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Research Article

In vitro germination and micropropagation of the Balkan endemic *Lilium chalcedonicum* L., a potential ornamental lily

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Abstract

Lilium chalcedonicum L., a bulbous perennial endemic to the Balkans, is recognized for its considerable ornamental potential. In this study, seeds were gathered from a wild population and subjected to surface disinfection. Subsequently, they were placed in Petri dishes containing a half-strength Murashige and Skoog (MS) nutrient substrate (MS/2), with temperature conditions at 15 °C and 20 °C, followed by cycles of 5 °C and return to 15 °C and 20 °C, respectively. The *in vitro*-cultivated seedlings were then transplanted for further growth in MS medium enriched with either 30 g L^{-1} or 60 g L^{-1} sucrose and 0.1 mg L^{-1} 6-N-benzyladenine (BA). In the following stage, the resulting bulblets were separated and cultivated in MS/2, either without hormones (Hf) or with BA at concentrations of 0.2 mg L^{-1}, 0.5 mg L^{-1}, or 1 mg L^{-1}. Moreover, the combined influence of 1-naphthaleneacetic acid (NAA) at a concentration of 1.0 mg L^{-1} and 0.2 mg L^{-1} BA was examined. This stage was succeeded by a further division of the bulblets, and the explants were cultured in Hf, MS/2 or with the inclusion of 0.5 mg L^{-1} BA or a combination of BA and NAA at ratios of 0.2/1 and 0.5/0.05 mg L^{-1}; zeatin at 0.5 mg L^{-1} was also used, combined with 0.05 mg L^{-1} NAA. Notably, all seeds exhibited a 100% germination rate following alternating temperature regimes including periods of low temperature. During the initial cultivation phase, each seedling gave rise to two bulblets measuring 0.6 cm in diameter. Subsequent subcultures on Hf substrates and those containing BA without NAA, resulted in the highest number of bulblets (1.6-2.2 bulbs/plant) of the largest diameter (0.8-0.9 cm). Particularly noteworthy was the substantial increase in the average number of produced bulblets during the subsequent subcultures (multiplication stage), where 16.9 bulbs were formed in MS/2 medium containing 0.5 mg L^{-1} BA, with a median diameter of 1.7 cm.

Keywords: BA; bulblets; *in vitro* rooting; NAA; zeatin

Introduction

The genus *Lilium* (family Liliaceae) consists of more than 110 species of bulbous perennials, grouped in seven sections (Van Tuyl *et al.*, 2018) widespread in the temperate and subtropical climate zones of the

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Holarctic floristic kingdom, most of them with intrinsic and well-established ornamental value (Gong *et al.,* 2017). *Lilium* species are widespread and of high economical value due to their impressive, fragrant flowers (Younis and Lim, 2014; Bakhshaie *et al*., 2016). In Europe there can be found 12 native *Lilium* species, of which seven grow naturally in the Balkan peninsula, some of them being found exclusively in this floristically rich region (İkinci *et al.,* 2006; Rešetnik *et al.,* 2007).

Lilium chalcedonicum L. is endemic to the southwestern corner of the Balkan peninsula, being found in southern Albania, extreme southwestern Northern Macedonia and south and western Greece as a characteristic floristic component of montane fir forests, mixed woodland, *Buxus* scrub and ravines, preferring moist locations in limestone (Strid, 2016). It belongs in the *Lilium carniolicum* group, resolved as a sister species of the similarly Balkan endemics *L. bosniacum*, *L. carniolicum* and *L. jankae* (Rešetnik *et al.,* 2007). In Greece *L. chalcedonicum* is one of five native lilies, occurring in semi-shaded habitats in the mountains from Taygetos and Parnonas in the south to the NW borders, extending into southern Albania (Bergmeier, 2002; Dimopoulos *et al.,* 2013). It takes its name from Chalcedon, now Kadiköy, a district of Istanbul (Turkey) (İkinci, 2010).

L. chalcedonicum exhibits distinctive morphological features that set it apart from its congeners, being characterized by numerous, all alternate, lanceolate leaves, erecto-patent below, appressed above, enveloping the entire stem. Attaining a stature ranging from 60 to 120 cm, it bears remarkable large, red flowers, typically one-six per stem but sometimes up to 12, each measuring 5-8 cm in diameter, with a flowering period extending from July to August (Figure 1). These attributes endow it with noteworthy ornamental value (Woodcock and Steam, 1950; Strid 2016). The name *Lilium heldreichii* has been applied to one-flowered plants with supposedly wider and less crowded leaves but there are no consistent differences (Dimopoulos *et al.,* 2013).

Figure 1. A *L. chalcedonicum* individual flowering in Ano Chora, Nafpaktia, Greece, July 2021 (Photo: Dr. Nikolaos Moustakas)

A visually striking species, ancient botanical literature historically identified it as 'κρίνον το πορφυρούν,' as mentioned by Theophrastus. Being a showy plant, *L. chalcedonicum* was taken into cultivation early; it appears in German Kräuterbücher from the 16th and 17th centuries and may have been introduced into central Europe via Turkey. A healthy-looking plant with seven flowers, apparently cultivated in England, was illustrated in Elwes's *Lilium* monograph (Strid, 2016). It has been also used as one of the parents for the creation of the hybrid *Lilium* × *testaceum*. More recently, the species, while not widespread in trade, is currently available for purchase in specialized bulb nurseries in Europe, with its bulbs fetching prices of up to 50 euros

per piece and most of the original collections tracing their origin to Greek native populations, a concerning trend for many native and endemic geophytes (Menteli *et al.,* 2019; Krigas *et al.,* 2014, 2021).

It is important to note that native species within the genus are subject to protective measures and local conservation initiatives (van de Kasteele, 1974; Krigas *et al.,* 2022). In Greece, local populations of *L. chalcedonicum* such as those located in Parnitha National Park face significant pressures and threats (Aplada *et al.*, 2007), while they can be found in other protected areas (Mertzanis *et al.,* 2016). *L. chalcedonicum* and five more *Lilium* species, i.e. *L. polyphyllum, L. pomponium, L. jankae, L. ciliatum,* and *L. rhodopeum,* have been listed by recent studies as extinct in their habitat (Ved *et al*., 2005; Bilz, 2011; Petrova and Bazos, 2013; İkinci, 2014; Gargano, 2015; Saha *et al.,* 2015; Lansdown, 2018). In Albania the population is healthy and stable, and considered stable in Northern Macedonia, although in the southern parts, in the gorge of the River Vardar near the Demir Kapija, the population of this species is very small. This species occurs in Natura 2000 sites GR2410002 "Oros Parnassos", GR2530001 "Oros Zireia (Kyllini)", GR2440007 "Ethnikos Drymos Oitis–Koilada Asopou", as well as the UNESCO World Heritage Site "Natural and cultural heritage of the Ohrid region". There are no other conservation measures in place throughout its native range, while there are credible reports about the removal of mature bulbs of the species in order to be replanted and propagated in gardens and nurseries.

However, a conspicuous lacuna exists in the existing literature pertaining to the *in vitro* propagation of *L. chalcedonicum* through seeds, as previous research conducted by Papafotiou and Rappou (2003) primarily focused on the potential of *in vitro* propagation using bulb scale shoots. *In vitro* scale culture is the most productive and reliable vegetative propagation method of *Lilium* species and hybrids thanks to its ability to mass produce healthy and uniform plants from only a limited amount of plant material (Youssef *et al.,* 2019). At the same time, the use of seeds as an explant source highly can enhance the genetic diversity of the propagated material, permitting the selection and proliferation of elite genotypes both concurrently and after the completion of the propagation procedure (Sarasan *et al.,* 2011; Silva *et al.,* 2018; Bertsouklis *et al.,* 2022a,b; Pipinis *et al.,* 2023).

In view of this, the primary objective of the current study is to investigate the *in vitro* germination of seeds of *L. chalcedonicum* and explore the feasibility of bulb production. In particular, the effects of: a) an alternating warm-cold-warm stratification treatment on *in vitro* germination in solid, MS/2 medium, b) cytokinin and auxin concentrations on bulblet formation and the *ex-vitro* acclimatization of plantlets were studied. The outcomes of this research are expected to contribute to the dissemination and cultivation of the species in the ornamental plant and cut flower industries and aid its *in situ* and *ex situ* conservation by lessening the collection pressure on wild populations while facilitating the introduction and survival of new or re-established population in its native range.

Materials and Methods

Plant material

Dry, ripe capsules of *L. chalcedonicum* were collected in August 2021 from a native population (Figure 1) of the species located in the vicinity of Ano Chora, Nafpaktia, Greece (38°34'34.64" N, 21°55'35.87" E). Afterwards, they were transferred to Agricultural University of Athens' Laboratory of Floriculture and Landscape Architecture, where ripe, fully formed and healthy seeds were extracted from the fruits and then dry stored (relative humidity of 30%) in the darkness, at room temperature (25 °C). *In vitro* germination and propagation did not take place until 12 months had elapsed after seed storage.

Surface sterilization and in vitro germination

Seeds were surface disinfected with 20% (v/v) aqueous solution of commercial bleach (4.6% w/v sodium hypochlorite) containing 0.1% Tween-20 [polyxyethylene (20) sorbitan monolaurate, MERCK], for 10 min and then rinsed with sterile distilled water three times for three minutes each time. After disinfection, the seeds were sown in plastic Petri dishes (9 cm) with solid (8 g L^{-1} agar), half-strength, Murashige and Skoog (MS/2) media (Murashige and Skoog, 1962). The seeds were incubated in growth chambers set at two different temperatures (15 or 20 °C), with 16 h photoperiod under 37.5 μmol m^{-2} s^{-1} fluorescent light for 40 days. Then three different treatments were performed: a) stayed for 40 more days at 15 °C or 20 °C, with 16 h photoperiod under 37.5 μmol m^{-2} s^{-1} fluorescent light for 40 days (control); b) transferred to darkness at 5 °C for 40 days; c) transferred back to 15 °C or 20 °C. Germination was defined as the appearance of a 2 mm long radicle (ISTA, 1999). T_{50} was defined as the time for 50% of the final percentage of germination (Soltani *et al.*, 2001).

Micropropagation procedure

After germination, the *in vitro* produced seedlings were transferred for further growth on MS medium containing 30 or 60 g L^{-1} sucrose and 0.1 mg L^{-1} 6-N-benzyladenine (BA). The *L. chalcedonicum* seedlings produced bulblets which were divided in two explants and transferred on MS/2 media containing BA at a concentration of 0.2, 0.5 or 1 mg L^{-1}. The combined effect of 1-naphthaleneacetic acid (NAA) 1 mg L^{-1} with 0.2 mg L^{-1} BA was also tested in this stage (establishment). Low concentrations of BA have been used previously for the *in vitro* propagation of *L. longiflorum* (Nhut, 1998).

The following multiplication stage consisted of two subcultures on MS/2 media, pooled data of which are presented in the following section. The effects of BA at 0.2 or 0.5 mg L^{-1}, with or without the addition of NAA (1 or 0.05 mg L^{-1}) were assessed. Moreover, the use of the naturally occurring cytokinin, zeatin, was also evaluated at a concentration of 0.5 mg L^{-1} in combination with 0.05 mg L^{-1} NAA (He *et al.,* 2020; Jameson, 2023). *In vitro* cultures were carried out in glass vessels with a metal cap (7.2 cm × 7.2 cm × 10 cm), with four explants per vessel.

The cultures were maintained at 25 °C. Cool-white, fluorescent lamps provided a 16 h photoperiod at 37.5 μmol m^{-2} s^{-1}. Media of establishment and multiplication stage contained 30 g L^{-1} sucrose. All *in vitro* culture media were solidified with 8 g L^{-1} agar (M. Roumboulakis SA, Athens, Greece), had their pH adjusted to 5.7–5.8 and were autoclaved at 121 °C, for 20 min. The collection of data (percentage, number and diameter of regenerated bulblets, percentage of leaflets and rooting, root number) took place after 60 days of culture.

Acclimatization

All bulblets produced measuring 1.5-2 cm in length were thoroughly rinsed under running, tap water. They were then placed into containers (500 mL, eight bulblets/container) that included both peat (pH 5.5-6.5, Klasmann-Delimann GmbH, Geeste, Germany) and perlite (particles diameter 1–5 mm, Perloflor, Isocon S.A., Athens, Greece) substrate 1:1 (v/v). In order to control the humidity, we covered all containers with plastic wrap (Sanitas; Sarantis S.A., Athens, Greece). Next, the containers were moved into a growth chamber for seven days (chamber temperature: 25 °C; 16 h cool-white, fluorescent light 37.5 μmol m^{-2} s^{-1}/8 h dark photoperiod). Followingly, the containers stayed uncovered for a period of seven days and then were transferred onto a heated glasshouse bench for another seven days (37°58′58.0″N, 23°42′19.2″E). The acclimatization percentage was recorded 30 d after plantlets were transferred to *ex vitro* conditions. After acclimatization, the plants were transplanted into 500 mL plastic pots containing peat:perlite (1/1, v/v) and were fertilised monthly with 2 g L^{-1} complete water-soluble fertiliser (Nutrileaf 60, 20-20-20; Miller Chemical and Fertilizer Corp., Hanover, PA, USA). The last step, which took place two months later, involved the calculation of the plants' survival rate.

Experiment plan, examined criteria and statistical analysis

The experiments of seed germination consisted of six replications of 10 seeds each, i.e., 60 seeds per treatment. The results, for each treatment are shown as the mean ± SE (standard error mean). As shown in the *in vitro* propagation data tables, the number of replicates per treatment differed among the experiments. A completely randomized design was used during every stage of the experimental procedure, the significance of the results was assessed by one-way analysis of variance (ANOVA) and the treatment means were compared using Tukey-Kramer HSD at $p \leq 0.05$ (JMP 14.0 software, SAS Institute Inc., Cary, NC, USA, 2013). Data on percentages were arcsine-transformed prior to the statistical analysis.

Results and Discussion

In vitro germination

One year old, seeds germinated (100%) three days after their final transfer to 15 °C or 20 °C; no fungal or bacterial growth was observed in any of them before or during their germination (Figure 2 a, 3). A similar effect of alternating medium and low temperatures on seed germination was reported in other *Lilium* species (Roh and Sim, 1996; Zhang *et al.*, 2010; Mascarello *et al.,* 2011; Ruffoni *et al.,* 2011; Dhyani *et al.,* 2014, 2019). T_{50} was completed in one day, while control seeds did not germinate. This, particular, germination ecophysiology is assumed to be the result of a morphophysiological dormancy (MPD) localized in the seed epicotyl, as is the case of *L. polyphyllum* (Boeken and Gutterman, 1990; Dhyani *et al.,* 2014). De Hertog and Le Nard (1993) reported that a series of warm-cold-warm periods peculiar for each *Lilium* species are needed, and Pelkonen (2005) divided the species in four germination combinations. Thus, as in other warm-temperate perennials such as *Paeonia ostii* or *Yunnanopilia longistaminea*, in addition to an underdeveloped embryo in need of post-dispersal growth to a critical size, a physiological component of dormancy is also present, with germination occurring after a cycle of warm and cold stratification, mimicking the seasonal fluctuation of temperatures in their habitat (Zhang *et al.,* 2010; Baskin and Baskin 2014; Yang *et al.,* 2017; Ma *et al.,* 2023).

In the present study *in vitro* grown seedlings were transferred for further growth in MS medium containing 30 or 60 g L^{-1} sucrose and 0.1 mg L^{-1} BA (Figure 2 b,c). In previous studies *in vitro* grown seedlings have been successfully used for other *Lilium* species, i.e., *L. martagon* var. *cattaniae* Vis. (Glamočlija *et al.,* 2010). The concentration of sucrose in the propagation medium, as well as the explants carbohydrate reserves, had a significant influence on the stimulation of bulb growth, bulblet weight being positively affected by explant size and sucrose concentration in three different *Lilium* cultivars (Langens-Gerrits *et al.,* 2003). Yang *et al.,* (2019) recorded significant differences in MS media containing either 30 g L^{-1} or 60 g L^{-1} sucrose for *L. fargesii,* growth being higher for higher sucrose concentration. Sucrose at a concentration of 60 g L^{-1} was also used for the establishment of a seedling-derived *in vitro* culture of *L. candidum* (Saadon and Zaccai, 2013). On the other hand, the growth medium for *in vitro*-seedling derived explants of *L. michiganense* (Ault and Siquiera, 2008) and 15 *Lilium* species and cultivars (Mori *et al.,* 2005) contained 30 g L^{-1} sucrose, accordingly to our data: two bulblets, 0.6 cm in diameter, were formed in both treatments, without any significant difference observed between them. Still, Okazaki and Koizumi (1995) used even lower sucrose concentration (1 mg L^{-1}). In the present study, all the seedlings had well growth, sprouted and formed two rooted bulblets, the rooting percentage and root number being with no differences among the different treatments (Figure 4 a,b). Hence, it could be assumed that a broad range of sucrose concentration could be used for *in vitro* culture of various *Lilium* species.

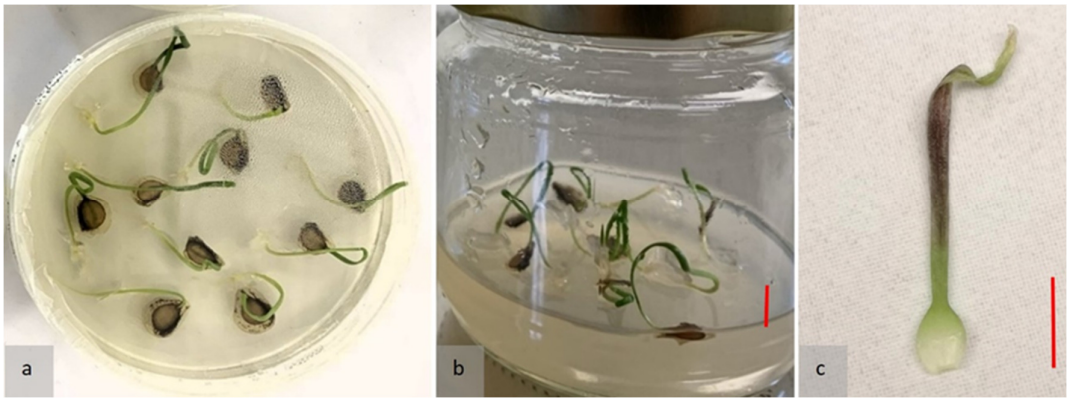


Figure 2. Germination of *L. chalcedonicum* seeds in Petri dishes with half strength Murashige and Skoog medium (MS/2), at 20 °C (a); growth stage of *L. chalcedonicum* seedlings in MS media containing 60 g L^{-1} sucrose and 0.1 mg L^{-1} 6-N-benzyladenine (BA) (b); *in vitro* growth of a bulblet 30 days after its transfer (c)
Red bar represents a length of 1 cm

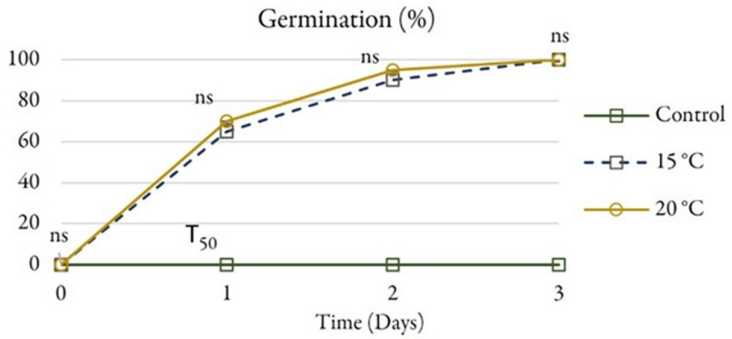
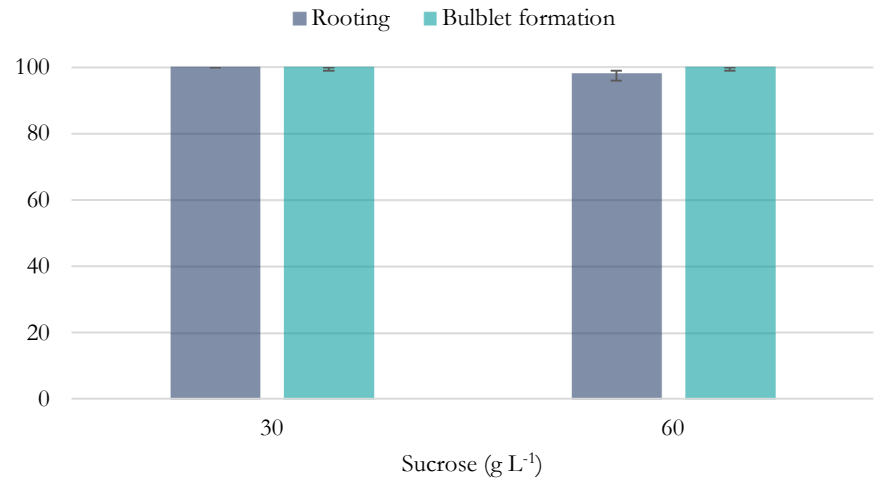
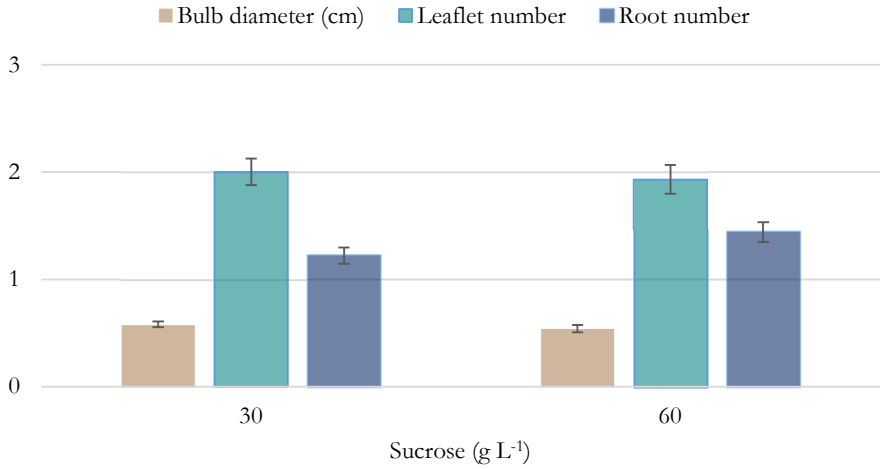


Figure 3. Seed germination of *L. chalcedonicum* on MS/2 medium, at 15 °C and 20 °C, after cycles of 5 °C and return to 15 °C and 20 °C, respectively
F values indicated non-significant differences (ns) at $p \leq 0.05$; n = 6; 6 Petri dishes, 10 seeds/dish



(a)



(b)

Figure 4. *L. chalcedonicum* seedling bulblet production in MS medium containing 30 or 60 g L^{-1} sucrose, supplemented with 0.1 mg L^{-1} BA: rooting (%) and bulblet formation (%) (a); bulb diameter (cm), leaflet number, root number (b)
F values indicated non-significant differences at $p \leq 0.05$; each error bar is constructed using 1 standard error from the mean; n = 55-60

At the following establishment stage, the percentage of bulblet formation was 100% for all treatments; the highest number of bulblets (1.6-2.2 bulblets/plant) had been formed in media absent of NAA, which were also the treatments with the largest diameter of bulblets reported (0.8-0.9 cm) (Table 1, Figure 5a). The leaflet formation was 100% for all the treatments, while rooting percentage and root number presented no differences among the various treatments (Table 1). High rooting percentages on media without indole-3-acetic acid (IAA) or indole-3-butyric acid (IBA) or containing low concentration of NAA (0.1 mg L^{-1}) have been previously reported by Pelkonen and Kauppi, 1999; Chang *et al.,* 2000; Kim *et al.*, 2003; Mori *et al.,* 2005; Ault and Siqueira 2008) for other members of the genus *Lilium*.

Table 1. Establishment stage: culture of bulblet explants excised from *in vitro*-grown, young seedlings of *L. chalcedonicum,* on MS/2 media without hormones (control) or supplemented with (BA) at 0.2, 0.5 or 1.0 mg L^{-1}, with or without 1-naphthaleneacetic acid (NAA) at 1.0 mg L^{-1}

BA (mg L^{-1})	NAA (mg L^{-1})	Bulblet formation (%)	Number of bulblets	Diameter (cm)	Leaflet formation (%)	Rooting (%)	Root number
Control		100	2.2±0.2 a	0.8±0.1 a	100	65.6±4.8	1.5±0.1
0.2	-	100	2.1±0.1 a	0.8±0.1 a	100	56.0±4.0	1.3±0.1
0.2	1.0	100	1.4±0.2 b	0.4±0.1 b	100	66.0±4.8	1.6±0.1
0.5	-	100	1.6± 0.1 ab	0.9±0.1 a	100	62.4±6.0	1.4±1.1
1.0	-	100	2.0±0.2 a	0.9±0.1 a	100	59.2±4.6	1.2±1.0
$F_{\text{one-way ANOVA}}$		ns	**	***	ns	ns	ns

ns, **, ***, non-significant or significant at $p \leq 0.01$ or $p \leq 0.001$, respectively; mean separation in columns by Tukey-Kramer HSD at $p \leq 0.05$; mean values followed by the same letter are not significantly different at $p \leq 0.05$; n = 30

During the multiplication stage, the average number of bulblets produced increased and 16.9 bulbs were formed in MS/2 medium containing 0.5 mg L^{-1} BA, with a median diameter of 1.7 cm, while the treatment with the highest auxin/cytokinin ratio (0.2 mg L^{-1} BA and 1 mg L^{-1} NAA) produced significantly less bulblets (10.8) (Table 2, Figure 5b). No differences were observed among the various treatments in terms of bulblet and leaflet formation, rooting percentage and root number (Table 2). Papafotiou and Rappou (2003), starting from bulb scale explants, reported that the highest number of bulblets (3.0) was formed in media containing 2,4-D and BA at equal concentrations, with a high ratio of NAA/BA also leading to good results (1.7-2.1). The differences reported may be provisionally attributed to the physiological and anatomical particularities of scale explants from *in situ* collected bulbs (Skoric *et al.,* 2011). Similarly, the exceptionally high increase of the number of bulblets compared to the establishment stage can be correlated with the increased bulblet size and carbon reserve mobilization of the explants, as well as their interaction with acclimatization to *in vitro* culture conditions and cytokinin carry over effects (Langens-Gerrit*set al.,* 2003; Youssef *et al.,* 2019).

Table 2. Effect of various concentrations of BA, NAA and zeatin (ZEA) on MS/2 media, on bulblet proliferation of *L. chalcedonicum*, during the multiplication stage (merged data of two subcultures are presented)

BA (mg L^{-1})	ZEA (mg L^{-1})	NAA (mg L^{-1})	Bulblet formation (%)	Number of bulblets	Diameter (cm)	Leaflet formation (%)	Rooting (%)	Root number
Control			100	12.2±0.4 b	1.4±0.1 b	100	56.4±2.6	3.7±0.2
0.2	-	1.0	100	10.8±0.5 b	1.9±0.1 a	100	60.4±4.7	3.9±0.2
0.5	-	-	100	16.9±0.7 a	1.7±0.1 a	100	52.2±3.1	3.7±0.2
0.5	-	0.05	100	12.4±0.3 b	1.4±0.1 b	100	58.0±2.3	4.1±0.2
-	0.5	0.05	100	12.2±0.3 b	1.4±0.1 b	100	56.0±2.8	4.0±0.2
$F_{\text{one-way ANOVA}}$			ns	***	***	ns	ns	ns

ns, ***, non-significant or significant at $p \leq 0.001$, respectively; mean separation in columns by Tukey-Kramer HSD at $p \leq 0.05$; mean values followed by the same letter are not significantly different at $p \leq 0.05$; n = 42

Figure 5. Bulblet formation of *L. chalcedonicum* on MS/2 media containing 0.5 mg L^{-1} BA, during the establishment and multiplication stage (a and b, respectively); fully acclimatized plantlets *L. chalcedonicum* plantlets on peat: perlite substrate (1:1) after one month in *ex vitro* conditions (c, d).
Red bar represents a length of 1 cm

The acclimatization of the bulblets, 30 days after their transfer *ex vitro*, was completely successful (100%), in agreement with the results of a previous study concerning the micropropagation of the species from *ex vitro* collected bulb scales (Papafotiou and Rappou, 2003). All the bulbs produced roots and grew vigorous leaves after one month from their transfer into *ex vitro* conditions, continuing their growth as uniform and healthy plants with burgeoning scaly bulbs (Figure 5 c, d). Acclimatization success was very high in the vast majority of described micropropagation protocols, with results of over 90% acclimatized plantlets reported for most species of *Lilium* (Mori *et al.,* 2005; Sahoo *et al.,* 2018; Rafiq *et al.,* 2021). Finally, all the plantlets produced survived for at least two months following their successful acclimatization.

Conclusions

In the present study, an effective *in vitro* germination and regeneration protocol for a Balkan endemic ornamental lily species, *L. chalcedonicum,* was established. All seeds germinated after an alternating warm-cold-warm stratification protocol, with the produced seedlings transferred for further *in vitro* growth in solid MS medium. Successful establishment of the cultures was accomplished after transferring the initial *in vitro* culture of seedling-derived bulblets in solid MS/2 media, either Hf or containing BA, producing upwards to 2.2 bulblets per explants, with a median diameter of 0.8-0.9 cm. The number of bulblets produced increased significantly during the multiplication stage (16.9 bulblets per explants), on MS/2 media supplemented with 0.5 mg L^{-1} BA. All the bulblets readily produced leaves and roots after a month of their successful transfer in *ex vitro* conditions, leading to 100% acclimatization. In the future, the currently described protocol is expected to enable the reveal and the utilization of possibly considerable intra- and inter-popular morphological variation, suitable for the selection of new clones with desirable ornamental traits for the floriculture market.

Authors' Contributions

Conceptualization: KB, AA-I; Data curation: KB, MT and A-EB; Investigation: KB, MT; Methodology: KB, MT, A-EB; Supervision: KB; Visualization: KB, MT and AA-I; Writing - original draft: KB, MT, A-EB; Writing - review and editing: KB, A-EB. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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