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Effect of temperature and light on seed germination of *Erysimum naxense* and *Erysimum krendlii*

Research Article

Paraskevi I. Gkika[1], Nikos Krigas[2,*] George Menexes[3], Ilias G. Eleftherohorinos[3], Eleni Maloupa[4]

[1]*Forest Department of Arnea, 63074 Arnea, Chalkidiki, Greece*

[2]*Department of Botany, School of Biology, Aristotle University of Thessaloniki, 541 24 Thessaloniki, Greece*

[3]*Laboratory of Agronomy, School of Agriculture, Aristotle University of Thessaloniki, 541 24 Thessaloniki, Greece*

[4]*Laboratory of Conservation and Evaluation of Native and Floricultural Species-Balkan Botanic Garden of Kroussia, Hellenic Agricultural Organization "Dimitra", 57001 Thermi, Thessaloniki, Greece*

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Abstract: Seed germination of two local Greek endemics was studied (*Erysimum naxense, Erysimum krendlii*). Seed viability was determined by using the tetrazolium method and germination was studied in synchronized cycles of five and four alternating temperatures [10/5 (for *E. naxense* only) and 15/10, 20/15, 25/20, and 30/25°C for both species, in cycles of 16 h day/8 h night], and in five light regimes (red, blue, green, white, and dark). Germination of *E. naxense* and *E. krendlii* seeds was determined daily for six and five weeks, respectively, with the data analyzed as viability adjusted accumulative seed germination at the end of each week. *E. naxense*'s seed viability was higher (90%) than that of *E. krendlii* (64%); seed germination (%) of both increased at low alternating temperatures (10/5°C, 15/10°C, 20/15°C). Germination of *E. naxense* seeds at low temperatures was light-independent, whereas at high temperatures it was increased with red light. Germination of *E. krendlii* seeds was inconsistently affected by light at the temperatures studied. Percentages of seed germination of both species were higher in experimental conditions similar to the ones of their natural habitats during autumn and/or spring (facilitated with Geographic Information Systems). These conclusions provide guidelines for species-specific propagation protocols and *ex situ* conservation.

Keywords: *Propagation • Ex situ • Brassicaceae • Greece • Endemic • Geographic Information Systems • GIS*

1. Introduction

Under the Convention of Biological Diversity, the updated Global Strategy for Plant Conservation 2011-2020 (http://www.cbd.int/gspc/) prioritizes scientific research on rare and threatened species. More specifically, target 8 suggests the development of species-specific propagation and cultivation protocols aiming to achieve "at least 75% of the threatened plants under *ex situ* conservation, preferably in the country of origin, and at least 20% available for recovery and restoration programs" (https://www.cbd.int/gspc/). In order to approach this global target in the European context, it has been estimated that 60% of threatened species should be stored in seed banks and research should be initiated into storage and propagation methods [1].

* *E-mail: nkrigas@bio.auth.gr*

If such a demanding target is to be achieved, basic research is required regarding the biology of every species threatened with extinction.

Investigation and understanding of the biological cycle of conservation priority species has been considered the key factor for successful propagation efforts [2,3]. For seed conservation of these species, studies regarding their germination ability and dormancy status are essential for seed bank storage, reproduction, and future re-introduction of plants in the wild [4-6]; such research is also recommended by target 8.1 of the European Strategy for Plant Conservation [1]. In this framework, the conservation efforts of the Balkan Botanic Garden of Kroussia, N Greece, are focused on the rare, threatened, and endemic plants of the Greek and Balkan flora [7] and applied research is conducted concerning the propagation of European priority species [8-10].

Current scientific literature regarding the biology of many European threatened plant species is quite limited. The same applies for the species investigated in this study, viz. *Erysimum naxense* Snogerup and *Erysimum krendlii* Polatschek (Brassicaceae), two species of sectio *Cheiranthus* (L.) Wettst. and *Erysimum* L., respectively. A major search of bibliographic databases such as Scopus, Web of Science, and Google Scholar in May of 2013 did not reveal any new records; hence, our knowledge is quite limited [11,12]. Both of the studied species are local endemics of Greece that are restricted to a single island of the Aegean Archipelago [Naxos and Samothraki, respectively]. *E. naxense* is nationally [11] and globally [13] considered "Rare" and is protected by the Greek Presidential Decree 67/1981, while *E. krendlii* is nationally considered "Vulnerable" [12]. *E. naxense* is a perennial plant (rarely biennial) growing mainly as a rock-dweller at 100-500(-800) m above sea level [11]; *E. krendlii* is a biennial (or short-living perennial) plant growing in phrygana, roadsides, and rocky areas from 250-1,000(-1,500) m [12]. The germination of both species *in situ* probably occurs during autumn, although no specific data exist.

The objective of this study was to examine the seed viability along with the effect of temperature and light on seed germination of *Erysimum naxense* and *Erysimum krendlii,* so that the knowledge obtained might be used to develop propagation guidelines for the long-term *ex situ* conservation of these species.

2. Experimental Procedures

2.1 Seed collection

Two botanical expeditions were organized in 2005 and 2008 in order to collect plant material of *Erysimum naxense* and *Erysimum krendlii,* respectively, from the wild (Figure 1). This was done using a special permit issued by the Greek Ministry of Rural Development and Foods (renewed yearly). The plant material collected has been fully documented (Table 1) and obtained an accession number that follows the numbering of the International Plant Exchange Network (IPEN, http://www.bgci.org/resources/ipen/). Due to the limited population sizes of both species [11,12], seeds were collected from wild *E. krendlii* individuals and >1,000 seeds from wild *E. naxense* individuals. During seed collection in the wild, ripe siliquae were considered those with valves opened at least partially and ripe seeds those with brown colour. In order to produce enough material of *E. naxense* for experimentation, we raised a few plants *ex situ*, which flowered and produced ripe seed in 2008.

All seeds used in the experiments were stored at the seed bank of the Laboratory of Conservation and Evaluation of Native and Floricultural Species of the Hellenic Agricultural Organization "Dimitra" at controlled conditions of 4-5°C and <5% relative humidity. It is worth mentioning that, before their inclusion at the seed bank, the seeds had been stored for one month under controlled conditions (temperature 18-20°C coupled with dehydrator) to reduce relative moisture to a desirable level (20-25%) for storage.

2.2 Detection of temperatures and precipitation in natural habitats

In order to detect the temperature and available moisture outline of the species' natural habitats, the geographical coordinates of the original collection sites of *E. naxense* and *E. krendlii* (Table 1) were imported into the Geographical Information Systems (GIS) application developed by Krigas *et al*. [9,10]. The exact collection spots were then linked accordingly with the WorldClim database [14,15] and this link furnished quantitative data (mean values for the last 50 years) regarding the precipitation and temperature of the original collection spots (Table 1).

2.3 Seed weight and viability

The average weights of 100 *E. naxense* and *E. krendlii* seeds were determined by using three 100-seed samples randomly taken from each species. To determine the seed viability of *E. naxense* and *E. krendlii*, the 2,3,5-triphenyltetrazolium chloride (TTC, tetrazolium method) test was performed on a random sample of 100 seeds per species, using 0.5% and 1% solutions w/v (pH 6.8), respectively [16-18]. As a result of preliminary experimentation testing different concentrations (data not shown), different tetrazolium

Figure 1. Exemplified *ex situ* conservation of *Erysimum naxense* (left column pictures) and *Erysimum krendlii* (right column pictures), illustrating the connection between the wild material surveyed (up: flowering individuals in natural habitats), the individual seeds (mm, middle) used in experimental procedures and the seedlings finally produced for long-term cultivation (down: young plants grown in pots) in the Balkan Botanic Garden of Kroussia (Greece).

Taxon	Origin	Altitude (m, a.s.l.)	Geographical coordinates	IPEN accession number	Rainfall outline (mean values in mm)	Temperature outline (mean values in °C)
Erysimum naxense	Mt Koronos, Naxos Island	578	N37°07'52'' E25°31'50''	GR-BBGK-1-05.2957	Annual: 610, Min: 2 (July), Max: 120 (December)/ Summer: 1.70, Autumn: 30.83, Spring: 24.33, Winter: 62.63	Annual: 14.4, Min: 4.6 (February), Max: 25.7 (July)/ Summer: 21.6, Autumn: 16.3[1], Spring: 12.6[2], Winter: 5.4
Erysimum krendlii	Chora, Samothraki Island	250	N40°28'28'' E25°31'32''	GR-BBGK-1-06.3648	Annual: 621, Min: 12 (August), Max: 98 (December)/ Summer: 20.10, Autumn: 54.04, Spring: 40.02, Winter: 66.30	Annual: 13.6, Min: 1.8 (January), Max: 28.4 (July)/ Summer: 21.5, Autumn: 14.2[3], Spring: 11.1[4], Winter: 8.2

Table 1. Origin and documentation of wild *Erysimum naxense* and *Erysimum krendlii* seeds collected from Greece with an outline of prevailing rainfall and temperature conditions in their natural habitats. Annual and seasonal minimum and maximum data (values represent means of 50 years) were extracted from the WorldClim database [14,15], using the geographical coordinates of the species' original collection sites in Geographic Information Systems (GIS); for methodology see [9,10].

[1]Mean min: 9.7-16.0, Mean max: 15.1-22.9, [2]Mean min: 5.8-12.0, Mean max: 11.7-19.9, [3]Mean min: 6.9-14.6, Mean max: 13.6-24.3, [4]Mean min: 3.8-11.0, Mean max: 11.0-20.8

solutions were used for the two species. Two samples of seeds were taken for each species and one (negative control) was killed by boiling for 3 min [19]. After soaking seeds of *E. naxense* in deionised water for 24 hours, embryos were extracted under stereomicroscope. The embryos were placed on filtered paper with TTC solution for 8 hours at a temperature of 30°C in the dark. Seeds of *E. krendlii* were placed directly on filtered paper with TTC solution for 16 hours at a temperature of 30°C in the dark. Embryos that turned red were considered viable [17]. The TTC test was also used on seeds that did not germinate during our trials.

2.4 Seed germination experiments

All seed germination experiments were carried out in 2009, using 1,400 seeds of *E. naxense* and 1,120 seeds of *E. krendlii*. Seed germination was studied in synchronized cycles of five and four alternating temperatures for *E. naxense* and *E. krendlii,* respectively, and in five light regimes (red, blue, green, white, and dark).

Seeds were soaked in a 2% solution of sodium hypochloride for 10 min [19,20]. Germination tests were performed in plastic Petri dishes (8.5 cm in diameter) lined with two filter papers and moistened with 3 ml distilled water [21]. The criterion of germination was the visible radicle or cotyledon protrusion [22-26]; this was measured daily under stereoscope in semi-darkness for 6 weeks in *E. naxense* and 5 weeks in *E. krendlii*, respectively. For both species, the accumulative germination percentages at the end of each week were corrected for seed viability according to the following formula [27]:

$$\frac{\%\ \text{germination}}{\%\ \text{viability}} \times 100$$

Viability Adjusted Germination (VAG) percentages which exceeded the value of 100% were fixed to 100%. In addition, the obtained data at the end of each week were subjected to Probit regression analysis in order to estimate the GR_{50} (time-weeks required for 50% seed germination). Combined treatment comparisons (temperature x light regimes) within each species were also made only at the last week's accumulative germination percentages. In both experiments, there were seven replicates: Petri dishes with eight seeds per replicate for each combined treatment. The number of replicates used for both experiments was chosen after conducting preliminary experiments for both species at low temperatures (<20°/15°), which showed that the variability of germination percentages was extremely low when seven replicates were used. This was also confirmed by the final experiments in which at these temperatures, for some light treatments, germination percentages reached almost 100%, meaning that substantial variation was absent. Eight seeds per replicate were used in order to avoid impact to the natural populations of the taxa under study (both rare and/or endangered globally, [11,12]). At the end of each experiment, the non-germinated seeds were tested for viability with the TTC method.

Following the natural autumn and spring temperatures prevailing at the original habitats of the studied species (Figure 1, Table 1), the alternating temperatures selected for experimentation were 10/5 (for *E. naxense* only) and 15/10, 20/15, 25/20 and 30/25°C (for both species), all in cycles of 16 h day/8 h night. White light was provided by 12 fluorescent light lamps (OSRAM L36W/10). Colored plastic membranes were used to provide the light quality variation of red (>600 nm), green (500-600 nm) and blue light (400-500 nm). Darkness was provided by enclosing the Petri dishes in aluminum foil. All the experiments were conducted in a temperature and light controlled growth chamber (Ing Clima, Spain, model AGP/HR). All seedlings produced after experimentation were transplanted into 1.5 L pots and were maintained under *ex situ* conservation in the Balkan Botanic Garden of Kroussia (Figure 1), where seeds are collected annually. This long-term cultivation aims to provide a stock of plants and enough seeds to guaranty the species' effective *ex situ* conservation, and will provide material for future re-introduction of individuals into wild habitats, if deemed necessary [7].

2.5 Statistical analysis

The viability adjusted accumulative germination percentages at the sixth week were subjected to an analysis of variance (ANOVA) in order to test, in a 5x5 split-plot factorial experiment, the effects of five temperature regimes (main plots) and five light regimes (sub plots) on *E. naxense* seed germination. Likewise, an ANOVA was performed to test, in a 4x5 split-plot factorial experiment, the effects of four temperature regimes (main plots) and five light regimes (sub plots) on *E. krendlii* seed germination at the fifth week. As the ANOVA indicated that seed germination of both species was significantly affected by the combined effect of temperature and light, the interaction means are presented and their comparison was made using the Least Significant Difference (LSD) criterion at $P \leq 0.05$. In addition, for each species, the seed adjusted germination data per week were subjected to Probit regression analysis ($\log_{10}$ transformation) in order to estimate the GT_{50} for each combined treatment. In this regression equation, seed germination (averaged over seven replications) was the dependent variable (y)

and the duration period (weeks) was the independent variable (*x*). The statistical analyses of the data were performed using SPSS (version 15.0).

3. Results

3.1 Seed weight and viability

The average weights of 100 *E. naxense* and *E. krendlii* seeds (Figure 1) were 0.144 g and 0.080 g, respectively. The tetrazolium method (TTC test) revealed that seed viability of *E. naxense* was higher (90%) than that of *E. krendlii* (64%) (data not shown).

3.2 Effect of temperature and light on viability adjusted seed germination

The viability adjusted germination of *E. naxense* seeds at the end of the sixth week indicated significant light and temperature interaction. In particular, 80-100% of *E. naxense* seeds were germinated for all light regimes at 10/5, 15/10, and 20/15°C while the seed germination for white, blue and dark was less than 50% at 25/20°C (Figure 2). In addition, seed germination for white, blue, green, and dark was less than 50% at 30/25°C. On the basis of the estimated GT_{50} values (time required for 50% seed germination), the results showed that this parameter increased with increasing temperature regimes (Table 2). More specifically, GT_{50} values ranged from 1.5 to 1.7 and 1.2 to 1.7 at temperatures of 10/5 and 15/10°C, respectively, whereas the values at 20/15°C were 1.7 to 3.0. Regarding GT_{50} values at 25/20 and 30/25°C, these were very high and ranged from 5.4 to 9.0 and 4.4 to 9.3, respectively. Regarding GT_{50} values for light regimes, these ranged from 1.7 to 5.4 and 1.5 to 9.3 under red and blue light, respectively, whereas the values under green light were 1.7 to 9.1. The GT_{50} values under dark conditions and white light ranged from 1.2 to 7.6 and 1.7 to 7.0, respectively.

All non-germinated *E. naxense* seeds exposed at temperatures of 10/5 and 15/10°C and averaged over light regimes were not viable according to the seed viability test, whereas 79, 93, and 52% of the non-germinated seeds exposed at 30/25, 25/20, and 20/15°C, respectively, were viable (data not shown).

The viability adjusted germination of *E. krendlii* seeds at the end of the fifth week showed that 80-100% of seeds were germinated for all light regimes at 10/5, 20/15, 25/20°C, while the respective seed germination for red light and dark was less than 50% at 30/25°C (Figure 3). On the basis of the estimated GT_{50} values, the results showed that this parameter was less than one week at 10/5 and 20/15°C and increased with

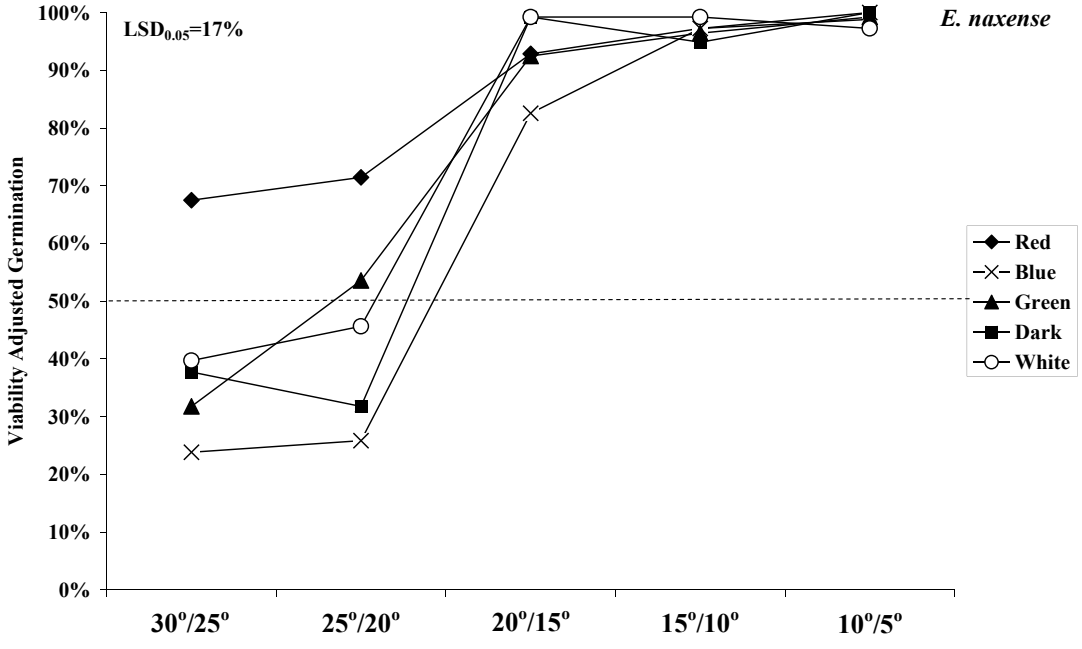

Figure 2. Effect of light regimes on mean viability adjusted accumulative germination percentages (at 6th week) of *Erysimum naxense* seeds per alternating temperature regimes. Seven replicates - Petri dishes with eight (8) seeds per replicate - were used for each combined treatment and their presented means are compared with $LSD_{0.05}$=7%.

increasing temperature regimes (Table 3). In particular, GT_{50} values ranged from 0.3 to 1.3 and 3.8 to 10.1 at temperatures of 25/20 and 30/25°C, respectively. Regarding GT_{50} values for light regimes, these ranged from 0.0 to 3.9 under both red and blue light, and 0.0 to 3.8 under green light. Finally, the GT_{50} values under dark conditions and white light ranged from 0.0 to 10.1 and 0.0 to 4.0, respectively.

Treatments			95% CI	
Temperature	Light	GT_{50} (weeks)	LB	UB
30°/25°	Red	4.4	3.9	5.0
	Blue	9.3	7.8	11.5
	Green	9.1	7.7	11.3
	Dark	7.6	6.5	9.2
	White	7.0	6.0	8.3
25°/20°	Red	5.4	4.6	6.5
	Blue	9.0	7.2	12.3
	Green	6.8	5.7	8.6
	Dark	6.9	5.8	8.8
	White	6.8	5.7	8.6
20°/15°	Red	2.4	2.2	2.7
	Blue	3.0	2.8	3.3
	Green	2.3	2.1	2.5
	Dark	1.7	1.5	1.9
	White	1.8	1.6	2.0
15°/10°	Red	1.7	1.5	1.8
	Blue	1.7	1.6	1.9
	Green	1.7	1.6	1.9
	Dark	1.2	1.1	1.3
	White	1.7	1.5	1.8
10°/5°	Red	1.7	1.6	1.9
	Blue	1.5	1.4	1.7
	Green	1.7	1.6	1.8
	Dark	1.5	1.4	1.6
	White	1.7	1.6	1.9

Table 2. Estimated GT_{50} (weeks required for 50% viability adjusted seed germination of *Erysimum naxense*) values with 95% confidence intervals (CI) from the fitted Probit regression analysis for each combined treatment (light regime x alternating temperature). Seven replicates - Petri dishes with eight (8) seeds per replicate - were used for each combined treatment, and the measurements used for the Probit analysis were expressed as mean accumulative germination data at the end of each week of the six-week experiment.

Note: CI: Confidence interval, LB: Lower bound, UB: Upper bound

All non-germinated *E. krendlii* seeds exposed at temperatures 15/10 and 20/15°C, averaged over light regimes, were not viable, whereas 56 and 36% of the non-germinated seeds exposed at 30/25 and 25/20°C, respectively, were viable (data not shown).

4. Discussion

This study presents, for the first time, a GIS-facilitated germination protocol for the *ex situ* conservation of two local endemic plant species of Greece (*Erysimum naxense* and *E. krendlii*) contributing, at a European level, to the implementation of target 8 of the Global Strategy for Plant Conservation (http://www.cbd.int/gspc/).

Treatments			95% CI	
Temperature	Light	GT_{50} (Weeks)	LB	UB
30°/25°	Red	3.9	3.2	5.0
	Blue	3.9	3.2	5.0
	Green	3.8	3.0	4.8
	Dark	10.1	7.5	15.0
	White	4.0	3.2	5.2
25°/20°	Red	1.3	0.7	1.8
	Blue	1.3	0.8	1.9
	Green	0.3	0.1	0.6
	Dark	0.6	0.3	1.0
	White	0.3	0.1	0.6
20°/15°	Red	0.0	na	na
	Blue	0.0	na	na
	Green	0.0	na	na
	Dark	0.0	na	na
	White	0.0	na	na
15°/10°	Red	0.2	0.1	0.5
	Blue	0.4	0.2	0.5
	Green	0.5	0.3	0.6
	Dark	0.2	0.1	0.4
	White	0.6	0.4	0.8

Table 3. Estimated GT_{50} (weeks required for 50% viability adjusted seed germination of *Erysimum krendlii*) values with 95% confidence intervals (CI) from the fitted Probit regression analysis for each combined treatment (light regime x alternating temperature). Seven replicates—Petri dishes with eight (8) seeds per replicate—were used for each combined treatment, and the measurements used for the Probit analysis were expressed as mean accumulative germination data at the end of each week of the five-week experiment.

Note: CI: Confidence interval, LB: Lower bound, UB: Upper bound, na: not applicable

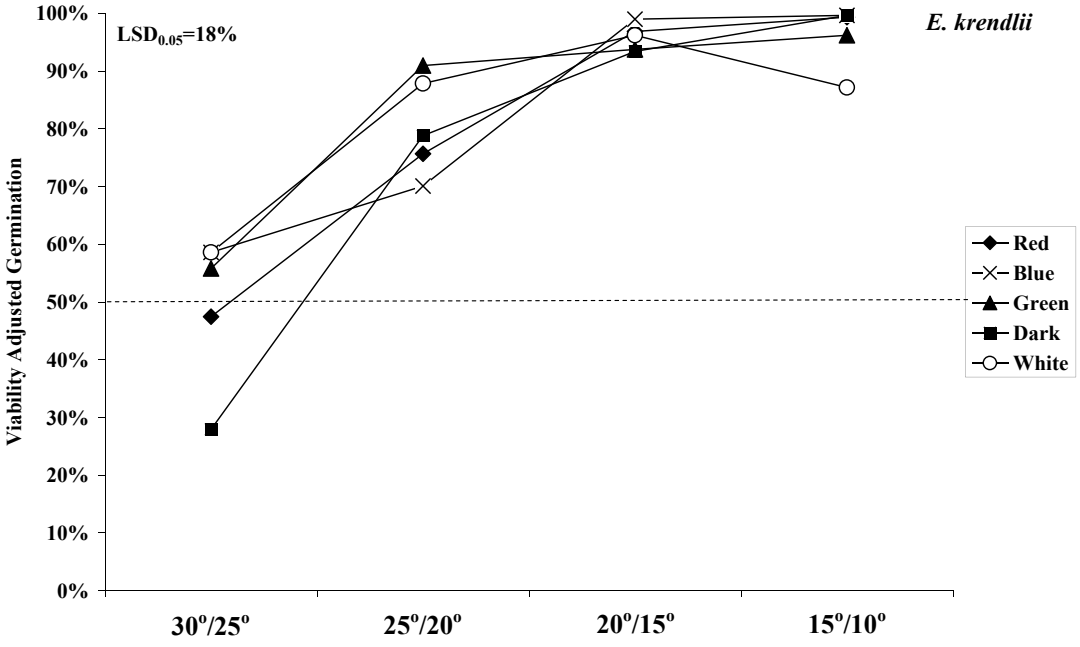

Figure 3. Effect of light regimes on mean viability adjusted accumulative germination percentages (at 5th week) of *Erysimum krendlii* seeds per alternating temperature regimes. Seven replicates - Petri dishes with eight (8) seeds per replicate - were used for each combined treatment and their presented means are compared with $LSD_{0.05}$=18%.

The lower percentage of viable seeds of *E. krendlii* (64%) compared to those of *E. naxense* (90%) may be attributed to possible necrosis of some seeds due to immaturity at the time of harvest. In our study, to comply with seed maturity, we put emphasis on collecting visibly brown-coloured seeds of *E. krendlii* and *E. naxense* from siliquae with valves at least partially opened. For *E. krendlii* especially, the seeds used for experimentation were collected from wild individuals in full fruiting (siliquae valves were clearly opened). Although it has been reported that seeds should be harvested before fully ripe (because they will not grow and will usually die when dehydrated), some species represent exceptions to this rule as their immature seeds germinate quickly and efficiently [26].

The higher germination percentage and the lower GT_{50} values for *E. naxense* and *E. krendlii* seeds at low alternating temperature (10/5-20/15°C and 15/10-20/15°C, respectively) was expected, since these conditions are similar to the ones prevailing during the end of autumn and/or beginning of spring in their natural habitats (see temperature outline in Table 1). The increased seed germination of the studied species at the end of autumn and/or beginning of spring may be associated with the lower temperatures combined with sufficient water availability during these periods. By contrast, the lower germination during late summer and early autumn could be attributed to the higher temperatures coupled with only temporal water availability in their natural habitats (see temperature and rainfall outline in Table 1). Similar results were previously reported [24], indicating that a narrow range of cool temperatures for germination is typical for several widespread Mediterranean species, such as *Muscari neglectum*, *M. comosum*, *M. weisii*, and *M. commulatum* (all germinated at 10-15°C). In addition, other studies [21,28-31] have revealed that other species (*i.e. Allium staticiforme, Cakile maritima*, *Origanum vulgare, Origanum dictamnus, Coridothymus capitatus* and *Satureja thymbra*) germinated better at temperatures ranging from 5 to 20°C. Lastly, it was reported that some rare East Mediterranean species with restricted distribution, such as *Alyssum akamasicum, Phlomis cypria, Origanum cordifolium* and *Ferulago cypria*have optimal germination at 10-20°C [32].

A previous study suggests that a soil seed bank is not expected to be formed for *E. naxense*, and thus the species will depend mainly on continued suitable conditions in natural habitats [11]. Although primary dormancy (immediately after collection) was not evaluated in our study, and assays to determine the possible existence of secondary dormancy were not performed, the very high germination percentages found for both *E. naxense* and *E. krendlii* seeds at low temperatures suggest a possible absence of endogenous seed dormancy. This seems plausible,

as nearly 50% of the seeds germinated within 2-3 weeks at low temperatures. The seed germination of the studied species differs noticeably from those reported for *Erysimum cheiranthoides* and *Erysimum capitatum* subsp. *angustatum* - which produce dormant seeds at low temperatures (autumn-winter) and germinate slowly in spring, when the soil temperature increases and subsequently ensures the survival of seedlings [33,34] - or from those reported for some other Brassicaceae species (*i.e. Arabis serotina, Lesquerella lyrata, Iberis pectinata*, and *Sisymbrium cavanillesianum*), which also form a soil seed bank [35-38]. However, in accordance with our findings, the lowland Mediterranean species *Matthiola tricuspidata* (Brassicaceae) shows no dormancy [39].

Light may have diverse effects on seed germination in different species. Even between congeners, *e.g. Isatis tinctoria* and *Isatis indigotica* (Brassicaceae), different germination sensitivity to light may be detected [40]. In the case of *E. naxense*, light does not seem to significantly affect seed germination at low temperatures, where germination is favored and was similar for all light regimes tested. However, at higher temperatures, germination appears sensitive to one light regime: the final percentage of seed germination was higher under red light conditions in comparison with the other four light regimes tested. A similar response was reported [41] in other species, suggesting the presence of phytochrome [42] and a comparable effect of red light and low temperatures, or of high temperatures and infrared light, to seed germination. These findings indicate that seed positioning of the studied species at the soil layer does not seem to affect their germination in natural habitats, where it may actually take place under or at the surface of the soil, under humus or in vegetation openings [25,42,43].

Light does not seem to significantly affect germination of *E. krendlii* seeds at low temperatures, where germination was similar for all light regimes tested. However, the fact that the seeds of *E. krendlii* were found to respond inconsistently to light regimes does not imply the absence of phytochrome, but could be explained by the presence of a very low active phytochrome level (Pfr). In addition, it is likely that the seed germination of *E. krendlii* is induced differently by particular combinations of temperature and light conditions. In *E. naxense* seeds, where the red light sensitivity has suggested the presence of phytochrome, the detected inhibition of germination at high temperatures may be due to thermal inversion of active phytochrome Pfr to inactive Pr, while the presence of red light promotes the formation of Pfr. The reversible status of phytochrome Pr↔Pfr can be explained by the presence of phytochrome B (phyB) [44,45].

5. Conclusions

Our GIS-facilitated study offers understanding of the biological cycle of conservation priority species, which is the key factor for any successful propagation effort [2,3,5,7]. The results of the present study indicate that the seeds of *E. naxense* and *E. krendlii* probably do not feature innate dormancy and, consequently, a soil seed bank is not expected to form in natural habitats. Seed germination of *E. krendlii* was not affected consistently by light at all temperatures studied, whereas seed germination of *E. naxense* was light-independent at low temperatures but increased with red light at high temperatures. The germination percentages of both species were higher in experimental conditions similar to the ones prevailing at their natural habitats in autumn and/or spring. Responses to different temperature and light regimes of the studied species indicated that seed positioning at the soil layer does not seem to affect their germination *in situ*. These conclusions can provide useful guidelines for their *ex situ* conservation.

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