for planting in containers and in rock gardens. It produces balls of white flowers on 20 cm stems in May and June. The stems are held above rosettes of wide, pleated, fleshy and leathery, silver-grey leaves.

There are few articles on *in vitro* propagation of ornamental *Allium*. They concern mainly two species: *Allium aflatunense* [Evenor et al. 1997a, b, Subotić et al. 2006] and *Allium giganteum* [Inagaki et al. 1992, 1994, Šušek et al. 2002].

Very important step in culture *in vitro* is sterilization of explants for aseptic culture establishment. Explant contamination depends on many factors as species, plant age, explants source, weather condition. Underground organs, like bulbs, tubers or rhizomes are very difficult to reduce contaminations [Custers and Bergervoet 1994, Sochacki and Orlikowska 1997, McCartan and Van Staden 1998, Kozak 1999].

In vitro regeneration of *Allium* species was initiated from a range of explant sources, including: shoot tips [Ziv et al. 1983, Pandey et al. 1992, Mohamed-Yasseen et al. 1994, Uzman et al. 1998, Gantait et al. 2009], stem discs [Ayabe and Sumi 1998, Xu et al. 2008], young leaves [Inagaki et al. 1992, Bockish et al. 1997, Fereol et al. 2002, Xu et al. 2009], flower organs [Evenor et al. 1997a, b], basal plates [Barringer et al. 1996, Evenor et al. 1997a, b, Cho et al. 2007, Yan et al. 2009], root tips [Haq et al. 1998, 2000, Robledo-Paz et al. 2000, Khar et al. 2005].

The way of explants position was suggested to influence the rate of regeneration of geophytes. It was found that inflorescence stem pieces of *Gladiolus* [Ziv et al. 1970], leaves explants of *Narcissus* [Seabrook et al. 1976, Kozak 1991] and peduncle-pedicel junctions of *Clivia* [Ran and Simpson 2005] produced more shoots in inverted position than in the non-inverted ones.

For the initiation of most *Allium* species mainly the presence of NAA and BA is required. Shoot regeneration was obtained on Murashige and Skoog [1962] medium (MS) with addition of NAA 0.1–1 mg·dm⁻³ and BA 2–5.6 mg·dm⁻³ [Seabrook 1994, Evenor et al. 1997a, b, Ziv and Lilien-Kipnis 1997, Haq et al. 2000]. For shoot multiplication of

Allium was used MS medium containing: NAA 0.1–1 mg·dm⁻³ and BA 2–4.5 mg·dm⁻³ [Seabrook 1994, Evenor et al. 1997a, b, Haque et al. 2000], IAA 0.5 mg·dm⁻³ and BA 1 mg·dm⁻³, IAA 2 mg·dm⁻³ and kinetin 10 mg·dm⁻³ [Evenor et al. 1997a, b].

The objectives of the study were to elaborate an efficient method of explants sterilization and evaluate the responses of different explants of *Allium aflatunense* 'Purple Sensation' and *Allium karataviense* 'Ivory Queen' on media supplemented with different auxins and cytokinins and subsequent multiplication of obtained shoots. No reports on the *in vitro* propagation of *Allium karataviense* 'Ivory Queen' have been published so far.

MATERIAL AND METHODS

Sterilization of explants. *Allium aflatunense* B.Fedtsch. 'Purple Sensation' and *Allium karataviense* Regel. 'Ivory Queen' bulbs of 18–20 cm circumference obtained from the M. Thoolen B.V. Velsbroek – 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 min and then kept till beginning of October in a storage room with air circulation at the temperature of 20–22°C. At the beginning of experiments many *Allium* explants were discarded because of infection. For this reason sterilization experiment was employed. The bulbs were first thoroughly washed under running tap water and then outward scales were stripped off and bulb fragments were thoroughly washed in water with addition of detergent, disinfected with 0.4% Topsin solution for 60 min, dipped in 70% ethyl alcohol for a few seconds and then dipped in a solution of sodium hypochlorite. Following treatments of sterilization were checked: 2 and 4% of active chlorine for 30 minutes – one-step sterilization, 2 and 4% of active chlorine for 30 minutes and 1% of active chlorine for 15 min – two-step sterilization, 2% of active chlorine for 30 min and 0.5% of active chlorine for 15 min – two-step sterilization (tab. 1). During the first step of sterilization bulb fragments were shaken in a sodium hypochlorite solution. Inflorescence buds surrounded by leaves were excised aseptically and placed on Murashige and Skoog [1962] medium. The cultures were kept in Erlenmeyer flasks of 100 ml capacity. Each treatment consisted of 1 explant (1 flask) with 15 repetitions. Data on contamination percentage was recorded after 6 weeks of culture. Experiment was repeated twice.

Table 1. Methods of sterilizations used in experiment

Sterilization methods	Concentration of Cl ⁻ (%)	Time (min)	Concentration of Cl ⁻ (%)	Time (min)
1	2	30	–	–
2	4	30	–	–
3	2	30	1	15
4	4	30	1	15
5	2	30	0.5	15

1 and 2 – one-step sterilization

3, 4, 5 – two-step sterilization

Basal medium and culture conditions. Murashige and Skoog [1962] (MS) basic medium containing mineral salts, thiamine – $0.4 \text{ mg}\cdot\text{dm}^{-3}$, pyridoxine – $0.5 \text{ mg}\cdot\text{dm}^{-3}$, nicotinic acid – $0.5 \text{ mg}\cdot\text{dm}^{-3}$, glycine – $2 \text{ mg}\cdot\text{dm}^{-3}$, myo-inositol – $100 \text{ mg}\cdot\text{dm}^{-3}$, sucrose – $30 \text{ g}\cdot\text{dm}^{-3}$, and Agar-Agar (Lab-AgarTM Biocorp) – $6.5 \text{ g}\cdot\text{dm}^{-3}$ was used. The pH of the media was adjusted to 5.7 before autoclaving. The cultures were maintained at 22°C , with light intensity of $35 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ from 40 W fluorescent lamps LF and in 16-h photoperiod.

Shoot induction from inflorescence stem sections and basal part of leaves. Inflorescence buds surrounded by 5–6 leaves after sterilization with the 3rd method were placed on basal MS medium and after 4 weeks inflorescence stem and base of leaves were isolated (fig. 1). The flowering stems were cut into 2 mm thick slices and (due to limited number) placed only in inverted position on MS medium containing BA $2 \text{ mg}\cdot\text{dm}^{-3}$ plus NAA $1 \text{ mg}\cdot\text{dm}^{-3}$. The leaf bases, about 3 mm long, were placed in normal and inverted position on MS medium supplemented with BA $2 \text{ mg}\cdot\text{dm}^{-3}$ plus NAA $1 \text{ mg}\cdot\text{dm}^{-3}$, BA $5 \text{ mg}\cdot\text{dm}^{-3}$ plus NAA $1 \text{ mg}\cdot\text{dm}^{-3}$ or TDZ $1 \text{ mg}\cdot\text{dm}^{-3}$ plus NAA $1 \text{ mg}\cdot\text{dm}^{-3}$. The cultures were kept in Erlenmeyer flasks of 250 ml capacity. Each combination consisted of 5 explants (1 flask) with 4 repetitions. The experiment was repeated twice.



Fig. 1. Young leaf bases and inflorescence bud surrounded by leaves taken from *Allium aflatumense* 'Purple Sensation' bulb in October (left-to-right)

Shoot multiplication. Adventitious shoots initiated from primary explants were proliferated on the medium supplemented with BA $0.5 \text{ mg}\cdot\text{dm}^{-3}$ and NAA $0.1 \text{ mg}\cdot\text{dm}^{-3}$ in Erlenmeyer flasks to produce plant stock. Cultures were subcultured every 6 weeks. After several passages multiple shoot clusters were divided into 2–3 segments, each consisting of 2 shoots and used in experiments. The multiplication rate was tested after six weeks on medium supplemented with BA 0.5, 1, 2 $\text{mg}\cdot\text{dm}^{-3}$ with NAA $0.1 \text{ mg}\cdot\text{dm}^{-3}$

or BA 2 mg·dm⁻³ with IAA 0.1 mg·dm⁻³. The multiplication rate during four subcultures with 8 weeks intervals on medium with BA 0.5 mg·dm⁻³ and NAA 0.1 mg·dm⁻³ was examined. The cultures were kept in Erlenmeyer flasks of 250 ml capacity. Each combination consisted of 5 explants (1 flask) with 4 repetitions.

Data collection and statistical analysis. All measurements were made under laminar air flow chamber. In all experiments shoot length was measured from shoot base to leaf peak. 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

Sterilization of explants. Sterilization of explants is one of the very important steps in tissue culture. The influence of sterilization method on contamination rate in tissue culture of *Allium aflatunense* 'Purple Sensation' was observed. The amount of visually clean cultures ranged from 66.6% to 13.3% (tab. 2). The least contaminated explants was noted when disinfection with NaOCl solution was done in two-steps, first in solution containing 2.0% of Cl⁻ for 30 min and then in solution containing 1.0% of Cl⁻ for 15 min. When the method 4 was applied (4% NaOCl – bulbs and 1% NaOCl – explants) softening of tissues, especially basal plate and adjoining layers, was observed.

Table 2. Effect of sterilization method on cultures purity of *Allium aflatunense* 'Purple Sensation' inflorescence buds surrounded by leaves

Sterilization methods	One-step sterilization		Two-step sterilization		Bacterial contaminations		Fungal contaminations		Pure cultures	
	concentration of Cl ⁻ (%)	time (min.)	concentration of Cl ⁻ (%)	time (min.)	(pcs)	(%)	(pcs)	(%)	(pcs)	(%)
	1	2	30	–	–	2	6.7	24.0	80.0	4
2	4	30	–	–	0	0	22.0	73.3	8	26.7
3	2	30	1	15	2	6.7	8.0	26.7	20	66.6
4	4	30	1	15	6	20.0	12.0	40.0	12	40.0
5	2	30	0.5	15	6	20.0	14.0	46.7	10	33.3

1 and 2 – one-step sterilization

3, 4, 5 – two-step sterilization

While observing cleanness of *Allium karataviense* 'Ivory Queen' cultures, the most contaminations were observed when the 1st (2% of NaOCl – bulbs) or the 2nd (4% NaOCl – bulbs) methods of sterilization were used (tab. 3). The use of two-step sterili-

zation increased purity of *in vitro* cultures of examined species. The most advantageous method of sterilization was the 3rd, when NaOCl containing 2.0% of Cl⁻ for 30 min and 1.0% Cl⁻ for 15 min were used for sterilization. Application of the 4th method of disinfection, that is 4% NaOCl – bulbs and 1% NaOCl – explants, caused similar effects as in the case of *Allium aflatunense* ‘Purple Sensation’ softening of tissues, especially those surrounding basal plate.

Table 3. Effect of sterilization method on cultures purity of *Allium karataviense* ‘Ivory Queen’ inflorescence buds surrounded by leaves

Sterilization methods	One-step sterilization		Two-step sterilization		Bacterial contaminations		Fungal contaminations		Pure cultures	
	concentration of Cl ⁻ (%)	time (min)	concentration of Cl ⁻ (%)	time (min)	(pcs)	(%)	(pcs)	(%)	(pcs)	(%)
1	2	30	–	–	10	33.3	18	60.0	2	6.7
2	4	30	–	–	6	20.0	16	53.3	8	26.7
3	2	30	1	15	0	0	6	20.0	24	80.0
4	4	30	1	15	2	6.7	10	33.3	18	60.0
5	2	30	0,5	15	0	0	20	66.7	10	33.3

1 and 2 – one-step sterilization

3,4,5 – two-step sterilization

It is very difficult to disinfect underground organs, like bulbs, [Hussey 1975, Girmen and Zimmer 1988] or tubers [Custers and Bergervoet 1994]. Custers and Bergervoet [1994] observed vast contaminations during initiation of gloriosa cultures, which reached 50% for explants from tuber tips isolated during the first month of tubers storage and 90–100% for tubers stored for 3–5 months. Kozak [1999] obtained the best effect of disinfection of gloriosa – 88.3% of visually pure cultures, when tip buds were used. Fragments of tubers occurred very difficult to disinfect, the effectiveness of disinfection was 6.7%. Kapoor et al. [2008] obtained 70–80% of not contaminated lily explants when they were isolated from bulbs growing in containers in a greenhouse. Contaminations caused many difficulties while initiating narcissus cultures and they were especially high when bulbs were stored for longer time and cooled (10–52% for bulbs stored for 3 months) [Sochacki and Orlikowska 1997]. Squires and Langton [1990] noted that the amount of contaminated narcissus tissues initiated from bulbs stored for one month ranged from 6.7 to 42.4%, depending on the variety. McCartan and van Staden [1998] observed that approximately 20–30% of primary bulb explants of *Scilla natalensis* were sterile.

The solution of sodium hypochlorite effectively sterilized the examined ornamental species. It is commonly used to disinfect plant material: Seabrook [1994], Evenor et al. [1997a, b], Izquierdo et al. [1997], Kozak [1999], Haque et al. [2000], Hidayat [2005].

Shoot induction from inflorescence stem sections and basal part of leaves. No significant differences in regeneration capabilities of inflorescence stems explants taken from bulbs of *Allium aflatunense* 'Purple Sensation' and *Allium karataviense* 'Ivory Queen' were found (tab. 4). Also growth of adventitious shoots was similar in both genotypes.

Table 4. Comparison of regenerative capabilities of inflorescence stem slices of *Allium aflatunense* 'Purple Sensation' and *Allium karataviense* 'Ivory Queen', placed in an inverted position on medium containing BA 2 mg·dm⁻³ plus NAA 1 mg·dm⁻³, after 8 weeks of *in vitro* culture

Taxon	Number of adventitious shoots	Length of adventitious shoots (mm)
<i>Allium aflatunense</i> 'Purple Sensation'	5.4 a	8.6 a
<i>Allium karataviense</i> 'Ivory Queen'	4.8 a	9.3 a

*Means followed by the same letter are not significantly different at $\alpha = 0.05$

The results of the study concerning leaf base explants showed that the average number of adventitious shoots regenerating on this explants depended on plant genotype and growth regulators added to the media (tab. 5). Orientation of explants did not influence significantly adventitious shoots number but the tendency for slight better regeneration (0.2–1 shoot) from inverted explants was observed. The more responsive genotype, *Allium karataviense* 'Ivory Queen', formed 7.1 shoots from 1 inverted explant and 6.4 shoots from 1 explant normally positioned on the medium containing BA 5 mg·dm⁻³ and NAA 1 mg·dm⁻³. This results are not significantly different from those obtained in the presence of TDZ 1 mg·dm⁻³ and NAA 1 mg·dm⁻³ (fig. 2). The greatest number of *Allium aflatunense* 'Purple Sensation' shoots was also obtained on medium supplemented with BA 5 mg·dm⁻³ and NAA 1 mg·dm⁻³. This value was significantly different from shoots number achieved in presence of BA 2 mg·dm⁻³ and NAA 1 mg·dm⁻³.

Analyzing the length of *Allium karataviense* 'Ivory Queen' regenerating shoots it was observed that shoots arising from leaves bases were slightly longer in comparison to shoots of *Allium aflatunense* 'Purple Sensation'. The longest shoots from leaves bases of *Allium karataviense* 'Ivory Queen' placed in a normal position were formed on the media supplemented with BA 5 mg·dm⁻³ and NAA 1 mg·dm⁻³ but this value was significantly different only from the one obtained on the media with addition of BA 2 mg·dm⁻³ and NAA 1 mg·dm⁻³ for the same genotype. The influence of media and explant orientation on growth of *Allium aflatunense* 'Purple Sensation' was not proven.

Table 5. Comparison of regenerative capabilities of leaf bases of *Allium aflatunense* ‘Purple Sensation’ and *Allium karataviense* ‘Ivory Queen’, depended on explant orientation and growth regulators, after 8 weeks of *in vitro* culture

Taxons	Growth regulators (mg·dm ⁻³)	Explants orientation	Number of adventitious shoots	Length of adventitious shoots (mm)
<i>Allium aflatunense</i> ‘Purple Sensation’	BA 2 + NAA 1	natural	2.9 f*	6.9 b
		base up	3.1 ef	7.4 b
	BA 5 + NAA 1	natural	4.4 cd	8.8 ab
		base up	5.4 bc	8.1 ab
	TDZ 1 + NAA 1	natural	4.3 cde	7.9 b
		base up	4.7 cd	8.3 ab
<i>Allium karataviense</i> ‘Ivory Queen’	BA 2 + NAA 1	natural	3.5 def	7.8 b
		base up	4.2 cde	9.2 ab
	BA 5 + NAA 1	natural	6.4 ab	11.9 a
		base up	7.1 a	10.1 ab
	TDZ 1 + NAA 1	natural	5.9 ab	9.5 ab
		base up	6.2 ab	8.3 ab

*Means followed by the same letter are not significantly different at $\alpha = 0.05$

Experiments conducted in this work comparing two types of explants showed high usability of inflorescence stem fragments for micropropagation of *Allium aflatunense* ‘Purple Sensation’ and *Allium karataviense* ‘Ivory Queen’. This is consistent with studies of many authors. Pieces of immature inflorescence stems were used in studies on micropropagation of: *Allium cepa* [Mohamed-Yasseen et al 1993, Mohamed-Yasseen 2002, Marinangeli 2013], *Allium ampeloprasum* [Mohamed-Yasseen 2001], *Allium sativum* [Ayabe and Sumi 1998, Keller and Senula 2013], *Amaryllis belladonna* [De Bruyn et al. 1992], *Narcissus* [Kozak 1991]. Ayabe and Sumi [1998] obtained more than 15 shoots differentiated from a single stem disc of *Allium sativum* on LS medium containing BA 0.1 mg·dm⁻³ and NAA 0.1 mg·dm⁻³. Tubić et al. [2011] comparing effect of 3 cytokinins on shoots induction from an inverted stalk of *Allium schoenoprasum* obtained 4.6 shoots on BA 1.1 mg·dm⁻³, 5.7 on TDZ 1.1 mg·dm⁻³ and 7 on 1.1 mg·dm⁻³ kinetin. Podwyszyńska and Marasek [2003] reported that in presence of TDZ the number of shoots regenerating from flower stalk slices of *Tulip* was twice or 4–5 times higher in comparison to control. According to Kozak [1991], it is possible to obtain 10.4–15.8 adventitious shoots of *Narcissus* ‘Carlton’ from 1 inflorescence stem piece on the media supplemented with 5.6 mg·dm⁻³ BA and 1 mg·dm⁻³ NAA, depending on their position.

To overcome explant contamination many authors use mature inflorescences as a source of explants. Mature inflorescence stalks or floral stems were used for: *Allium aflatunense* and *Allium ampeloprasum* [Ziv and Lilien-Kipnis 1997, 2000], *Allium sati-*

vum [Xu et al. 2005], *Bowiea volubilitis* [Hannweg et al. 1996], *Crinum macowanii* [Slabbert et al. 1995], *Lachenalia* [Niederwieser and Vcelar 1990]. Xu et al. [2005] studied influence of different sizes of inflorescence pieces of *Allium sativum* (< 5 mm, 0.5–1 mm, > 1 mm) on shoot induction. Their results indicated that the bigger garlic inflorescence diameter the larger number of formed shoots (12.8–24.8).

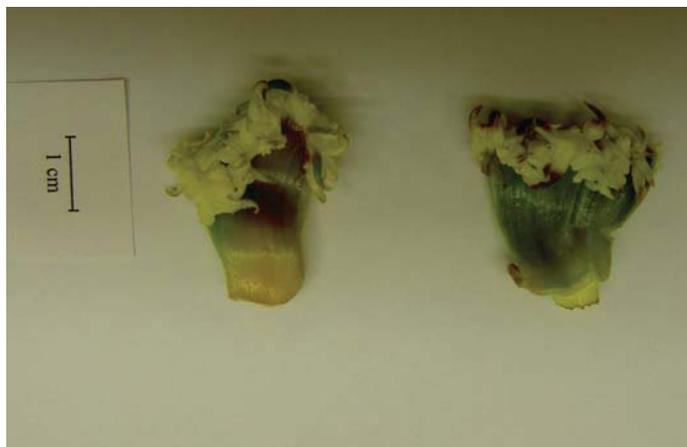


Fig. 2. Adventitious shoots regenerating from young leaf bases of *Allium karataviense* 'Ivory Queen' on MS medium containing BA 5 mg·dm⁻³ and NAA 1 mg·dm⁻³ or TDZ 1 mg·dm⁻³ and NAA 1 mg·dm⁻³, after 4 weeks of *in vitro* culture (left-to-right)

In the presented research a good source of explants were also leaf bases isolated from *Allium aflatanense* 'Purple Sensation' and *Allium karataviense* 'Ivory Queen' bulbs. It is confirmed by results of many works. Hosoki and Asahira [1980] investigating the growth of *Narcissus* 'Geranium' in tissue culture obtained 3 shoots from 1 leaf after 8 weeks of culture on the media supplemented with BA 5 mg·dm⁻³ and NAA 1, while Kozak [1991] obtained 10.2 shoots of *Narcissus* 'Carlton' after 10 weeks of culture in the presence of BA 5.6 mg·dm⁻³ and NAA 1 mg·dm⁻³.

Leaf pieces were a good source of explants to micropropagate: *Allium* [Inagaki et al. 1992, Bockish et al. 1997, Fereol et al. 2002, Chen and Hao 2003, Xu et al. 2008], *Hyacinthus* [Bach 1989], *Lachenalia* [Niederwieser and Vcelar 1990], *Lilium* [Kapoor et al. 2008], *Narcissus* [Chow et al. 1993], *Scilla* [McCartan and Van Staden 1998, Chaudhuri and Sen 2002].

In the presented experiments, satisfactory results of regeneration were obtained from inflorescence stem pieces and leaf bases positioned upside down. In the available literature on the subject there are reports about stimulating multiplication rate by placing bulbous plants explants with apical side down. According to Seabrook et al. [1976] this polarity effect of the tissues may be related to polar transport of auxins that exists in such tissues. Ziv et al. [1970] observed that regeneration of *Gladiolus* from inflorescen-

ce stem pieces depended on its position on the media. Explants placed in an apolar way had better adventitious shoots regenerative capabilities. In the research of Seabrook et al. [1976] and Kozak [1991] leaves explants of *Narcissus* placed on the media with apical side characterized with high regeneration rate. The inverted position of peduncle-pedicle junction explants of *Clivia* resulted in producing more shoots than if they were placed in a natural position [Ran and Simpson 2005]. There are a few reports about positive effects of inverting stem explants on rooting [Pierik and Steegmans 1975, Van der Krieken et al. 1993, Pierik 1997]. Pierik and Steegmans [1975] suggest that oxygen supply at the basal part is much better when the explants are inverted. There are also informations about lack of beneficial effect of inverting explants on shoot regeneration. Slabbert et al. [1995] reported that the most shoots were formed when discs of floral stems of *Crinum macowanii* were placed on the medium with their morphologically basal end. Chow et al. [1993] reported, that leaf explants of *Narcissus* positioned upside down regenerated in a very low rate in comparison to those placed in a natural way. De Bruyn et al. [1992] noted that the orientation of the twin-scale explants of *Amaryllis belladonna* did not have a significant effect on the number of plantlets.

Table 6. Effect of BA and auxins (NAA, IAA) on development and growth of *Allium aflatunense* 'Purple Sensation' and *Allium karataviense* 'Ivory Queen', after 6 weeks of *in vitro* culture

Taxons	Growth regulators (mg·dm ⁻³)	Number of adventitious shoots	Length of adventitious shoots (mm)
<i>Allium aflatunense</i> 'Purple Sensation'	BA 0.5 + NAA 0.1	5.7 bc	24.6 bc
	BA 1 + NAA 0.1	7.5 a	19.6 d
	BA 2 + NAA 0.1	6.7 ab	17.6 d
	BA 2 + IAA 0.1	7.2 ab	22.8 c
<i>Allium karataviense</i> 'Ivory Queen'	BA 0.5 + NAA 0.1	5.1 c	27.9 ab
	BA 1 + NAA 0.1	4.7cd	28.6 a
	BA 2 + NAA 0.1	4.2 cd	25.0 abc
	BA 2 + IAA 0.1	3.5 d	28.1 ab

*Means followed by the same letter are not significantly different at $\alpha = 0.05$

Shoot multiplication. The most shoots occurred on medium supplemented with BA 1 mg·dm⁻³ and NAA 0.1 mg·dm⁻³ for *Allium aflatunense* 'Purple Sensation', although results from this treatment for this genotype were significantly different only from BA 0.5 mg·dm⁻³ and NAA 0.1 mg·dm⁻³ (tab. 6). Multiplication of *Allium karataviense* 'Ivory Queen' shoots was the best on medium supplemented with BA 0.5 mg·dm⁻³ and NAA 0.1 mg·dm⁻³ and statistical differences in comparison to medium with addition of BA 2 with IAA 0.1 mg·dm⁻³ for this genotype were found.

It was observed that BA used in concentrations from 0.5 to 2.0 mg·dm⁻³ together with NAA in concentration of 0.1 mg·dm⁻³ on *Allium aflatunense* 'Purple Sensation' inhibited growth of shoots (tab. 6). Significantly shorter shoots were obtained in the presence of 2 mg·dm⁻³ BA plus 0.1 mg·dm⁻³ NAA in comparison to length of shoots cultured on the media with addition of 0.5 mg·dm⁻³ BA. Comparing the effect of type of cytokinin added to the media already supplemented with 2 mg·dm⁻³ BA it was observed that IAA in concentration of 0.1 mg·dm⁻³ had much more positive influence than addition of BA in concentration of 2 mg·dm⁻³ together with NAA in concentration of 0.1 mg·dm⁻³. There were not significant differences in the length of *Allium karataviense* 'Ivory Queen' shoots on the studied media (tab. 6).

During the 4 subsequent subcultures on MS medium supplemented with BA 0.5 mg·dm⁻³ and NAA 0.1 mg·dm⁻³ increase in number of shoots of both examined genotypes was observed (tab. 7). Analyzing multiplication of *Allium aflatunense* 'Purple Sensation' shoots after the first passage the number of shoots was 18.8 and increased to 31.1 after 32 weeks of culture, that is by 12.3 shoots (fig. 3). The length of shoots in single subcultures also increased and ranged from 13.5 mm (passage 1st) to 25.8 mm (passage 4th).

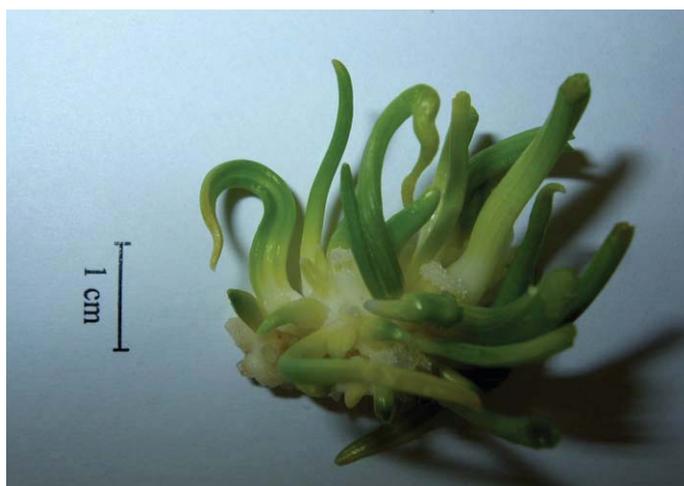


Fig. 3. Adventitious shoot cluster of *Allium aflatunense* 'Purple Sensation' obtained on MS medium containing BA 0.5 mg·dm⁻³ and NAA 0.1 mg·dm⁻³ after 1st passage

After the first subculture of *Allium karataviense* 'Ivory Queen' there were 12.8 shoots and the number of shoots increased to 26.6 after 32 weeks of culture, that is by 13.8 shoots (fig. 4). The length of shoots in single passages was similar. The results showed that regeneration potential of two studied genotypes is similar and that the increasing number of subculture cycles promoted the induction of new shoots. Increase in number of shoots for two studied genotypes was high after the first subculture then after the second and the third ones it was small, and then it increased again. Kahane et al.

[1992] observed that, the number of *Allium cepa* shoots increased after the first subculture and remained stable after 2 months. They suggest that after one month each shoot restored an individual apical dominance, prohibiting further regeneration.



Fig. 4. Adventitious shoot cluster of *Allium karataviense* 'Ivory Queen' obtained on MS medium containing BA $0.5 \text{ mg} \cdot \text{dm}^{-3}$ and NAA $0.1 \text{ mg} \cdot \text{dm}^{-3}$ after 1st passage

Table 7. Effect of subcultures number on multiple shoot formation of *Allium aflatunense* 'Purple Sensation' and *Allium karataviense* 'Ivory Queen' on MS medium supplemented with BA 0.5 and NAA 0.1 $\text{mg} \cdot \text{dm}^{-3}$

Taxons	Passage	Number of adventitious shoots	Increase in number of adventitious shoots	Length of adventitious shoots (mm)
<i>Allium aflatunense</i> 'Purple Sensation'	initial	4.1 e	–	7.9
	1 st	18.8 bcd	4.7	13.5
	2 nd	21.3 b	2.5	16.3
	3 rd	25.6 ab	4.3	21.4
	4 th	31.1 a	5.5	25.8
<i>Allium karataviense</i> 'Ivory Queen'	initial	5.6 e	–	9.5
	1 st	12.8 d	7.2	17.8
	2 nd	15.4 cd	2.6	18.1
	3 rd	17.6 bcd	2.2	17.6
	4 th	26.6 ab	9.0	17.3

* Means followed by the same letter are not significantly different at $\alpha = 0.05$

During multiplication of *Allium aflatunense* 'Purple Sensation' and *Allium karataviense* 'Ivory Queen' shoots the differences in number of regenerating shoots depending on the concentration of growth regulators added to the MS media and species were observed. Evenor et al. [1997, b] cultured shoots of *A. aflatunense* and *A. ampeloprasum* regenerating from different explants on media with growth regulators in different concentrations (BA 2 mg·dm⁻³ plus NAA 1 mg·dm⁻³, BA 4.5 mg·dm⁻³ plus NAA 1 mg·dm⁻³, BA 3 mg·dm⁻³ plus NAA 1 mg·dm⁻³, BA 1 mg·dm⁻³ plus IAA 0.5 mg·dm⁻³, KIN 10 mg·dm⁻³ plus IAA 2 mg·dm⁻³). They noted different regeneration rates depending on cultivar and growth regulators used. They obtained about 600 bulblets from one inflorescence of *Allium aflatunense* 'Lucy Ball' on MS medium supplemented with BA 2–3 mg·dm⁻³ and NAA 1 mg·dm⁻³ during 9 months. For *Allium aflatunense* 'Purple Sensation' it was possible to obtain 200 plants from a single bulb during 9 months on medium containing BA 1 mg·dm⁻³ and IAA 0.5 mg·dm⁻³. Seabrook [1994] multiplied shoots of *A. sativum* and *A. ampeloprasum* and noted that the most optimal media was the one with addition of BA 2 mg·dm⁻³ plus NAA 0.1 mg·dm⁻³ on which 6.1 to 9.5 shoots were formed per explant, depending on the cultivar. Tubić et al. [2011] observed the highest shoot multiplication (33.6) of *Allium schoenoprasum* on the medium with 1.1 mg·dm⁻³ TDZ, while on the medium with 1.1 mg·dm⁻³ BA number of shoots was 3 times lower (11.3).

CONCLUSIONS

Contaminations are very frequent when underground storage organs of bulbous plants are used as a source for explants. The use of two – step sterilization procedure (shaking of bulb fragments in 2% Cl⁻ for 30 min and soaking explants in 1% Cl⁻ for 15 min) gave the best results to receive clean cultures of *Allium aflatunense* 'Purple Sensation' and *Allium karataviense* 'Ivory Queen'. Explants isolated from young inflorescences and from immature leaf bases were effective to form shoots (4.8–5.4 shoots from 1 explant of inflorescence and 3.5–7.2 shoots from 1 leaf base, depending on genotype and growth regulators). The orientation of leaf explants did not have a significant effect on the number of regenerating shoots. 5.7 to 7.5 shoots of *Allium aflatunense* 'Purple Sensation' and 3.5 to 5.1 shoots of *Allium karataviense* 'Ivory Queen' were obtained on studied media during 6 weeks of culture. Multiplication factor of 31.3 shoots of *Allium aflatunense* 'Purple Sensation' and 26.6 shoots of *Allium karataviense* 'Ivory Queen' was possible to obtain on medium supplemented with BA 0.5 mg·dm⁻³ and NAA 0.1 mg·dm⁻³ after 32 weeks of culture.

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PORÓWNANIE ZDOLNOŚCI REGENERACYJNYCH *Allium aflatunense* B. Fedtsch. ‘PURPLE SENSATION’ I *Allium karataviense* Regel. ‘IVORY QUEEN’ W KULTURZE *in vitro*

Streszczenie. *Allium aflatunense* i *Allium karataviense* to cenne rośliny cebulowe do uprawy w ogrodach. Ponadto *Allium aflatunense* jest jednym z najbardziej dekoracyjnych gatunków uprawianym na kwiat cięty. Rośliny te charakteryzują się niskim współczynnikiem rozmnażania. Źródłem materiału do zapoczątkowania kultur były fragmenty młodych pędów kwiatostanowych i nasady liści izolowane w październiku z *Allium aflatunense* ‘Purple Sensation’ i *Allium karataviense* ‘Ivory Queen’. Zastosowanie sterylizacji

dwukrotnej (2% Cl⁻ – 30 min i 1% Cl⁻ – 15 min) dało lepsze rezultaty niż sterylizacja pojedyncza. Eksplantaty z pędów kwiatostanowych układano w pozycji odwrotnej do naturalnej i kultywowano na pożywce Murashige i Skooga [1962] zawierającej BA 2 mg·dm⁻³ i NAA 1 mg·dm⁻³. Podstawy liści układano w pozycji naturalnej i odwróconej i inkubowano na pożywce MS z dodatkiem: BA 2 mg·dm⁻³ + NAA 1 mg·dm⁻³, BA 5 mg·dm⁻³ + NAA 1 mg·dm⁻³ i TDZ 1 mg·dm⁻³ + NAA 1 mg·dm⁻³. W kolejnym doświadczeniu zespoły pędów zawierające 2 pędy namnażano na pożywkach MS uzupełnionych: BA 0,5; 1; 2 mg·dm⁻³ oraz NAA 0,1 mg·dm⁻³ i BA 2 mg·dm⁻³ oraz IAA 0,1 mg·dm⁻³ podczas 6 tygodni. Oceniono również współczynnik namnażania pędów podczas 4 pasażu na pożywce z dodatkiem BA 0,5 mg·dm⁻³ i NAA 0,1 mg·dm⁻³. Fragmenty młodych pędów kwiatostanowych i podstawa liści wykazywały duże zdolności regeneracyjne pędów. Sposób ułożenia eksplantatów nie wywierał istotnego wpływu na liczbę regenerujących pędów. Na badanych pożywkach uzyskano od 5,7 do 7,5 pędów *Allium aflatunense* 'Purple Sensation' i od 3,5 do 5,1 pędów *Allium karataviense* 'Ivory Queen' podczas 6 tygodni namnażania. Podczas 32 tygodni namnażania pędów na pożywce zawierającej BA 0,5 mg·dm⁻³ i NAA 0,1 mg·dm⁻³ uzyskano 31,3 pędów *Allium aflatunense* 'Purple Sensation' i 26,6 pędów of *Allium karataviense* 'Ivory Queen'.

Słowa kluczowe: czosnki ozdobne, pęd kwiatostanowy, podstawa liści, polarność, namnażanie pędów

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