

Vegetative propagation and *ex-situ* conservation of *Acantholimon androsaceum* and *Limonium chersonesum*, two promising local endemics of Crete (Greece) available for floricultural and pharmaceutical sustainable exploitation

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Abstract

The continual search for new attractive ornamentals and innovative natural medicinal products is the main focus of floricultural and pharmaceutical industries worldwide. Aiming to introduce two new Cretan endemic species in the commercial trade, *Acantholimon androsaceum* (Jaub. & Spach) Boiss. and *Limonium chersonesum* Erben & Brullo (Plumbaginaceae) have been selected in the current study. These were derived from a total of 223 local endemic species and subspecies of Crete, which constitute an exceptional wild treasure of the Mediterranean flora. Prior to any commercialization, efficient massive propagation protocols are required. Although sexual propagation using seeds collected from the wild was unsuccessful for these two taxa, the results of vegetative propagation experiments were satisfactory. Wild plant material was used for cuttings in order to develop a reasonable and homogenous number of stock mother plants for propagation trials. As a result of experiments conducted in the current research, two vegetative propagation protocols were developed, which could be used commercially for the massive production of elite clonal plants of *A. androsaceum* and *L. chersonesum*. These protocols provide 71.43% successful rooting for *A. androsaceum* within 40 days, using 2000 ppm IBA, and 80.95% rooting for *L. chersonesum* within 30 days, using 1000 ppm IBA. This study is part of a strategy and research methodology aiming at the selection of new, rare and endemic native industrial crops for the ornamental and pharmaceutical sector, exploiting sustainably the rich phylogenetic resources of Crete.

Keywords: cuttings; horticulture; phylogenetic resources; seed germination; sustainability

Introduction

Over the last two decades, medicinal and horticultural trade has been on the rise, driven by the growing interest of society for new innovative products. Consequently, there is enhanced attention to extant

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opportunities derived from new native plant species with an essential role as potential new innovative medicinal and/or new ornamental crops. These unexplored native phylogenetic resources grow in self-maintaining natural populations in wild habitats (Pascale and Romano, 2019). Although these resources are often neglected and underused, they play an important role in generating new medicinal products and are successfully used in floriculture as cut flowers or potted-plants, in sustainable landscaping with native plants (Antrop, 2006; Ahern, 2013) and in xeriscaping (Sari and Karaşah, 2015); such applications provide substantial advantages over other commercially available crops, such as lower water consumption, reduced demand for pesticides, lower need for fertilizers, reduced maintenance and labor costs (Helfand *et al.*, 2006).

In this framework, the thorny cushion-like *Acantholimon* spp. of Plumbaginaceae family are used in landscaping of mountain area (Vainoriene, 2010; Kazemi and Abbasi, 2018), in restoration of steppe ecosystems (Dilaver, 2013) and in semi-arid or sub-humid regions (Jankju and Noedoost, 2014), while *A. acerosum* (Willd.) Boiss., *A. armenum* Boiss. & A. Huet and *A. litvinovii* Lincz. have been planted and adapted well on green roofs in a semi-arid climate (Schneider *et al.*, 2014). Many *Acantholimon* spp. (ca. 200 species in the genus, see Kubitzki, 1993) are of high economic importance, primarily in floriculture because of the coloured flowers, long period of flowering and concomitant fruiting of similar appearance (Muvaffak *et al.*, 2001).

Limonium spp. (Plumbaginaceae) are wild-growing halophytes or rock-dwellers in nutrient-poor, rocky, sandy or dry soils (Ančev, 1982). These plants are quite popular in the global ornamental industry, mainly as cut-flowers (among the top-20 worldwide) (Mebakerlin and Chakravorty, 2015) but also as potted plants (Mercuri *et al.*, 2001), and represent suitable and reliable choices for sustainable landscaping in arid areas suffering from increased salinity and/or limited water availability (Pascale and Romano, 2019). *Limonium* spp. are popular for their distinct ornamental characteristics, such as the attractive flower colour, the branched inflorescences (many different types) and attractive post-harvest appearance, thus making them as valuable genetic resources for breeding and suitable ground cover plants in urban landscaping (Burchi *et al.*, 2006). Among *Limonium* spp., several new flower species have been successfully introduced to the horticultural cut-flower market. These species or hybrids are grown for their flowers, the attractive calyx which remains open and coloured on the plant long after the true flowers have senesced, thus offering the potential to be also used as dried flowers. *Limonium* spp. are also used as a cheap 'filler' or ornament in modern bouquets, competing with other famous crops such as *Gypsophila* spp. It is estimated that the total value and turnover around them are constantly increasing in the Dutch flower market during the last decade, reaching 15 million euro in 2014 (Hanks, 2015; Morgan and Funnell, 2018).

With regards to medicinal interest, several members of the Plumbaginaceae family contain anthocyanins, flavonoids, tannins and quinines (Trabelsi *et al.*, 2014) and have been used as effective natural remedies in Chinese, Mediterranean and Iranian folk medicine (Dhale and Markandeya, 2011) due to anti-plasmodial and insecticidal properties (Sunil *et al.*, 2012), while *A. lycopodioides* (Girard) Boiss. has been used in the trans-Himalaya region as a medicinal plant for cardiac disorders (Bhadrecha *et al.*, 2017).

Reproduction-wise, vegetative propagation is a common and relatively inexpensive method used for many medicinal or ornamental plants as it overcomes the difficulties of seed propagation (Elhaak *et al.*, 2015). Initially, unsuccessful propagation by cuttings has been reported for *A. glumaceum* (Jaub. & Spach.) Boiss. using the auxins IBA and NAA (Metcalfe and Templeman, 1939), thus discouraging further research in this direction. Propagation of different *Acantholimon* spp. has been mainly achieved to date via seeds, germination varying depending on the species studied, seed age and quality or storage conditions. Usually, their seed germination is achieved at 20 °C within three months (or after storage at -4 °C to 4 °C for 2-4 weeks and repeated sowing at 20 °C) as reported in *A. acerosum*, *A. albanicum* O. Schwarz & F. Mey., *A. armenum*, *A. bracteatum* (Girard) Boiss., *A. dianthifolium* Bokhari, *A. glumaceum*, *A. kotschyi* (Jaub. & Spach) Boiss., *A. ulicinum* (Willd. ex Schult.) Boiss. (Clothier, 2003) or *A. raddeanum* Czerniak. (Jankju and Noedoost, 2014), while germination problems have been reported for several *Acantholimon* spp. (Deno, 1993). Vegetative propagation of *Limonium* spp. through shoot and root cuttings usually requires long periods and ensures

limited success, i.e. 20-30% (Anandamoy *et al.*, 2012). A wide range of *in vitro* techniques have been also developed for their asexual massive propagation (Hosni *et al.*, 2000), serving primarily conservation purposes (Casazza *et al.*, 2002) or breeding needs through embryo culture, chromosome duplication, mutagenesis and transformation (Morgan and Funnell, 2018). Seed germination of *L. meyeri* (Boiss.) Kuntze, *L. bulgaricum* (Cav.) Kuntze, *L. vulgare* Mill., *L. asterotrichum* (C. E. Salmon) C. E. Salmon and *L. gmelini* (Willd.) Kuntze harvested from *ex-situ* grown plants and from *in-situ* wild-growing plants seems to be compromised (in laboratory conditions, *in vivo* and *in vitro*), with germination ability reported as species-specific (Kaninski *et al.*, 2012). Other studies report high but time-varying and species-dependent germination, i.e. either timely 100% germination in 3-7 days for *L. californicum* (Boiss.) A. Heller (Woodell and Mooney, 1970; SID-Kew) and *L. cornarianum* Kypr. & R. Artelari (Markaki, 2006), or slow (in 33 days) germination (100%) for *L. dichroanthum* (Rupr.) Ikonn. -Gal. (SID-Kew) and very slow (in 151 days) for 86% germination in *L. popovii* Kubansk. (SID-Kew).

In this study we focused on two native Cretan, local (single-island) endemic, rock-dwelling species with potentially valuable medicinal properties, which are universally rare. Additionally, these species have interesting features and natural adaptations that could be exploited in the horticultural field. These native local endemic plants provide exclusive characteristics, i.e. combination of uniqueness, impressive plant features, rarity, adaptability and utility, which are well appreciated by the global online floricultural-ornamental market (Dee *et al.*, 2019). Moreover, such plants are already associated with high prices in the electronic ornamental trade of rare, threatened and unusual plants with conservation implications (Krigas *et al.*, 2014; Menteli *et al.*, 2019). Hence, the development of propagation protocols (asexual and vegetative) of *A. androsaceum* (Jaub. & Spach.) Boiss. and *L. chersonesum* Erben & Brullo, species studied herein for the first time, aims to facilitate their *ex situ* conservation and pave the way for their sustainable exploitation as valuable and exceptional phylogenetic resources.

Materials and Methods

Plant material

Two botanical collections were organized on the island of Crete (Greece) during the end of August and mid-October of 2018. The collections were made using a special permission for the Balkan Botanic Garden of Kroussia (BBGK) which is issued and renewed every year by the Greek Ministry of Environment and Energy endorsing the provisions of the Convention of Biological Diversity, Nagoya Protocol and EU Directive 511/2014. Plant material composed of young individuals, seeds and annual stems was collected carefully from wild-growing populations of *A. androsaceum* and *L. chersonesum* (Figure 1), avoiding damages for the wild plants and was then transferred to the facilities of BBGK in Thermi, Thessaloniki. This wild plant material was taxonomically identified and finally obtained an IPEN (International Plant Exchange Network) accession number endorsing the provisions of the Convention of Biological Diversity, Nagoya Protocol and EU Directive 511/2014 (Table 1).

Table 1. *A. androsaceum* and *L. chersonesum* plant material (seeds, cuttings, living individuals) collected from wild habitats on the island of Crete for *ex situ* conservation and propagation of clonal plants at the facilities of the Balkan Botanic Garden of Kroussia

Taxon (IPEN accession number)	Geographical areas	No of seeds	Weight of 10 seeds (mg)	No of cuttings	No of living individuals
<i>Acantholimon androsaceum</i> (GR-1-BBGK-19,1)	Mt Psiloritis (Skinakas peak)	115	64	168	1
<i>Limonium chersonesum</i> (GR-1-BBGK-19,2)	West Stalida	10	2.5	-	1



Figure 1. Wild-growing and rock-dwelling individuals of *L. chersonesum* (left) and *A. androsaceum* (Photo: M. Avramakis, Natural History Museum of Crete, University of Crete) (right) of Plumbaginaceae family in their natural habitats in coastal and mountain areas, respectively.

The young plants collected from the wild habitats (Table 1, Figure 1) were transplanted in pots and acclimatized for 6-9 weeks. The pots with the wild plants were placed outdoors on benches under shade without direct sunlight at the Institute of Plant Breeding and Phylogenetic Resources (Thermi, Thessaloniki), following the local seasonal climatic conditions of the area. Two months after collections from wild, shoot tips of 4-6 cm in length were excised from the collected plants and were treated with 0.2% powder indole-3-butyric acid (IBA) (Radicin, Fytorgan SA, Greece); then, they were transferred in multi-cell propagation trays using a substrate mixture of peat moss (Terrahum, Klasmann): perlite (1:3 v/v) and were placed on a heated bench ($\pm 19^\circ\text{C}$) under mist in 80-90% relative humidity (RH).

The rooted cuttings were transplanted after three weeks in 1 L plastic pots, containing a mixture of peat (TS2, Klasmann) and perlite (3:1 v/v), for further growth and this transfer was repeated monthly for six months (September 2018 until February 2019), in order to produce the stock plant material required for further propagation experiments (Table 2).

Table 2. Initial rooting trials of the cuttings collected *in-situ* and/or *ex-situ* in different seasons from *A. androsaceum* and *L. chersonesum* Cretan endemic plants

Taxon	September 2018			October 2018			February 2019			Total mother stock plants (Sep+Oct+Feb)
	No of cuttings	Rooting (%)	Period (days)	No of cuttings	Rooting (%)	Period (days)	No of cuttings	Rooting (%)	Period (days)	
<i>Acantholimon androsaceum</i> (Oct: <i>in-situ</i> ; Feb: <i>ex-situ</i>)	-	-	-	168	23	60	74	55	75	78 (0+38+40)
<i>Limonium chersonesum</i> (All <i>ex-situ</i>)	2	100	13	12	100	24	21	74	30	30 (2+12+16)

The young stems collected from wild-growing plants of *A. androsaceum* and *L. chersonesum* (Table 1), were transported in a portable fridge to the BBGK's laboratory, were transferred and maintained then in a walk-in cold room (2-4 °C) until experimentation for rooting, following the same procedure as described above.

The fruiting calyces and the inflorescences collected from wild were transferred for drying in a walk-in dark room with stable temperature (15 °C) and reduced humidity (RH 15%). After one month, the seeds were separated, weighed and used in the experiments (Table 1).

Vegetative propagation by cuttings

Propagation of *A. androsaceum* and *L. chersonesum* for the production of clonal plants was planned for experimentation using 0, 1000, 2000 and 4000 ppm of IBA (Duchefa Biochemie, The Netherlands) during June-July 2019. Softwood tip cuttings of *A. androsaceum* and *L. chersonesum* (both 1.5-2 cm) derived from mother stock plants conserved in the facilities of BBGK, were immersed for 10 sec in IBA solutions (dissolved in 50% ethanol) and finally were placed in multi-cell propagation trays using a 1:3 v/v peat moss (TS1, Klasmann): perlite substrate. The trays were then transferred on a heated bench ($\pm 19^\circ\text{C}$) under mist (80-90% RH). The number of roots per cutting and root length was evaluated for *A. androsaceum* after 40 days and for *L. chersonesum* after 15 and 30 days, while the rooting rate was calculated as percentage (%). The rooted plants were then transplanted in 0.33 L plastic pots and subsequently in 2.5 L after 4 weeks, following the procedure described previously. A number of the excessive clonal plants of *A. androsaceum* and *L. chersonesum* was transferred for long-term *ex situ* conservation in special garden beds at the BBGK's sea level and mountain facilities (botanic gardens in Thermi, prefecture of Thessaloniki and in Pontokerassia, prefecture of Kilkis at 650 m altitude).

Propagation by seeds

The experiments for seed germination were performed in autumn (November 2018). The seeds ($n=50$ for *A. androsaceum* and $n=10$ for *L. chersonesum*) were first saturated in distilled water overnight and then were sowed (4-5 mm in depth) in plastic trays using a substrate of peat (Terrahum, Klasmann): perlite (1:1 v/v) and finally the seeds were covered with a layer of vermiculite (2-3 mm). These plastic trays were then placed on a heated bench ($\pm 19^\circ\text{C}$) under mist (80-90% RH). The germination rate of the seeds was calculated every two weeks, counting the emergence of the visible sprouts for a period of two months. Finally, the seedlings were transplanted in multi-cell propagation trays following the procedure described above for cuttings.

Statistical analysis

The experiments on the induction of rhizogenesis of *A. androsaceum* and *L. chersonesum* included four treatments with 21 replicates (3 groups of 7 repetitions) and were repeated twice. Data generated from these experiments were analyzed according to the completely randomized design (CRD) and the means were subjected to analysis of variance (ANOVA) using SPSS 17.0 (SPSS Inc, Chicago, Illinois, USA). Comparison of means was performed with the Duncan's multiple range test at significance level $\alpha = 0.05$ ($P \leq 0.05$). For *A. androsaceum*, a monofactorial experiment was organized where the influence of IBA concentration on the analyzed indicators was studied. For *L. chersonesum*, a bifactorial experiment was organized, with the main effect of two factors (rooting period and IBA concentration) as well as their interaction was evaluated by the General Linear Model (2-way ANOVA).

Results

Propagation and development of stock mother plants

The plant material collected from *A. androsaceum* plants from wild habitats (October 2018) for the production of clonal stock mother plants led to a rooting rate of 23% within 60 days. This value was lower compared to that obtained in the case of plant material collected in winter (end February) from *ex-situ* cultivated plants, namely a rooting rate of 55% within 75 days. In total, 78 new clonal plants of *A. androsaceum* were produced within seven months (Table 2). Following the same procedure for *L. chersonesum*, the cuttings collected in September 2018, October 2018 and the end of February 2019 from *ex-situ* cultivated plants s formed roots in proportion of 100%, 100% and 74%, respectively, obtaining a total production of 30 new clonal stock mother plants (Table 2).

In the case of both species studied (*A. androsaceum* and *L. chersonesum*), the seeds did not germinate.

Effect of IBA on rooting

The 2000 ppm IBA treatment was the most effective best, resulting in a rooting rate of 71.43% of *A. androsaceum* cuttings within 40 days, with an average root length of 0.86 cm and 2.65 roots per cutting. Similar results were achieved in the case of the highest concentration of IBA (4000 ppm), however with no statistical difference (Table 3, Figure 2).

Table 3. The influence of IBA on rooting rate (%), number of roots and root length (cm) of *A. androsaceum* cuttings obtained from stock mother plants after 40 days

Treatments	Rooting rate (%)	Number of roots per cutting	Root length (cm)
Control	9.53 c	1.50 ± 0.03 b	0.53 ± 0.00 a
1000 ppm IBA	38.10 b	3.13 ± 0.37 a	0.72 ± 0.11 a
2000 ppm IBA	71.43 a	2.65 ± 0.21 ab	0.86 ± 0.20 a
4000 ppm IBA	71.43 a	3.13 ± 0.29 a	0.61 ± 0.06 a
<i>P</i> -values	0.001**	0.000***	0.224 ns

Means (n=21) ± standard error (S.E.) with the same letter in a column are not statistically significant different from each other according to the Duncan's multiple range test at $P \leq 0.05$; ns: $P \geq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

In the case of *L. chersonesum* species, the 1000 ppm IBA treatment was the most effective, resulting in a rooting rate of 80.95% of cuttings within 30 days, with an average root length of 2.75 cm and 4.93 roots per cutting. Similar but not statistically different results were obtained for 2000 and 4000 ppm IBA treatments, although the rooting rate at 2000 ppm was 84.21%. During the first fortnight, 4000 ppm IBA treatment resulted in higher rooting percentage (68.42%) with an increased number of roots and root length (3.69 roots and 2.00 cm), but at the end of the experimental period, the concentrations of 1000 and 2000 ppm IBA produced a significant increase of rooting rate (Table 4; Figure 3).

Table 4. Effect of IBA on rooting rate (%), number of roots and root length (cm) of *L. chersonesum* cuttings obtained from stock mother plants depending to the rooting period

Rooting period (days under mist)	IBA (ppm)	Rooting rate (%)	Number of roots per cutting	Root length (cm)
15	Control	45.00 e	2.33 ± 0.15 c	1.22 ± 0.13 d
	1000	57.14 d	3.00 ± 0.26 bc	1.28 ± 0.13 d
	2000	55.00 d	3.36 ± 0.25 b	1.30 ± 0.09 d
	4000	68.42 c	3.69 ± 0.39 b	2.00 ± 0.13 c
30	Control	72.22 bc	4.64 ± 0.25 a	2.06 ± 0.12 c
	1000	80.95 ab	4.93 ± 0.29 a	2.75 ± 0.15 b
	2000	84.21 a	4.56 ± 0.35 a	2.59 ± 0.18 b
	4000	73.68 bc	4.51 ± 0.18 a	3.40 ± 0.10 a
<i>P</i> -values (2-way ANOVA)				
IBA Concentration (A)		0.001**	0.000***	0.000***
Number of days in the mist (B)		0.000***	0.122 ns	0.000***
(A)*(B)		0.002**	0.031*	0.079 ns

Means (n=21) ± standard error (S.E.) with the same letter in a column are not statistically significant different from each other according to the Duncan's multiple range test at $P \leq 0.05$; ns: $P \geq 0.05$; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.



Figure 2. The influence of IBA on rooting of *A. androsaceum* (above) and *L. chersonesum* (below) cuttings from stock mother plants after 40 and 30 days, respectively

Acclimatization in different environments

The propagated plants of the Cretan local endemic taxa are currently cultivated and evaluated *ex-situ* with regards to their acclimatization potential in different environments at the grounds of the BBGK, such as outdoors in garden beds, both at sea level (Thermi, Thessaloniki) and at intermediate altitude of 650 m (Pontokerasia, Kilkis prefecture) as well as outdoors and greenhouse conditions cultivation in pots. To date, almost two years after *ex-situ* cultivation, the propagated plants of *A. androsaceum* and *L. chersonesum* presented a high adaptability to the man-made environment of the BBGK without any problems observed (Figure 4).

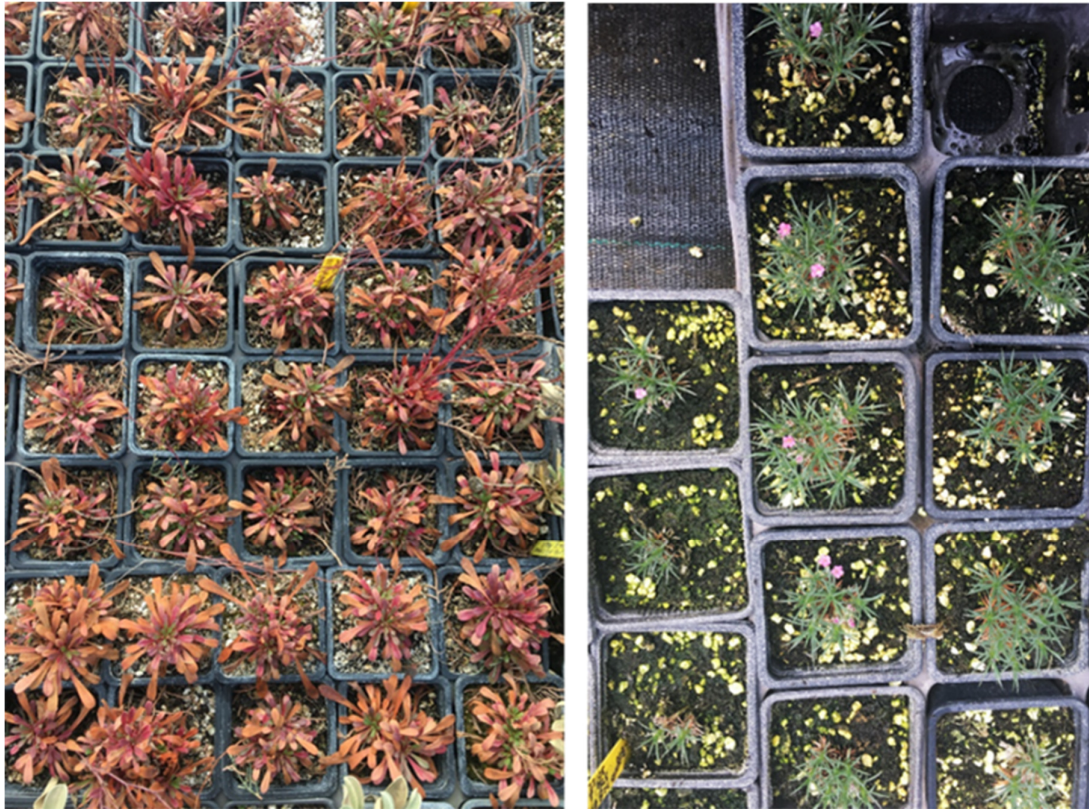


Figure 3. Rooted individuals of the local Cretan endemics *L. chersonesum* (left, with seasonal brownish-reddish leaves) and *A. androsaceum* (right, flowering) in spring during their acclimatization and *ex-situ* conservation at the premises of the Balkan Botanic Garden of Kroussia in Thermi, Thessaloniki (Northern Greece)



Figure 4. Wild-growing, cushion-forming individual of *A. androsaceum* during flowering with densely arranged flowers (photos: M. Avramakis, Natural History Museum of Crete, University of Crete) and close-up of spiny leaves, corolla and fruiting calyces (photo: F. Samaritakis), a local endemic of Cretan high mountains, Greece.

Discussion

The local Cretan endemic plants studied herein have some impressive features. *A. androsaceum* presents intense cushion-forming habit in the wild habitats and under *ex-situ* cultivation with rather symmetrical growth; this combined with limited nutrient demands, increased frost hardiness, impressive flowering, high flower density and lasting fruiting of similar appearance and dense spiny-tipped leaves result in its ranking in average to high positions among 223 local Cretan, Tunisian and Moroccan local endemics after multifaceted ornamental evaluation (Krigas *et al.*, 2021, Figure 4). On the other hand, *L. chersonesum* shows increased salt resistance and limited water requirements in combination with impressive and long summer flowering, while long-lasting fruiting of similar appearance and seasonal differences in leaf colour may enhance and differentiate its ornamental potential (Krigas *et al.*, 2021; Figures 3 and 5). On the other hand, *L. chersonesum* has increased salt resistance and limited water requirements in combination with an impressive and long summer flowering, while long-lasting fruiting of similar appearance and seasonal differences in leaf colour may enhance and differentiate its ornamental potential (Krigas *et al.*, 2021; Figures 3 and 5). These attractive and useful features combined with uniqueness (local endemics of Crete), the exotic character (brand name Crete, Mediterranean) and existing commercial interest (Menteli *et al.*, 2019) offers these plants considerable potential allowing for their sustainable exploitation (Krigas *et al.*, 2021) and their introduction in commercial floriculture and/or the medical field (Dee *et al.*, 2019).



Figure 5. Young stems (late spring), flowering (summer) and fruiting (autumn) stems of *L. chersonesum*, a local endemic plant of Stylida area in Crete (Greece)

Nevertheless, research on unknown plant taxa is a difficult task to achieve mainly due to the lack of information available in the literature. Moreover, the development of an efficient propagation protocol for the massive production of clonal plants is a difficult procedure that requires long experimentation and explicit technical skills. The current study demonstrates for the first-time successful experiments for vegetative propagation of the wild Cretan local endemics *A. androsaceum* and *L. chersonesum* aiming to be used for their *ex-situ* conservation and further evaluation as new medicinal and/or ornamental species.

Preliminary experiments on the germination of seeds collected from the wild Cretan endemics *A. androsaceum* and *L. chersonesum* showed no seed germination for these two taxa. Similar unsuccessful results as those reported herein are described for several *Acantholimon* spp., sowing at 20 °C and cold stratification at -4 to 4 °C for 2-4 weeks was proposed as likely essential for their germination (Kootenay Local Agricultural Society, 2008). However, the limited number of seeds obtained from the wild did not allow any further

experimentation. The same unsuccessful results delivered for *L. chersonesum*, are possible due to the immaturity of the collected seeds before their natural ripening, which normally occurs during October. This may also be deduced from another study concerning the well-ripen seeds of the local Cretan endemic *L. cornarianum* (another local Cretan endemic), that showed full germination (100%) within 2-4 days, at various temperatures tested (Markaki, 2006). According to Reyes-Betancort *et al.* (2008), the variation in the germination of seeds of *Limonium* spp. is partially determined genetically but also environmentally determined. Unfortunately, the limited number of wild seeds collected did not allow any further investigation.

The results of propagation experiments for *A. androsaceum* during winter using wild plant material for the production of mother-stock plants showed a lower rooting rate (23-55% within 2-2.5 months), compared to those repeated during summer (June-July) using cuttings from the conserved stock mother plants, (rooting rate of 71.43% at 2000 ppm IBA). It is known that IBA is commonly used to stimulate rhizogenesis, especially when endogenous auxin production is insufficient for root induction and development (Fragoso *et al.*, 2017). This seems to apply for *A. androsaceum*, where the absence of IBA led to low rooting rates (9.53%). Better results were obtained for *L. chersonesum* (100% rooting rate during September in 13 days and in October within 24 days). In addition, for *L. chersonesum*, the current study showed that 1000 ppm of IBA was optimum (80.95%) for rhizogenesis within a month compared to 2000 ppm of IBA (84.21% rooting) with no statistically difference. The highest concentration of IBA (4000 ppm) led to a rooting rate of 73.68% but had phytotoxic effect on cuttings, an aspect also reported in the literature.

There are several factors and parameters affecting the development of an optimum vegetative propagation protocol for a plant species, such as the quality and quantity of irradiance (Hoffman *et al.*, 2016; Lee *et al.*, 2016), microbial associations (Assis *et al.*, 2004; Zavattieri *et al.*, 2016), gene expression (Delker *et al.*, 2017), enzyme activity and phytohormonal responses (Sung *et al.*, 2003; Assis *et al.*, 2004). As the initial goal of this study was the achievement of a fast and efficient propagation protocol for the massive production of clonal plants of *A. androsaceum* and *L. chersonesum*, the most important parameters examined were the type of material used as explant, and the amounts and type of exogenous auxin used for root induction (De Almeida *et al.*, 2017). These propagation protocols are above commercially needed standards and therefore may be used for the sustainable commercial exploitation of the studied local endemic species.

Seasonal differences and the origin of material used for the cuttings were also factors examined in this study. The season of the botanical collections and further experiments always depend on the plant species under investigation and especially on the stage of their biological cycle. Many times, the stage of obtaining the plant material from the wild is inappropriate but sometimes this cannot be avoided; in such cases, it is more difficult to develop the mother stock plants and special technical skills with experience are required. The development of mother stock plants after botanical collections is a standard procedure followed by the BBGK in order to obtain adequate and homogenous plant material for experimentation; this was also done for the Cretan endemics *A. androsaceum* and *L. chersonesum*. The mother plant material is grown in controlled environmental conditions in man-made habitats and the explants used for cuttings are all juvenile with fast-growing meristematic shoots which are ideal for experimentation. This has been broadly reported in the literature in massive propagation studies (Guerreira *et al.*, 2010; Pijut *et al.*, 2011; Beemnet and Solomon, 2012; Denaxa *et al.* 2012; Wendling *et al.*, 2014b; Elhaak *et al.* 2015). Hence, most of the vegetative protocols developed for unknown native plant species in the BBGK use *ex-situ* cultivated material i.e., mother stock plants in the greenhouse, in plastic pots, garden beds or *in vitro* plants. This research methodology allows the development of vegetative propagation protocols derived from homogenic cultivated explant material and therefore can be further used for commercial nurseries.

Conclusions

The increase of human needs and the demanding market to date request new ornamental plants and new natural medicinal products. The present study demonstrates:

1) The development of two successful vegetative propagation protocols via cuttings for the local Cretan endemics *Acantholimon androsaceum* and *Limonium chersonesum*.

2) The rooting rates for both species are more than adequate for commercialization (71.43% for *A. androsaceum* and 80.95% for *L. chersonesum*), which may deliver potentially new industrial crops and may serve as an example of exploiting sustainably wild phylogenetic resources in horticulture and the cosmetic-medicinal sector.

3) In search of new profitable crops, the evaluation and domestication of many new wild taxa derived from natural resources is a promising way to enhance the income of local communities addressing global commercial needs. The strategy and research methodology presented herein, is aiming at the selection of new, rare and endemic native industrial crops from the rich phylogenetic resources of Crete.

Authors' Contributions

Conceptualization: NK, KG, GT; Data curation: KG, NK, VS; Formal analysis: KG, NK, VS; Funding acquisition: KG, NK, GT; Investigation: KG, VS; Methodology: KG, NK, VS; Supervision: KG, EM; Validation: KG, EM, NK, GT; Visualization: KG, NK, VS; Writing - original draft preparation: KG, NK, VS, GT; Writing - review & editing: KG, NK, VS, GT; Project administration: KG, GT. All authors read and approved the final submitted manuscript.

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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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