

Propagation and *ex-situ* conservation of *Lomelosia minoana* subsp. *minoana* and *Scutellaria hirta* - two ornamental and medicinal Cretan endemics (Greece)

Katerina GRIGORIADOU*, Nikos KRIGAS,
Virginia SARROPOULOU, Eleni MALOUPA,
Georgios TSOKTOURIDIS

Hellenic Agricultural Organization (HAO)-DEMETER, Institute of Plant Breeding and Genetic Resources, Balkan Botanic Garden of Kroussia, Laboratory of Protection and Evaluation of Native and Floriculture Species, GR-570 01 Thermi, Thessaloniki, P.O. Box 60458, Greece; kgrigoriadou@jggrb.gr (*corresponding author); nikoskrigas@gmail.com; vsarrop@gmail.com; maloupa@bbgk.gr; gtsok1@yahoo.co.uk

Abstract

Human needs and concomitant commercial trade to date trigger the demand of new ornamental plants and new natural medicinal products. The current study includes preliminary seed germination trials and presents the development of effective vegetative propagation protocols for *Lomelosia minoana* subsp. *minoana* (Dipsacaceae) and *Scutellaria hirta* (Lamiaceae), both globally rare, local endemics of Crete (Greece) with potential ornamental and medicinal value. Based on material collected directly from the wild, seed germination was succeeded (38%, T_{50} : 10.74) only for *L. minoana* subsp. *minoana*. The optimal indole-3-butyric acid concentrations for effective rooting of cuttings were 2000 mg L⁻¹ for *L. minoana* subsp. *minoana* (85%) and 4000 mg L⁻¹ for *S. hirta* (50%). Seasonal differences were observed in respective rooting rates of the studied taxa. The detected rooting rates for *L. minoana* subsp. *minoana* cuttings are above standards to allow possible commercial application in the ornamental industry. Although the detected rooting rate (50%) for *S. hirta* was adequate for its *ex situ* conservation, it seems almost marginal for future commercial application and further investigation is needed. The sustainable utilization of these Cretan endemics studied herein provides new input for the ornamental-horticultural and cosmetic-pharmaceutical industries. This study shows how rare and endemic wild plant species can be progressively domesticated and introduced into sustainable cultivation systems in order to avoid the depletion of unique phylogenetic resources.

Keywords: cuttings; floriculture; germplasm preservation; phylogenetic resources; rooting; seed germination; sustainable utilization

Introduction

The medicinal and horticultural industries are in continuous search for new natural products and new floricultural or ornamental crops. This trend is coupled to date with the need for ecological plantings, landscaping with native plants (Beck, 2013), sustainable landscaping (Antrop, 2006; Ahern, 2013) and

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sustainable exploitation of phylogenetic resources (Krigas and Maloupa, 2008; Maloupa *et al.*, 2008). In the frame of sustainability and modern consumerism addressing commercial/economic needs, such driving forces have triggered to date many contemporary studies focusing on many plant groups which are native across different scales.

From an ornamental/horticultural point of view, members of the genus *Lomelosia* (including the closely related *Scabiosa*) of the Dipsacaceae family have been dynamically introduced in ornamental horticulture as new flower crops, such as *L. caucasica* (M. Bieb.) Greuter & Burdet (Beisheim and Otte, 2017); as xerophytes in landscaping such as *L. crenata* (Cirillo) Greuter & Burdet subsp. *dellaportae* (Boiss.) Greuter & Burdet (Krigas *et al.*, 2017), *L. graminifolia* (L.) Greuter & Burdet (Barth, 2020), *L. hymettia* (Boiss. & Spruner) Greuter & Burdet and *L. cretica* (L.) Greuter & Burdet (Papafotiou *et al.*, 2017b); for use on green roof plantations such as *L. prolifera* (L.) Greuter & Burdet (Cristaudo *et al.*, 2015). Amongst >300 species in the genus *Scutellaria* of Lamiaceae family (Joshee *et al.*, 2002), some members have been used in landscaping for harboring ecosystem diversity (Joshee *et al.*, 2013); as ornamental plants or as new flower crops (Cantor *et al.*, 2009; Morgan and Pearson, 2018); in plantations of green roofs such as *S. wrightii* A. Gray (Getter and Rowe, 2008), *S. scordifolia* (Schltdl. & Cham.) Benth. (Schneider *et al.*, 2014) and *S. pontica* K. Koch (Sari and Karaşah, 2015); for xeriscaping such as *S. pontica* (Sari and Karaşah, 2015), *S. rupestris* Boiss. & Heldr. (Maloupa *et al.*, 2005) and *S. alpina* L. (Calhoun, 2012).

Successful propagation in members of *Lomelosia* has been reported both via seeds and vegetative methods. Propagation with cuttings for *L. hymettia* using IBA results to a high rate of rooting (92.5-100%) during autumn, compared to 50-67.5% during experimentation in spring (Vlachou *et al.*, 2019). *In vitro* propagation has been successfully employed for *L. argentea* (L.) Greuter & Burdet (Panayotova *et al.*, 2008) and the closely related *Scabiosa comosa* Fisch. ex Roem. & Schult. (= *S. tschiliensis* Grunning) (Wang *et al.*, 2013). *Scabiosa* spp. which are closely related with *Lomelosia* may show as low as 50-53% germination e.g., *S. triniifolia* Friv. and *S. africana* L. (SID-Kew), or up to 100% in *S. canescens* Waldst. Kit. (Csonthos *et al.*, 2007) and *S. columbaria* L. (Cerabolini *et al.*, 2003).

With increased annual consumption of scullcaps (*Scutellaria* spp.) during the last decades (Greenfield and Davies, 2004), there is a high need for pathogen-free biomass production of these ornamental and medicinal plants at least in the USA (Tascan *et al.*, 2010). In general, seed germination, cuttings and root division are the main propagation methods for members of *Scutellaria* (Similien, 2009). In addition, successful propagation through *in vitro* techniques has been reported (Joshee *et al.*, 2002; Tascan *et al.*, 2010). Advanced propagation systems *in vitro* have been developed to study model-members of the genus *Scutellaria*, involving simple shoot proliferation techniques, bioreactors, and gene transformation protocols (Cole *et al.*, 2007, 2009; Joshee *et al.*, 2013). Propagation of *Scutellaria* spp. by seeds can be highly successful and fast (100% germination within 8 days for *S. pycnoclada* Juz. and *S. tomentosa* Bertol.), intermediate and rather slow, 52% within 42 days for *S. ramosissima* Popov (SID-Kew) or slow with very low success, 10% within a month for *S. incana* Spreng. (O'Brien, 2013).

With regards to medicinal interest, most species of genus *Scutellaria* are usually rich in scutellarin, a substance with anti-cancer activity and for treatment of cardiovascular diseases (Cui *et al.*, 2010), and they contain essential oils and phenolic compounds with antioxidant, anti-carcinogenic, anti-mutagenic, anti-bacterial and anti-inflammatory action (Heo *et al.*, 2004). *L. hymettia* is also considered as a medicinal plant (Grigoriadou *et al.*, 2019) with antimicrobial properties (Christopoulou *et al.*, 2008).

In this study we focused on Greek native rock-dwelling plants which are globally rare and exclusively confined to the island of Crete (local single-island endemics). Additionally, the focal herbaceous perennial plants studied have useful medicinal properties as well as interesting features and natural adaptations that could be exploited in floriculture, horticulture, and landscaping. Such features refer to seasonal colour variations as in *Scutellaria hirta* Sm.; impressive inflorescence (compound heads of many lingulate florets) combined with vigorous growth and natural adaptation to arid conditions allowing (among others) for use as ground cover as in *Lomelosia minoana* (P. H. Davis) Greuter & Burdet subsp. *minoana*. The combination of rarity, uniqueness,

usefulness and impressive plant features or adaptations are known to be extremely appreciated by the international online floricultural-ornamental market (Dee *et al.*, 2019) and consequently such endemic native plants are usually associated with high prices (Krigas *et al.*, 2014; Menteli *et al.*, 2019).

In this context, the vegetative and sexual propagation of the selected plants are studied herein for the first time with the aim to facilitate their *ex situ* conservation and further sustainable exploitation as valuable and unique phylogenetic resources of Greece in the global context.

Materials and Methods

From botanical collections in the wild to stock mother plants for experimentation

Two botanical collections were performed on the island of Crete (Greece) during late summer (end of August) and autumn (mid-October) of 2018 using a special permit of the Balkan Botanic Garden of Kroussia (BBGK), which is issued yearly by the Greek Ministry of Environment and Energy (renewed every year). The selected plant material (young individuals, seeds, and cuttings) was collected carefully in order to avoid any damage to the extant wild growing populations of the taxa (species and subspecies) and was transferred to the facilities of the BBGK in Thermi, Thessaloniki, where finally received an IPEN (International Plant Exchange Network) accession number after taxonomical identification (Table 1).

The living plants collected from the wild (Table 1; Figure 1) in Crete were transplanted and maintained in a greenhouse.



Figure 1. Mature individuals of *Lomelosia minoana* subsp. *minoana* (left) and *Scutellaria hirta* (right) in their wild habitats at mountain areas on the island of Crete, Greece

A month later, shoot tip cuttings were excised from the collected plants, were treated with 2000 mg L⁻¹ indole-3-butyric acid (IBA) (Radicin, Fytorgan SA, Greece), were transferred in multi-cell propagation trays using a substrate mixture of peat moss (Terrahum, Klasmann): perlite (1 : 3 v / v) and were placed under mist in 80-90% relative humidity (RH). Three weeks later, the rooted cuttings were transplanted in 1 L plastic pots. This procedure was repeated every month from September 2018 until February 2019, allowing the production of adequate stock material for experimentation.

The cuttings collected from the wild grown plants (Table 1) were initially kept in a fridge, they were transported using a portable one to the BBGK's laboratory and were treated with the same rooting procedure described previously. The inflorescences collected from the wild-growing plants were placed for drying in a dark chamber at 15 °C and RH 15% and one month later, the seeds were separated and weighed, determining the average weight of ten seeds (Table 1). The cleaned seeds were used in experimentation. Cuttings and young wild individual plants were collected for *S. hirta*, because the season was not appropriate for seed collection.

Table 1. Basic information about the original plant material (seeds, cuttings, living individuals) of the local Cretan endemics collected from the natural environment of the island of Crete (Greece) for *ex situ* conservation in 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 individuals
<i>Lomelosia minoana</i> subsp. <i>minoana</i> (GR-1-BBGK-19,16)	Ano Viannos	1500	60	132	1
<i>Scutellaria hirta</i> (GR-1-BBGK-19,3)	Skinakas	-	-	30	1

Seed germination

Seed germination was determined in autumn (November 2018) right after their collection. The seeds (n= 50 for *L. minoana* subsp. *minoana*) were saturated in di-ionized water (dH₂O) for 24 h and were then sowed (4-5 mm depth) in plastic trays containing a substrate of peat (Terrahum, Klassman): perlite (1 : 1 v / v) covered with a layer of vermiculite (2-3 mm). The trays were placed on a heated-bench mist-chamber (19 ± 2 °C soil temperature and 80-90% RH). The seed germination rate was recorded every fortnight for two consecutive months by measuring the number of seeds with visible sprouting and T₅₀ (the time required for 50% germination of the final germination rate of the seeds) was calculated (Bewley and Black, 1994; Genmedoc, 2006). After seed germination and growth for 60 d, the seedlings were transferred in multiple cell propagation trays and followed the procedure described below for rooted plants.

Rooting of cuttings

The rooting of cuttings obtained from mother stock plants were treated with four different concentrations (0, 1000, 2000 and 4000 mg L⁻¹) of IBA (Duchefa Biochemie, The Netherlands) during June-July 2019. Softwood tip cuttings of *L. minoana* subsp. *minoana* (3-4 cm) and *S. hirta* (3.5-4 cm long) were immersed for 10 sec in the above IBA solutions (dissolved in 50% ethanol), were transferred in multi-cell propagation trays using a 1 : 3 v / v peat moss (TS1, Klasmann): perlite substrate and were placed in a mist chamber (soil temperature 18-25 °C, air temperature 18-30 °C depending on local weather conditions, and RH 70-85%) within the BBGK's non-heated greenhouse. The number of roots per cutting and root length was measured for *L. minoana* subsp. *minoana* after 40 d and for *S. hirta* after 15 d. The rooting of cuttings was expressed as percentage. Rooted plants from all the above treatments were transplanted in 0.33 L pots and subsequently in 2.5 L after four weeks, containing a mixture of peat (TS2, Klasmann) and perlite (3 : 1 v / v) for further growth. The excess plant material produced was planted 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). One potted mother stock plant of both *L. minoana* subsp. *minoana* and *S. hirta* was depicted visually under *ex situ* conservation at the BBGK, each species separately which represent the potential of the two taxa for field cultivation. (Figures 2c, 3c).

Statistical analysis

All rooting experiments were conducted in a completely randomized design and were repeated twice. The means were subjected to analysis of variance (ANOVA) using the statistical package SPSS 17.0 (SPSS Inc, Chicago, Illinois, USA) and were compared by using the Duncan's multiple-range test. The experiments consisted of four treatments where each value was the mean of 21 replicates (three groups of seven repetitions). To compare the means, the Duncan's multiple range test was used at P ≤ 0.05 to establish significant differences among the treatments. The main effect of factors (rooting period and IBA concentration) as well as their interaction were determined by the General Linear Model (two-way ANOVA).

Results

Vegetative propagation and development of stock mother plants

The cuttings of *L. minoana* subsp. *minoana* collected from the wild (cuttings from the wild growing plants transferred to the *ex situ* facilities of BBGK) presented 86-88% rooting, resulting in a total of 186 stock mother plants. High rooting success (96-100%) was achieved for *S. hirta* cuttings obtained from wild-growing individuals, producing a total of 237 plants within seven months (Table 2).

Table 2. Preliminary rooting experiments of cuttings from the Cretan local endemic plants *Lomelosia minoana* subsp. *minoana* (September: *in situ* collected cuttings; February: *ex situ* collected cuttings) and *Scutellaria hirta* (all *ex situ*), originated from wild-growing populations, using 2000 mg L⁻¹ IBA for the initial development of stock clonal mother 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>Lomelosia minoana</i> subsp. <i>minoana</i>	132	86	23	-	-	-	84	88	60	186 (113+0+73)
<i>Scutellaria hirta</i>	24	100	40	54	100	24	166	96	28	237 (24+54+159)

Seed germination

Seed germination for *L. minoana* subsp. *minoana* was observed within the first six days (first signs of sprouting). During the first fortnight, 20% of the seeds germinated and after 30 days, the number of germinated seeds increased to 34% and reached 38% in 60 days ($T_{50} = 10.74$) (Table 3).

Table 3. Effect of sowing days (15, 30, 45, 60) on germination (%) of *Lomelosia minoana* subsp. *minoana* seeds in peat: perlite (1:1 v / v), after pre-treatment in ddH₂O for 24 h prior to sowing (20/11/2018).

Days after sowing	Germination (%)
15	20 b
30	34 a
45	36 a
60	38 a
<i>p-values</i>	0.009**

Different letters between different days of sowing within the Germination (%) column denote significant differences (Duncan test, $p > 0.05$, ** $p \leq 0.01$).

Effect of IBA in rooting

The use of IBA independently of concentration was the best treatment for the vegetative propagation of cuttings in *L. minoana* subsp. *minoana*. IBA at 2000 mg L⁻¹ was rather the best treatment considering mean number of roots (17.17) (Table 4; Figure 2).

Table 4. Effect of different concentrations of IBA on rooting (%), number and length (cm) of roots obtained from *Lomelosia minoana* subsp. *minoana* stock mother plants after 40 d in mist, using a substrate composed of peat: perlite at 1:3 v / v

Treatments	Rooting (%)	Number of roots / rooted cutting	Root length (cm)
Control	42.86 b	5.33 ± 0.48 c	2.62 ± 0.43 b
1000 mg L ⁻¹ IBA	71.43 a	14.30 ± 2.17 ab	4.42 ± 0.27 a
2000 mg L ⁻¹ IBA	85.71 a	17.17 ± 2.20 a	3.61 ± 0.34 ab
4000 mg L ⁻¹ IBA	85.71 a	10.83 ± 1.58 b	4.54 ± 0.35 a
<i>P</i> -values	0.019*	0.000***	0.001**

Different letters between different IBA concentrations within a column denote significant differences (Duncan test, $p > 0.05$, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$).

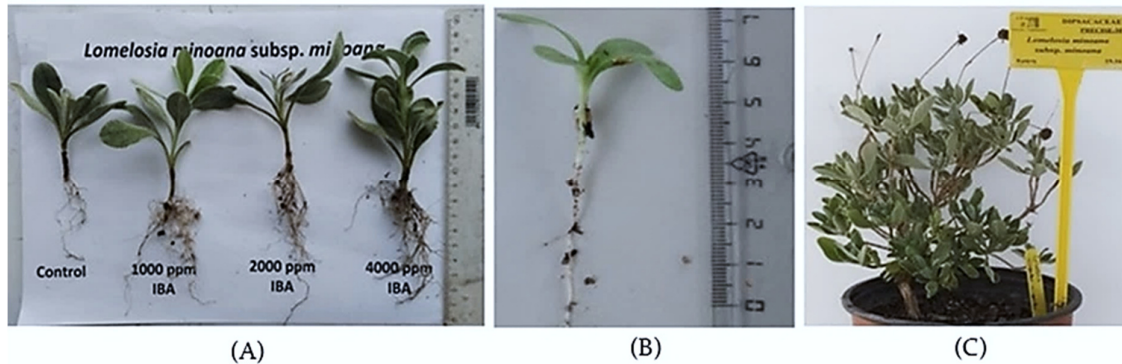


Figure 2. Effect of IBA (0, 1000, 2000 and 4000 mg L⁻¹) on rooting of cuttings of *Lomelosia minoana* subsp. *minoana*

(A) after 40 d under mist using a substrate composed of peat moss (TS1, Klasmann): perlite at 1 : 3 v / v; (B) individual rooted cutting; (C) potted mother stock plant under *ex situ* conservation at the Balkan Botanic Garden of Kroussia

The best application of IBA was achieved at 4000 mg L⁻¹ in *S. hirta* cuttings, by increasing the rooting percentage from 5.26% (control) to 52.38%. The two lower IBA concentrations also increased the percentage of rooted cuttings to 22.22%. Symptoms of browning from the base towards the apex of the cuttings were evident in all treatments including the control (19.05-23.81%) irrespectively of IBA concentration, thus resulting gradually in total necrosis at the end of the experimental rooting period (15 d) due to gradual degradation of the cuttings not forming any roots. IBA concentration did not affect root length, since treated cuttings exhibited similar results with the untreated ones (1.05 and 0.92 cm, respectively) (Table 5; Figures 3A-C).

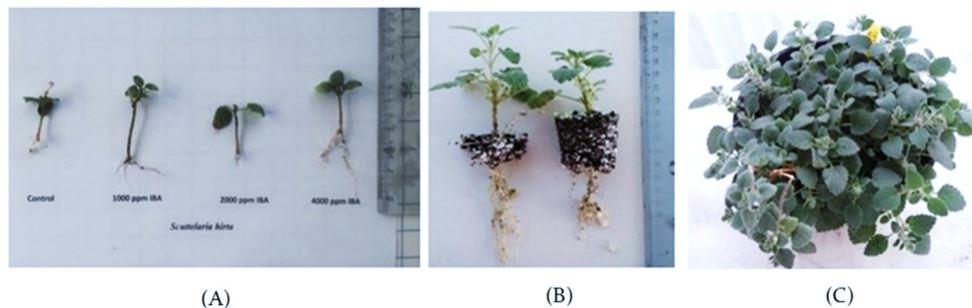


Figure 3. Effect of IBA (0, 1000, 2000 and 4000 mg L⁻¹) on rooting of cuttings of *Scutellaria hirta*;

(A) after 15 d under mist using a substrate composed of peat moss (TS1, Klasmann): perlite at 1 : 3 v / v; (B) individual rooted cuttings; (C) potted mother stock plant under *ex situ* conservation at the Balkan Botanic Garden of Kroussia

Table 5. Effect of different concentrations of IBA on rooting (%), number and length (cm) of roots obtained from *Scutellaria hirta* stock mother plants after 15 d in mist, using a substrate composed of peat: perlite at 1:3 v / v

Treatments	Rooting (%)	Number of roots / rooted cutting	Root length (cm)	Necrosis (%)
Control	5.26 c	6.00 ± 0.00 a	0.92 ± 0.00 ab	23.81 ab
1000 mg L ⁻¹ IBA	22.22 b	1.75 ± 0.08 c	1.05 ± 0.07 a	33.33 a
2000 mg L ⁻¹ IBA	22.22 b	1.50 ± 0.08 c	0.75 ± 0.02 b	19.05 b
4000 mg L ⁻¹ IBA	52.38 a	3.63 ± 0.31 b	1.05 ± 0.11 a	23.81 ab
<i>P</i> -values	0.000***	0.000***	0.003**	0.044*

Different letters between different IBA concentrations within a column denote significant differences (Duncan test, $p > 0.05$, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$).

Discussion

The propagation study of plants with no available data is a tedious and challenging practice in search for the best protocol allowing massive production of elite clonal plants. Our study presents propagation experimentation using seeds and cuttings of the wild Cretan local endemics *Lomelosia minoana* subsp. *minoana* and *Scutellaria hirta* for the first time.

The present study firstly describes preliminary experimentation on germination of seeds collected from the wild growing Cretan endemic *L. minoana* subsp. *minoana* in autumn right after collection from wild. With germination starting at the 6th d and T50: 10.71 d, the germination rate was 34% on d 30, and slightly increased (38%) till the end of the experimental period. Low germination rates (<5% or <30%) were also reported for *L. graminifolia* (Kupferschmid *et al.*, 2000) and *Scabiosa atropurpurea* indicating the necessity of storage period (ten months under alternating 20/10 °C conditions) to allow increased final germination from approximately 40-50% (fresh seeds) to 70-80% (stored ones), thus suggesting innate dormancy and a dormancy-breaking effect (Frischie *et al.*, 2018). In the current study, the mature seeds of *L. minoana* subsp. *minoana* were directly harvested from the wild 50 d prior to experimentation, were only saturated for 24 h in dH₂O at room temperature in dark, without previous storage in a fridge. The absence of dry storage of its seeds might be the reason for the low germination percentage (38%) obtained within a 60-d period during autumn-winter, indicating a possible occurrence of primary normal dormancy.

The propagation experiments using cuttings for *L. minoana* subsp. *minoana* presented 86% rooting during September in 23 d and for *S. hirta* 100% rooting during September within 40 d. The experiments for *L. minoana* subsp. *minoana* showed no statistical difference on rooting percentage regarding all IBA concentrations used. However, the treatment of 2000 mg L⁻¹ IBA solution was the optimum, producing the highest number of roots (17.17) and 85.71% rooted plants within 40 d. High induction of roots (100%) after a two-week period in the mist has been also described for *L. hymettia* cuttings collected in autumn and winter and treated with 1000 mg L⁻¹ IBA (Vlachou *et al.*, 2019). Asexual propagation using IBA is also reported in *L. crenata* subsp. *dallaportae* softwood tip cuttings delivering 60-80% rooting rates (Krigas *et al.*, 2010). In the case studied herein, IBA presented a promoting effect on rooting (irrespective of concentration), increased rooting rate (1.66-two-fold) as well as increased number of roots (2 to >3-fold) and root length (1-2 cm). In the herbaceous perennial ornamental *Scabiosa* 'Pink Mist' (closely related to *Lomelosia* spp.), drench of stem cuttings with 1000 and 3000 mg L⁻¹ IBA solutions as a dip or foliar spray is reported to increase the root number, rooting percentage and rooting area of the generated plants (Hoover, 2018). The effective response of *L. minoana* subsp. *minoana* cuttings treated with IBA could be possibly due to the promoting effect of auxins on the root formation process, resulting in high enzyme activity and translocation rate of nutrients from the upper part of the cuttings to their basal ends, which in turn, leads to carbohydrates' hydrolysis, providing enough energy for root cells' formation (Paradikovic *et al.*, 2013).

The IBA concentration of 4000 mg L⁻¹ was the optimum rooting treatment for *S. hirta* (52.38% rooting, 3.63 roots, 1.05 cm long) within 15 days. This treatment was the highest concentration of IBA used for rooting and the most effective one, with a 10-fold rooting increase compared to control. The abrupt termination of the propagation procedure after 15 days due to infections and degradation of the fragile shoot tips used for cuttings was possibly the reason for the low rooting rate (52.38%) observed, thus not allowing enough time for a clear IBA effect. This percentage of rooting resulted in the development of a good propagation protocol for conservation purposes regarding *S. hirta* but not good enough for its massive production. Consequently, further experimentation is required using various auxins in different seasonal periods. IBA was selected due to its positive rooting effects in other species of Lamiaceae, resulting to 5-10-fold increase of rooting percentage and number of roots (Venugopal *et al.*, 2018) in *Thymbra capitata* (L.) Cav., *Thymus serpyllum* L. and *T. vulgaris* L. (Iapichino *et al.*, 2006).

Seasonal differences were observed regarding the Cretan local endemics studied during the experimentation for the development of vegetative propagation protocols using cuttings. The experiments performed during summer resulted to lower rooting percentage compared to the autumn and winter treatments. The mother stock plants were maintained in controlled environmental conditions and the cuttings obtained from them were characterized as juvenile fast-growing apical shoots. The use of such juvenile shoots for cuttings may increase the rooting rate as has been extensively reported in the literature and the same observed for hardwood cuttings (Ferreira *et al.*, 2010; Pijut *et al.*, 2011; Beemnet and Solomon, 2012; Denaxa *et al.*, 2012; Wendling *et al.*, 2014b; Elhaak *et al.*, 2015). Another factor affecting rooting (perhaps also associated with the current study) is the age of stock plant material used for the cuttings. It is known that aged plant material (such as the well-developed mature plants from which we excised the cuttings in the wild) tends to be less responsive to the application of IBA in comparison to cuttings obtained from younger plants, which are more responsive to IBA application in terms of root length (Pijut *et al.*, 2011).

In this study, only shoot tip cuttings were used as the ideal cutting type for successful vegetative propagation. The juvenile softwood tip cuttings used in the experimentation process were excised during the end of May or end of August, after continuous pruning of the upper vegetative part of the mother stock plants throughout the previous winter and spring. The reason for this choice is that tip cuttings exhibit higher rooting rates than stem (decapitated) cuttings and this could be attributed to the hypothesis previously reported by Sabatino *et al.* (2014), suggesting that the endogenous auxin IAA (being basipetally transferred from the apex meristems) plays an essential role in the formation of adventitious roots even when an exogenous auxin type (IBA, NAA, IAA) is also applied. However, root induction of cuttings during vegetative propagation is a complex and multifactorial procedure, where different basic parameters should be optimized for the massive production of elite clonal plants.

The amount of exogenous auxin for rooting induction, initiation, and expression (De Almeida *et al.*, 2017) depends on the species studied and the concentration of auxins present in plant tissues (Paulus *et al.*, 2016). For example, in both *Rosmarinus officinalis* L. (Paulus *et al.*, 2016) and *Aloysia triphylla* Paláu (Paulus *et al.*, 2014) the use of higher amounts than 2500 mg L⁻¹ and 1500 mg L⁻¹ of IBA, respectively, is reported to diminish the percentage of rooted cuttings. *L. minoana* subsp. *minoana* was proved to require low IBA concentration (2000 mg L⁻¹) for the highest rooting rate in comparison to *S. hirta* that needs higher IBA concentration (4000 mg L⁻¹), depending on season. Therefore, this study suggests that there are species-specific differences with respect to the degree of easiness/difficulty of rooting of cuttings amongst species in different seasons.

Conclusions

The current study presents the development of vegetative propagation protocols for the Cretan local endemics *L. minoana* subsp. *minoana* and *S. hirta* which have potential ornamental and medicinal value. The

concentration of 2000 mg L⁻¹ IBA is the optimum for rooting the cuttings of *L. minoana* subsp. *minoana* at 85% within 40 days and 4000 mg L⁻¹ IBA was found appropriate for *S. hirta* cuttings at 50% within 15 days. The rooting rates for *L. minoana* subsp. *minoana* cuttings treated with 2000 ppm were above standards to allow possible commercial application in the ornamental industry. The respective percentage of rooting for *S. hirta* was almost marginal (50%) for commercialization and further experimentation is necessary.

The growth of human needs and the concomitant commercial trade to date trigger the demand of new ornamental plants and new natural medicinal products. The sustainable utilization of the two Cretan endemics studied herein provides new inputs for the ornamental and pharmaceutical industry, given the over-exploitation and direct marketing of wild phylogenetic resources in the horticultural sector (Krigas *et al.*, 2014, 2017; Menteli *et al.*, 2019) and the cosmetic-medicinal sector (Joshee *et al.*, 2013; Grigoriadou *et al.*, 2020). Under this perspective, it is imperative to urgently domesticate many wild plant species and introduce them into sustainable cultivation systems in order to avoid the depletion of phylogenetic resources. This is an important conservation strategy alleviating the effect of harvesting from the wild populations for satisfaction of commercial needs, allowing for or facilitating at the same time the long-term sustainable use of the phylogenetic resources and their steady supply.

Authors' Contributions

Conceptualization: K.G., N.K., E.M. and G.T.; Data curation: K.G., N.K. and V.S.; Formal analysis: K.G., N.K. and V.S.; Funding acquisition: K.G., N.K. and G.T.; Investigation: K.G. and V.S.; Methodology: K.G., N.K. and V.S.; Supervision: K.G. and E.M.; Validation: K.G., N.K., E.M. and G.T.; Visualization: K.G., V.S. and N.K.; Writing - original draft preparation: K.G., V.S., N.K. and G.T.; Writing - review and editing: K.G., N.K. and G.T.; Project administration: K.G. and G.T. All authors read and approved the final 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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