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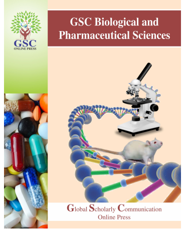

(RESEARCH ARTICLE)

Vegetative propagation of three prioritized Greek endemics with potential commercial interest: *Erysimum naxense* Snogerup, *Erysimum krendlii* Polatschek and *Centaurea paxorum* Phitos & Georgiadis

Sarropoulou Virginia * and Maloupa Eleni

Hellenic Agricultural Organization (HAO)-DEMETER, Institute of Plant Breeding and Genetic Resources, Laboratory of Protection and Evaluation of Native and Floriculture Species, Balkan Botanic Garden of Kroussia, P.C. 570 01 Thermi, Thessaloniki, P.O. Box 60458, Greece.

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Abstract

The Balkan Botanic Garden of Kroussia has formulated a conservation strategy for the collection and documentation of wild plant material for sustainable utilization, prioritizing the Greek rare - threatened endemics. In this framework, the species studied were the endangered *Erysimum krendlii* Polatschek, the vulnerable *Centaurea paxorum* Phitos & Georgiadis and the rare *Erysimum naxense* Snogerup: all are range-restricted, local Greek endemics with small populations in the wild, therefore with conservation priority, showing also potential commercial interest (aromatic-medicinal properties, edible parts or as pot plants for gardening). The effect of the immersion of the base of the cuttings for 1 min in indole-3-butyric acid (IBA) solutions at different concentrations (0, 1000, 2000, 4000 ppm) on root formation was tested for all species. A peat moss: perlite substrate (1:3 ratio) was used for the experiment. *E. krendlii* had better performance with 4000 ppm IBA showing 100% rooting, 30.86 roots 3.71 long after 5 weeks in the mist. Rooting of *C. paxorum* cuttings was optimum after 3 weeks exhibiting 100% rooting, 47.86 roots 2.61 cm long with 1000 ppm IBA. *E. naxense* gave 85.71% rooting, 19 roots 3.06 cm long with 4000 ppm IBA after 8 weeks. All the young individuals produced were transplanted in bigger pots (0.33, 1 Lt) until their final transplanting pot size (2.5 Lt), each time in a peat moss: perlite: soil substrate (2:½:½ v/v), for their subsequent growth. The successful asexual propagation of the studied wild species allowed the creation of mother plants, facilitated their *ex situ* conservation and domestication.

Keywords: Cuttings; Greek flora; Rooting; Propagation protocols; *Erysimum* spp.; *Centaurea paxorum*

1. Introduction

Plant diversity in Greece and the Balkans is exceptionally rich and unique, present higher degree of endemism in relation to surface than any other comparable area of Europe or the Mediterranean region [1, 2]. About 15–20% of the plant taxa (species and subspecies) found in Greece are unique, found nowhere else in the world (Greek endemics), and therefore the country is considered to be among the most valuable regions for plant conservation in Europe and the Mediterranean [3]. The uniqueness and rarity of the Greek and the Balkan flora demands effective conservation efforts as it is under increasing threat from climatic change, fires, land reclamation, over-grazing and urban and tourist development. Botanic gardens play a key role in ensuring that plant resources are not only conserved but are also used sustainably for the benefit of all people, in order to improve human well-being [4, 5]. With these threats to the region's vegetation the Balkan Botanic Garden of Kroussia (BBGK) has formulated a conservation strategy [6] and has adopted the Mission Statement to "support research, maintenance, propagation, evaluation, conservation and sustainable use of the native plants of Greece and the Balkans, combined with raising the environmental awareness of the public. BBGK's

* Corresponding author
E-mail address: vsarrop@gmail.com

mission includes eight different complementary policies; only native plants, important plant Species (IPS), explicit plant documentation, propagation of the IPS, DNA barcoding, combined *ex situ* and *in situ* conservation, evaluation for sustainable exploitation and the environmental awareness on native plants [7].

In the context of the global efforts to halt biodiversity loss by 2010 and beyond, emphasis is also given to the creation of a link between the *ex situ* and the *in situ* conservation actions regarding the IPS of Greece and the Balkans. Consideration has also been given by adjusting BBGK's individual conservation actions in light of the targets of the Global Strategy for Plant Conservation (GSPC) [5] and the European Plant Conservation Strategy (EPCS) [8]. With this in mind, the BBGK has undertaken initiatives and has developed a strategy for the maintenance of extensive mother plantations, development of protocols for large-scale propagation and cultivation, estimation of the market potential for possible introduction of new products in ornamental floriculture and horticulture, cosmetology, pharmaceuticals, food flavourings, and landscaping [6].

Current scientific literature regarding the biology of many European threatened plant species is quite limited. The same applies for the species investigated in this study, viz. *Erysimum naxense* Snogerup and *Erysimum krendlii* Polatschek (Brassicaceae), two species of section *Cheiranthus*(L.) Wettst. and *Erysimum* L., respectively [9]. Both of the studied *Erysimum* species (*E. naxense, E. krendlii*) are local endemics of Greece that are restricted to a single island of the Aegean Archipelago [Naxos and Samothraki, respectively]. *E. naxense* is nationally [10] and globally [11] considered "Rare" and is protected by the Greek Presidential Decree 67/1981, while *E. krendlii* is nationally considered "Vulnerable" [12]. *E. naxense* is a perennial plant (rarely biennial) growing mainly as a rock-dweller at 100-500(-800) m above sea level [10]; *E. krendlii* is a biennial (or short-living perennial) plant growing in phrygana, roadsides, and rocky areas from 250-1,000(-1,500) m [12].

Centaurea paxorum is assessed as Vulnerable [IUCN criteria B1a,b(ii),C2a(i),D2] due to: single-island endemic (Paxoi-Antipaxoi), small population size (<5000 individuals), chasmophytic (niche restriction, need for suitable habitat conditions), high probability of habitat alteration /destruction imposed by natural phenomena (earthquakes, rock erosion due to winds and waves) and by human activities (overgrazing, tourist development, construction works, land use changes) [13]. The specific name of the plant comes from the Centaur, which is a mythological monster in the shape of a horse with a human head, the existence of which was proposed by Hippocrates. Probably as a result of this similarity, the plant was named *Centaurea* by Linnaeus [14]. Studies on the propagation of *Centaurea* species is limited, as they were mainly focused on early flowering, increasing the number of flowers and suitable sowing period for *C. montana* [15] and *C. moschata* var*. imperials* [16]. Davis et al. [17] conducted studies on the seed storage times for *C. masculosa*.

Cuttings are still the most important means of plant propagation. Many new plants can be started available in a limited space from a few stock plants. Cutting propagation is fast, simple and does not require the special techniques necessary in grafting, budding, or micropropagation. Greater uniformity is obtained and the parent plant is usually reproduced exactly, with virtually no genetic change [18].

The propagation of these 3 species, for reconstitution of degraded ecosystems and recovery of other areas concern so many researchers and developers in order to establish a sustainable management of theses natural resources with promising potential. Two species of the genus *Erysimum* (*E. naxense and E. krendlii*), and one of the genus Asteraceae (*C. paxorum*) were studied. Plant material was collected from its natural habitats and maintained in mother plantations.

2. Material and methods

2.1. Plant material

All native plants maintained at the nursery of the Laboratory derived from natural populations as a result of botanic expeditions conducted at floristically important areas (e.g., National Parks, NATURA 2000 sites and other protected areas). For each plant, site specific information was kept (location, region, altitude, longitude and latitude) as well as a detailed habitat description. All plants collected, received immediate care in the nursery. They are designated as stock plants, planted at big containers or special places according to their needs, taken special treatments since they recover from transplantation shock.

2.2. Asexual propagation

Research on the asexual propagation of the three species was conducted. Softwood tip cuttings of 1.5-6 cm were taken during early-mid autumn from mother plants developed inside the greenhouse (1.5-2.5 cm for *E. naxense*, 2-4 cm for *E. krendlii*, and 5-6 cm for *C. paxorum*). The effect of the auxin indole-3-butyric acid (IBA) at four different concentrations

(0, 1000, 2000, and 4000 ppm) on root formation was tested. Cuttings after immersion for 1 min in solutions of different IBA concentrations were placed in propagation trays in a substrate of peat (Terrahum) and perlite (Geoflor) (1:3 v/v) 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 weather conditions. Relative humidity was approximately 70-85% (mist). Experiments lasted 8 weeks for *E. naxense*, 5 weeks for *E. krendlii*, and 3 weeks for *C. paxorum* followed a randomized design with 7 replications per treatment for the 2 *Erysimum* species (*E. naxense* and *E. krendlii*) and 8 replications per treatment for *C. paxorum*. At the end of the experimental period, different for each species, the number of roots per rooted cutting and root length were measured. Rooting was expressed as %. Produced rooted plants from all 3 species were then transplanted in pots of 0.33 Lt (8x8x7 cm) and subsequently in 2.5 Lt containing a mixture of peat (Klasmann, TS2), perlite and soil (2:1:1 v/v). Plants were maintained at the nursery with the aim of creating adequate initiation material for future experiments on sexual or asexual propagation.

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 *E. naxense* by cuttings was successfully achieved within 8 weeks. All IBA concentrations (1000-4000 ppm) increased the number (19-26.75) and length of roots (2.15-3.21 cm) compared to the control (2 roots 0.39 cm long). Rooting was highest (85.71%) and almost doubled when cuttings treated with 4000 ppm IBA compared to the control's cuttings (42.86%). No browning and necrosis symptoms were observed in cuttings treated with IBA, with respect to the control (14.29%) (Table 1, Figure 1A-1J).

Figure 1 Asexual propagation of *E. naxense* cuttings: (A) Control, (B) 1000 ppm, (C) 2000 ppm, (D) 4000 ppm IBA, (E) vegetative growth of transplanted rooted cuttings into 0.33 and 2.5 Lt pots after one week, (F) 6, (G) 8, (H)10, (I) 12 and (J) 16 weeks

Table 1 Effect of IBA concentration (0, 1000, 2000 and 4000 ppm) on rooting percentage (%), root number/rooted cutting and root length (cm) in *E. naxense* cuttings after 8 weeks

IBA (ppm)	Rooting (%)	Root number	Root length (cm)	Necrosis (%)
Control	42.86 ^{a}	2.00 ± 0.22 ^{a}	0.39 ± 0.04 ^{a}	14.29 ^{b}
1000	42.86 ^{a}	19.00 ± 4.46 ^{b}	2.15 ± 0.41 ^{b}	0 ^{a}
2000	57.14 ^{b}	26.75 ± 3.25 ^{b}	3.21 ± 0.39 ^{c}	0 ^{a}
4000	85.71 ^{c}	19.00 ± 1.71 ^{b}	3.06 ± 0.24 ^{c}	0 ^{a}
P-values	0.000***	0.000***	0.000***	0.000***

Means ± 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 ≤ 0.05. *** P ≤ 0.001.

In *E. krendlii* cuttings, rooting was optimum with 2000 and 4000 ppm IBA. Root length (4.28 cm) was higher with 2000 ppm IBA and root number with 4000 ppm IBA. Rooting was 100% in all IBA treatments, compared to control (85.71%) (Table 2, Figure 2A-2F).

Table 2 Effect of IBA concentration (0, 1000, 2000 and 4000 ppm) on rooting percentage (%), root number/rooted cutting and root length (cm) in *E. krendlii* cuttings after 5 weeks

IBA (ppm)	Rooting (%)	Root number	Root length (cm)	Necrosis (%)
Control	85.71 ^{a}	11.67 ± 2.21 ^{a}	2.45 ± 0.38 ^{a}	14.26 ^{b}
1000	100 ^{b}	14.57 ± 2.95 ^{a}	2.88 ± 0.41 ^{a}	0 ^{a}
2000	100 ^{b}	18.29 ± 1.06 ^{a}	4.28 ± 0.38 ^{b}	0 ^{a}
4000	100 ^{b}	30.86 ± 4.85 ^{b}	3.71 ± 0.50 $^{a, b}$	0 ^{a}
P-values	0.000***	0.001**	0.023*	0.000***

Means ± 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 ≤ 0.05. * P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001.

Figure 2 Asexual propagation of *E. krendlii* cuttings: (A) Control, (B) 1000 ppm, (C) 2000 ppm, (D) 4000 ppm IBA, (E) vegetative growth of transplanted rooted cuttings into 0.33 Lt pots after 4 weeks and (F) into2.5 Lt pots after another 4 weeks

Asexual propagation of *C. paxorum* cuttings was successfully performed within 3 weeks. IBA (1000-4000 ppm) positively affected rooting (100%) giving 27.14-47.86 root number, while the control exhibited 71.43% rooting with

17.4 roots. Cuttings treated with 1000 and 2000 ppm IBA gave similar root lengths (2.61-2.67 cm) to the control (2.44 cm), whereas 4000 ppm IBA decreased root length by 0.87 cm. Optimum rooting was achieved with 1000 ppm IBA (47.86 roots 2.61 cm long, 100% rooting) (Table 3, Figure 3A-3G).

Table 3 Effect of IBA concentration (0, 1000, 2000 and 4000 ppm) on rooting percentage (%), root number/rooted cutting and root length (cm) in *C. paxorum* cuttings after 3 weeks

IBA (ppm)	Rooting (%)	Root number	Root length (cm)
Control	71.43 [a]	17.40 ± 2.53 [a]	2.44 ± 0.32 [a]
1000	100 [b]	47.86 ± 2.35 [c]	2.61 ± 0.19 [b]
2000	100 [b]	45.75 ± 3.66 [c]	2.67 ± 0.26 [b]
4000	100 [b]	27.14 ± 4.46 [b]	1.57 ± 0.18 [b]
P-values	0.000***	0.000***	0.013*

Means ± 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 $P \leq 0.05$. * $P \leq 0.05$, *** $P \leq 0.001$.

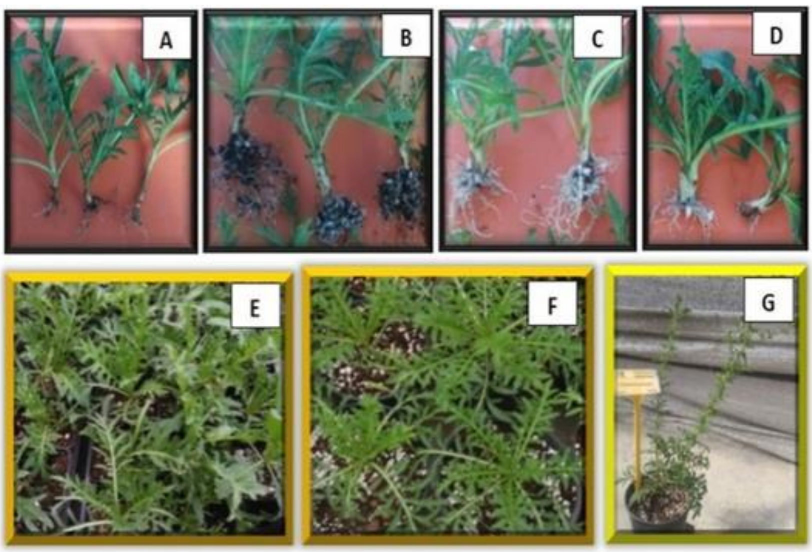

Figure 3 Asexual propagation of *C. paxorum* cuttings: (A) Control, (B) 1000 ppm, (C) 2000 ppm, (D) 4000 ppm IBA, (E) vegetative growth of transplanted rooted cuttings into 0.33 Lt pots after 4 weeks, (F, G)2.5 Lt pots after another 4 and 8 weeks, respectively

4. Discussion

In general, for the successful *ex situ* cultivation of IPS, the plant medium must be similar to that of the substrate in the plant's natural environment, so that similar root aeration and drainage conditions are provided; the improved drainage conditions during cultivation can inhibit fungal disease risks, induce greater rooting depth and enhance general plant health and vigour [19].

Considering the recent challenges in regional conservation planning, it becomes apparent that there is an urgent need for increased applied research in order to develop propagation and cultivation protocols for target plants threatened with extinction, towards species recovery and populations' reinforcements [20, 21, 22].

In the present study with *E. naxense*, best rooting results were obtained when cuttings treated with 4000 ppm IBA. It has widely been documented that auxins promote adventitious root development of stem cuttings through their ability to promote the initiation of lateral root primordia [23].

In the other studied *Erysimum* species (*E. krendlii*), the auxin "IBA" had a significant effect on rooting performance of cuttings with 2000 and 4000 ppm concentrations to be the most effective. Enhancing rate of adventitious roots development with auxin application has been found to increase the number of roots initiated per rooted cutting in a number of species [24]. This study, thus presents for the first time a facilitated asexual propagation by cuttings protocol for the ex situ conservation of two local endemic plant species of Greece (*E. naxense* and *E. krendlii*) contributing, at a European level, to the implementation of target 8 of the Global Strategy for Plant Conservation (http://www.cbd.int/gspc/).

With respect to studied *C. paxorum*, the application of 1000 and 2000 ppm IBA caused a 3-fold increase in root number whereas IBA, irrespective of concentration led to 100% rooting. Increased number of roots is important for the plants to increase their ability to exploit soil water and nutrients, which in turn increases their overall growth [25]. The same authors reported that juvenile *Centaurea tchihatcheffii* cuttings treated with 500 ppm IBA for 10 min gave the highest frequency of rooting, root number and root length along with normal flowering [25]. In relation to *C. paxorum* under study, no symptoms of browning and necrosis were evident. Different response was noted in another *Centaurea* species (*C. tchihatcheffii*) where cutting mortality was associated with increase in IBA concentration, maybe due to depletion in foliar nutrient contents and the consequent onset of leaf senescence [25].

Additional research is needed on the rooting mechanism and to know the functions of adventitious roots in the functional biology of the plant. Knowledge of the behavior and relative contribution of these to the total performance of the plant would be beneficial.

5. Conclusion

All 3 endemic species (*E. naxense, E. krendlii* and *C. paxorum*) were successfully propagated and effective species-specific protocols have been produced for their asexual propagation (rooting: 85-100%) to back up their *ex situ* conservation at the Balkan Botanic Garden of Kroussia and to facilitate their sustainable exploitation. It was shown that there is a correlation between optimum rooting performance and IBA concentration, species-depended. The concentration of IBA must be carefully chosen in order to avoid induced after effects, depended on plant species. This study is a step forward towards the regeneration, sustainable utilization and conservation of these (critically) endangered, and/or vulnerable rare-indigenous species.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors of this article declare that there is no conflict of interest.

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