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RESEARCH

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GIS-facilitated seed germination of six local endemic plants of Crete (Greece) and multifaceted evaluation in three economic sectors

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Abstract

In the context of conservation and sustainable exploitation of neglected and underutilized plant genetic resources (NUPs), this study focused on six Cretan local endemic plants i.e., three monocots (*Allium bourgeaui* subsp. *creticum*, *Allium dilatatum* and *Muscari spreitzenhoferi*) and three dicots (*Alyssum baldaccii*, *Campanula saxatilis* subsp. *saxatilis* and *Silene antri-jovis*). We aimed at determining the ecological conditions needed for these plants to thrive based on their natural preferences which define their germination requirements and allow the development of species-specific propagation protocols. Secondly, we overviewed the potential of the targeted species for sustainable exploitation in three economic sectors (ornamental-horticultural, medicinal-cosmetic, agro-alimentary). The ecological profiles of each species were constructed using Geographic Information Systems and climate data from WorldClim. Four temperatures were examined in seed germination trials (10, 15, 20, 25°C) and respective germination percentages (GP) were calculated. Seed germination of monocots showed preference in more cold temperatures (70.0%, 40.0% and 71.25% at 10°C for *A. bourgeaui* subsp. *creticum*, *A. dilatatum* and *M. spreitzenhoferi*, respectively) while in two of the dicots it exhibited a wider temperature range (83.75 - 86.25% at 10, 15, 20°C for *A. baldaccii* and 90 - 98.75% at all temperatures tested for *S. antri-jovis*) while in *C. saxatilis* subsp. *saxatilis* at lower temperatures (85% and 71.25% at 10 and 15°C, respectively). The assessed taxa showed interesting values in terms of potential mainly for the ornamental and agro-alimentary sectors, and their prospective is discussed herein in detail (first-time for *A. baldaccii*). Exploiting all the above results, we re-evaluated the feasibility and readiness timescale for sustainable exploitation in three economic sectors for the targeted NUPs and their upgraded assessments are first-time presented herein in detail.

Keywords: biodiversity; phytogenetic resources; seed germination; GIS; sustainable exploitation; neglected and underutilized plants; plant endemism

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Introduction

The anthropogenic activities have resulted worldwide in fragmentation and degradation of ecosystems and concomitant loss of biodiversity, bringing many species at the verge of extinction and accelerating extinction patterns [1, 2]. This complex problem can be challenged or may be counteracted at least in part or at local scales by the implementation of targeted restoration programs which aim at halting biodiversity loss, re-enforcing declined species populations and restoration of natural vegetation [3, 4, 5]. Still, such ambitious efforts are rather limited compared to the urgent biodiversity crisis faced to date and the anticipated results of such actions are usually compromised due to social, scientific and political factors coupled with other complexities stemming from discrepancies in species prioritization and/or selection of focal areas [4, 6].

The success in all biodiversity restoration efforts is mainly based on prioritized conservation strategies, efficient management schemes, availability of resources, appropriate species selection (suitable to local environmental conditions) and prioritization schemes, and depend on the knowledge of successful propagation and acclimatization of the focal species [4, 5]. To this end, the sexual propagation represents the most adequate method to maintain the genetic diversity for many species and this is also the most common and the easiest method used in nurseries for further utilization [7]. Therefore, the knowledge of species-specific germination requirements is the first essential step for successful propagation of focal species that are either prioritized conservation-wise or will be utilized for various purposes. Unfortunately, specific information on seed germination requirements is often unknown or remains scarce for most of the native plant species of certain regions, especially regarding those that are rare, threatened and/or local endemics in different areas [5, 8]. To this end, Geographical Information Systems (GIS) have been employed during the last decade to unveil the abiotic environmental conditions required by prioritized endemic plants in their local wild habitats, thus furnishing insights into their seed germination requirements [9, 10, 11, 12, 13, 14, 15]. Unless bridged, this common research gap generally hinders any attempt for sustainable exploitation of NUPs and in particular impedes the utilization of local endemic phytogenetic resources of given areas in different economic sectors such as the ornamental-horticultural industry [8], the medicinal-cosmetic [16] or the agro-alimentary sectors [17].

From biological viewpoint, germination and seedlings' establishment are considered to be the most critical phases in the life cycle of plants [18], and local climatic factors in different areas affect seed germination and seedling growth [19]. Although soil moisture is critical for seedling emergence and survival, temperature is the most important environmental factor which synchronizes seed germination with the most suitable environmental conditions for seedling establishment [19]. Depending on their origin, different species have varied temperature requirements for seed germination [20]. In Mediterranean-type ecosystems, the optimal temperature for seed germination is relatively low, ranging between 10 and 20°C; in the natural environment, the season with this temperature range usually coincides with early winter when rainfall is sufficient for seed germination [21, 22, 23, 24].

In this framework, the study herein firstly aimed to explore comparatively the potential of six local endemic plants of Crete (Greece) in different economic sectors (ornamental-horticultural, medicinal-cosmetic, agro-alimentary). The present study furthermore aimed to investigate how temperature affects their germination under controlled conditions, employing Geographical Information Systems (GIS) that offer insights into their abiotic environmental conditions required in their respective wild habitats [9, 10, 12, 13, 14, 15]. The focal taxa (species or subspecies) of conservation importance [25] studied herein include: (i) Three dicots of different families, i.e. *Alyssum baldaccii* Nyár. (Brassicaceae) currently known as *Odontarrhena baldaccii* (Vierh. ex Nyár.) Španiel (https://portal.cybertaxonomy.org/flora-greece/, accessed on 13 December 2021), *Campanula saxatilis* L. subsp. *saxatilis* (Campanulaceae), and *Silene antri-jovis* Greuter & Burdet (Caryophyllaceae); as well as (ii) Three monocots of two families, namely *Allium bourgeaui* Rech. f. subsp. *creticum* Bothmer, *Allium dilatatum* Zahar. (Alliaceae) and *Muscari spreitzenhoferi* (Heldr.) H. R. Wehrh. (Hyacinthaceae). These taxa are also characterized as neglected and underutilized local endemic species of Crete with potential value in different economic sectors [8, 16, 17]. The knowledge of their life cycle and their germination requirements offer stepping stones for their effective *in-situ* and *ex-situ* conservation as well as for their sustainable utilization and possible exploitation [8, 16, 17]. In addition, the GIS-derived ecological profiles can provide significant insights regarding the climate conditions under which the studied taxa thrive in their wild habitats, thereby facilitating future integrated conservation efforts including population reinforcement or restoration.

Methods

Basic biological features of the focal plants

The unique plant genetic resources of Crete (Greece) studied herein include plants that are rare and range-restricted and are thus assessed as threatened with extinction [25]. The chamaephyte *Alyssum baldaccii* or *Odontarrhena baldaccii* (Figure 1A) is assessed as Endangered [25], the hemicryptophyte *Silene antri-jovis* (Figure 1C) is assessed as Critically Endangered [25] and the geophytes *Allium bourgeaui* subsp. *creticum* (Figure 2A) and *Allium dilatatum* (Figure 2B) are also assessed as Endangered [25]; the latter is additionally protected by the Greek Presidential Decree 67/1981. The geophyte *Muscari spreitzenhoferi* (Figure 2C) and the hemicryptophyte *Campanula saxatilis* subsp. *saxatilis* are assessed as Vulnerable [25]; the latter is additionally protected by the Greek Presidential Decree 67/1981 (Figure 1B).

Alyssum baldaccii (Figure 1A) occurs exclusively in five

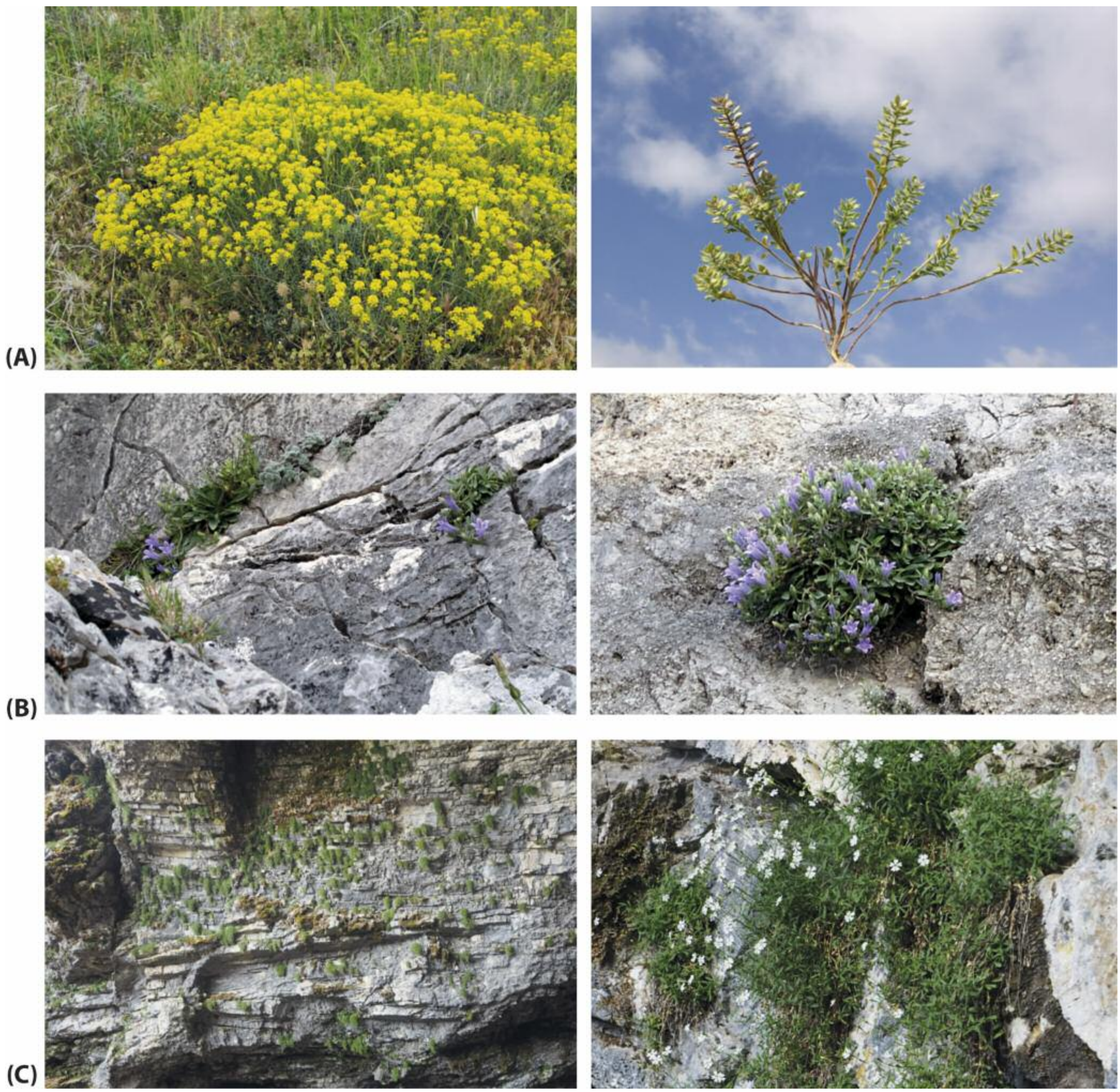

Figure 1. Habitats (left) and habit (right) of the studied dicot local endemic plants of Crete): (A) *Alyssum baldaccii* (Endangered; Brassicaceae), (B) *Campanula saxatilis* subsp. *saxatilis* (Vulnerable; Campanulaceae), and (C) *Silene antri-jovis* (Critically Endangered; Caryophyllaceae). Extinction risk assessments according to Kougioumoutzis et al. [25].

Figure 2. Habitats (left) and habit (right) of the studied monocot local endemic plants of Crete: (A) *Allium bourgeaui* subsp. *creticum* (Endangered; Alliaceae; Photos: F. Samaritakis, reproduced with permission), (B) *Allium dilatatum* (Endangered; Alliaceae; Photos: G. Palimetakis, reproduced with permission), and (C) *Muscari spreitzenhoferi* (Vulnerable; Hyacinthaceae; Photos: F. Samaritakis, reproduced with permission). Extinction risk assessments according to Kougioumoutzis et al. [25].

localities of Mt Psiloritis in Central Crete [26]. *Campanula saxatilis* subsp. *saxatilis* (Figure 1B) is restricted to 11 rocky sites of Western and Central Crete [26]. *Silene antri-jovis* (Figure 1C) and *A. dilatatum* are found in 15 and 11 localities, respectively, across the main mountain massifs of Central and Eastern Crete and of Western, Central and Eastern Crete, respectively [26]. *Allium bourgeaui* subsp. *creticum* (Figure 2A) and *M. spreitzenhoferi* (Figure 2C) are found in about 30 or more scattered localities of varied altitudes across the island of Crete [26].

Five of the focal threatened plants studied herein thrive in hardy habitats as facultative or obligatory rock-dwellers (chasmophytes) on limestone or on ultramafic substrates (such as *A. baldaccii*), while *M. speitzenhoferi* occurs in a variety of sandy and rocky habitats, phrygana and mountain dolines ([26], Figures 1 and 2). Four of the studied taxa occur in different altitudes below 1000 m above sea level; *S. antri-jovis* occurs in higher altitudes mainly above 1000 m while *M. speitzenhoferi* has a wide altitudinal range (0 - 2200 m) [26]. The studied dicots are perennial herbaceous plants that flower in late spring and early summer, while the studied monocots are bulbous plants with seasonal appearance flowering mainly during summer, depending on the altitudes in which their populations thrive [26]. For example, *M. speitzenhoferi* occurs in a wide altitudinal range and thus may be in flower from spring at low altitudes until summer at high altitudes [26].

In terms of Ellenberg indicator values as defined for the south Aegean flora [27], *A. bourgeaui* subsp. *creticum* and *A. baldaccii* are characterized as light-demanding plants with natural adaptation to strong sunlight while the rest of the studied taxa are semi-shade plants; most of the taxa grow in xerothermic arid conditions and occur in fairly hot areas with the exception of *A. baldaccii* and *S. antri-jovis* that are relatively hygrophilous thriving in relatively cooler areas; most plants prefer substrates of relatively alkaline pH while *A. baldaccii* prefers relatively high acid conditions; most of them are found in soils with average content in nutrients while *A. dilatatum* and *S. antri-jovis* prefer substrates with low nutrient content and most of them are relatively salt-tolerant while *A. baldaccii* and *S. antri-jovis* are halophobic [27].

Multifaceted evaluation in three sectors of economy

The developed methodology for the multifaceted evaluation of the targeted plants in three sectors of the economy (Level I evaluation) is presented in detail in previous own studies exploring in a comparative way the potential of 399 endemic plants of Crete, Morocco and Tunisia [8, 16, 17]. In brief, the potential value of the taxa was individually assessed for each economical sector based on: (i) twenty parameters associated with the ornamental-horticultural sector [8], (ii) seven parameters related with the agro-alimentary sector [17], and (iii) nine parameters linked with the medicinal-cosmetic sector [16]. Moreover, further taxon-specific evaluations were made regarding the associated feasibility for value chain creation (Level II evaluations) [8]. To envisage the potential of these NUPs in comparison with other commercial crops, the individual evaluation scores were converted into relative percentages (%) of the maximum possible scores that could be obtained [8, 16, 17]. Thus, the divergence from the absolute theoretical value (100%, maximum score in all parameters) can illustrate in any case the relative potential of an evaluated taxon in a given economic sector or the associated feasibility for value chain creation [8].

Although the evaluations of five of the local endemic plants of Crete (*A. bourgeaui* subsp. *creticum*, *A. dilatatum*, *M. spreitzenhoferi*, *C. saxatilis* subsp. *saxatilis* and *S. antri-jovis*) have been sought from previous own studies [8, 16, 17], they are presented herein in detail for the first time. Using the same methodological scheme, the evaluation of *A. baldaccii* in three economic sectors was originally performed herein, since it represents a newly added species to the endemic flora of Crete [28].

The readiness timescale designations for the sustainable exploitation of the herein focal plants (Level III evaluation) were based on previous SWOT (Strengths, Weaknesses, Opportunities, Threats) and gap analyses [8]; this assessment was re-evaluated in the light of the seed germination data obtained in this study.

GIS ecological profiling

The distribution points of the focal taxa were georeferenced from consolidated floristic sources [26, 29]. Minimum, maximum and average temperatures of the distribution sites as well as 19 bioclimatic values were calculated using the historical climate data of 30 sec pixel size from the website WorldClim (https://www.worldclim.org/data/worldclim21.html, accessed on 13 December 2021). The taxon-specific ecological profiles were created with GIS, linking the respective values of the following layers:

(a) WorldClim version 2.1 with minimum, maximum, and average temperature (°C), as well as precipitation values (mm) and data for 19 bioclimatic variables for every month derived from 1970 - 2000 in raster resolution of 1 km^2, and

(b) Raster files of taxon's distribution points [26, 29].

Seed collection and documentation

Mature seeds of the six local endemic taxa of Crete were collected by hand in 2018 - 2020, just before dispersion, from several (10 - 20) wild-growing individuals of each taxon (Table 1, Figure 3). The seeds were collected using a special permission by the national competent authority (Greek Ministry of Environment and Energy), thus representing authorized original collections of Greek native plant material in the wild for conservation purposes, i.e., Permit 82336/879 of 18 May 2019 & 26895/1527 of 21 April 2021 of the Institute of Plant Breeding and Genetic Resources, Hellenic Agricultural Organization "Demeter" (issued yearly after detailed reporting). After collection and cleaning, the seeds of each taxon were stored in glass containers at 4 - 5°C within a low humidity chamber (<15%) before germination experiments were carried out (16 - 40 months storage period, Table 1).

All seed collections (seeds collected from populations and habitats as in Figures 1 and 2; individual seeds in Figure 3) and their respective vouchers were deposited in the herbarium of the Balkan Botanic Garden of Kroussia (BBGK) and were taxonomically identified based on Strid [26].

Consequently, the collected seeds were coded with a unique IPEN (International Plant Exchange Network) accession number assigned to each population sample collected from a single Cretan locality on a given date. The IPEN numbering is commonly used for plant exchanges between botanic gardens defining the country of origin (GR), the terms of use (1: restrictions of use or 0: no restriction), the original collecting institution (BBGK), the collection year (two digits), along with a basic number identifier (https://www.bgci.org/wp/wpcontent/uploads/2019/04/Manual_IPEN_Numbers.pdf, accessed on 13 June 2022).

Germination tests

Germination experiments were initiated in December 2021 and were conducted in the laboratory of Floriculture, School of Agriculture, Aristotle University of Thessaloniki (Thermi, Greece). Seed germination of each taxon (Table 1) was investigated using temperature controlled CRW-500SD growth chambers (Chrisagis, Athens, Greece) with relative humidity (RH) at 75 ± 1%. Specifically, their germination responses at four constant temperatures of 10, 15, 20, and 25°C were evaluated. The selection of temperature intervals was facilitated by the GIS ecological profiles generated for each taxon (Supplementary Material S1). In each temperature, four replications of 20 seeds were used. The seeds were placed in 9-cm sterile plastic Petri dishes and they germinated on two filter papers moistened with dH_2O. The Petri dishes were randomly arranged on the shelves of the growth chambers, with a 12-h light/12-h dark photoperiod and filter paper was kept moisten, as required along the whole experimental period. The germinated seeds were counted and removed every five days for a period of 45 days, except those of *M. spreitzenhoferi* for which the germination test lasted 65 days in total. A seed was considered as germinated upon evident radicle protrusion from the seed coat.

Statistical analysis

For each taxon, a completely randomised design was used. To evaluate the effect of germination temperature on final germination percentage of each taxon, the data were subjected to analysis of variance (one-way ANOVA), followed by Duncan's test for the comparisons of the means at significance level of $p \leq 0.05$ [30]. Prior to the ANOVA, the germination percentage data was transformed to arc-sine square root values [31]. All statistical analyses were carried out using SPSS 21.0 (SPSS, Inc., Chicago, IL, USA).

Results

Potential in three sectors of economy

In the agro-alimentary sector, the two taxa of the genus *Allium* (*A. bourgeaui* subsp. *creticum* and *A. dilatatum*) achieved 54.76% of the optimum possible overall score due to their aromatic properties and type of aroma, their food additive potential, bee attraction potential and the fact that occasionally are collected from the wild as edible greens; all other taxa scored very low (> 35%) (Table 2).

In the medicinal-cosmetic sector, all evaluated taxa scored very low with *M. spreitzenhoferi* scoring slightly higher due to identified ethnobotanical uses, medicinal potential as well as absence of poisonousness or toxicity (37.04%) (Table 3).

In the ornamental-horticultural sector, *M. spreitzenhoferi* received 60.83% of the maximum possible score (Table 4) achieving maximum individual values in seven variables (frost hardiness, shade preference, altitudinal and environmental tolerance, attraction for botanical holidays, seasonal phenotypic changes and extant e-trade) and high values in another three variables (salt tolerance, blooming and wild collections interest). *Allium bourgeaui* subsp. *creticum*, *A. dilatatum* and *S. antri-jovis* showed limited or below average interest in this sector (39.17 - 45%) while the rest of the taxa had very low (< 30%) scores (Table 4).

Table 1. Documentation concerning the seeds collected from wild-growing populations of six local endemic plants in Crete (Greece) including dates defining their storage period prior to experimentation (months in parenthesis). Information for different IPEN (International Plant Exchange Network) accession numbers are separated with stroke (/). The accession numbers marked with asterisk (*) were used in seed germination trials

Cretan local endemic taxa	**IPEN accession number (*used in seed germination trials)**	**Collectors' taxonomic verification**	**Coordinates (Greek geothetic system)**	**Altitude (m)**	**Date of collection (seed storage till germination trials)**	**Cretan prefecture, area, locality**
Allium bourgeaui subsp. *creticum*	GR-0-BBGK-19,81 / *GR-1-BBGK-19,1062	Krigas N.	35.020356, 26.051311/ 35.020356, 26.051311	6 / 6	29-8-2018 (40) / 6-8-2019 (29)	Lasithi, Moni Kapsa, Pervolakia gorge / Lasithi, Moni Kapsa, Pervolakia gorge
Allium dilatatum	*GR-1-BBGK-19,1133	Krigas N., Anestis I., Avramakis M.	35.298657, 23.960998	350	21-8-2019 (28)	Chania, Samaria gorge, close to Samaria village
Muscari spreitzenhoferi	*GR-0-B-2045597-19,247 / GR-0-B-2045597-19,248 / GR-0-B-2790684-19,249	Berlin-Dahlem Botanical Garden (Böhling N.)	Not provided	1250 / 1490 / 450	2019 (30) / 2019 (30) / 2019 (30)	Rethimno, Mt Kedros / Rethimno, Mt Kedros / Chania
Alyssum baldaccii	*GR-1-BBGK-19,133	Krigas N., Anestis I., Avramakis M.	35.301369, 24.910353	580	29-8-2020 (16)	Rethimno, Mt. Psiloritis, Gonies to Sisarcha
Campanula saxatilis subsp. *saxatilis*	GR-1-BBGK-19,1071 / *GR-1-BBGK-19,1110 / GR-1-BBGK-20,409	Krigas N., Anestis I., Avramakis M.	34.925490, 24.777870 / 34.925490, 24.777870 / 35.180015, 24.400951	6 / 5 / 1	9-8-2019 (29) / 21-8-2019 (29) / 23-8-2019 (29)	Herakleion, Ayiofaraggo / Herakleion, Ayiofaraggo / Rethimno, Plakias
Silene antri-jovis	*GR-1-BBGK-19,1169	Krigas N., Anestis I., Avramakis M.	35.208321, 24.828868	1486	26-8-2019 (28)	Rethimno, Mt Psiloritis, Idaion Andro

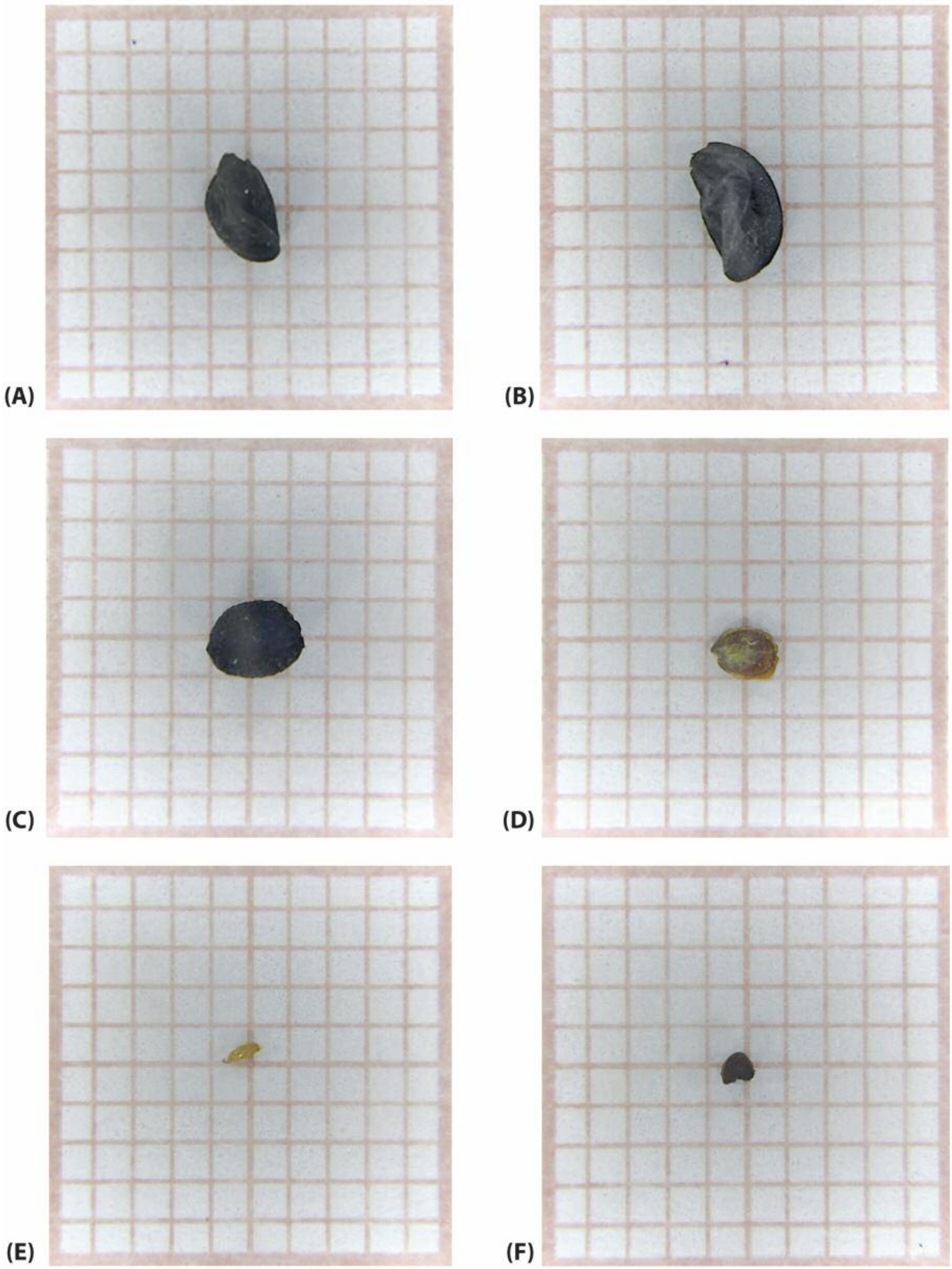

Figure 3. Seed morphology of the six threatened local endemic plants of Crete including three monocots: (A) *Allium bourgeaui* subsp. *creticum* GR-1-BBGK-19,1062; (B) *Allium dilatatum* GR-1-BBGK-19,1133; (C) *Muscari spreitzenhoferi* GR-0-B-2045597-19,247; and three dicots: (D) *Alyssum baldaccii* GR-1-BBGK-19,133; (E) *Campanula saxatilis* subsp. *saxatilis* GR-1-BBGK-19,1110; (F) *Silene antri-jovis* GR-1-BBGK-19,1169.

Table 2. Scoring regarding the agro-alimentary potential of the studied local endemic plants of Crete with percentage (%) of the maximum possible scores achieved

Variables	*Allium bourgeaui* subsp. *creticum*	*Allium dilatatum*	*Muscari spreitzenhoferi*	*Alyssum baldaccii*	*Campanula saxatilis* subsp. *saxatilis*	*Silene antri-jovis*
Aromatic properties	6	6	0	0	0	0
Beverage potential	0	0	0	0	0	0
Food additive potential	4	4	4	0	4	0
Wild edible greens	4	4	4	0	0	0
Bee attraction	6	6	6	6	6	6
Spicy element	0	0	0	0	0	0
Type of aroma	3	3	0	0	0	0
Percentage (%) of the maximum possible score	54.76	54.76	33.33	14.29	23.81	14.29

Nonetheless, examining how feasible the sustainable exploitation was for the studied local endemic taxa of Crete after SWOT analysis (Level II evaluation, Table 5), *C. saxatilis* subsp. *saxatilis* reached an above average to high score (59.72% of the maximum possible) while all other taxa had below average to low (37.50 - 45.38%) or very low scores (27.78%). With respect to the assessment of readiness timescale, sustainable exploitation for *C. saxatilis* subsp. *saxatilis* can be achieved in the short-term while and in the long-term for the rest of the taxa; however, this timescale still remains indeterminable for *A. dilatatum* (Table 5).

Table 3. Scoring regarding the medicinal-cosmetic potential of the studied local endemic plants of Crete with percentage (%) of the maximum possible scores achieved

Variables	*Allium bourgeaui* subsp. *creticum*	*Allium dilatatum*	*Muscari spreitzenhoferi*	*Alyssum baldaccii*	*Campanula saxatilis* subsp. *saxatilis*	*Silene antri-jovis*
Super food potential	0	0	0	0	0	0
Identified ethnobotanical uses	6	5	6	0	5	5
Absence of poisonousness-toxicity	6	6	6	4	6	6
Identified phytochemical compounds	0	0	0	0	0	0
Monograph of European Medicines Agency (EMA)	0	0	0	0	0	0
Number of approved EMA indications	0	0	0	0	0	0
Medicinal potential	0	0	6	0	0	0
Number of ethnobotanical uses	1	0	1	0	0	0
Number of medicinal properties	0	0	0	0	0	0
Percentage (%) of the maximum possible score	22.22	20.37	37.04	7.41	20.37	20.37

Table 4. Scoring regarding the ornamental-horticultural potential of the studied local endemic plants of Crete with percentage (%) of the maximum possible scores achieved

Variables	*Allium bourgeaui* subsp. *creticum*	*Allium dilatatum*	*Muscari spreitzenhoferi*	*Alyssum baldaccii*	*Campanula saxatilis* subsp. *saxatilis*	*Silene antri-jovis*
Frost hardiness	3	3	6	3	1	6
Shade preference	6	6	6	6	6	6
Wild collections	0	0	4	5	5	1
Altitudinal range	1	3	6	3	1	6
Blooming period	5	4	5	3	3	4
Compactness of form	0	0	0	0	0	3
Environmental tolerance	3	5	6	5	4	4
Height	6	5	2	3	2	1
Possibility for breeding	6	6	0	0	0	6
Attraction for botanical holidays	0	0	6	0	0	0
Eligibility for cut flower	6	4	1	0	1	0
Salt tolerance estimation	6	1	5	1	1	1
Impressive flowers	3	3	3	0	3	0
Leaf colour	3	3	3	4	4	3
Plant symmetry	0	0	2	0	0	2
Seasonal phenotypic changes	6	6	6	0	0	0
Attractiveness of leaf shape	0	0	3	3	3	3
Eligibility as foliage plant	0	0	0	1	1	1
Extant e-trade	0	0	6	0	0	0
Shining of leaf texture	0	0	3	0	0	0
Percentage (%) of the maximum possible score	45.00	40.83	60.83	30.83	29.17	39.17

Table 5. Hierarchically arranged percentages (%) of the maximum possible scores achieved regarding the feasibility (Level II evaluation) and readiness timescale (Level III evaluation) for sustainable exploitation of the studied local endemic plants of Crete

	Sustainable exploitation	
Local endemic taxa of Crete	**Feasibility (Level II)**	**Readiness timescale (Level III)**
Campanula saxatilis subsp. *saxatilis*	59.72	Short-term
Alyssum baldaccii	45.83	Long-term
Silene antri-jovis	40.28	Long-term
Allium bourgeaui subsp. *creticum*	37.50	Long-term
Muscari spreitzenhoferi	37.50	Long-term
Allium dilatatum	27.78	Indeterminable

GIS Ecological profiling of focal plants

Six GIS ecological profiles were first-time developed on the basis of the natural occurrences of each taxon in Crete, thus depicting detailed information on taxon-specific climatic conditions in terms of temperature and precipitation (Supplementary Material, Figures S1-S6).

Temperature-related attributes

Based on the produced GIS ecological profiles, the highest mean values of the average temperatures are peaked in mid- and late-summer for all studied taxa (i.e., July and August), although there were differences between them (Supplementary Material, Figures S1-S6). For example, the values of *A. bourgeaui* subsp. *creticum*, *A. dilatatum*, *M. speitzenhoferi* and *A. baldaccii* ranged from 23.23 ± 1.55°C to 24.20 ± 1.04°C (July) while those of *C. saxatilis* subsp. *saxatilis* were relatively higher (25.01 ± 0.99°C) and those of *S. antri-jovis* were relatively lower (21.18 ± 1.66°C). After the peak of temperatures, a gradual decrease in mean temperatures was evidenced from September (20.5 ± 1.60 - 21.58 ± 1.08°C for the first group of taxa; 22.37 ± 1.03°C for *C. saxatilis* subsp. *saxatilis;* 18.40 ± 1.67°C for *S. antri-jovis*) to December (9.8 ± 1.79 to 10.99 ± 1.22°C for the first group; 11.76 ± 1.13°C for *C. saxatilis* subsp. *saxatilis;* 7.30 ± 1.87°C for *S. antri-jovis*), showing similar temperature differences between taxa. With the exception of February's lowest mean temperatures shown for *A. bourgeaui* subsp. *creticum* (9.41 ± 1.24°C), the rest of the taxa were adapted to lowest mean temperatures in January, i.e., 8.26 ± 1.80 to 9.21 ± 1.15°C for *A. dilatatum*, *M. spreitzenhoferi* and *A. baldaccii*; 10.23 ± 1.10°C for *C. saxatilis* subsp. *saxatilis*; 5.65 ± 1.89°C for *S. antri-jovis*. From the end of February, monthly mean temperatures were shown to start rising gradually from March (9.79 ± 1.78 to 10.85 ± 1.19°C for *A. bourgeaui* subsp. *creticum*, *A. dilatatum*, *M. speitzenhoferi* and *A. baldaccii*; 11.66 ± 1.13°C for *C. saxatilis* subsp. *saxatilis*; 7.32 ± 1.86°C for *S. antri-jovis*) to May (16.98 ± 1.06 to 17.76 ± 1.06°C for *A. bourgeaui* subsp. *creticum*, *A. dilatatum*, *M. speitzenhoferi* and *A. baldaccii*; 18.73 ± 1.03°C for *C. saxatilis* subsp. *saxatilis*; 14.64 ± 1.66°C for *S. antri-jovis*), reaching in early summer (June) 21.33 ± 1.56 to 22.05 ± 1.03°C for *A. bourgeaui* subsp. *creticum*, *A. dilatatum*, *M. speitzenhoferi* and *A. baldaccii*; 23.10 ± 1.00°C for *C. saxatilis* subsp. *saxatilis* and 19.03 ± 1.64°C for *S. antri-jovis*.

Regarding temperature extremes, the data from the sites where the six plants are wild-growing have shown that they do not naturally thrive in extremely low (below 0°C) or very high (above 30°C) temperatures (T_{mean} of T_{min} = 2.89 ± 1.80°C was the lowest value for *S. antri-jovis* and T_{mean} of T_{max} = 29.15 ± 0.97°C was the highest value for *C. saxatilis* subsp. *saxatilis*).

Precipitation-related attributes

The historical precipitation data showed that the six threatened and local endemic plants originated from wild habitats characterized by seasonal fluctuations of precipitation (Supplementary Material S1, Figures S1-S6). However, the amount of precipitation varied between taxa. In particular, the highest precipitation values were evidenced during mid-winter in January (136.89 ± 18.03 to 158.80 ± 20.93 mm for *A. bourgeaui* subsp. *creticum*, *A. dilatatum*, *M. speitzenhoferi* and *C. saxatilis* subsp. *saxatilis*, 182.33 ± 26.78 to 190.12 ± 12.23 mm for *A. baldaccii* and *S. antri-jovis*) and then shown to decrease gradually during early spring in March (84.30 ± 10.84 to 97.01 ± 13.01 mm for *A. bourgeaui* subsp. *creticum*, *A. dilatatum*, *M. speitzenhoferi* and *C. saxatilis* subsp. *saxatilis*, 108.67 ± 13.59 to 114.16 ± 8.41 mm for *A. baldaccii* and *S. antri-jovis*) and more intensively till the end of spring in May (1.90 ± 0.62 to 3.00 ± 1.28 mm for *A. bourgeaui* subsp. *creticum*, *A. dilatatum*, *M. speitzenhoferi* and *C. saxatilis* subsp. *saxatilis*, 23.67 ± 3.82 to 3.98 ± 1.48 mm for *A. baldaccii* and *S. antri-jovis*). Summer (especially June) was shown as the driest period with August being the driest month (highest amount of rainfall was 3.98 ± 1.47 mm for *S. antri-jovis*). After the driest period, rainfall was shown to increase until November, introducing the raining season.

Germination tests

A considerable difference was observed in the germination behavior between the studied monocot and dicot taxa. More precisely, in all three monocot taxa, the highest germination was observed in seeds incubated at 10°C (70.0, 40.0 and 71.25% for *A. bourgeaui* subsp. *creticum*, *A. dilatatum* and *M. spreitzenhoferi* seeds, respectively) (Figure 4). The seeds of *A. bourgeaui* subsp. *creticum* and *A. dilatatum* germinated after the 10th and the 15th day and their germination at 10°C was completed by the 30th day from the onset of the germination test. In *M. spreitzenhoferi*, germinated seeds were recorded at the 30th day and their germination at 10°C was completed after 55 days from the beginning of the germination test.

In contrast, the seeds of the three dicot taxa germinated over a wide temperature range (Figure 5). Specifically, in *A. baldaccii*, seeds incubated at 10, 15 and 20°C exhibited the highest germination percentage (86.25, 85.00 and 83.75%, respectively) while the increase of temperature resulted in a significant reduction of germinated

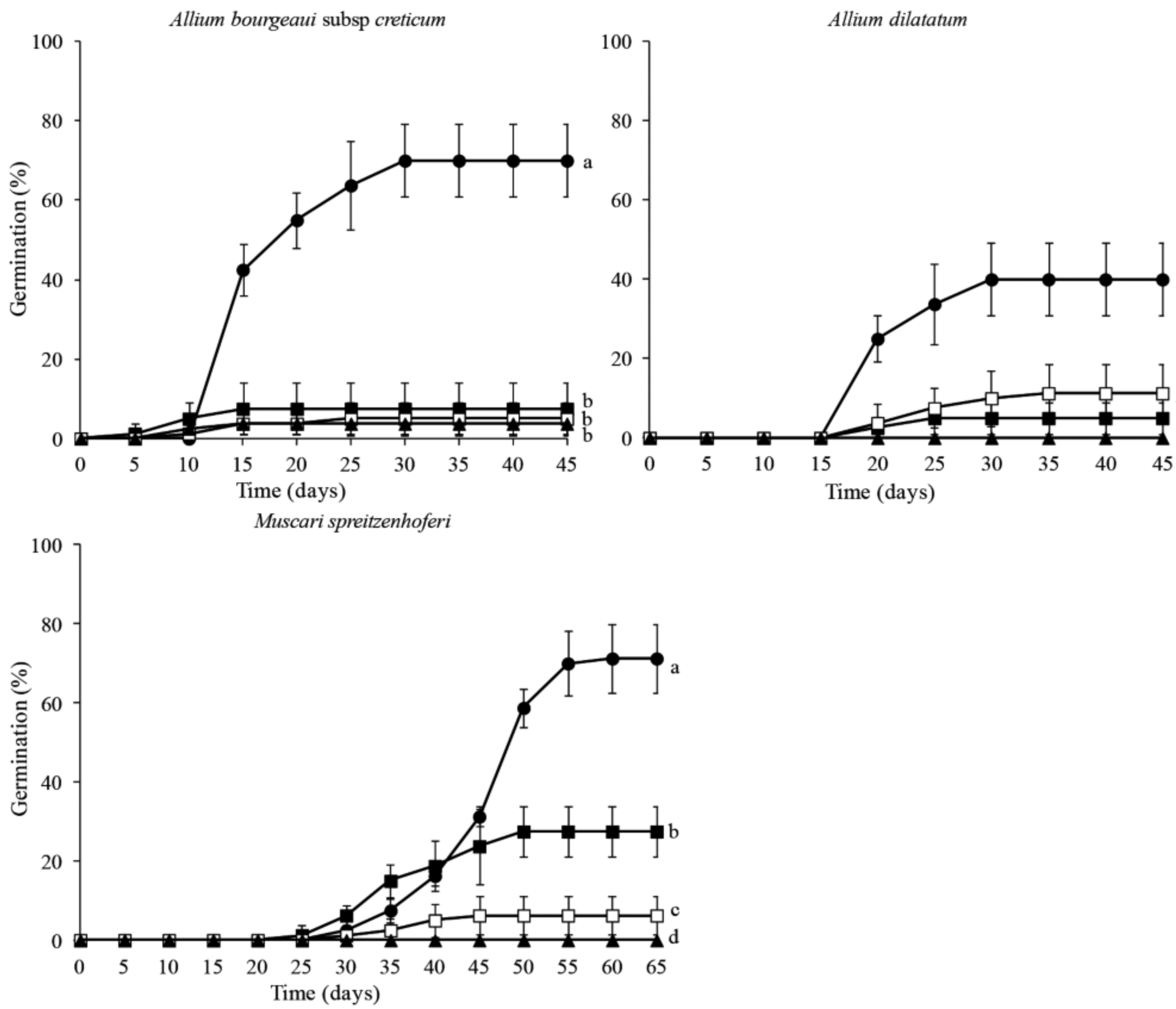

Figure 4. Cumulative seed germination percentage diagrams of the three monocot local endemic taxa of Crete, Greece (*Allium bourgeaui* subsp. *creticum*, *Allium dilatatum*, and *Muscari spreitzenhoferi*) incubated at 10 (●), 15 (■), 20 (□), and 25°C (▲). For each taxon, means were statistically different at $p \leq 0.05$, when not sharing a common letter. The comparisons were made using the Duncan's test.

seeds (58.75% at 25°C). Although, in the first five days the germination rate of *A. baldaccii* seeds was faster at 15 and 20°C compared to 10°C, in all three temperatures the germination was completed in one month from the beginning of the germination test. *Campanula saxatilis* subsp. *saxatilis* seeds incubated at 10°C exhibited the highest germination percentage (85.00%). The first germinated seeds in this temperature were recorded at the 20th day from the beginning of the test and the germination was completed at the 40th day.

In *C. saxatilis* subsp. *saxatilis* seed incubated at 15°C, the germination was reduced to 71.25% and was completed five days earlier compared to seeds incubated at 10°C. The increase of incubation temperature at 20 and 25°C resulted in a significant decrease of the percentage of germinated seeds (17.00 and 8.75%, respectively).

The seeds of *S. antri-jovis* exhibited high germination percentages (90.00 - 98.75%) at all temperatures tested. In seeds incubated at 10, 15 and 20°C, germination started after the 10th day and was completed at the 35th day from the beginning of the test whereas the germination at 25°C started five days earlier and was completed five days later compared to other temperatures.

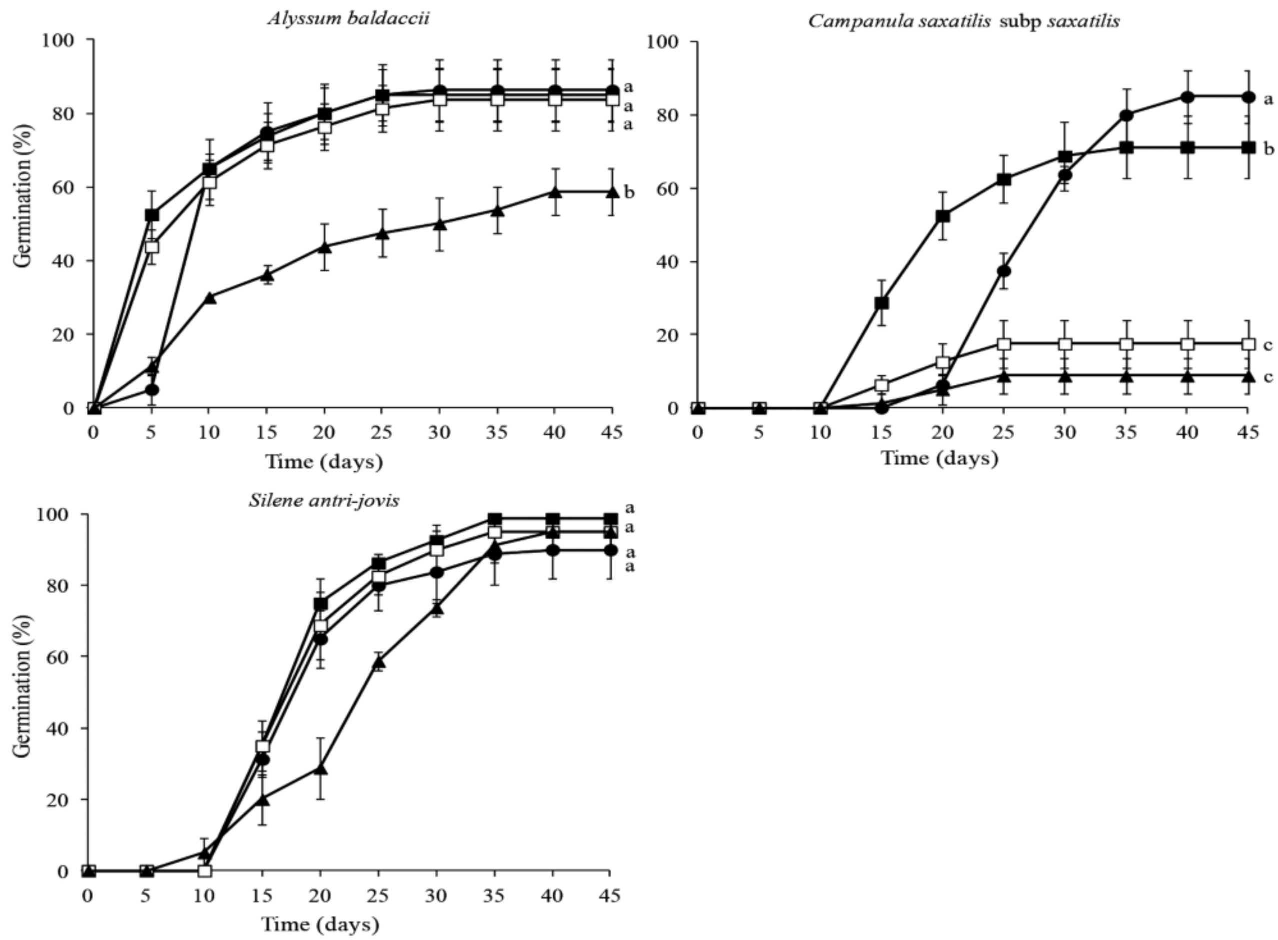

Figure 5. Cumulative seed germination percentage diagrams of the three dicot local endemic taxa of Crete, Greece (*Alyssum baldaccii*, *Campanula saxatilis* subsp. *saxatilis*, and *Silene antri-jovis*) incubated at 10 (●), 15 (■), 20 (□), and 25°C (▲). For each species, means were statistically different at $p \leq 0.05$, when not sharing a common letter. The comparisons were made using the Duncan's test.

Discussion

Seed germination

After 28 - 30 months of seed storage of the studied taxa (16 months for *Alyssum baldaccii*, Table 1), the tested seeds of all taxa exhibited high germination percentages without any pretreatment. However, the results of the present study clearly showed that temperature affects the germination of seeds in five out of six examined taxa. Since, freshly seeds of the studied taxa were not subjected to any germination test, it still remains unknown if their seeds have some type of dormancy. According to Baskin and Baskin [32], seeds of the most herbaceous matorral species have physiological dormancy and in many species with non-deep physiological dormancy, the seeds may easily come out of dormancy when they are placed dry at temperatures of 20°C or higher.

Seed germination of monocot local Cretan endemics

In the present study, the seeds of the three monocot taxa showed similar germination behavior. The seeds of all three monocot local Cretan endemics exhibited exclusively the highest germination percentage at low temperature (10°C), and further increase of temperature resulted in a sharply reduction of germinated seeds. For example, in *A. dilatatum* and *M. spreitzenhoferi*, no seed germinated at 25°C. The optimal seed germination of *Allium staticiforme* Sm., a Greek endemic littoral geophyte, was recorded at a temperature range of 5 - 20°C [33]. Furthermore, in two *Allium* species namely *A. truncatum* (Feinbrun) Kollmann & D. Zohary and *A. rothii* Zucc. of the Negev Desert highlands of Israel, seed germination takes place at 5 - 15°C [34]. Similarly, seed germination of four widespread *Muscari* species namely *M. comosum* (L.) Mill., *M. neglectum*

Guss. ex Ten., *M. commutatum* Guss. and *M. weissii* Freyn occurs at a narrow range of low temperatures (10 - 15°C) and is characterized by a slow germination rate [23]. Compared to the germination rate of the herein studied *Allium* taxa, the seeds of *M. spreitzenhoferi* needed a longer time to germinate; therefore, this may indicate a preference for a habitat which should be moist for a longer period of time. The results of the present study indicate that the germination mechanism of the three monocot taxa represents an adaption to the climatic conditions of their natural habitats. Given that these taxa are wild-growing in similar rocky habitats (limestone calcareous cliffs and rocky places), analogous germination behavior should be expected [26]. According to the GIS ecological profiles of *A. bourgeaui* subsp. *creticum*, *A. dilatatum*, and *M. spreitzenhoferi* (see Supplementary material Figures S1, S2 and S3, respectively), the seeds of these monocots in their natural habitats are probably wet enough during the autumn months (October, November) when rather mild temperatures prevail, thus they may easily germinate during early winter (December) when the mean monthly air temperature is around 10°C. However, a low percentage of them may even germinate in October and November, when the mean monthly air temperature is below 20°C and above 10°C. The relatively mild winter temperatures may probably allow the growth of emerging seedlings, and their growth should be expected to intensify during spring due to favorable temperature and soil moisture, thereby allowing the completion of their life cycle in summer.

Seed germination of dicot local Cretan endemics

A different germination behavior was detected for the seeds of the three dicot taxa studied herein. The maximum germination of *A. baldaccii* seeds was obtained in a temperature range of 10 - 20°C. The seeds of this species germinated very fast, particularly at 15 and 20°C. The GIS ecological profile of *A. baldaccii* (Supplementary material, Figure S4) has shown that such temperatures may prevail in October in its natural habitats. In parallel, during this month, the mean amount of precipitation is 103.50 mm. Therefore, the seeds of this species may take advantage of suitable conditions for very fast germination. Possibly, this trait may enable its seedlings to grow sufficiently during the autumn months, thus making them also capable to withstand the relatively low winter temperatures. In turn, this trait probably makes *A. baldaccii* – a relatively hygrophilous species [27] – able to survive also in semidry conditions, under which seed germination has to be completed before the soil becomes too dry (below critical germination levels). According to Baskin and Baskin [20], freshly collected seeds of the widespread *Alyssum alyssoides* (L.) L. are conditionally dormant and they may germinate only at low temperatures (5 - 15°C); however after a period of dry storage for one month at 30/15°C, its seeds fully after-ripen and may germinate over a wide range of constant temperatures (5 - 25°C) or alternating temperatures (15/6 - 35/20°C) [35].

In *C. saxatilis* subsp. *saxatilis*, seed germination was promoted at the low temperature of 10°C. Although, the germination at 15°C was slightly reduced, the rate was faster than that observed at 10°C. Furthermore, the optimal temperatures for germination in most studied taxa of the genus *Campanula* are 10 and 15°C [36, 37]. The GIS ecological profile of *C. saxatilis* subsp. *saxatilis* (Supplementary Material, Figure S5) unveiled that in its natural habitat such favorable temperatures for seed germination prevail in late autumn and winter, a period during which high soil moisture due to increased amount of precipitations probably creates the optimum conditions for seed germination. Later on, the relatively mild winter temperatures may probably allow the growth of emerging *C. saxatilis* subsp. *saxatilis* seedlings.

On the other hand, the seeds of *S. antri-jovis* germinated at high percentage at all constant temperatures (10, 15, 20 and 25°C). However, the seeds germinated more rapidly at 10, 15 and 20°C compared to those incubated at 25°C. The germination behaviour which was observed in *S. antri-jovis* in the present study indicates that although seed germination in its natural habitats may be possible in summer after dispersion, this is unlikely to occur due to absence of rainfall. It has been shown that seeds of various *Silene* populations occurring in arid regions with hot and dry summers may germinate optimally at low temperatures associated with the rainfall period [38]. Furthermore, fresh seeds of *S. hicesiae* Brullo & Signor., a narrow endemic species of Southern Italy, may only germinate over a narrow range of low temperatures (5 - 15°C) and the dry storage of seeds at room temperature can enlarge this range up to 20 and 25°C [39]. This germination behavior indicates that fresh seeds of *S. hicesiae* exhibit non-deep physiological dormancy [39]. Similarly, seeds of three Mediterranean coastal species in *S. mollissima* (L.) Pers. aggregate – namely *S. velutina* Pourr. ex Loisel, *S. badaroi* Breistr., and *S. hicesiae* Brullo & Signor. – may germinate at high percentage at low temperatures (5 - 15°C), whereas their germination is significantly reduced at higher temperature (25°C) [40]. The seeds of these species mature and disperse during summer. In case that the seeds of the herein studied species are characterized by physiological (non-deep) dormancy, their exposure to high temperature and dry conditions of summer (optimal conditions for the after-ripening of seeds) should probably result in dormancy breaking, and therefore to widening of temperature range for seed germination. This remains to be studied in future investigations. According to the GIS ecological profile of *S. antri-jovis* species (Supplementary Material, Figure S6), it was shown that in early to mid-autumn (September to October), the mean amount of precipitation in its natural habitat starts to increase from 22.99 to 98.20 mm, respectively. Therefore, favorable conditions are probably created for the germination of *S. antri-jovis* seeds. According to ecological profiles of the three dicots studied herein, the winter temperatures in the natural habitat of *S. antri-jovis* are lower compared to those prevailing in the natural habitats of the other two dicots. Possibly, the earlier seed germination in autumn of *S. antri-jovis* allows seedlings to growth enough to withstand the low winter temperatures.

The aforementioned dicot and monocot local endemic taxa of Crete have probably developed a germination mechanism which ensures that in the harsh Mediterranean conditions their seeds will germinate at the right time when the established seedlings will have more possibilities to survive. Due to this mechanism, seed germination is avoided after sporadic rain events during summer or in early autumn and seed germination is probably delayed until mid-autumn or early winter when the available soil moisture after increased rainfall is sufficient to ensure seedling emergence, growth and survival. The efficient seed germination protocols developed herein may facilitate targeted conservation actions in the future for these threatened local endemic plants of Crete aiming at populations' re-enforcement or restoration.

Multifaceted evaluation and sustainable exploitation possibilities

The possibility of original genotypic selection and targeted pilot cultivation of selected neglected and underutilized plants (NUPs) along with new processing techniques for specific applications may provide an unprecedented potential for them to be used as a valuable novel phytogenetic resources, especially in the Mediterranean regions [41], and in particular when being local endemics to specific areas [8].

With regard to the agro-alimentary sector, the herein studied taxa of the genus *Allium* (*A. bourgeaui* subsp. *creticum*, *A. dilatatum*) have been ranked in the top-10 neglected and underutilized plants among 223 Cretan local endemics or in the top-25 ones among 399 local endemics of selected Mediterranean regions of Greece, Morocco and Tunisia [17]. This aspect shows evidence of an interesting agro-alimentary potential for *A. bourgeaui* subsp. *creticum* and *A. dilatatum* mainly as wild edible greens.

In the medicinal-cosmetic sector, all the herein evaluated taxa have been ranked relatively low compared to other Cretan endemics such as dittany of Crete (*Origanum dictamnus* L.) and Cretan mountain tea locally called 'malotira' (*Sideritis syriaca* L. subsp. *syriaca*) which are both recognized medicinal perennial herbs of high value associated with finalized monographs by the European Medicines Agency and approved indications as traditional herbal medicinal products [16]. However, despite the limited research and medicinal knowledge about the local endemic NUPs of specific regions, *M. spreitzenhoferi* has been ranked in the top-16 NUPs among 223 Cretan local endemics or in the top-22 ones among 399 local endemics of selected Mediterranean regions of Greece, Morocco and Tunisia [16].

In the ornamental-horticultural sector, *M. spreitzenhoferi* scoring 60.83% has been ranked fourth among 223 Cretan local endemics or 399 local endemics of selected Mediterranean regions of Greece, Morocco and Tunisia [8], thus outlining its remarkable ornamental-horticultural potential.

The feasibility assessment for the sustainable exploitation of *C. saxatilis* subsp. *saxatilis* (59.72% of the maximum possible) after SWOT and gap analyses (Level II evaluation) has ranked this taxon in the top-10 cases among both 223 Cretan endemics or 399 local endemics of selected Mediterranean regions of Greece, Morocco and Tunisia [8] showing that its sustainable exploitation can be achieved in the medium-term (Table 5).

The knowledge generated and the sexual propagation protocols developed in the present investigation for the six threatened local endemic plants of Crete may actually contribute to bridging extant gaps of applied research associated with their sustainable exploitation and may also permit their re-evaluation in terms of feasibility and readiness timescale for future sustainable exploitation and value chain creation. When re-evaluation is attempted for the attributes previously scored [8], the feasibility for value chain creation and sustainable exploitation concerning the six Cretan taxa is considerably improved as follows:

Regarding the studied monocots, the upgrading for *A. bourgeaui* subsp. *creticum* (from 37.5% to 44.44%), *A. dilatatum* (from 27.78% to 33.33%) and *M. spreitzenhoferi* (from 37.5% to 44.44%) had no noteworthy impact on the readiness timescale category designated for these taxa which remained unchanged (35 - 50%, 'long-term'; > 35%, 'indeterminable'; 35 - 50%, 'long-term', respectively). The difference in the Level II re-evaluation in all cases has been generated due to the higher seed germination success presented herein for the studied taxa providing score 5 or score 3 (61 - 75%) compared to no data (0 score).

Regarding dicots, no update was generated for *C. saxatilis* subsp. *saxatilis* and *S. antri-jovis* as these two taxa had already high scores in terms of seed germination success (score 6) while that for *A. baldaccii* was from 45.83% to 54.17%, thus upgrading taxon's readiness timescale category (50 - 55%, 'medium-term'). The difference in the Level II re-evaluation has been generated due to the higher seed germination success presented herein providing score 6 (> 75%) compared to no data (0 score).

However, to sustainably promote new NUPs such as those outlined in this study, further important knowledge gaps need to be bridged in the first place [42] as well as enhanced marketing strategies should be in place and aligned with increased consumer awareness regarding the benefits associated with selected NUPs [43].

Conclusion

This study investigated the climatic conditions in which thrive naturally the wild-growing populations of six threatened local endemic plants of Crete and revealed their seed germination requirements (effect of temperature). In this way, this study delivered six efficient propagation protocols for targeted conservation actions such as populations' re-enforcement or restoration.

The findings of this study also fill in extant research gaps associated with these neglected and underutilized local endemic plants of Crete and may contribute meaningfully to *in-situ* and *ex-situ* conservation strategies, while facilitating sustainable exploitation schemes at the same time.

The current investigation explored comprehensively the extant potential of six threatened local endemic plants of Crete in different economic sectors (ornamental-horticultural, medicinal-cosmetic, and agro-alimentary). Herein promising cases emerged such as *M. spreitzenhoferi* and *C. saxatilis* subsp. *saxatilis* with strong ornamental-horticultural potential as well as *A. bourgeaui* subsp. *creticum* and *A. dilatatum* with interesting agro-alimentary potential. Basic knowledge gaps should be further filled in for these local endemic plants of Crete including targeted scientific research on agronomical aspects, assessment of breeding potential, efficient cultivation techniques for product development and post-harvest handling, allowing the establishment of future value chains for these valuable local phytogenetic resources of Crete.

Authors' Contributions

Conceptualization, SH, NK and GT; data curation, NK, IA, GT, SK, MA, ED, and EP; formal analysis, EP, NK, IA, SK, and SH; investigation, NK, SH, IA, EP, SK, VG, NK, and GT; methodology, NK, SH, EP, SK, IA, and GT; project administration, NK, GT, and SH; resources, NK, SH, and SK; software, IA, and EP; supervision, NK, SH, and GT; validation, IA, EP, GT, MA, ED, VG, and SK; visualization, NK, IA, MA, ED, VG, and EP; writing—original draft, EP, IA, VG, and NK; writing—review and editing, all authors. All authors have read and agreed to the final version of the manuscript.

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Availability of data and materials

The data presented in this study are available on request from the corresponding author.

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Conflicts of Interest

The authors declare no conflict of interest.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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