Influence of plant origin on propagation capacity and alkaloid biosynthesis during long-term *in vitro* cultivation of *Leucojum aestivum* L.

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Abstract The investigation deals with *in vitro* clonal propagation of *L. aestivum* L. (summer snowflake), a threatened Amaryllidaceae plant species in Bulgaria used in the pharmaceutical industry as raw material for production of galanthamine-based medicines. Plants of known origin and with different alkaloid profile were taken from the living collection of the Institute of Botany, Sofia. Bulbs were used to initiate *in vitro* cultures and 24 clones were multiplied. The influence of the clone origin on the propagation coefficient, shoot and bulblet morphology, alkaloid profile and content of galanthamine, lycorine, and four related alkaloids was evaluated. Clones kept stable alkaloid profiles and for most of them, high regeneration rates were noted. Galanthamine content of some clones was commensurable with that of Bulgarian populations of *L. aestivum* of commercial importance. Five clones: four

galanthamine-type and one lycorine-type were selected as promising for further investigation.

Keywords Amaryllidaceae · Bulb regeneration · Galanthamine · Lycorine · Summer snowflake

Introduction

Reliable and environment-friendly production of natural active substances is a constant challenge for the pharmaceutical industry. Galanthamine (Gal) is one of the alkaloids specific for the Amaryllidaceae plants and known as acetylcholinesterase inhibitor. It has been successfully used for treatment of poliomyelitis, post-polio paralysis, myasthenia gravis, facial nerve paralysis, and other neuromuscular disorders, as well as for traumatic brain injuries, and schizophrenia (Paskov 1955; Irwin and Smith 1960). Furthermore, its commercial application in Alzheimer's disease therapy has been widely adopted (Dal-Bianco et al. 1991; Marco and Carreiras 2006). Another alkaloid of interest found in several Amaryllidaceae species, lycorine (Lyc), has been studied for its inhibitory activities against human immunodeficiency virus (HIV-1), severe acute respiratory syndrome-associated coronavirus (SARS-CoV), poliovirus, coxsackie virus, measles virus, and herpes simplex virus type 1 (HSV-1; Ieven et al. 1983; Szlavik et al. 2004; Li et al. 2005).

In Bulgaria, the commercial production of galanthamine-based patented medicine Nivalin® was initially based on *Galanthus nivalis* L. (Paskov 1955). Subsequent investigations have shown that *Leucojum aestivum* was a more productive Amaryllidaceae species, as compared to *Galanthus nivalis*, *Galanthus woronowii*, and *Sternbergia*

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colchiciflora (Bubeva-Ivanova and Daleva 1970). Furthermore, in a wide survey of numerous Bulgarian natural populations of *L. aestivum*, Stefanov (1990) observed different alkaloid profiles and galanthamine content ranging from 0.1 to 0.56% DW and suggested the existence of three geographically differentiated chemotypes, with galanthamine, lycorine or lycorenine as main alkaloid. However, exploitation reserves and possible annual harvest have decreased extremely during the period 1960–1990 due to overutilization of the populations and habitat destruction (Stoyanov and Savchev 1964; Mitrev 1995). Therefore, *ex situ* strategy for conservation of this species has been developed in Bulgaria, including *in vivo* and *in vitro* approaches (Gushev et al. 2003).

Studies on *in vitro* propagation of *L. aestivum* were carried out earlier (Popov and Cherkasov 1984; Stanilova et al. 1994; Ptak and Cierniak 2003). Identification of eight alkaloids in callus, clumps, and bulblets of *L. aestivum* by CGS-MS confirmed the capacity of *in vitro* cultures to produce alkaloids (Berkov et al. 2005). However, the few investigations of the alkaloid content have revealed absence or low concentrations of galanthamine (Diop et al. 2006; Diop et al. 2007; Georgieva et al. 2007; Pavlov et al. 2007). Comparison was done concerning the alkaloid pattern of wild plants from 18 Bulgarian populations and several *in vitro* obtained shoot-clumps (Georgieva et al. 2007). Nevertheless the relation to mother plants features was not previously examined. *In vitro* cultures of *Narcissus confusus* and *Ungernia victoris* were also studied as potential producers of galanthamine (Aleksandrova et al. 1994; Sellés et al. 1999).

Our studies were aimed (1) at testing the stability of the propagation rate and the alkaloid profile of *in vitro* organ cultures initiated from *L. aestivum* plants of known origin, and (2) at selecting high galanthamine or lycorine producing clones by means of a long-term *in vitro* cultivation.

Materials and Methods

Plant material. A living *ex situ* collection of *L. aestivum* consisting of about 3,500 individuals and representative of the species diversity in Bulgaria was established in 2001 at the experimental plot of the Institute of Botany, Sofia. Plant clusters gathered from 27 natural populations were transferred to controlled conditions and assessed for galanthamine and lycorine content after 2 y. Twenty-five individuals (one per cluster) coming from 11 selected populations of different chemotypes were used as mother plants for initiation of *in vitro* clones. Clone label refers to the population (first number) and to the cluster in the living collection (second number), for example: 5.2; 7.6. The selected bulbs were picked up in June 2003, at the end of the vegetation phase.

Bulbs were either sterilized immediately (13 of them) or got a chilling treatment, i.e., they were stored for 2–3 mo in a refrigerator at 5°C in paper bags (12 bulbs).

Bulb sterilization and culture conditions. Bulbs were washed with tap water and detergent, soaked consequently in 70% ethanol for 1 min and 50% Domestos (<5% chlorine) for 20 min, then halved, put in 30% Domestos for another 10 min and rinsed three times with sterile distilled water. Twin-scales were excised and used as primary explants (an average of 25 explants per bulb), placed vertically in tubes, the basal part slightly dipped into the medium. Sterilization efficiency was assessed after 4 wk of cultivation by Excel ANOVA Single factor tool.

The initial medium contained salts and vitamins after MS (Murashige and Skoog 1962) and 30 g/l sucrose, with pH 5.7, and was solidified with 6 g/l plant agar (Duchefa, NL). The explants with morphogenic response were further grown *in Vitro* Vent containers (Duchefa, NL) on solid MS medium modified with a ten-fold increase of thiamine-HCl and supplemented with 30 g/l sucrose, 2 mg/l BAP and 0.15 mg/l NAA, transfer to fresh medium every 4 wk. Temperature and illumination were: 23±1°C and 16/8 h light/dark period, 1,600 lux. Clones were grown for at least 3 y under the same conditions.

Propagation rate of *in vitro* clones. Regenerated bulblets with diameter larger than 6–8 mm were regularly detached from the explants and considered as “bulblets”. Smaller bulblets and shoot-clumps were noted as “shoots” and transferred to fresh medium, together with the explants. After an initial period of 6 mo, subcultivation of detached bulblets was started by cutting them into four sectors. The propagation rate was assessed by counting bulblets and shoots of 12 clones three times: in 2003, 6 mo after the cultures initiation; in 2004, when the cultures were 1-y old; and in 2006, under long-term cultivation conditions. The initial *in vitro* propagation coefficient of each clone was calculated as an average of regenerated bulblets (iPC_b) and an average of bulblets and shoots ($iPC_{b\&sh}$) per twin-scale for a period of 6 mo. PC of 1-y old cultures (yPC_b) was presented as an average of the bulblets per twin-scale. PC of subcultivation was evaluated in 2006, after 3 y of *in vitro* cultivation, as an average of regenerated bulblets (sPC_b) and an average of bulblets and shoots ($sPC_{b\&sh}$) per bulblet sector for 6 mo, on the basis of six subcultured bulblets per clone. PC of the clones was compared by grouping the values under and over the average, and the differences were assessed by Excel ANOVA Single factor tool.

Alkaloid determination. Each sample of *in vitro* plant material included shoot-clumps and bulblets. Leaf samples of *in vivo* cultivated plants represented three to five leaves

per cluster, depending on cluster volume. Each analysis was carried out in two repetitions.

Alkaloids were extracted from dried (65°C) and powdered plant material. Samples (50 mg DW) were macerated with methanol (3 ml) in ultrasonic bath, three times for 30 min every 8 h at 25°C. After filtration (FILTRAK 390Ø, Wiesenbad, Germany), centrifugation at 9,500×g for 10 min and evaporation with liquid nitrogen, the total methanol extract was diluted with a mixture of 2.5% methanol and 1.7% acetonitrile in water, and filtrated through a 0.45 µm filter (Waters, Milford, MA).

Chromatographic analysis was carried out on Waters HPLC system supplied with quaternary pump 600E and PDA 996 detector; Alltech Ultrashere-Octyl RP-C8 column (150×4.6 mm i.d., 5 µm) protected by a Symmetry Guard column C8 (20×3.9 mm i.d., 5 µm). The mobile phase consisted of acetonitril/methanol/water (containing 7.5 mM triethanolamine, pH up to 6.9 with phosphoric acid; 20/15/65); column temperature: 35°C; flow rate: 1.0 ml/min; injection volume: 20 µl). Data acquisition and analysis were made by Empower chromatographic software. Determination was done for galanthamine, lycorine and 4 other related alkaloids: norgalanthamine, homolycorine, galanthaminone, and unguiminorine. The standard of galanthamine was ensured by Sopharma JFCo (Sofia, Bulgaria). The other standards were isolated from aboveground mass of *L. aestivum*, purified by CPC, confirmed by H1 and C13 NMR spectra and placed by JH Renault from the University of Reims, France.

Alkaloid content of *in vitro* cultures was analyzed every 3 mo during 18 mo, beginning in October 2004. Comparison of average alkaloid contents of clones was carried out by Excel ANOVA Single Factor tool.

Localization of the two main alkaloids galanthamine and lycorine in bulblets and leaves was determined for three clones of different alkaloid type, 24 samples totally.

Alkaloid content of the corresponding plant clusters from the living collection was determined during the flowering stage in April (harvesting season), in two consecutive years: 2003 and 2004.

Results

Sterilization of bulbs. We succeeded in initiating cultures from 24 of 25 initial bulbs (Fig. 1). The primary explants obtained from nine chilled bulbs were free of any contamination. Furthermore, the percentage of the necrotic explants after chilling was low (8.86%) and, in the case of two bulbs, all explants survived and manifested morphogenesis. Most explants from non-chilled bulbs dropped out due to contamination with variable fungi and bacteria (49.55%), or necrosis (32.43%). The average axenic explants of chilled and non-chilled bulbs represented

68.67% and 36.64%, respectively. The cold storage of *L. aestivum* bulbs improved significantly the survival of the explants ($P<0.001$).

Morphological features of *in vitro* clones. Shoots were observed between the scales of all survival explants after 4 wk of cultivation. In general, shoot base swelled and shaped out a bulblet; however, morphological differences were noted among the cultures during all cultivation period. Clones 3.9, 5.2, 5.77, 7.26, 7.6, 7.80, 9.6, 10.4, and 11.3 formed mostly well-distinguished countable shoots and the bulblets consisted of several scales, thick base and basal plate (Fig. 2a). Shoots obtained from clones 1.19, 2.72, 4.45 and 6.31 were crispate and often fused, and the bulblets had usually few scales (Fig. 2b). There were also differences among the well-formed bulblets: shoot-clumps of clones 10.4 and 11.3 were needle-shaped (Fig. 2c) and developed into bulblets with long leaves, up to 5 cm, whereas bulblets of clones 7.6, 7.80, and especially 9.6 were conical and formed large basal plates (Fig. 2d). Bulb diameter reached 20 mm and adventitious shoots regenerated densely between the outer scales (Fig. 2e). They developed into bulblets on the periphery of a large bulblet exhausting its reserve substances (Fig. 2f).

Propagation coefficient of *in vitro* clones. Subcultivation stimulated shoot formation (Fig. 3). The initial propagation coefficient $iPC_{b\&sh}$ and the subcultivation propagation coefficient $sPC_{b\&sh}$ concerning both bulblets and shoots were on the average 3.16 and 7.52, respectively, which represented a significant difference ($P<0.001$). Shoots needed 5 to 10 mo to develop into bulblets with a diameter over 6 mm, therefore, iPC_b and sPC_b were relatively low.

The morphological features of *in vitro* clones reflected on their propagation rate. Although the sPC differed from the iPC , each clone kept its own expressed morphogenic potential (Fig. 4a,b). Higher PC , considering both cultivation of primary explants and subcultivation, was established for clones 5.2, 9.6, and 7.6. Their well-shaped bulblets reached rapidly the size for subcultivation and the increased number of new explants resulted in higher number of shoots and bulblets at the end of the first y (Fig. 4a). Lower iPC and sPC were noted for the clones with crispate shoots and bulblets with few scales: 6.31, 2.72, 1.19, and 4.45 (Fig. 4a, b). The needle-shaped bulblets of clones 10.4 and 11.3, as well as the bulblets of clones 2.72 and 7.26 were slow-growing and subcultivation was possible only after a longer period of growth (8 to 10 mo). Some shoots from these clones remained small or deteriorated and did not reach a size for subcultivation, which resulted in low yPC (Fig. 4a).

Alkaloid profile and content. Galanthamine and/or lycorine were the main alkaloids in all *in vitro* cultures and in their

Figure 1. Survival rate of the initial explants excised from bulbs belonging to different *L. aestivum* clusters of the living collection (chilled and non-chilled bulbs).

Figure 2. Morphological differences between *in vitro* cultures of *L. aestivum* concerning the shape and the size of the shoot-clumps and the bulblets (bar=1 cm) (a) well-formed shoot-clump from clone 5.2 3 mo after subcultivation; (b) crispate shoot-clumps from clone 4.45 3 mo after subcultivation; (c) needle-shaped shoot-clumps from clone 11.3 2 mo after subcultivation; (d) large conical bulblets from clone 9.6 6 mo after subcultivation; (e) section of *in vitro* bulblet with adventitious shoots growing between the outer scales, clone 7.6; (f) adventitious bulblets on the periphery of a large *in vitro* bulblet, clone 9.6.

Figure 3. Average initial and subcultivation propagation coefficient (PC) of 12 tested clones for 6 mo, considering the number of detached bulbets (iPC_b and sPC_b) and the total number of bulbets and shoots (iPC_{b&sh} and sPC_{b&sh}).

corresponding plant clusters from the living collection of *L. aestivum*. Furthermore, bulbets have kept their inherent alkaloid profile throughout 3 y of *in vitro* cultivation (Fig. 5). Clone origin was found to be a factor of statistical significance for the content of galanthamine ($P < 0.01$). Samples from Gal-type plant clusters and *in vitro* clones 5.2, 4.45, 3.9, and 5.9 contained usually only galanthamine in a high concentration (Fig. 5a), and occasionally low amounts (up to 0.03 mg/g DW) of norgalanthamine and galanthaminone, two related alkaloids from the Gal biosynthetic pathway. In the leaves of the Lyc-type clusters 9.6 and 9.15, only lycorine was detected, whereas in the *in vitro* cultures, besides high Lyc quantity, low concentration of Gal (less than 0.1 mg/g DW) and a relatively high content of norgalanthamine (up to 1.34 mg/g DW) were also found. In the mixed-type *in vivo* plant clusters and *in vitro* clones 7.80, 7.26, 7.73, 2.72, 7.6, 1.19, 6.31, and 5.77, Gal and Lyc were presented in different proportions. Leaves of clusters 10.4 and 11.3 contained homolycorine (up to 0.2 mg/g DW), while *in vitro* cultures produced lycorine (over 1 mg/g DW) and unguinorine (up to 0.94 mg/g DW) as well.

In general, galanthamine content in long-term *in vitro* cultures was lower, as compared to that in plant clusters from the living collection (Fig. 5a). The most productive Gal clone was 5.2, with an average of 1.83 mg/g DW and in four other clones (4.45, 7.80, 7.26, and 3.9) the average Gal content was over 1 mg/g DW. The Lyc content of clone 9.6 reached an average of 2.85 mg/g DW (Fig. 5b).

Alkaloid localization in organs of *in vitro* plantlets was irregular. Gal content was almost twice higher in the leaves than in the bulbets of clone 5.2 (2.15 and 1.19 mg/g DW respectively). In clone 9.6, Lyc was in equal amounts in these organs (3.75 and 3.52 mg/g DW respectively). Two alkaloids determined in clone 10.4, unguinorine and lycorine, predominated in the leaves (1.06 and 1.64 mg/g DW respectively), as compared to their content in the bulbets (0.57 and 0.25 mg/g DW).

Discussion

The origin of *L. aestivum in vitro* clones was proved to be of crucial importance for their main culture characteristics: morphology, PC and capacity to produce alkaloids. Mother plants were chosen according to their alkaloid profile and content, in a way to cover all three chemotypes of the species described for Bulgaria by Stefanov (1990). The author suggested that the alkaloid biosynthesis and especially the content of galanthamine depended only on the geographical latitude of populations and the soil characteristics. However, the long-term *in vitro* cultivation on a medium with unchanged composition, under fixed temperature and illumination regime, has demonstrated that all propagated clones have kept the inherent features of their corresponding plant clusters. Pure Gal-type clones 3.9, 4.45, 5.2 and 5.9 produced only Gal. Their *in situ* clusters of origin gathered in April 2001 from populations Petkaki, Vessellie, and Arkutino along the Bulgarian southern Black sea coast contained only Gal (unpublished data). Similarly, the mother plant of the most productive Lyc-type clone 9.6 belonged to a cluster taken from the sole Bulgarian Lyc-type population Baltata close to the northern Black sea coast. Clones 10.4 and 11.3 were established from plants originating from populations along the Danube and they also did not produce Gal. On the other hand, the mixed-

Figure 4. Propagation coefficient (PC): (a) iPC_{b&sh} for 6 mo, and yPC_b for 12 mo—grouping under and over the average (3.43). (b) sPC_{b&sh} for 6 mo: grouping under and over the average (7.52).

Figure 5. Content of main alkaloids [mg/g DW] in *L. aestivum* *in vitro* clones (samples of shoot-clumps and bulblets) and their corresponding plant clusters (samples of several leaves per cluster): (a) galanthamine; (b) lycorine. Clones are presented in descending order of their Gal content.

type *in vitro* clones 7.80, 7.26, 7.73, and 7.6, with a common population of origin Kalinata, produced Gal and Lyc in quite different contents and proportions: galanthamine between 0.44 and 1.12 mg/g DW, and lycorine between 0.12 and 2.03 mg/g DW. The alkaloid contents of their corresponding plant clusters on the field plot also varied strongly. This was in accordance with the reported normal distribution of plants concerning their Gal content (from 0.1 to 4 mg/g DW) within one sole population in South Bulgaria (Poulev et al. 1993). Significant variation of the Gal content between and within seven Bulgarian populations permitted for industrial use was revealed by Gushev et al. (2007) as well. Georgieva et al. (2007) reported that the alkaloid patterns of 35 dormant bulbs gathered from 18 Bulgarian populations (two bulbs from population) also varied a lot comprising two to twelve alkaloids. All these results referred to genetic determination of the alkaloid biosynthesis that is a precondition for selection of high alkaloid producing clones. Although the populations pertain to three chemotypes according to their major alkaloids it is important to notice that the selection of high Gal producing cultures should be based on clonal level because of the population heterogeneity regarding both the alkaloid pattern and the content of the alkaloids.

The Gal content in the *in vitro* cultures seemed lower, as compared to the plants from the corresponding clusters grown in the field plot. This, however, was due to the alkaloid localization in plant organs. Our analyses demon-

strated that during the long-term *in vitro* cultivation Gal content remained much higher in the leaves than in the bulblets, as it was noted for the organs of the wild plants (Stefanov et al. 1974). Thus, the average content of Gal in the leaves of *in vitro* and *in vivo* cultivated plants of clone 5.2 was similar: 2.15 and 2.18 mg/g DW respectively. Gal richest *in vitro* clones, were those originating from clusters with high Gal content and either developing long leaves, or remaining with small bulblets.

During a 3-y *ex situ* cultivation under equal conditions (2001–2004 *in vivo* and 2003–2006 *in vitro*), all investigated *L. aestivum* clones and plant clusters have kept their biosynthetic capacity and alkaloid profile. In five *in vitro* clones Gal content was over 1 mg/g DW, which is considered a borderline for the industrial importance of the biomass. Commercially harvested wild populations contain Gal between 0.94 and 1.87 mg/g DW (Gushev et al. 2007).

Other investigations of *L. aestivum* oriented toward *in vitro* galanthamine production have mentioned much lower alkaloid concentrations. A correlation between the state of tissue differentiation and the accumulation of Gal was observed by Diop et al. (2006) and highest concentration was found in bulblets: 0.2 mg/g DW. Diop et al. (2007) used market bulbs and succeeded in hairy root induction; however, no Gal was detected in the cultures. Bulblets obtained from embryogenic callus produced Gal from 0.01 to 0.07 mg/g DW, depending on the presence of the growth regulators NAA and BAP in the medium. Pavlov et al. (2007)

reported up to 0.2 mg/g DW Gal in shoots regenerated from callus. Gal content between 0.03 and 0.45 mg/g DW was determined in shoot-clump cultures obtained by Georgieva et al. (2007) from plants picked up from eighteen wild Bulgarian populations including the richest one, Arkutino. These low concentrations of Gal were probably due to the low alkaloid content in the mother plants, or to the processes of dedifferentiation and redifferentiation.

Besides *L. aestivum*, Gal was extracted on commercial scale from bulbs of *Narcissus confusus* and *Lycoris squamigera*, and from leaves of *Galanthus woronowii* and *Ungernia victoris* (Chercasov and Tolkachev 2002). Since bulb production of *N. confusus* is a slow process and the limited supply of *U. victoris* has been exhausted, these species were also *in vitro* initiated for Gal biosynthesis. Cell cultures of *U. victoris* produced lycorine, galanthamine and norgalanthamine, with total alkaloid content of 0.42 mg/g DW, Gal being only 0.03 mg/g DW (Aleksandrova et al. 1994). Alkaloid content in cultures of *N. confusus* increased with tissue differentiation: in calli Gal was about 0.15 mg/g DW, whereas in liquid shoot-clump cultures it reached up to 1.42 mg/g DW for the best medium composition (Codina 2002).

Effectiveness of *in vitro* galanthamine production should depend also on biomass multiplication. The rate of proliferation of direct adventitious shoots and their growth to harvestable size does not always stay constant for long periods (Bigot 1981). The morphogenic potential of *L. aestivum* cultures was not affected under long-term *in vitro* cultivation. Three years after cultures initiation, direct shoot proliferation was ever more intensive and from 7.6 to 14.3 shoots and bulblets per explant were obtained in half of the clones. One possible reason for the retained regeneration potential was the suitable balance of the growth regulators in the medium. Popov and Cherkasov (1984) obtained callus and shoots of *L. aestivum*, *G. woronowii*, and *N. hybridus* on media supplemented with BAP, kinetin and NAA in different concentrations and noted that domination of cytokinins limited callus formation. In case of higher auxin concentration, 10 μ M NAA and 0.5 μ M BAP, the organogenesis turned to root formation with 1.5 bulblets and 5.6 roots per primary explant (Diop et al. 2007). Another reason for the increased PC was the larger meristem zones of the bulblet sectors consisting of five to eight scales compared to the primary twin-scale explants. The PC of subcultivation of all clones was higher than the PC of *L. aestivum* in our earlier studies: 2.72 bulblets per explant (Stanilova et al. 1994); and in those of some other Amaryllidaceae species: 3.5 and 2.0 bulblets per initial explant for *Galanthus nivalis* and *Galanthus elwesii*, respectively (Staikidou et al. 2006), and three to four adventitious bulblets per twin-scale for *Pancratium maritimum* (Dragassaki et al. 2003).

Clones with highest galanthamine or lycorine content (5.2, 9.6, 4.45, 7.80) excelled at PC too (with 6.9 to 14.3

shoots and bulblets per explant), although no strong relationship was found between the propagation rate of the clones and their capacity to produce alkaloids. Squires and Langton (1990) also emphasized the large differences between the PC of *Narcissus* cultivars, with an average of 5.4 bulblets per *in vitro* bulblet.

The heavy microbial contamination of the wild *L. aestivum* plants gathered from different Bulgarian populations was discussed previously (Stanilova and Damianova-Kirilova 1998). Initiation of *in vitro* cultures was easier when bulbs were sterilized 6 wk after their picking up, although the percentage of surviving explants remained relatively low (up to 35.7%) and explants with organogenesis were even less because of the toxicity of HgCl₂ used as sterilizing agent. The present results showed that cold storage of bulbs previous to their sterilization was crucial for the efficiency of the procedure, regardless of their population of origin. Domestos® as a soft sterilizing agent caused limited or no damage of tissues. Improved reliability of bulb disinfection will be of practical importance for rapid multiplication of high-quality *L. aestivum* individuals by means of *in vitro* micropropagation.

Selection of clones was completed after assessment of their shoot-clump and bulblet quality, value and stability of PC and alkaloid content during 3 y of *in vitro* cultivation. Five clones were chosen for further investigation as promising alkaloid producers: clones 5.2, 4.45, 3.9, and 7.80 for biosynthesis of galanthamine and clone 9.6 for biosynthesis of lycorine. The high galanthamine concentration of the *in vitro* clones, commensurable with those of the commercially important wild populations, is a good prerequisite for their use as an alternative alkaloid source. Moreover, clones 5.2, 4.45, and 3.9 originated from Gal-type populations Arkutino, Vessellie, and Petkaki that are not permitted for industrial harvest. Effectiveness of *in vitro* galanthamine production of the selected clones could be additionally enhanced via improvement of the medium composition and culture conditions.

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