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Vegetative propagation of the range-restricted Greek endemic *Silene fabaria* (L.) Sm. in Sibth. & Sm. subsp. *domokina* Greuter (Caryophyllaceae)

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Abstract
Maintaining the genotype through asexual propagation is necessary for species with conservation priority (rare and threatened). *Silene fabaria* subsp. *domokina* (Caryophyllaceae) is a range-restricted Greek endemic of ornamental value. In autumn, softwood top cuttings (2-3 cm) were cut from mother plants kept inside the greenhouse and their base was immersed for 1 min in IBA (0, 1000, 2000 and 4000 ppm) solutions. Cuttings were placed in multiple disks on a peat: perlite (1:3) substrate on a heated greenhouse mist. The measurements taken were root number/rooted cutting, root length and rooting percentage (%). IBA at 1000 ppm gave the optimum rooting (100%) with 20.75 roots 4.84 cm long (four weeks). The survival percentage of rooted transplanted plants in pots containing a mixture of peat moss, perlite and soil after nine weeks was 100%. This study contributes to the successful *ex situ* conservation of *S. fabaria* subsp. *domokina* and enables its sustainable exploitation.

Keywords: auxins, cuttings, ex situ conservation, greek flora, rooting

1. Introduction
The family Caryophyllaceae is well known for ornamental flowering plants such as *Dianthus* spp. (Carnations) and *Silene* spp. (Campions) which form a big fraction of the world's cut flower trade [1]. The genus *Silene* includes ca. 700 species worldwide [2], many of which can be extensively used as landscape ornamentals in rock gardens, herbaceous borders and green areas [3] due to their drought tolerance, perennial characteristics and profuse flowering (showy, bright colored flowers either magenta, red, orange, crimson, pink, or white) [4]. In Greece alone (major centre of the genus diversity [2]), 136 *Silene* species and subspecies are presently found, of which 53 are endemic to the country [5, 6].

Silene fabaria (L.) Sm. in Sibth. & Sm. subsp. *domokina* Greuter (Caryophyllaceae) is a perennial herbaceous plant with erect stems, whitish flowers and purplish filaments and somewhat fleshy, elliptic-obovate, glaucous leaves (possibly edible), which occurs predominately on serpentine (also on flysch) and blossoms from April to August [7]. It is a range-restricted Greek endemic subspecies with disjunct distribution only in three out of 13 phytogeographical regions of Greece i.e. small parts of South Pindos, East Central Greece (Mt Geraneia) and Sterea Hellas (Domokos area) [5].

Although it is generally preferred to conserve threatened species *in situ* (evolutionary processes are more likely to remain dynamic in natural habitats), *ex situ* conservation is becoming increasingly important considering the rate of habitat loss worldwide [1]. Propagating wild rare species in greenhouses and botanic gardens for restoration or reintroduction in wild habitats can be an effective method of improving the size and viability of rare or threatened populations [9, 10]. However, for most of the rare and threatened species data about their sexual or asexual propagation are quite limited.

Plenty of cutting propagation techniques and commercial rooting products are available to facilitate better root of cuttings for floricultural crops [8], and these are also used for rare and threatened species [12, 13]. Indole-3-butyric acid (IBA) is the common active ingredient either formulated in water (potassium salt, K-IBA), alcohol (free acid of IBA), or talc depending on the chemical formulation and rooting requirements of the cuttings [14]. The mechanism underlying the promotion of adventitious root formation by exogenous hormone treatment has been largely investigated. Several studies have shown that exogenous hormone treatment accelerates cell division, promotes synthesis of endogenous hormones (e.g., auxins, cytokines, and gibberellins) and salicylic acid, stimulates carbohydrate accumulation, and consequently induces rooting [15, 16, 17].

In this context, the aim of this study is to investigate for the first time the propagation of *Silene fabaria* subsp. *domokina* by softwood cuttings in order to facilitate its *ex situ* conservation and potential sustainable exploitation as ornamental plant at the Balkan Botanic Garden of Kroussia. This is done in the frame of its focused conservation efforts on the rare, threatened and endemic plants of Greece, with the aim to contribute to the implementation of Targets 8 and 9 of the Global and European Strategies for Plant Conservation across local, regional and international scales [18].

2. Materials and Methods
2.1 Plant Material
Plant material was collected from the natural habitats of the selected species as a result of Plant material (living plants)

was collected from ophiolithic screes of Omvriaki mining area (Domokos, Sterea Hellas), at an altitude of 500 m above sea level during a botanic expedition conducted (accession number GR-BBGK-1-98,456 linked with site description, habitat information and treatments received). In total, six plant individuals were placed in 1.5 L pots right after collection and were transferred at the Balkan Botanic Garden of Kroussia-Laboratory for the Conservation and Evaluation of Native and Floricultural Species (Institute of Plant Breeding and Genetic Resources, Hellenic Agricultural Organization Demeter) for immediate care to recover from transplanting shock.

2.2 Asexual Propagation

In early-mid autumn, softwood top cuttings (2-3 cm) of *S. fabaria* subsp. *domokina* were cut from mother plants that were maintained outdoors (Thermi, Thessaloniki), followed by immersion of their base for 1 min in solutions of four IBA concentrations (0, 1000, 2000 and 4000 ppm). Afterwards, the cuttings were placed in propagation trays in a substrate of peat moss (Terrahum) and perlite (Geoflor) (1:3 v/v ratio) and maintained at bottom heat benches in a plastic greenhouse. Soil temperature was kept at 18-21°C, while air temperature was 15-25°C, depending on local weather conditions. Relative humidity was approximately 70-85% (mist). The experiment lasted for four weeks and consisted of four treatments with 12 replications per treatment. At the end of the experimental period, the number of roots per rooted cutting and root length were measured. Rooting was expressed as percentage (%). Produced rooted plants were then transplanted into pots of 0.33 L (8x8x7 cm) containing a mixture of peat moss (Klasmann, TS2), perlite and soil (2: ½ : ½ v/v ratio) and subsequently into pots of 2.5 L containing a mixture of peat moss (Klasmann, TS2), perlite and soil (2:1:1 v/v ratio). The survival percentage followed each transplantation of rooted plants into pots of larger capacity was recorded.

2.3 Statistical analysis

Analysis of variance was performed with the SPSS 17.0 statistical package and mean separation with Duncan's Multiple Range Test. Significance was recorded at $P \leq 0.05$.

3. Results

Vegetative propagation of *S. fabaria* subsp. *domokina* by cuttings was successfully achieved within four weeks. Cuttings treated with 1000 ppm IBA showed the longest roots (4.84 cm) and with 2000 ppm IBA more roots (28.75) were produced, compared to control (13 roots, 2.84 cm long). All IBA treatments (1000-4000 ppm) resulted in 100% rooting with respect to control (75%) (Table 1). Absolute survival percentage (100%) was recorded after three and another six weeks for rooted cuttings transplanted into 0.33 L and subsequently 2.5 L pots containing a peat moss, perlite and soil substrate (2: ½ : ½ v/v ratio for 0.33 L pots and 2:1:1 v/v ratio for 2.5 L pots) (Fig. 1).

4. Discussion

Rooting powders containing auxins are used to increase the percentage of rooted cuttings, to improve root ball development and to shorten the production cycle, as the auxins enhance the initiation of roots and increase their number [19]. Benefits of auxin treatments depends on auxin concentration, condition of the cutting plants, application methods (such as the quick-dip method and foliar spray method), substrates and other factors [20-27]. In this study rooting of cuttings of *S. fabaria* subsp. *domokina* was promoted by IBA application, being optimum with 1000 and 2000 ppm concentrations. In other *Silene* species (*S. chalcedonica*), the rooting percentage of cuttings was improved by Dip 'N' Grow treatment (1,000 mg/L) containing 1% IBA + 0.5% NAA, and Hormex Rooting Powder No. 1 (1,000 mg/L) containing 0.1% IBA [4]. Different results to ours were obtained in *S. coronaria*, where 89% of cuttings were rooted readily without an auxin treatment in a vermiculite substrate [4].

5. Conclusions

To our best knowledge, there is no prior information on the vegetative propagation of *S. fabaria* subsp. *domokina*. Effective rooting (100%) was achieved for *Silene fabaria* ssp. *domokina* (local Greek endemic) in mid-autumn within four weeks, with the application of 1000 ppm IBA. This propagation protocol allows its back up in the frame of the *ex situ* conservation efforts of the Balkan Botanic Garden of Kroussia, facilitating also its sustainable exploitation. In early-June 2018, after the end of experimentation and recording of the final survival percentage of the plants outside the greenhouse, >100 plants was transferred for transplantation and *ex situ* conservation to the Balkan Botanic Garden of Kroussia (BBGK), N. Greece (600 m above sea level) where their development is being monitored.

Table 1: Effect of IBA concentrations (0, 1000, 2000 and 4000 ppm) on rooting percentage (%), root number per rooted cutting and root length (cm) in *Silene fabaria* subsp. *domokina* softwood top cuttings after four weeks in mid-autumn

IBA (ppm)	Rooting (%)	Root number	Root length (cm)
Control	75 a	13.00 ± 1.51 a	2.84 ± 0.11 a
1000	100 b	20.75 ± 5.00 ab	4.84 ± 0.66 b
2000	100 b	28.75 ± 6.08 b	3.77 ± 0.38 ab
4000	100 b	24.00 ± 1.66 ab	4.11 ± 0.38 ab
P-value	0.000***	0.074 ns	0.024*

Means ± standard error (S.E.) followed by the same letters within each column are not significantly different at $P \geq 0.05$ based on Duncan's multiple range test. ns $P > 0.05$, * $P \leq 0.5$, *** $P \leq 0.01$

Fig 1: Asexual propagation of *Silene fabaria* subsp. *domokina* cuttings: (a) mother plants; (b) cuttings prior to experimentation; (c) cuttings in multiplate discs inside mist; (d) control; (e) cuttings with 1000 ppm IBA; (f) with 2000 ppm IBA; (g) with 4000 ppm IBA; (h, i, j) vegetative growth of transplanted rooted cuttings into 0.33 L pots at day 1, 14 and 29, respectively, and (k) into 2.5 L pots inside greenhouse

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