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Seed Germination Trials and Ex Situ Conservation of Local Prioritized Endemic Plants of Crete (Greece) with Commercial Interest

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Abstract: The in vivo germination course (15, 30, 45, and 60 days) of nine threatened local endemic plants of Crete (Greece) was studied due to conservation concerns and commercial interest in economic sectors. We used seeds directly collected from the wild sown in peat (Terrahum):perlite (1:1 *v*/*v* ratio)covered with coarse-grained vermiculite in a greenhouse mist bench with a substrate temperature of 19 ± 2 °C. The onset of in vivo germination was defined by the visible radicle protrusion (6th–9th day from sowing). After 60 days, 100% germination was observed for seeds of *Campanula cretica*, *Dianthus fruticosus* subsp. *creticus*, and *Petromarula pinnata*, followed by *Draba cretica* (91%) and *Sanguisorba cretica* (86%). Average–low germination capacity was observed for seeds of *Calamintha cretica* (26%), *Lomelosia minoana* subsp. *minoana* (38%), and *Origanum microphyllum* (23%), and very low capacity was observed for *Onopordum bracteatum* subsp. *creticum* (1.5%). After keeping seeds at 4–5 °C for three months, pre-treatments were performed (immersion in 50 or 250 ppm of gibberellic acid solution for 24 h) on three taxa with low germination capacity, thus resulting to the germination acceleration of *Calamintha cretica* seeds and increased germination capacity of *Onopordum bracteatum* subsp. *creticum* seeds. Apart from the facilitation of future species-specific conservation actions, the study showcases that the obtained results herein may permit an upgrade in the feasibility and readiness timescale assessments for the sustainable exploitation of studied taxa in different economic sectors.

Keywords: sexual propagation; seed dormancy; GA_3; biodiversity; Mediterranean; sustainable exploitation; neglected and underutilized plants

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1. Introduction

In situ plant biodiversity conservation is often but should always be supported by ex situ conservation actions that involve: storing seed lots in seed banks to secure the enhanced representation of the genetic diversity of prioritized plant species; employing different propagation trials with varied methods to aid the development of species-specific propagation protocols; using acclimatization methods regarding high-cost and valuable plant stocks raised ex situ destined for future reintroduction; and reinforcing the population of targeted species in the wild habitats [1–3]. Nevertheless, to date severe knowledge gaps (e.g., largely unknown biological cycle in man-made settings, appropriate species-specific propagation techniques, ex situ acclimatization potential) are still extant for the majority of plant species that are of conservation concern, such as insular local endemic plants [3–9]. Foregoing studies on the sexual reproduction of several conservation priority plants have focused on the settings or conditions that promise a satisfactory germination of seeds, thus introducing effective propagation protocols [3,10–15]. Nonetheless, the seeds of many plant species often present varied dormancy types with innate mechanisms securing the appropriate timing of seed germination; therefore, species-specific research is required [16]. To overcome seed dormancy for conservation purposes or sustainable exploitation needs,

a plethora of stratification and pre-treatment regimes have been proposed to date in the literature [17–20].

Neglected and underutilized plants (NUPs) often encompass scarcely known plant species, subspecies, or crop varieties with interesting or remarkable potential in economic sectors, but only infrequently do they refer to local endemic floristic elements of small geographical territories, namely from an island [4]. The native phytogenetic resources of given regions are globally and locally valuable, meriting both conservation and new ventures to establish value chains, and they are also used or are appreciated at local scales. However, they have never been mainstreamed and they hardly appeal to research communities, politicians, and stakeholders [4,21,22]. Given this shortcoming, the critical step for the sustainable exploitation of NUPs is the development of species-specific propagation and cultivation protocols which are both effective and affordable; upon such development, agronomical approaches and agro-processing procedures may be facilitated [23], thus unlocking the commercialization potential of NUPs and permitting value chain creation [24]. Furthermore, both evidence and storytelling regarding high-added value products of unique identity are needed for effective commercial prospecting and fascination of consumers, stakeholders, producers, wholesalers, and retailers. Hence, multidisciplinary approaches have been suggested for the sustainable exploitation of NUPs in conjunction with applied research methods, political and consumer preferences, and policy awareness [24]. The availability, development, and/or revival of knowledge on NUPs may also trigger the mitigation of extant barriers hindering the sustainable exploitation of unique resources [24].

In geographical terms, the present study was focused on the wild flora of Crete (south Aegean Archipelago) which represents an important center of endemism in Greece as well as in the Mediterranean and European contexts [25]. The flora of Crete includes at least 223 local endemic species and subspecies (taxa) that grow exclusively to this island [26], among which some have already been sustainably managed to date, which has allowed for the establishment of value chains [4]. For example, the dittany of Crete (*Origanum dictamnus*) and recently the Cretan mountain tea (*Sideritis syriaca* subsp. *syriaca*) have already established value chains in at least Crete and Greece, which formed after the domestication and effective crossing of all barriers for the species, starting as exclusively wild-growing species in natural habitats, ending up being threatened species in the wild, and finally being extant commercial crops cultivated for marketed products in man-made settings [4,27].

Primary dormancy and the imposition of secondary dormancy, seed photosensitivity, and the range of germination temperatures in relation to habitat, life-form, and size of seeds appear to be related to the specific adaptation strategies that the Mediterranean plants have developed among others, ensuring that seed germination is achieved under appropriate conditions that are favorable for the appearance and survival of seedlings [28]. Dormancy in species native to Mediterranean climate areas can be overcome through cold or hot stratification [29], widening the range of temperatures at which seeds germinate, alternating day/night temperatures, reducing the level of abscisic acid through the application of growth regulators such as gibberellic acid (GA_3) or other chemicals such as nitrate ions (i.e., KNO_3) and smoke substances, keeping seeds in dry conditions (pre-ripening or dry storage), and increasing seed photosensitivity in the light regime [30].

In the context of the plant conservation efforts of threatened local endemic plants and in the frame of sustainable exploitation strategies of Greek native NUPs, this study focused on exploring the in vivo seed germination of some perennial Cretan endemic plants (mostly rock-dwellers) that have been threatened with extinction [31]. Apart from conservation concerns, for these plants there are both extant applied research gaps and distinctive interest in various economic sectors [4–6]. The herein focal endemic plants of Crete which are threatened with extinction [31] include: *Campanula cretica* (A. DC.) D. Dietr. (Cretan bellflower), *Dianthus fruticosus* L. subsp. *creticus* (Tausch) Runemark (Cretan fruticose carnation), *Draba cretica* Boiss. & Heldr., *Lomelosia minoana* (P.H. Davis) Greuter & Burdet subsp. *minoana*, *Calamintha cretica* (L.) Lam. (Cretan calamint), *Origanum*

microphyllum (Benth.) Vogel (Cretan marjoram), *Petromarula pinnata* (L.) A. DC. (Cretan rock lettuce), *Sanguisorba cretica* Hayek (Cretan burnet), and *Onopordum bracteatum* L. subsp. *creticum* Franco (Cretan wild artichoke). Furthermore, this study attempted to re-examine the feasibility of value chain creation and readiness timescale [4] for the sustainable exploitation of the studied Cretan endemic plants.

2. Materials and Methods

2.1. Focal Local Endemic Plants of Crete (Greece)

The selection of the focal threatened endemic plants of Crete (n = 223) [26] with interest in different economic sectors [4–6] incorporated several criteria, such as extant rarity in the wild, endemism, valuable properties (aromatic and/or medicinal, floricultural and/or ornamental, edible and/or flavoring value), and took also into consideration the estimated accessibility of their wild-growing populations for the acquisition of propagation material. Additionally, previous own propagation experience in related Greek native plants of similar life-forms such as annuals, biennials, and herbaceous perennials were incorporated [3,4,32–35]. The focal local endemic plants of Crete (Greece) are as follows (Table 1).

Dianthus fruticosus subsp. *creticus* (Carophyllaceae, Figure 1) is a range-restricted and endangered [31] chamaephyte [36]. *Draba cretica* (Brassicaceae) (Figure 1) is a range-restricted and critically endangered [31] dwarf hemicryptophyte [36]. *Sanguisorba cretica* (Rosaceae) is a range-restricted and critically endangered [31] hemicryptophyte (Figure 1) [36].

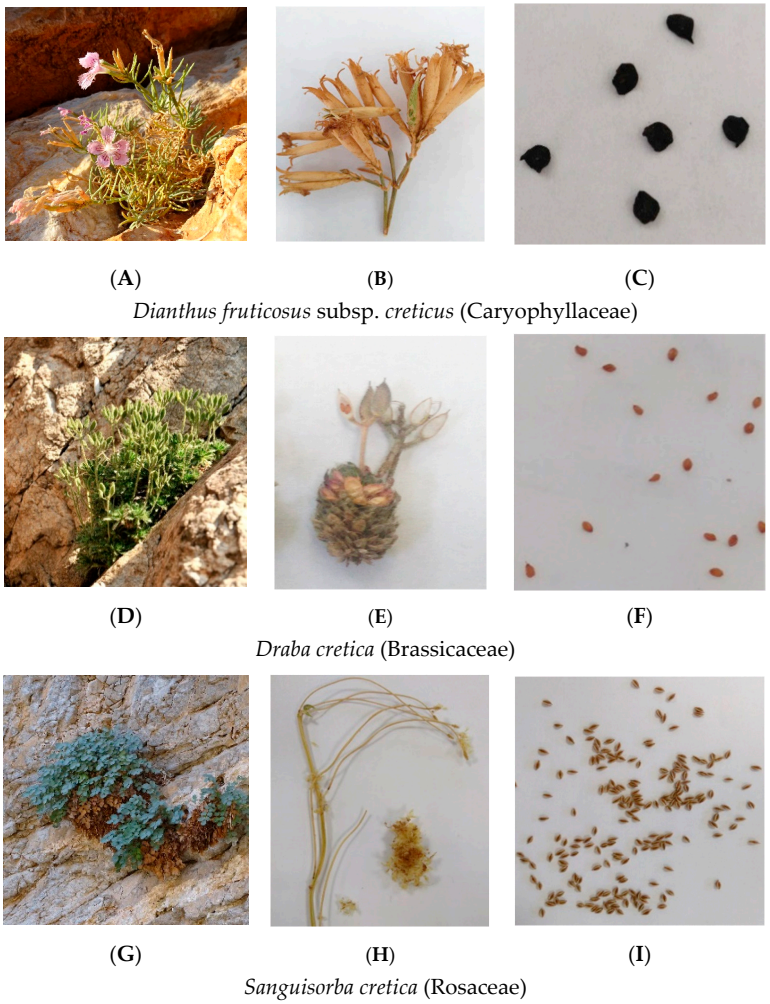


Figure 1. Studied Cretan endemic members of Brassicaceae, Caryophyllaceae, and Rosaceae in their wild habitats (**A**,**D**,**G**) and illustration of collected parts (**B**,**E**,**H**) with mature seeds (**C**,**F**,**I**).

Table 1. Extinction risk, protection status, and seed collection details for the studied Cretan endemic plants (ordered alphabetically according to scientific name) including the IPEN (International Plant Exchange Network) accession numbers stored and accessions used in different periods of this investigation (A: autumn; S: spring). Extinction risk assessments according to Kougioumoutzis et al. [31]: CR, critically endangered; EN, endangered; VU, vulnerable.

Extinction Risk	Presidential Decree 67/1981	Scientific Name	Family	Seed Accessions Stored	IPEN Accession Number Used (Period)	Collection Date	Collection Site	Prefecture	Latitude (North)	Longitude (East)
EN	-	*Campanula cretica* (A. DC.) D. Dietr.	Campanulaceae	2	GR-BBGK-1-19,147 (A)	29/8/2018	Samaria Gorge	Chania	35.513536	24.017463
CR	YES	*Calamintha cretica* (L.) Lam.	Lamiaceae	4	GR-BBGK-1-19,146 (A,S)	29/8/2018	Samaria Gorge	Chania	35.513536	24.017463
EN	-	*Dianthus fruticosus* L. subsp. *creticus* (Tausch) Runemark	Caryophyllaceae	5	GR-BBGK-1-19,17 (A.S)	29/8/2018	Agios Georgios Selinaris	Lasithi	35.284803	25.540993
CR	-	*Draba cretica* Boiss. & Heldr.	Brassicaceae	3	GR-BBGK-1-19,10 (A,S)	29/8/2018	Skinakas	Heraklion	35.21146	24.89459
CR	-	*Lomelosia minoana* (P.H. Davis) Greuter & Burdet subsp. *minoana*	Dispacaceae	4	GR-BBGK-1-19,16 (A)	7/10/2018	Ano Viannos	Heraklion	35.05421	25.40998
EN	-	*Onopordum bracteatum* Boiss. & Heldr. subsp. *creticum* Franco	Asteraceae	1	GR-BBGK-1-19,1 (A,S)	29/8/2018	Zomynthos	Rethymno	35.24257	24.88549
CR	-	*Origanum microphyllum* (Benth.) Vogel	Lamiaceae	5	GR-BBGK-1-19,31 (A.S)	7/10/2018	Dikti Mountain	Lasithi	35.118191	25.492043
VU	-	*Petromarula pinnata* (L.) A. DC.	Campanulaceae	27	GR-BBGK-1-19,124 (A)	29/8/2018	Gorge of Agia Irini	Chania	35.2995	23.8346
CR	YES	*Sanguisorba cretica* Hayek	Rosaceae	4	GR-BBGK-1-19,154 (A,S)	29/8/2018	Samaria Gorge	Chania	35.51353	24.017463

Campanula cretica (Campanulaceae, Figure 2) is a range-restricted and critically endangered [31] hemicryptophyte [36]. *Petromarula pinnata* (Campanulaceae) (Figure 2) is a widespread but locally endemic hemicryptophyte of Crete which has been assessed as vulnerable [31].



(**A**) (**B**) (**C**)

Campanula cretica



(**D**) (**E**) (**F**)

Petromarula pinnata

Figure 2. Studied Cretan endemic members of Campanulaceae in their wild habitats (**A**,**D**) and illustration of collected parts (**B**,**E**) with mature seeds (**C**,**F**).

Calamintha cretica (Lamiaceae) (Figure 3) is a critically endangered [31] chamaephyte [36].

Lomelosia minoana subsp. *minoana* (Dipsacaceae, Figure 4) is a range-restricted and critically endangered [31] chamaephyte [36]. *Onopordum bracteatum* subsp. *creticum* (Asteraceae, Figure 4) is an endangered [31] and range-restricted hemicryptophyte [36].



(**A**) (**B**) (**C**)

Origanum microphyllum



(**D**) (**E**) (**F**)

Calamintha cretica

Figure 3. Studied Cretan endemic members of Lamiaceae in their wild habitats (**A**,**D**) and illustration of collected parts (**B**,**E**) with mature seeds (**C**,**F**).



(**A**) (**B**) (**C**)

Lomelosia minoana subsp. *minoana* (Dipsacaceae)



(**D**) (**E**) (**F**)

Onopordum bracteatum subsp. *creticum* (Asteraceae)

Figure 4. Studied Cretan endemic members of Dipsacaceae and Asteraceae in their wild habitats (**A**,**D**) and illustration of collected parts (**B**,**E**) with mature seeds (**C**,**F**).

2.2. Seed Collections

The seed collections were performed during botanical expeditions in Crete (Table 1) using a special permit of the Balkan Botanic Garden of Kroussia (BBGK) that is issued yearly by the Greek Ministry of Environment and Energy (Permits 154553/1861/13-7-2017 & 182336/879). Seeds of the targeted taxa (species and subspecies) were collected from wild-growing populations, and the collected material was promptly transferred to the facilities of the Balkan Botanic Garden of Kroussia (BBGK), Institute of Plant Breeding and Genetic Resources (IPBGR), Hellenic Agricultural Organization Demeter in Thessaloniki (Thermi) for taxonomic identification. Seed cleaning (removal of soil, foreign matter, and perishable materials) was then performed, followed by storage at 15 °C at a relative humidity of <15% for approximately 30 days. Upon taxonomic identification, all collected seed lots received a unique International Plant Exchange Network (IPEN) accession number (Table 1). The visited habitats and illustrations of the seed collections performed per Cretan endemic plant are illustrated in Figures 1–4.

2.3. Seed Germination Trials

The first in vivo sowing of the seeds of the nine Cretan endemic taxa took place during autumn and winter (20 November 2018–20 January 2019) without any pre-treatment of the seeds or prior storage in a cold room (Table 2).

Table 2. In vivo seed germination procedure of nine local endemic plants of Crete without any pre-treatments during autumn.

Plant Taxon	Days after Sowing	Germination (%)	t_{50} (Days)	*p*-Values
Calamintha cretica	15	2.0 b	24.17	0.000 ***
	30	20.0 a		
	45	26.0 a		
	60	26.0 a		
Campanula cretica	15	40 c	30.00	0.000 ***
	30	50 c		
	45	70 b		
	60	100 a		
Dianthus fruticosus subsp. *creticus*	15	93 b	10.00	0.001 **
	30	100 a		
	45	100 a		
	60	100 a		
Draba cretica	15	50 b	12.16	0.000 ***
	30	90 a		
	45	90 a		
	60	91 a		
Lomelosia minoana subsp. *minoana*	15	20 b	10.74	0.009 **
	30	34 a		
	45	36 a		
	60	38 a		

Table 2. *Cont.*

Plant Taxon	Days after Sowing	Germination (%)	t_{50} (Days)	*p*-Values
Onopordum bracteatum subsp. *creticum*	15	1.5 a	10.50	1.000 ns
	30	1.5 a		
	45	1.5 a		
	60	1.5 a		
Origanum microphyllum	15	8.3 b	18.14	0.017 *
	30	22.7 a		
	45	22.7 a		
	60	22.7 a		
Petromarula pinnata	15	50 b	15.00	0.000 ***
	30	100 a		
	45	100 a		
	60	100 a		
Sanguisorba cretica	15	6 b	21.94	0.000 ***
	30	86 a		
	45	86 a		
	60	86 a		

Statistical analysis was separately conducted for each taxon. Means with the same letter in the germination (%) column are not statistically significant different from each other according to the Duncan's multiple range test at $p \leq 0.05$; n.s.: $p > 0.05$, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

The second in vivo sowing took place during spring (6 February 2019–6 April 2019) after 80 days of storing the seeds in a cold room (4–5 °C, relative humidity <5%) for the six selected taxa (*Calamintha cretica*, *Dianthus fruticosus* subsp. *creticus*, *Draba cretica*, *Onopordum bracteatum* subsp. *creticum*, *Origanum microphyllum*, and *Sanguisorba cretica*) (Table 3). The six taxa were selected due to their low germination capacity during the first (autumn-winter) sowing and, therefore, the procedure was repeated during spring using pre-treatments to increase their germination. Two different 24 h pre-treatments took place prior to sowing in the case of *Calamintha cretica* seeds (0 and 50 ppm of GA_3), and three pre-treatments were performed for *Onopordum bracteatum* subsp. *creticum* seeds (0, 50, and 250 ppm of GA_3). The seeds of *Origanum microphyllum* were only pre-treated in 50 ppm of GA_3 solution for 24 h prior to sowing (24 h immersion in H_2O was not tested due to very low availability of seeds). For *Calamintha cretica*, *Onopordum bracteatum* subsp. *Creticum*, and *Origanum microphyllum*, the 24 h immersion of seeds in dH_2O (same or equivalent to 24 h in 0 ppm of GA_3 solution) served as the control treatment to the increased concentrations of GA_3 (50 and 250 ppm for *O. bracteatum* subsp. *creticum*, but only 50 ppm for *C. cretica* and *O. microphyllum*); thus, 24 h in a 0 ppm GA_3 solution is equivalent to 24 h in dH_2O (same pre-treatment). In the other three taxa, including *Dianthus fruticosus* subsp. *creticus*, *Draba cretica*, and *Sanguisorba cretica*, pre-treatment was performed by immersing the seeds in dH_2O for 24 h (Table 3). The reason behind the additional pre-treatment of *Calamintha cretica*, *Onopordum bracteatum subsp. creticum*, and *Origanum microphyllum* seeds with GA_3 with the exception of dH_2O in spring sowing was the significantly lower germination percentages that were detected during the previous autumn sowing period combined with the limited availability of seed material for each taxon.

Table 3. In vivo seed germination (%) of six local endemic plants of Crete collected from wild habitats after storage for 80 days in a cold chamber (4–5 °C, RH < 5%) and pre-treatment with dH_2O for 24 h or GA_3 solution for 24 h prior to spring sowing.

Cretan Taxon/ (Pre-Treatment)	Days after Sowing	Germination (%)	t_{50} (Days)	*p*-Values
Calamintha cretica (dH_2O for 24 h)	15	5 b	22.45	0.025 *
	30	18 a		
	45	20 a		
	60	22 a		
Calamintha cretica (50 ppm GA_3 solution for 24 h)	15	25 b	9.23	0.044 *
	30	36 a		
	45	36 a		
	60	36 a		
Dianthus fruticosus subsp. *creticus* (dH_2O for 24 h)	15	79 a	9.30	0.992 ns
	30	80 a		
	45	80 a		
	60	80 a		
Draba cretica (dH_2O for 24 h)	15	60 b	13.27	0.026 *
	30	71 a		
	45	74 a		
	60	74 a		
Onopordum bracteatum subsp. *creticum* (dH_2O for 24 h)	15	18 a	8.42	1.000 ns
	30	18 a		
	45	18 a		
	60	18 a		
Onopordum bracteatum subsp. *creticum* (50 ppm GA_3 solution for 24 h)	15	23 a	10.41	0.411 ns
	30	24 a		
	45	24 a		
	60	24 a		
Onopordum bracteatum subsp. *creticum* (250 ppm GA_3 solution for 24 h)	15	23 a	9.61	0.323 ns
	30	24 a		
	45	25 a		
	60	25 a		
Origanum microphyllum (50 ppm GA_3 solution for 24 h)	15	16 a	12.00	0.587 ns
	30	17 a		
	45	17 a		
	60	21 a		
Sanquisorba cretica (dH_2O for 24 h)	30	17 a	20.61	0.000 ***
	45	17 a		
	60	21 a		
	60	89 a		

Statistical analysis separately conducted for each plant taxon and pre-treatment prior to sowing. Means with the same letter in the germination (%) column (individually per taxon and per pre-treatment) are not statistically significant different from each other according to the Duncan's multiple range test at $p \leq 0.05$; ns: $p > 0.05$, * $p \leq 0.05$, *** $p \leq 0.001$.

We assessed the seed germination ability of the studied plants in different seasons of the year (Tables 2 and 3). In all cases (autumn and spring sowing), single crates with peat substrate (Terrahum): perlite (1:1 *v/v*) were used, and their surface was covered with vermiculite. The crates were placed on a heated greenhouse bench (19 ± 2 °C, relative humidity 80–90%), and the time of in vivo seed germination was determined with regular measurements at 15-day intervals (15, 30, 45, and 60 days). The in vivo germination criterion in both trials was the radicle protrusion from the seed coat. In addition, the median germination time (t_{50}) was calculated according to the formula by Farooq et al. [37] as the time needed (in days) to reach 50% of the final/maximum germination (Tables 2 and 3). The unit measure used for "Germination onset day" was the number of days, and the onset (i.e., initiation) of germination was defined as the day when the first seed was germinated (Table 4).

Table 4. Overview of in vivo seed germination trials of nine local endemic plants of Crete (!: first-time data) studied right after collection from wild habitats in autumn (A) without any pre-treatment and in spring (S) after 80 days storage at 4–5 °C.

			Germination Rate (%) every Forthright (in Days) during Autumn (A)/Spring (S)			
Scientific Name	**Pre-Treatment (Spring)**	**Germination Onset Day A/S**	**15th A/S**	**30th A/S**	**45th A/S**	**60th A/S**
! Campanula cretica	-	9/-	40/-	50/-	70/-	100/-
! Calamintha cretica	24 h in H_2O	-/12	-/5	-/18	-/20	-/22
	50 ppm GA_3 (24 h)	13/9	2/25	20/36	26/36	26/36
! Dianthus fruticosus subsp. *creticus*	24 h in H_2O	6/5	93/79	100/80	100/80	100/80
! Draba cretica	24 h in H_2O	9/9	50/60	90/71	90/74	91/74
! Lomelosia minoana subsp. *minoana*	-	6/-	20/-	34/-	36/-	38/-
! Onopordum bracteatum subsp. *creticum*	0 ppm GA_3 (24 h in H_2O)	6/7	1.5/18	1.5/18	1.5/18	1.5/18
	50 ppm GA_3 (24 h)	-/6	-/23	-/24	-/24	-/24
	250 ppm GA_3 (24 h)	-/6	-/23	-/24	-/25	-/25
! Origanum microphyllum	50 ppm GA_3 (24 h)	6/9	8.3/16	22.7/17	22.7/17	22.7/21
Petromarula pinnata	-	9/-	50/-	100/-	100/-	100/-
! Sanguisorba cretica	24 h in H_2O	13/2	6/18	86/89	86/89	86/89

The two in vivo sowing experiments (first and second), considering that they were performed at different times of the year (autumn and spring) with a different age of the seeds (i.e., different physiological state, level of dormancy, humidity, etc.) cannot be eventually compared. Seeds of the first sowing trial in autumn received no previous cold storage or 24 h pre-treatment (nine taxa), whereas seeds of the second trial in spring were kept for 80 days in a cold chamber (4–5 °C, RH <5%) (six taxa) and were pre-treated for 24 h with dH_2O and/or GA_3 solutions. Due to the different seed storage conditions, different pre-treatment regimes, and different number of tested taxa between the two sowing seasons, no further comparisons were made.

The in vivo seed germination procedure in either autumn or spring took place in three single seat crates of 100 seeds each, comprising a total of 300 seeds per taxon (3 replicates × 100 seeds).

After the germination of the seeds and growth of the seedlings for 2–4 months (species- or subspecies-dependent), the seedlings were transplanted into multi-hole trays filled with

a substrate mixture of peat (Terrahum):perlite (1:1 *v/v* ratio), and were progressively filled into larger volume pots (0.33 and 1 L) with a mixture of peat (TS2, Klassmann):perlite (3:1 *v/v* ratio). The criterion for transplanting the plants into larger volume pots was the first emergence of the root system out of the container, thus indicating symptoms of intolerance and non-symmetrical growth of the underground part compared with the above ground part.

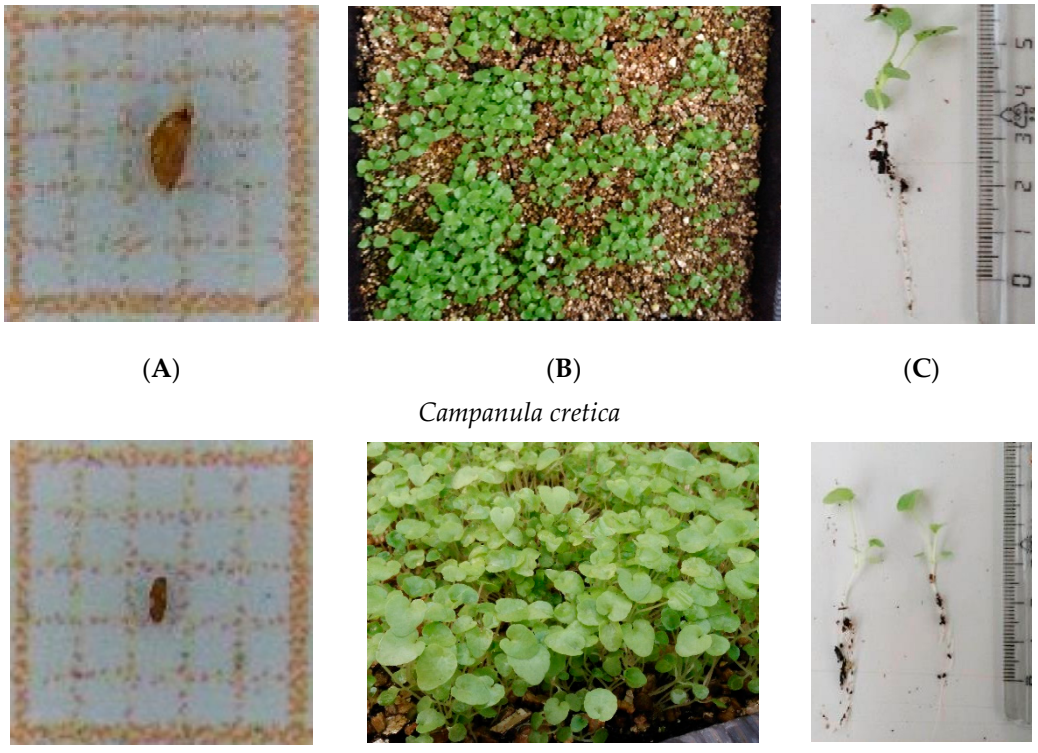
2.4. Experimental Design and Statistical Analysis

The experimental layout was completely randomized. The data related to the in vivo seed germination experiments were analyzed by using the statistical package SPSS 17.0 (SPSS Inc., Chicago, IL, USA) and Analysis of Variance (one-way ANOVA). The descriptive data were compared using the Duncan's multiple range test at a level of $p \leq 0.05$. Statistical analysis was conducted separately for each plant taxon as well as per pre-treatment.

3. Results

3.1. Seed Germination

After a period of 60 days during the first in vivo autumn–winter sowing, 100% germination was observed in *Campanula cretica*, *Dianthus fruticosus* subsp. *creticus* and *Petromarula pinnata* (Figures 5 and 6). The seeds of *Draba cretica* and *Sanguisorba cretica* also exhibited high germination rates, i.e., 91% and 86%, respectively (Figure 6). Intermediate germination capacity was evidenced in seeds of *Calamintha cretica* (26%), *Lomelosia minoana* subsp. *minoana* (38%), and *Origanum microphyllum* (23%) (Figure 7), while the germination rate of *Onopordum bracteatum* subsp. *creticum* was only 1.5% (Figure 8). The onset of germination for the nine studied Cretan plant taxa was defined by the visible growth of the radicle from the seed coat, and its appearance ranged from 6 to 9 days after sowing (Table 2, Figures 5–8).



(**A**) (**B**) (**C**)

Campanula cretica

(**D**) (**E**) (**F**)

Petromarula pinnata

Figure 5. Individual seeds (**A**,**D**), germinated seedlings (**B**,**E**), and transplanted individuals (**C**,**F**) of studied Cretan endemic members of Campanulaceae.



(**A**) (**B**) (**C**)

Dianthus fruticosus subsp. *creticus* (Caryophyllaceae)



(**D**) (**E**) (**F**)

Draba cretica (Brassicaceae)



(**G**) (**H**) (**I**)

Sanguisorba cretica (Rosaceae)

Figure 6. Individual seeds (**A**,**D**,**G**), germinated seedlings (**B**,**E**,**H**), and transplanted individuals (**C**,**F**,**I**) of studied Cretan endemic members of Brassicaceae, Caryophyllaceae, and Rosaceae.

In the second trial, the germination of *Calamintha cretica*, *Draba cretica*, and *Sanguisorba cretica* seeds was completed after 30 days, after being placed in cold storage for 80 days (4–5 °C, RH < 5%) and being pre-treated in dH_2O for 24 h prior to spring sowing, which exhibited the maximum germination rate. Further increase in the percentages of the germinated seeds after 30 and up to 60 days was non-significant, i.e., 89% for *Sanguisorba cretica*, 71–74% for *Draba cretica*, and 36% for *Calamintha cretica*. Regarding the other three Cretan endemic taxa studied in spring, the germination reached maximum competence after 15 days from sowing while no statistically significant changes were noticed in germination rate (%) in the subsequent period (15–60 days), i.e., 79–80% for *Dianthus fruticosus* subsp. *criticus*, 16–21% for *Origanum microphyllum*, and 18% for *Onopordum bracteatum* subsp. *creticum*. For all Cretan taxa studied in spring (n = 6), the t_{50} values ranged between 8.42 and 20.61 days, while the germination onset was recorded between 5 and 12 days, depending on species identity (Table 3).



(**A**) (**B**) (C)

Calamintha cretica



(D) (E) (F)

Origanum microphyllum

Figure 7. Individual seeds (**A**,**D**), germinated seedlings (**B**,**E**), and transplanted individuals (**C**,**F**) of Cretan endemic members of Lamiaceae.



(**A**) (**B**) (**C**)

Lomelosia minoana subsp. *minoana* (Dipsacaceae)



(**D**) (**E**) (**F**)

Figure 8. Individual seeds (**A**,**D**), germinated seedlings (**B**,**E**), and transplanted individuals (**C**,**F**) of studied Cretan endemic members of Dipsacaceae and Asteraceae.

Concerning the immersion of *Onopordum bracteatum* subsp. *creticum* seeds for 24 h in different concentrations of GA_3 solutions (0, 50, 250 mg/L), their germination process was initiated on day 7 for the GA_3-untreated seeds and on day 6 for the seeds pre-treated with GA_3, regardless of concentration. The germination rates obtained ranged between 18 and 25%. In particular, an 18% germination rate was recorded throughout the 60-day period (15, 30, 45, 60 days) in the case of dH_2O for 24 h (equivalent to 0 ppm GA_3 for 24 h) pre-treatment; 23–24% germination rate for seeds pre-treated with 50 mg/L of GA_3; and 23–25% for those imbibed for 24 h in a 250 mg/L GA_3 solution prior to sowing. The t_{50} values ranged between 8.42 (dH_2O for 24 h) and 10.41 days (50 mg/L GA_3) and was 9.61 days for seeds pre-treated with 250 mg/L GA_3 (Table 3).

According to Table 4 (combined overview of Tables 2 and 3), the sowing season of the year (autumn, spring), cold and non-previous storage of seeds, 24 h seed pre-treatment prior to sowing in dH_2O and/or GA_3 solutions, and taxon identity appear to be important factors that can affect the germination ability of the seeds either positively, negatively, or not at all. In specific, there was an acceleration in the germination onset from 9 to 13 days after sowing, and there were higher germination percentages for *Calamintha cretica* seeds sown in spring after a 24 h pre-treatment with 50 ppm GA_3 compared with autumn sowing under the same pre-treatment condition (24 h in 50 ppm GA_3) or spring sowing after a 24 h pre-treatment in dH_2O. Moreover, higher germination percentages were obtained for *Dianthus fruticosus* subsp. *creticus* and *Draba cretica* seeds sown in autumn compared to those sown in spring. In the case of *Origanum microphyllum* seeds pre-treated with 50 ppm of GA_3 for 24 h, the initiation of germination (in days) occurred earlier during autumn sowing than during spring (Table 4). The germination percentage of *Onopordum bracteatum* subsp. *creticum* seeds pre-treated for 24 h in dH_2O in the spring (18%) was 12-fold higher than that (1.5%) obtained in the autumn sowing, while the pre-treatment of spring-sown seeds with different concentrations of GA_3 solutions (0, 50, 250 ppm) did not manage to considerably raise the germination percentages (23–25%) of the control treatment (18%) (24 h in dH_2O or 0 ppm of GA_3) (Table 4, Figure 9).



Figure 9. *Onopordum bracteatum* subsp. *creticum* seedlings pre-treated with different GA_3 concentrations (0, 50, and 250 mg/L) for 24 h after 30 and 60 days of spring sowing.

Ex situ conservation efforts were successfully accomplished regarding all mother plants derived ex situ from the germination trials with seeds that were directly collected from wild-growing populations in their natural habitats. Therefore, an adequate amount of plant propagation material was obtained for each of the threatened local endemic species of Crete, and subsequent horticultural experience was gained; currently, all materials are cultivated ex situ for conservation purposes and future experimentations.

4. Discussion

4.1. From Seed Germination to Ex Situ Conservation and Sustainable Exploitation

This study presents first-time in vivo germination data for nine threatened local endemic plants of Crete (Greece), for which only scarce or limited documentation has existed to date [4–6]. This investigation employed seeds that directly originating from wild habitats, which were collected during maturity and are illustrated for the first time herein. The research was primarily focused on the exploration of seed dormancy, the appropriate season, and the temperatures for in vivo germination, as temperature is known as the most important environmental factor controlling germination of Mediterranean plant species [38]. This adaptation allows seeds to avoid harsh environmental conditions for seedling establishment, as low water availability in Mediterranean ecosystems often forces the seeds to germinate in autumn when the rainy season starts and temperatures are cool. The germination data generated in this study may bridge extant applied research gaps for the threatened Cretan local endemics studied herein; thus, the data are critical for in situ conservation actions targeting wild-growing endemic populations, can facilitate their ex situ conservation in botanic gardens, and may also pave the way for the sustainable exploitation of species in different economic sectors [4].

Many members of the genus *Campanula* are currently appreciated for their ornamental flowers mainly due to their typical flower color ranging from deep violet to the very palest milky blue or even bluish white [39]. They are often sold as garden plants or pot plants and as cut flowers. Despite the numerous species of this genus, only few are currently available on the market. Wild bellflowers can represent an important genetic resource for finding new crops and varieties that can contribute towards diversifying plant and flower production in the ornamental industry [39]. Most perennial *Campanula* species and cultivars are propagated by conventional vegetative propagation methods to produce homogeneous populations, such as shoot division and stem rooting [40]. It is known that many Campanulaceae seeds harvested in June or July have to be sown within the following 6–8 months to obtain maximum germination rate [40]. For example, in *Campanula trachelium* L., low temperatures are reported to delay germination time and to reduce germination percentages, whereas other species such as *C. sparsa* Friv. and *C. spatulata* Sibth & Sm. do not seem to have their germination prevented by low temperatures [41]. Light intensity can also affect seed germination as evidenced in *C. carpatica* Jacq., for which there are reports that enhanced light intensity combined with either no or low-salt levels may produce up to 89% more germinated seedlings [42]. However, no such data were extant for the critically endangered *Campanula cretica* studied herein, thus hindering future conservation actions and sustainable exploitation strategies for this threatened species. The in vivo seed germination of *Campanula cretica* was completed in our study within 60 days with a 100% germination rate after collection from the wild habitats and direct sowing in autumn, with visible signs of sprouting after 9 days (t_{50}: 30 days). Similar to our study, the application of plant growth regulators in other members of this genus such as *C. glomerata* L. [43] and *C. carpatica* [44] is not required for their seed germination. High germination percentages at 15 °C were also achieved in other local Cretan or south Greek endemic species of *Campanula* such as *C. hierapetrae* (>90%) having t_{50} = 11.9 days and *C. laciniata* L. (100%) having t_{50} = 4–5 days [28]. Currently, there is no indication of dormancy in *C. cretica*. This first-time seed germination data can possibly serve specific conservation actions if needed for *C. cretica* and may facilitate its sustainable exploitation in the future.

Petromarula pinnata (also Campanulaceae) is an attractive wild-growing Cretan endemic plant with ornamental value [4] and agro-alimentary potential, andit is often harvested in Crete directly from natural habitats to be used as an edible wild green [5]. The germination of the vulnerable *Petromarula pinnata* was completed (100%) in our study within 30 days during the autumn period with 5–15 °C mean temperatures. Other studies report similar performances for this species [45], indicating that the final germination percentage of *P. pinnata* seeds is higher in light conditions (61–76%) than in continuous dark

(3–22%). Herein, 50% of the *P. pinnata* seeds germinated within the first 15 days (t_{50}: 15 days) of the onset of germination were reported to occur after 9 days from sowing due to visible sprouting signs. It is known that fluctuating temperatures during the in vivo germination process can act as a sensing mechanism for both soil depth and vegetation cover due to direct sun exposure, and such soil temperature fluctuations can even be experienced by seeds buried at depths greater than those situated in upper soil layers [46]. Most members of Campanulaceae appear to have a light requirement for seed germination, which can be replaced by GA_3 and partly by nitrates such as KNO_3 [45]. Previous and current seed germination data can possibly play a role in conservation actions for *P. pinnata* and may further facilitate its sustainable exploitation in the future.

Calamintha cretica is a sweet-scented medicinal and aromatic plant of Crete that is marked as critically endangered [36]. Its leaves contain essential oil, with major compounds such as piperitenone, piperitone oxide, and p-menthane, thus rendering it a candidate plant for use in perfumery and in the pharmaceutical industry [47] due to piperitenone oxide being shown to have anticarcinogenic properties on human colon cancer cells [48]. In Crete, *C. cretica* is sometimes used as a herbal tea. This plant species could also be introduced in gardening and landscape applications as a ground cover for urban and peri-urban gardens, parks, and green roofs, which would be similar to a closely related species such as *Clinopodium nepeta* (K.) Kuntze [previously known as *Calamintha nepeta* (L.) Savi] that has been suggested for such uses [49,50]. In our study, seed germination of *Calamintha cretica* was completed earlier (within 30 days) and with a higher germination rate (36%) after cold storage of seeds in comparison to those that had direct sowing after collection from the natural environment. In a previous study conducted on the same species [51], *Calamintha cretica* seeds immediately after harvesting germinated at 100% and in a short period of time at 15 or 20 °C without any pre-treatment, and stored seeds for six months germinated equally well compared to those used directly after harvesting; seeds stored for 12 months had slightly reduced germination. Despite the lower germination percentage recorded in this study compared with previous ones (perhaps due to not fully ripened seeds harvested from the wild), the t_{50} value for *C. cretica* herein was three-fold higher for cold-stored seeds during the spring period compared with those directly sown after collection during autumn. In accordance with our findings, the germination of *Calamintha cretica* seeds was reported to be fastest at 20 °C [51]. The high germination without any seed pre-treatment that has been reported soon after seed harvest and for at least one year after indicates the absence of dormancy. The current in vivo seed germination data complement previous in vitro seed germination studies and can be exploited both for conservation actions and the sustainable exploitation of *Calamintha cretica.*

The critically endangered and sweet-scented Cretan marjoram has a noteworthy agro-alimentary potential as a spice, and it is often used in Crete as a local medicinal plant for tea making [5,6]; this is similar to its close relative *Origanum majorana* L., which has approved indications as a traditional herbal medicine with approved indications according to its monography of the European Medicines Agency (https://www.ema.europa.eu/en, accessed on 15 September 2022) [52]. In addition, Cretan marjoram also has interesting potential in the horticultural-ornamental sector [4]. Herein, *Origanum microphyllum* seeds directly sown without previous cold storage gave the highest germination rate (22.7%). On the contrary, Markaki [53] found that *O. microphyllum* seeds may germinate in vitro easily (100%) within 12 days at 20 °C under the influence of white light. Undoubtedly, the germination rates (16–21%) achieved after a 60-day period for *O. microphyllum* seeds pre-treated with cold storage and GA_3 were indeed low in our study. However, there are in vitro germination data [54] for nine different *Origanum* species, and the germination rates (50–100%) seem to be species-dependent (when examined in petri dishes containing 1% agar alone or with 250 mg/L of GA_3, or after mechanical scarification in different photoperiod regimes). Similarly to the findings of this study, a wide range of germination percentages have been reported in vitro for other *Origanum* species, including *O. minutiflorum* O. Schwartz & P. H. Davis (20%) [55], *O. acuti-*

dens (Hand.-Mazz.) Iestw. [56], *O. vulgare* L. (54–74%) [57], *O. compactum* Benth. (81%) [58], *O. cordifolium* (Montbret & Aucher ex Benth.) Vogel (80–100%) [59], and the closely related *O. majorana* (71–88%) [60]. Even though the onset of germination (6th day) was early for directly sown seeds of *Origanum microphyllum* with non-previous storage, the time finally needed for 50% of the seeds to germinate was increased (18.14 days) in relation to the cold storage and GA_3 pre-treated seeds (9 days, t_{50}: 12 days). Consistent with our results, the germination percentage of untreated *O. vulgare* seeds was only 25.8%; the percentage increased to 39% after chilling treatment at 4 °C for seven days, and when soaked for 36 h in a 100 mg/L GA_3 solution, their germination raised to 48%, with mean germination time ranging from 5.75 to 10 days. Thus, the results suggest that *Origanum* seeds have a rather combined dormancy that is both exogenous and endogenous [61]. Further research is suggested to fully master the germination potential of *Origanum microphyllum* prior to conservation efforts and sustainable exploitation attempts.

Many species (and numerous hybrids) of the genus *Dianthus* are used as ornamental plants of rocky gardens, owing to remarkably beautiful and often fragrant blossoms in spring, summer, or often deep into the autumn. Carnation plants are also often used as cut-flowers; given that the flower color of dry plants is retained, they are useful for pressed flower crafts and arrangements. From a horticultural viewpoint, species of particular significance are those that have beautiful and large flowers (preferably of different shades of pink to red) with long blossoming periods, capable of thriving on poor, acidic soils and being drought-resistant [62]. As such, carnation plants are commonly planted in parks, especially in rock gardens as the floral edges of seedbeds, or are used as covers of barren sites in settlements [63]. Some *Dianthus* species such as *D. barbatus* L., *D. chinensis* L., and *D. plumarius* L. have been easily reproduced with both seeds and cuttings [64]. Previous studies indicate that *Dianthus fruticosus* seeds (without reference to its geographically separated subspecies) can successfully germinate in vitro (97%) at 15 °C [65]. However, the germination rates of the endangered *Dianthus fruticosus* subsp. *creticus* in this study were notably lower (79–80%) after the cold storage of seeds (t_{50}: 9.3 days) compared with those directly sowed after collection from the natural environment (93–100%) (t_{50}: 10 days); the seed germination was optimum (100%) on the 30th day under autumn day-night fluctuating temperatures within a greenhouse temperature ranging between 5 °C and 15 °C. The current in vivo seed germination data may complement previous in vitro seed germination studies, and both can be exploited for conservation actions and the sustainable exploitation of *Dianthus fruticosus* subsp. *creticus* as a new and rare ornamental carnation.

In 13 different *Draba* species (including the studied *Draba cretica*), in vivo seed germination has been referred to occur in less than two weeks after sowing at 20 °C [66], which unfortunately does not have any further information available. According to the results of this study, the seed germination ability of the critically endangered high-mountain *Draba cretica* within a 60-day period ranged between 71% and 91% irrespective of the cold storage period or season of the year. In line with the present findings, similar or lower germination percentages were reported in other arctic species of the genus *Draba* [67]. The directly sown seeds of *Draba cretica* in autumn without previous cold storage showed better germination ability (50–91%) than the respective cold-stored ones sown during spring (60–74%). It is known that many *Draba* species have inferred physiological dormancy [68], and specifically *D. glabella* Richardson has been reported to germinate under alternating light and dark conditions following cold stratification for four weeks [69]; meanwhile, the conditional dormancy of *D. verna* L. seeds can be broken after ripening, as seeds seem to prefer lower temperatures and germinate in autumn temperatures in nature [70,71]. It has been postulated that *D. verna* seeds fall to the soil and remain there until germination in September or October as germination does not occur during the summer months due to the seeds being dormant and needing about three months to after-ripen before they can successfully germinate [70]; thus, if the seeds are covered before receiving 5–7 weeks of sunlight, they will not germinate well in the autumn, indicating that other factors affect its germination and light is the lowest influencer [72]. However, this is not the case with the high-mountain

rock-dweller *Draba cretica* studied herein. The germination process of *Draba cretica* under experimentation was completed after a 30-day period, and the onset of germination was observed after 9 days from sowing in both treatments. The seed germination data generated herein can possibly serve conservation actions for *Draba cretica* and may further facilitate its sustainable exploitation in the future.

Members of the closely related genera *Lomelosia* and *Scabiosa* also have attractive flowers, and their seeds may germination rates from as low as 50–53% (e.g., *Scabiosa triniifolia* Friv. and *S. africana* L.) [54] to easy germination (100%), such as in *S. canescens* Waldst. & Kit. [73] and *S. columbaria* L. [74]. In different *Lomelosia* and *Scabiosa* spp. [54], the above average-to-high germination success (50–100%) is reported in in vitro seeds treated with GA_3 or after mechanical scarification. The in vivo seed germination of the critically endangered *Lomelosia minoana* subsp. *minoana* in the present study was completed within 30 days and reached its maximum rate (34–38%) during autumn (t_{50}: 10.74 days). In agreement with our findings, low germination rates (<5% or <30%) such as those recorded herein have also been reported for *Lomelosia graminifolia* (K). Greuter & Burdet [75] and *Scabiosa atropurpurea* L., thus indicating the necessity of a storage period to successfully increase the final germination (from approximately 40–50% in fresh seeds to 70–80% in stored ones) and thus suggesting innate dormancy [76]. However, *Lolelosia minoana* subsp. *asterusica* (Greuter) Greuter & Burdet seeds, another critically endangered local endemic subspecies of Crete [30], may germinate well at all temperatures tested (10, 15, 20 °C), with an optimum (100%) of 15 °C after scarification [28]. The latter introduces the need for a new series of trials and scarification techniques to be attempted in the future with *Lomelosia minoana* subsp. *minoana* seeds, which would be conducted to aim at improving their in vivo germination capacity as observed to date to aid conservation efforts and sustainable exploitation attempts.

The germination rates of the endangered *Onopordum bracteatum* subsp. *creticum* under study were significantly higher (18%) after 15 days with cold storage of seeds as compared with those directly sowed after collection from the natural environment (1.5%). On the contrary, a previous in vitro study in the widespread *O. bracteatum* Boiss. & Heldr. subsp. *bracteatum* have revealed that seeds cultured in petri dishes can exhibit increased germination percentages (77–80%) [54]. Consistent with our findings, four populations of the closely related Scotch thistle (*O. acanthium* L.) exhibited a range of germination responses from readily germinable to strongly dormant in both freshly collected seeds and seeds stored for 67 days at room temperature; the study pointed out that the dry-stored seeds germinated faster than the fresh ones [77]. Despite the onset of seed germination of the studied *Onopordum bracteatum* subsp. *creticum* being recorded after 6 and 7 days from sowing and undergoing non-storage and cold storage, the time needed for half of the cold storage seeds to germinate was shorter (t_{50}: 8.42 days) compared with the non-stored ones (t_{50}: 10.5 days), thus indicating the key role effect of cold storage on its earlier germination and higher germination ability. Similarly, it has been reported that a higher percentage of *O. acanthium* seeds can germinate under controlled conditions (66–89%), but a significant number across treatments will not germinate until scarified [78], thus emphasizing the importance of cold scarification prior to seed germination. Therefore, further research is suggested to fully master the germination potential of *Onopordum bracteatum* subsp. *creticum* with the aim to facilitate conservation efforts and sustainable exploitation attempts.

Members of the genus *Sanguisorba* (generally called burnets) are used as leafy salads and can often be propagated by seeds and division, but the germination requirements for propagation in controlled environments and commercial production are still unclear. Small burnet (*Sanguisorba minor* Scop.) individuals can be established from the seed [79] or by sprouting from the caudex [80], and can occasionally even be regenerated from rhizomes [81]. To date, there are available records for nine *Sanguisorba* taxa showing a generally high germination success (56–100%) for seeds germinated in vitro within a varied periods (7 to 298 days) under a wide range of constant or alternating temperatures [54]. In various *Sanguisorba* species, namely *S. canadensis* L., *S. hakusanensis* Makino, *S. obtusa*

Maxim., *S. officinalis* L., and *S. tenuifolia* Fisch. ex Link, a treatment of cold stratification for 2–4 weeks has proven to be beneficial [66]. In the *Sanguisorba cretica* studies here for the first time, the germination process was completed on the 30th day and exhibited similar maximum germinations rates (86–89%) irrespective of previous cold storage. The seed germination data generated herein can possibly facilitate both conservation actions and the sustainable exploitation for *Sanguisorba cretica*.

4.2. Re-Evaluation of Feasibility and Readiness Timescale for Sustainable Exploitation

Ex situ conservation efforts were successfully accomplished regarding all mother plants derived ex situ from the germination trials, with seeds being directly collected from wild-growing populations in their natural habitats. Therefore, an adequate amount of plant propagation material was obtained for each of the threatened local endemic species of Crete, and horticultural experience was gained. Currently, all materials are cultivated ex situ for conservation purposes and future experimentations. The seed germination data generated in this study for the first time (Section 3.1) bridge extant applied research gaps for the studied local endemic plants of Crete, thus allowing their propagation and ex situ cultivation at pilot scales for conservation purposes. The latter application, when paired with recent publications [31], imposes the need for re-evaluating the studied Cretan endemic taxa in terms of feasibility for value chain creation and readiness timescale for sustainable exploitation in the future (Level II and III evaluations after [4]).

When the above-mentioned re-evaluation procedure is endeavored again for the attributes previously scored (see methodological scheme and scoring in Krigas et al., 2021) [4], in light of the data presented herein and/or recently published [31], the feasibility for value chain creation regarding the studied taxa is considerably increased, and the readiness timescale is remarkably upgraded in all cases. These increased scores and upgraded designations per studied taxon are summarized and outlined in Table 5. More specifically, it is shown that the estimated feasibility for sustainable exploitation regarding *Lomelosia minoana* subsp. *minoana* (30.55%), *Sanguisorba cretica* (33.33%), and *Calamintha cretica* (16.67%) are notably increased, and their respective readiness timescales can be considered "achieved", whereas previous assessments rendered them as either "achievable in the long-term or short-term (for the first two taxa and the last one, respectively). Similarly, the increased feasibility for the sustainable exploitation of *Campanula cretica* by 25.00%, *Draba cretica* by 33.33%, and *Petromarula pinnata* by 16.67% result in an upgrade to their readiness timescale for value chain creation, changing from "long-term" to "short-term"; the respective evaluations for *Origanum microphyllum* (increase by 9.72%) and *Dianthus fruticosus* subsp. *creticus* (increase by 36.11%) can upgrade both readiness timescales for sustainable exploitation from either "medium-term" (*O. microphyllum*) or "indeterminable" (*D. fruticosus* subsp. *creticus*) to "short-term" (Table 5). The feasibility for the sustainable exploitation of *Onopordum bracteatum* subsp. *creticum* is increased by 15.28%, and its readiness timescale for value chain creation is upgraded from "long-term" to "medium-term" (Table 5). It should be highlighted that the differences reported herein per taxon are due to the newly available extinction risk assessments [31], the increased representation of seed storage in ex situ conservation facilities (see Table 1 and Krigas et al. [4]), and their first-time pilot ex situ cultivations (as reported in this study after research on their asexual propagation) paired with the development of species-specific seed germination protocols. These advancements result in higher individual scores per attribute and higher percentages of respective optimum possible scores, thus enabling the process of value chain creation in the near future [4].

Table 5. Overview of the general and special interest in different economic sectors with a multifaceted evaluation of the feasibility and readiness timescale for sustainable exploitation regarding the nine studied local endemic plants of Crete. The table also includes the experimentation period (A: autumn; S: spring) and maximum in vivo germination percentage achieved after trials (in parentheses), which resulted in increased individual scores, pilot ex situ cultivations, and upgrade of previous assessments (Level II and III evaluations).

Cretan Endemic Plant	General Ornamental Interest [4]	* Special Ornamental Interest [4]	Agro-Alimentary Interest [5]	Medicinal Interest [6]	Level II & III Assessments ** [4]	Sowing Period (Maximum Germination Percentage Achieved)	Increase in Sum of Scores (+) and Upgraded Percentage (%)
Calamintha cretica	Low (37.50%)	Below average to very high (31.25%/43.90%/51.61%/63.24%)	High (59.52%)	Below average (46.30%)	69.44%/Short-term	A, S (36%)	+12 (86.11%)/Achieved
Campanula cretica	Average (50.00%)	Average (55.21%/49.09%/46.77%/51.07%)	Low (33.33%)	Low (33.33%)	36.11%/Long-term	A (100%)	+18 (61.11%)/Short-term
Dianthus fruticosus subsp. *creticus*	Low (36.67%)	Low (23.96%/21.04%/25.81%/28.06%)	Low (35.71%)	Very low(11.11%)	34.72%/Indeterminable	A, S (100%)	+23 (66.67%)/Short-term
Draba cretica	Average (53.33%)	Very high (59.38%/68.83%/67.74%/85.38%)	Very low (14.29%)	Very low (11.11%)	36.11%/Long-term	A, S (91%)	+24 (69.44%)/Short-term
Lomelosia minoana subsp. *minoana*	Average (51.67%)	Very high (62.50%/62.08%/68.28%/70.75%)	No (0)	Very low (20.37%)	40.28%/Long-term	A (38%)	+22 (70.83%)/Achieved
Onopordum bracteatum subsp. *creticum*	Low (34.17%)	Low to below average (31.25%/38.70%/45.70%/47.04%)	Low (38.10%)	Below average(46.39%)	38.89%/Long-term	A, S (25%)	+11 (54.17%)/Medium-term
Origanum microphyllum	Below average (44.17%)	Low to average (35.42%/44.42%/47.31%/54.35%)	Very high (80.95%)	High (59.26%)	52.78%/Medium-term	A, S (22.7%)	+7 (62.50%)/Short-term
Petromarula pinnata	Average (52.50%)	Low to average (54.17%/45.45%/50.54%/54.15%)	Below average (42.86%)	Low(37.04%)	45.83%/Long-term	A (100%)	+12 (62.50%)/Short-term
Sanguisorba cretica	Low (32.50%)	Low (31.25%/30.39%/39.25%/38.34)	Below average (45.24%)	Low (27.78%)	38.89%/Long-term	A, S (89%)	+24 (72.22%)/Achieved

* Special ornamental interest refers to pot/patio plant (1st percentage), home gardening (2nd percentage), landscaping (3rd percentage), and xeriscaping (4th percentage), modelled after Krigas et al. [4]. ** Assessments regarding the feasibility (Level II evaluation) and readiness timescale (Level III evaluation) for the sustainable exploitation of the studied taxa.

5. Conclusions

This paper presents new data regarding the in vivo seed germination for nine local Cretan endemic plants with conservation interests (all threatened); these plants were associated with sustainable exploitation potential and an enhanced feasibility to create new value chains for unique but neglected and underutilized phytogenetic resources of Crete. To this end, it was reported herein that the seeds of *Campanula cretica*, *Dianthus fruticosus* subsp. *creticus*, *Draba cretica*, *Petromarula pinnata*, and *Sanguisorba cretica* did not appear to have dormancy as they satisfactorily germinated under the examined in vivo conditions. Refrigeration and pre-treatment with GA_3 were reported to increase the in vivo germination rate in *Onopordum bracteatum* subsp. *creticum* and to accelerate the onset of germination in *Calamintha cretica* seeds. In this study, species-specific sexual propagation protocols through in vivo seed germination were developed for the studied taxa, thus facilitating conservation efforts as well as their sustainable exploitation; the latter may now be considered feasible in the near future or already achieved. At the same time, the data generated in this study have increased the feasibility and readiness timescale for the sustainable exploitation of these threatened, local endemic (unique), neglected, and underutilized phytogenetic resources of Crete, which considerably upgrades the possibility of paving the road toward the establishment of new and species-specific value chains in the future.

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References

1. Maxted, N.; Ford-Lloyd, B.V.; Hawkes, J.G. Complementary conservation strategies. In *Plant Genetic Conservation*; Maxted, N., Ford-Lloyd, B.V., Hawkes, J.G., Eds.; Springer: Dordrecht, The Netherlands, 2000; pp. 15–39. [CrossRef]
2. Krigas, N.; Menteli, V.; Vokou, D. Analysis of the ex situ conservation of the Greek endemic flora at national European and global scales and of its effectiveness in meeting GSPC Target 8. *Plant Biosyst.* **2016**, *150*, 573–582. [CrossRef]
3. Grigoriadou, K.; Sarropoulou, V.; Krigas, N.; Maloupa, E.; Tsoktouridis, G. GIS-facilitated effective propagation protocols of the Endangered local endemic of Crete *Carlina diae* (Rech. f.) Meusel and A. Kástner (Asteraceae): Serving ex situ conservation needs and its future sustainable exploitation as an ornamental. *Plants* **2020**, *9*, 1465. [CrossRef]
4. Krigas, N.; Tsoktouridis, G.; Anestis, I.; Khabbach, A.; Libiad, M.; Megdiche-Ksouri, W.; Ghrabi-Gammar, Z.; Lamchouri, F.; Tsiripidis, I.; Tsiafouli, M.; et al. Exploring the potential of neglected local endemic plants of three Mediterranean regions in the ornamental sector: Value chain feasibility and readiness timescale for their sustainable exploitation. *Sustainability* **2021**, *13*, 2539. [CrossRef]
5. Libiad, M.; Khabbach, A.; El Haissoufi, M.; Anestis, I.; Lamchouri, F.; Bourgou, S.; Megdiche-Ksouri, W.; Ghrabi-Gammar, Z.; Greveniotis, V.; Tsiripidis, I.; et al. Agro-alimentary potential of the neglected and underutilized local endemic plants of Crete (Greece), Rif-Mediterranean Coast of Morocco and Tunisia: Perspectives and challenges. *Plants* **2021**, *10*, 1770. [CrossRef] [PubMed]

6. Bourgou, S.; Ben Haj Jilani, I.; Karous, O.; Megdiche-Ksouri, W.; Ghrabi-Gammar, Z.; Libiad, M.; Khabbach, A.; El Haissoufi, M.; Lamchouri, F.; Greveniotis, V.; et al. Medicinal-cosmetic potential of the local endemic plants of Crete (Greece), Northern Morocco and Tunisia: Priorities for conservation and sustainable exploitation of neglected and underutilized phytogenetic resources. *Biology* **2021**, *10*, 1344. [CrossRef] [PubMed]
7. Paschalidis, K.; Fanourakis, D.; Tsaniklidis, G.; Tzanakakis, V.A.; Bilias, F.; Samara, E.; Kalogiannakis, K.; Debouba, F.J.; Ipsilantis, I.; Tsoktouridis, G.; et al. Pilot cultivation of the Vulnerable Cretan endemic *Verbascum arcturus* L. (Scrophulariaceae): Effect of fertilization on growth and quality features. *Sustainability* **2021**, *13*, 14030. [CrossRef]
8. Fanourakis, D.; Paschalidis, K.; Tsaniklidis, G.; Tzanakakis, V.A.; Bilias, F.; Samara, E.; Liapaki, E.; Jouini, M.; Ipsilantis, I.; Maloupa, E.; et al. Pilot cultivation of the local endemic Cretan marjoram *Origanum microphyllum* (Benth.) Vogel (Lamiaceae): Effect of fertilizers on growth and herbal quality features. *Agronomy* **2022**, *12*, 94. [CrossRef]
9. Grigoriadou, K.; Krigas, N.; Sarropoulou, V.; Maloupa, E.; Tsoktouridis, G. 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. *Not. Bot. Hort. Agrobot. Cluj-Napoca* **2021**, *49*, 12261. [CrossRef]
10. Hatzilazarou, S.; El Haissoufi, M.; Pipinis, E.; Kostas, S.; Libiad, M.; Khabbach, A.; Lamchouri, F.; Bourgou, S.; Megdiche-Ksouri, W.; Ghrabi-Gammar, Z.; et al. GIS-Facilitated seed germination and multifaceted evaluation of the Endangered *Abies marocana* Trab. (Pinaceae) enabling conservation and sustainable exploitation. *Plants* **2021**, *10*, 2606. [CrossRef]
11. Pipinis, E.; Hatzilazarou, S.; Kostas, S.; Bourgou, S.; Megdiche-Ksouri, W.; Ghrabi-Gammar, Z.; Libiad, M.; Khabbach, A.; El Haissoufi, M.; Lamchouri, F.; et al. Facilitating conservation and bridging gaps for the sustainable exploitation of the Tunisian local endemic Plant *Marrubium aschersonii* (Lamiaceae). *Sustainability* **2022**, *14*, 1637. [CrossRef]
12. Kostas, S.; Hatzilazarou, S.; Pipinis, E.; Bourgou, S.; Ben Haj Jilani, I.; Ben Othman, W.; Megdiche-Ksouri, W.; Ghrabi-Gammar, Z.; Libiad, M.; Khabbach, A.; et al. DNA barcoding, GIS-facilitated seed germination and pilot cultivation of *Teucrium luteum* subsp. gabesianum (Lamiaceae), a Tunisian local endemic with potential medicinal and ornamental value. *Biology* **2022**, *11*, 462. [CrossRef]
13. Mohammad Esmaeili, M.; Sattarian, A.; Bonis, A.; Bouzillé, J. Ecology of seed dormancy and germination of *Carex divisa* Huds.: Effects of stratification, temperature and salinity. *Intern. J. Plant Product.* **2012**, *3*, 27–40. [CrossRef]
14. Zarghani, H.; Mijani, S.; Nasrabadi, S.E.; Ghias-Abadi, M.; Khorramdel, S.; Azimi, R. Temperature effects on the seed germination of some perennial and annual species of Asteraceae family. *Plant Breed. Seed Sci.* **2014**, *69*, 3–14. [CrossRef]
15. Asha Rani, N.S.; Prasad, M.P. In-vitro studies on the germination of *Atropa belladonna* seeds under different conditions. *Intern. J. Sci. Res.* **2014**, *3*, 552–555. Available online: https://www.ijsr.net/get_abstract.php?paper_id=OCT14184 (accessed on 20 May 2022).
16. Weitbrecht, K.; Müller, K.; Leubner-Metzger, G. First off the mark: Early seed germination. *J. Exp. Bot.* **2011**, *62*, 3289–3309. [CrossRef]
17. Baskin, C.C.; Baskin, J.M. *Seeds: Ecology, Biogeography and Evolution of Dormancy and Germination*; Academic Press: Cambridge, MA, USA, 2001.
18. Paparella, S.; Araujo, S.S.; Rossi, G.; Wijayasinghe, M.; Carbonera, D.; Balestrazzi, A. Seed priming: State of the art and new perspectives. *Plant Cell Rep.* **2015**, *34*, 1281–1293. [CrossRef]
19. Statwick, J.M. Germination pretreatments to break hard-seed dormancy in *Astragalus cicer* L. (Fabaceae). *Peer J.* **2016**, *4*, e2621. [CrossRef]
20. Zhang, K.; Yao, L.; Zhang, Y.; Baskin, J.M.; Baskin, C.C.; Xiong, Z.; Tao, J. A review of the seed biology of *Paeonia* species (Paeoniaceae), with particular reference to dormancy and germination. *Planta* **2019**, *249*, 291–303. [CrossRef]
21. Rivera, D.; Obón, C.; Heinrich, M.; Inocencio, C.; Verde, A.; Fajardo, J. Gathered Mediterranean food plants—Ethnobotanical investigations and historical development. In *Local Mediterranean Food Plants and Nutraceuticals*; Heinrich, M., Müller, W.E., Galli, C., Eds.; Karger Publishing: Basel, Switzerland, 2006; Volume 59, pp. 18–74.
22. Baldermann, S.; Blagojevic, L.; Frede, K.; Klopsch, R.; Neugart, S.; Neumann, A.; Ngwene, B.; Norkeweit, J.; Schröter, A.; Schröter, D.; et al. Are neglected plants the food for the future? *Crit. Rev. Plant Sci.* **2016**, *35*, 106–119. [CrossRef]
23. Scariot, V.; Seglie, L.; Gaino, W.; Devecchi, M. Evaluation of European native bluebells for sustainable floriculture. *Acta Hortic.* **2012**, *937*, 273–280. [CrossRef]
24. Omotayo, A.O.; Ijatuyi, E.J.; Ogunniyi, A.I.; Aremu, A.O. Exploring the resource value of Transvaal Red Milk Wood (*Mimusops zeyheri*) for food security and sustainability: An appraisal of existing evidence. *Plants* **2020**, *9*, 1486. [CrossRef] [PubMed]
25. Georghiou, K.; Delipetrou, P. Patterns and traits of the endemic plants of Greece. *Bot. J. Linn. Soc.* **2010**, *162*, 130–422. [CrossRef]
26. Menteli, V.; Krigas, N.; Avramakis, E.; Turland, N.; Vokou, D. Endemic plants of Crete in electronic trade and wildlife tourism: Current patterns and implications for conservation. *J. Biol. Res. Thessalon.* **2019**, *26*, 10. [CrossRef] [PubMed]
27. Kloukina, C.; Tomou, E.M.; Krigas, N.; Sarropoulou, V.; Madesis, P.; Maloupa, E.; Skaltsa, H. Non-polar secondary metabolites and essential oil of *ex-situ* propagated and cultivated *Sideritis syriaca* L. subsp. syriaca (Lamiaceae) with consolidated identity (DNA Barcoding): Towards a potential new industrial crop. *Ind. Crop. Prod.* **2020**, *158*, 112957. [CrossRef]
28. Fournaraki, C. Conservation of Threatened Plants of Crete—Seed Ecology, Operation and Management of a Gene Bank. Ph.D. Thesis, National and Kapodistrian University of Athens, Faculty of Biology, Department of Botany, Athens, Greece, 2010. (In Greek with English Abstract)
29. Geneve, L.R. Some common misconceptions about seed dormancy. *Comb. Proc. Int. Plant Propag. Soc.* **2005**, *55*, 9–12.

30. Finch-Savage, W.E.; Leubner-Metzger, G. Seed dormancy and the control of germination. *New Phytol.* **2006**, *171*, 501–523. [CrossRef]
31. Kougioumoutzis, K.; Kokkoris, I.P.; Panitsa, M.; Strid, A.; Dimopoulos, P. Extinction risk assessment of the Greek endemic flora. *Biology* **2021**, *10*, 195. [CrossRef]
32. Gkika, P.I.; Krigas, N.; Menexes, G.; Eleftherohorinos, I.E.; Maloupa, E. Effect of temperature and light on seed germination of *Erysimum naense* and *Erysimum krendlii*. *Cent. Eur. J. Biol.* **2013**, *8*, 1194–1203. [CrossRef]
33. Grigoriadou, K.; Krigas, N.; Maloupa, E. GIS-facilitated ex situ conservation of the rare Greek endemic *Campanula incurva* Aucher: Seed germination requirements and effect of growth regulators on in vitro proliferation and rooting. *Plant Biosyst.* **2014**, *148*, 1169–1177. [CrossRef]
34. Krigas, N.; Mouflis, G.; Grigoriadou, K.; Maloupa, E. Conservation of important plants from the Ionian Islands at the Balkan Botanic Garden of Kroussia, N Greece: Using GIS to link the in situ collection data with plant propagation and ex situ cultivation. *Biodivers. Conserv.* **2010**, *19*, 3583–3603. [CrossRef]
35. Krigas, N.; Karapatzak, E.; Panagiotidou, M.; Sarropoulou, V.; Samartza, I.; Karydas, A.; Damianidis, C.K.; Najdovski, B.; Teofilovski, A.; Mandzukovski, D.; et al. Prioritizing plants around the cross-border area of Greece and the Republic of North Macedonia: Integrated conservation actions and sustainable exploitation potential. *Diversity* **2022**, *14*, 570. [CrossRef]
36. Strid, A. *Atlas of the Aegean Flora Part 1 (Text & Plates) & Part 2 (Maps)*, 1st ed.; Botanic Garden and Botanical Museum and Freie Universität: Berlin, Germany, 2016; Volume 33.
37. Farooq, M.; Basra, S.M.A.; Ahmad, N.; Hafeez, K. Thermal hardening: A new seed vigor enhancement tool in rice. *J. Integr. Plant Biol.* **2005**, *47*, 187–193. [CrossRef]
38. Probert, R.J. The role of temperature in the regulation of seed dormancy and germination. In *Seeds: The Ecology of Regeneration in Plant Communities*, 2nd ed.; Fenner, M., Ed.; CAB International: Wallingford, UK, 2000; pp. 261–292. [CrossRef]
39. Farina, E.; Dalla Guda, C. *Campanula. Guida Pratica Alla Coltivazione*; Ace International: Vernasca (PC), Italy, 2006; pp. 240.
40. Joung, Y.H.; Liao, M.S.; Roh, M.S.; Kamo, K.; Song, J.S. In vitro propagation of *Campanula glomerata* 'Aucalis' from leaf blade explants. *Sci. Hortic.* **2002**, *92*, 137–146. [CrossRef]
41. Blionis, G.J.; Vokou, D. Reproductive attributes of *Campanula* populations from Mt Olympos, Greece. *Plant Ecol.* **2005**, *178*, 77–88. [CrossRef]
42. Mork, E.K.; Sriskandarajah, S.; Serek, M. Influence of seed germination conditions on regenerative ability in *Campanula carpatica*. *Europ. J. Hortic. Sci.* **2005**, *70*, 173–176. Available online: https://www.pubhort.org/ejhs/2005/file_29850.pdf (accessed on 13 September 2022).
43. Tanaka, N.; Yamada, Y.; Shimomura, K.; Ishimaru, K. Polyacetilenes in tissue cultures of *Campanula glomerata*. *Plant Tissue Cult. Lett.* **1996**, *13*, 215–217. [CrossRef]
44. Sriskandarajah, S.; Frello, S.; Serek, M. Induction of adventitious shoots in vitro in *Campanula carpatica*. *Plant Cell Tissue Organ Cult.* **2001**, *67*, 295–298. [CrossRef]
45. Koutsovoulou, K.; Daws, M.I.; Thanos, C.A. Campanulaceae: A family with small seeds that require light for germination. *Ann. Bot.* **2014**, *113*, 135–143. [CrossRef]
46. Jankowska-Blaszczuk, M.; Daws, M.I. Impact of red:far red ratios on germination of temperate forest herbs in relation to shade tolerance, seed mass and persistence in the soil. *Funct. Ecol.* **2007**, *21*, 1055–1062. [CrossRef]
47. Karousou, R.; Kokkini, S.; Bessiere, J.M.; Vokou, D. *Calamintha cretica* (Lamiaceae), a Cretan endemic: Distribution and essential oil composition. *Nord. J. Bot.* **1996**, *16*, 247–251. [CrossRef]
48. Nakamura, Y.; Hasegawa, Y.; Shirota, K.; Noboru, S.N.; Nakamura, T.; Chomnawang, M.T.; Sato, K. Differentiation inducing effect of piperitenone oxide, a fragrant ingredient of spearmint (*Mentha spicata*), but not carvone and menthol, against human colon cancer cells. *J. Funct. Foods* **2014**, *8C*, 62–67. [CrossRef]
49. Caneva, G.; Kumbaric, A.; Savo, V.; Casalini, R. Ecological approach in selecting extensive green roof plants: A data-set of Mediterranean plants. *Plant Biosyst.* **2013**, *149*, 374–383. [CrossRef]
50. Casalini, R.; Bartoli, F.; Caneva, G. Investigation of seed germination of twelve Mediterranean wildflowers for evaluating their potential use on extensive green roofs. *Acta Hortic.* **2017**, *1189*, 263–266. [CrossRef]
51. Vlachou, G.; Papafotiou, M.; Bertsouklis, K.F. Seed germination, micropropagation from adult and juvenile origin explants and address of hyperhydricity of the Cretan endemic herb *Calamintha cretica*. *Not. Bot. Hort. Agrobot. Cluj Napoca* **2020**, *48*, 1504–1518. [CrossRef]
52. European Union Herbal Monograph on *Origanum majorana* L., Herba. European Medicines Agency, Committee on Herbal Medicinal Products, EMA/HMPC/166517/2015. 2 February 2016. Available online: https://www.ema.europa.eu/en (accessed on 15 September 2022).
53. Markaki, E. Out of the Place (Ex-Situ) Conservation of Endemic Plants of Crete. Graduate Dissertation, Higher Technological Educational Institution of Crete, Faculty of Agricultural Technology, Department of Plant Production, Heraklion, Greece, 2006. (In Greek)
54. SID-KEW (Seed Information Database-Royal Botanic Gardens, Kew), Wakehurst Place, Kew Gardens, 592 London, UK.
55. Özkum, D. In vitro shoot regeneration of oregano (*Origanum minutiflorum* O. Schwarz & Davis). *Hacet. J. Biol. Chem.* **2007**, *35*, 97–100.

56. Yildirim, M.U. Micropropagation of *Origanum acutidens* (Hand.-Mazz.) Ietsw. using stem node explants. *Sci. World J.* **2013**, *2013*, 276464. [CrossRef]
57. Liopa-Tsakalidi, A.; Zakynthinos, G.; Varzakas, T.; Xynias, I.N. Effect of NaCl and GA_3 on seed germination and seedling growth of eleven medicinal and aromatic crops. *J. Med. Plants Res.* **2011**, *5*, 4065–4073. [CrossRef]
58. Laghmouchi, Y.; Bouyahya, A.; Senhaji, N.S.; Abrini, J. Effect of temperature, salt stress and pH on seed germination of medicinal plant *Origanum compactum*. *Biocatal. Agric. Biotechnol.* **2017**, *10*, 156–160. [CrossRef]
59. Kadis, C.C.; Georghiou, K. The germination physiology of the endangered plants of Cyprus, *Alyssum akamasicum* and *Origanum cordifolium*. In Proceedings of the Fourth International Workshop on Seeds: Basic and Applied Aspects of Seed Biology, Angers, France, 20–24 July 1992; Volume 2, pp. 461–465.
60. Thanos, C.A. Physiology of seed germination in marjoram (*Origanum majorana* L.). *ISAFA Comun. Di Ric.* **2000**, *2*, 31–36.
61. Farashah, H.D.; Afshari, R.T.; Sharifzadeh, F.; Chavoshinasab, S. Germination improvement and α-amylase and β-1,3-glucanase activity in dormant and non-dormant seeds of Oregano (*Origanum vulgare*). *Aust. J. Crop Sci.* **2011**, *5*, 421–427.
62. Radojević, L.J.; Ćalić-Dragosavac, D.; Špirić, J.; Stevanović, B.; Stevanović, V. In vitro propagation of *Dianthus ciliatus* ssp. *dalmaticus and D. giganteus ssp. croaticus (Caryophyllaceae) from stem segment cultures. Bot. Serb.* **2010**, *34*, 153–161.
63. Radojević, L.J. Application of in vitro Culture in Flower Production: State and Possibilities of Floriculture Development in Serbia. In Proceedings of the Landscape Horticulture Seminar, Faculty of Forestry, Belgrade, Serbia, 8–9 February 2007; pp. 46–48. (In Serbian)
64. Hartmann, H.T.; Kester, D.E.; Davies, F.T., Jr.; Geneve, R.L. *Hartmann and Kester's Plant Propagation: Principles and Practices*, 7th ed.; Prentice Hall Inc.: Hoboken, NJ, USA, 2002.
65. Papafotiou, M.; Stragas, J. Seed germination and in vitro propagation of *Dianthus fruticosus* L. *Acta Hortic.* **2009**, *813*, 481–484. [CrossRef]
66. Kootenay Local Agricultural Society. Perennial Seed Germination Information. 2008. Available online: https://docplayer.net/46736765-Perennial-seed-germination-information-kootenay-local-agricultural-society-2008.html (accessed on 30 August 2022).
67. Baskin, C.C.; Baskin, J.M. *Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination*; Academic Press: San Diego, CA, USA, 1998. [CrossRef]
68. Alsos, I.G.; Muller, E.; Eidesen, P.B. Germinating seeds or bulbils in 87 of 113 tested Arctic species indicate potential for ex situ seed bank storage. *Polar Biol.* **2013**, *36*, 819–830. [CrossRef]
69. Murray, D.F.; Kelso, S. Chromosome numbers and notes on the taxonomy of selected Alaskan vascular plants. *Rhodora* **1997**, *99*, 33–55. Available online: http://www.jstor.org/stable/23313280 (accessed on 20 October 2022).
70. Baskin, J.M.; Baskin, C.C. Germination eco-physiology of *Draba verna*. *Bull. Torrey Bot. Club* **1970**, *97*, 209–216. [CrossRef]
71. Baskin, J.M.; Baskin, C.C. Effects of relative humidity on after ripening and viability in seeds of the winter annual *Draba verna*. *Bot. Gaz.* **1979**, *140*, 284–287. [CrossRef]
72. Baskin, J.M.; Baskin, C.C. The light factor in the germination ecology of *Draba verna*. *Am. J. Bot.* **1972**, *57*, 756–759. [CrossRef]
73. Csonthos, P.; Tamas, J.; Balogh, L. Thousand-seed weight records of species from the flora of Hungary, II. Dicotyledonopsida. *Stud. Bot. Hung.* **2007**, *38*, 179–189.
74. Cerabolini, B.; Ceriani, R.M.; Caccianiga, M.; De Andreis, R.; Raimondi, B. Seed size, shape and persistence in soil: A test on Italian flora from Alps to Mediterranean coasts. *Seed Sci. Res.* **2003**, *13*, 75–85. [CrossRef]
75. Kupferschmid, A.D.; Stampfli, A.; Newbery, D.M. Dispersal and microsite limitation in an abandoned calcareous grassland of the southern Prealps. *Folia Geobot.* **2000**, *35*, 125–141. [CrossRef]
76. Frischie, S.; Fernandez-Pascual, E.; Ramirez, C.G.; Toorop, P.; Gonzalez, M.H.; Jimenez-Alfaro, B. Hydrothermal thresholds for seed germination in winter annual forbs from old-field Mediterranean landscapes. *Plant Biol.* **2018**, *21*, 449–457. [CrossRef] [PubMed]
77. Qaderi, M.M.; Cavers, P.B. Interpopulation variation in germination responses of Scotch thistle, *Onopordum acanthium* L., to various concentrations of GA_3, KNO_3, and $NaHCO_3$. *Can. J. Bot.* **2000**, *78*, 1156–1163. [CrossRef]
78. Qaderi, M.M.; Cavers, P.B.; Bernards, M.A. Seed bank dynamics of *Onopordum acanthium*: Emergence patterns and chemical attributes. *J. Ecol.* **2002**, *90*, 672–683. [CrossRef]
79. Wieneke, S.; Prati, D.; Brandl, R.; Stocklin, J.; Auge, H. Genetic variation in *Sanguisorba minor* after 6 years in situ selection under elevated CO_2. *Glob. Chang. Biol.* **2004**, *10*, 1389–1401. [CrossRef]
80. Monsen, S.B. Selection of plants for fire suppression on semiarid sites. In *Proceedings—Ecology and Management of Annual Rangelands, Gen. Tech. Rep. INT-GTR-313, Boise, Idaho, 18–22 May 1992*; Monsen, S.B., Kitchen, S.G., Eds.; U.S. Department of Agriculture, Forest Service, Intermountain Research Station: Ogden, UT, USA, 1994; pp. 363–373.
81. Shaw, N.L. Production and use of planting stock. In *Restoring Western Ranges and Wildlands*; Monsen, S.B., Stevens, R., Shaw, N.L., Eds.; Gen. Tech. Rep. RMRS-GTR-136; U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station: Fort Collins, CO, USA, 2004; Volume 3, pp. 745–768.