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Seed dormancy and reproductive traits of the southern Balkan endemic plants *Jancaea heldreichii* (Gesneriaceae) and *Viola delphinantha* (Violaceae)

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Abstract: The Mediterranean region presents a significant number of plant species facing threats from habitat loss and climate change, particularly those with limited distribution and habitat scarcity, such as chasmophytes. The study represents the first investigation into the germination potential of *Jancaea heldreichii* (Gesneriaceae) and *Viola delphinantha* (Violaceae), shedding light on their seed dormancy characteristics and germination aiming to assist conservation efforts. Fruit capsules of both species were collected, and their seeds were either cold stratified or immersed in hormone solutions prior to a germination test. After the end of the germination test, the ungerminated seeds were subjected to a cutting test and those with a well-developed embryo were subjected to a viability staining test using two different dyes, 2,3,5-triphenyl tetrazolium chloride and Evans blue. The study revealed that hormone treatments, particularly gibberellic acid, significantly increased germination percentages (69.4% and 88.3% for *J. heldreichii* and *V. delphinantha*, respectively) compared to control (untreated) seeds. Kinetin also enhanced germination, with a significantly higher increase in *J. heldreichii* compared to *V. delphinantha* (50.1% and 6.4%, respectively), whereas cold stratification had a lesser effect (28% and 4.2%, respectively), and the control treatment showed negligible germination. An ungerminated viability test in both species revealed that in the gibberellic acid treatment no ungerminated seeds with a well-developed embryo (stained or unstained) were found, suggesting that all the seeds that could produce normal seedlings were germinated. The study underscores the importance of germination protocols for conserving chasmophytic endemics like *J. heldreichii* and *V. delphinantha*. Such protocols are essential for preserving these unique plant species and their habitats.

Keywords: chasmophytes, germination treatments, Gesneriaceae, Greece, *Jancaea heldreichii*, reproductive traits, seed dormancy, viability staining test, *Viola delphinantha*, Violaceae

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Introduction

Plant endemism in the Mediterranean region is significant and has been shaped by the unique climatic, geological and ecological conditions of the area (Mendoza-Fernández & al. 2022). Despite their ecological significance, many endemic plant species in the Mediterranean region are threatened by habitat loss and fragmentation (Manes & al. 2021). In addition, climate change poses a new significant threat as rising temperatures, changes in precipitation patterns, and more frequent extreme weather events can disrupt their habitats and affect their population dynamics and distribution (Vogiatzakis & al. 2016). Among the endemic plant species vulnerable to the impacts of climate change, chasmophytes hold a distinctive place. These plants, which thrive on cliffs, are characterized by their limited distribution and rarity primarily attributed to the scarcity of suitable habitats (Gaston 1994; Valli & al. 2021). These highly specialized habitats act as

refuges for chasmophytes benefiting from reduced interspecific competition and minimal grazing pressure (Larson & al. 2000). However, their limited dispersal ability, coupled with low habitat connectivity, may lead to population decline and a contraction in their geographic range under future climate change scenarios (Colas & al. 1997; Soriano & al. 2012).

Restoration efforts have become an increasingly critical issue, usually in the context of climate change, because they are essential for restoring degraded habitats and protecting biodiversity and ecosystem services (Lázaro-González & al. 2023). The restoration of degraded habitats usually requires the collection of significant quantities of seeds, which for various reasons might be limited (Broadhurst & al 2008). Large-scale implementation and cost-effectiveness of seed-based restoration programmes requires that seeds of the target plant species are readily available (Guerrant & Kaye 2007; Fenu & al. 2020). However, it is equally crucial to acknowledge the

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importance of producing seedlings and conserving them ex situ, in botanical gardens, for preserving germplasm from extinction (Nakahama & al. 2016; Godefroid & al. 2016; Diallo & al. 2021; Fenu & al. 2023). At the same time, urgent attention must be given to appropriate in situ species management, which involves enriching their habitats through reintroductions or restoration programmes, grounded on ex situ seed propagation (Guerrant & Kaye 2007; Godefroid & al. 2011; Draper & al. 2016; Commander & al. 2018). Ex situ conservation techniques, such as translocation or assisted colonization, are emerging as valuable and promising strategies to mitigate the impact of climate change on these crucial endemic plant species (Casazza & al. 2021).

A crucial step, in both in situ and ex situ management and conservation of endemic plant species, involves the research and development of effective seed viability estimation and germination protocols (Pedrini & Dixon 2020). Essential to all these management strategies is the assessment of seed dormancy, viability and germination capacity to ensure the production of healthy and vigorous seedlings (Abeli & al. 2016; Fenu & al. 2020). Seed viability testing is essential in plant research and conservation (Lamont 2022). A seed is classified as viable or nonviable based on its ability to germinate and develop into a healthy seedling (Baskin & Baskin 2001). While there are various methods for assessing viability, germinating seeds under optimal conditions is considered the most reliable, and the germination test is widely used for this purpose. It has become so universally accepted that germination and viability are often seen as synonymous (Copeland & McDonald 2001).

Dormancy is defined as the period during which a seed embryo is alive but inactive, waiting for the appropriate period of time to emerge from dormancy and to germinate (Finch-Savage & Leubner-Metzger 2006). Furthermore, it is an adaptive trait protecting young seedlings from emergence during unfavourable time periods (Baskin & Baskin 2004). To determine whether a plant species has dormant seeds, researchers subject them to various treatments aimed at facilitating water uptake and regulating the hormonal balance regulation between promoting agents such as gibberellic acid and suppressing agents with antagonistic behaviour such as abscisic acid (Gupta & Chakrabarty 2013). These treatments are compared to a control, in which seeds are placed to germinate immediately after collection. However, while this comparison can infer dormancy existence, a comprehensive understanding of seed ecology requires estimating the overall potential germination dynamic by conducting seed viability tests in non-germinated seeds. According to ISTA (2009), viable seeds are those that have the potential to germinate and produce normal seedlings under favourable exogenous and endogenous conditions. Seed viability tests can be conducted either before germination to determine the potential germination maxima or after germination test

to determine the degree of treatment success and dormancy reversal (Rami & Patel 2014).

The most referenced seed viability tests used are the staining ones in which the excised seed embryos are immersed in a dye and their viability status is estimated based on their staining pattern. The most common dye is the 2,3,5-triphenyl tetrazolium chloride (hereafter “TTZ”) at varying concentrations from 0.1 to 1% (e.g. Copeland & McDonald 2001; Gale & al. 2010; Sew & al. 2016). The TTZ staining method requires tissue metabolic activity, and the live tissues turn red (e.g. Ruf & Brunner 2003; Carvalho & al. 2010; Del Elgido & al. 2017; Li & al. 2023). However, if the tissue is deeply dormant then the TTZ staining might not happen, thereby presenting misleading results of seed viability (Busso & al. 2015). Another type of dye that does not presume tissue metabolic activity is Evans blue (hereafter “EvBl”) (Keßler & Furusaki 1997). EvBl penetrates the dead tissues giving a blue colour while it leaves the live ones unstained (Busso & al. 2015). Although EvBl is widely used in histochemical staining, its use for direct seed viability is little referenced (e.g. Busso & al. 2015; Tian & al. 2019; Pradhan & al. 2022). Therefore, the use of both the TTZ and EvBl techniques can provide a safe discrimination assay between metabolically active, dormant or dead seed embryos (Busso & al. 2015; Tian & al. 2019).

Jancaea heldreichii (Boiss.) Boiss. (*Gesneriaceae*) is a stenoendemic plant that occurs exclusively on Mount Olympus and thrives in somewhat damp and shady crevices of limestone cliffs at altitudes of 700–1500 m. This species is a relict from the late Neogene (Petrova & al. 2015). Although Plants of the World Online (POWO 2024) placed it in the genus *Ramonda* Rich. (also *Gesneriaceae*), as *R. heldreichii* (Boiss.) C. B. Clarke, Dimopoulos & al. (2013) placed it in the unispecific genus *Jancaea* Boiss. This herbaceous perennial forms basal rosettes of leaves covered with dense, long, soft hairs. The flowers are small and lavender-purple in colour (Dimopoulos & al. 2013). *Viola delphinantha* Boiss. (*Violaceae*) is a Balkan endemic and is considered a rare species that occurs on certain mountains in Greece and southern Bulgaria. It is an obligate chasmophyte that occurs in crevices of limestone cliffs at altitudes of (700–)1400–2500 m. This distinctive plant is a woody-based perennial with narrow, almost needle-like leaves and a pinkish lilac corolla with a long, slender spur (Dimopoulos & al. 2013).

The aim of the present study was to explore the seed dormancy and germination requirements of two chasmophytic plant species, *Jancaea heldreichii* and *Viola delphinantha*, in order to develop effective reproductive protocols for future ex situ or in situ conservation efforts. Both species are endemic to the southern Balkan Peninsula, with *J. heldreichii* restricted to the eastern and northern sides of Mount Olympus, and *V. delphinantha* having a disjunct distribution across mountains in southern Bulgaria and Greece (Dimopoulos & al. 2013).

Material and methods

Fruit capsules of *Jancaea heldreichii* and *Viola delphinantha*, in the fruiting stage, were collected from Mount Olympus in June 2022 (Fig. 1). Soon after their transfer to the laboratory, they were air-dried for two weeks. Subsequently, the seeds were extracted from the capsules and divided into petri dishes containing fifty seeds each. Superficial seed sterilization was performed by rubbing them between Watman filter papers moistened with pure ethanol, a common sterilizing agent for various plant species (Kodahl & al. 2016; Si & al. 2022).

Four distinct germination treatments, with four replicates of 50 seeds each, were applied to the seeds: control, cold moist stratification for two months at $0 \pm 1^\circ\text{C}$, and two different hormone treatments. In the hormone treatments, the seeds were subjected to immersion in either 250 ppm gibberellic acid (GA3) or 50 ppm kinetin for 48 hours and immediately placed to germinate. Immersion was achieved by placing the seeds between filter papers moistened with the respective hormone concentration. Seeds from the control treatment were immediately placed to germinate without any pretreatment.

Seeds were germinated under alternating temperature conditions ($+25^\circ\text{C}$ during the day and $+15^\circ\text{C}$ at night) with a 12-hour photoperiod. Seeds showing radicles protruding at least 2 mm in length were considered germinated. Germination percentage was assessed weekly over a total period of 18 weeks. Any rotten seed was removed and counted as dead (see definition below).

Upon completion of germination, the remaining ungerminated seeds underwent a cutting test and were categorized according to ISTA (2009) guidelines into three groups: empty (without any gametophytic tissue), dead

(mouldy or rotten), and intact seeds containing a firm, well-developed embryo with all its structure present. Assigned as dead seeds were those that were mouldy and did not produce seedlings, although they had gametophytic tissues (ISTA 2009). The seed cutting test aimed to compare the actual germination (i.e. the seeds that germinated) with the potential germination (i.e. the seeds with a well-developed and viable embryo capable of germination that were still dormant). Empty seeds were excluded and not considered in the final germination and viability estimates. Therefore, the actual germination was adjusted as follows:

$$\text{Germination (\%)} = \frac{N_{\text{germ}}}{N_{\text{total}} - N_{\text{empty}}} \times 100$$

where:

N_{germ} is the number of germinated seeds

N_{total} is the total number of seeds per repetition

N_{empty} is the number of empty seeds

The TTZ test, as recommended by ISTA rules (ISTA 2009), is commonly used to assess the viability of seeds that remain ungerminated at the end of the germination test. This test is essential for evaluating dormancy-breaking treatments and identifying dormant seeds that fail to germinate by the end of the germination period. Intact seeds were abraded on their testa using a dissecting needle and were soaked in water for 12 hours in order to make it easier to separate the embryos. Excised embryos were subjected initially to staining with TTZ (1% w/v) under dark conditions and room temperature for 12 hours (ISTA 2009). An ungerminated embryo was considered as viable in the TTZ test when at least 90% of its area stained red or pink, including hypocotyl, indicating

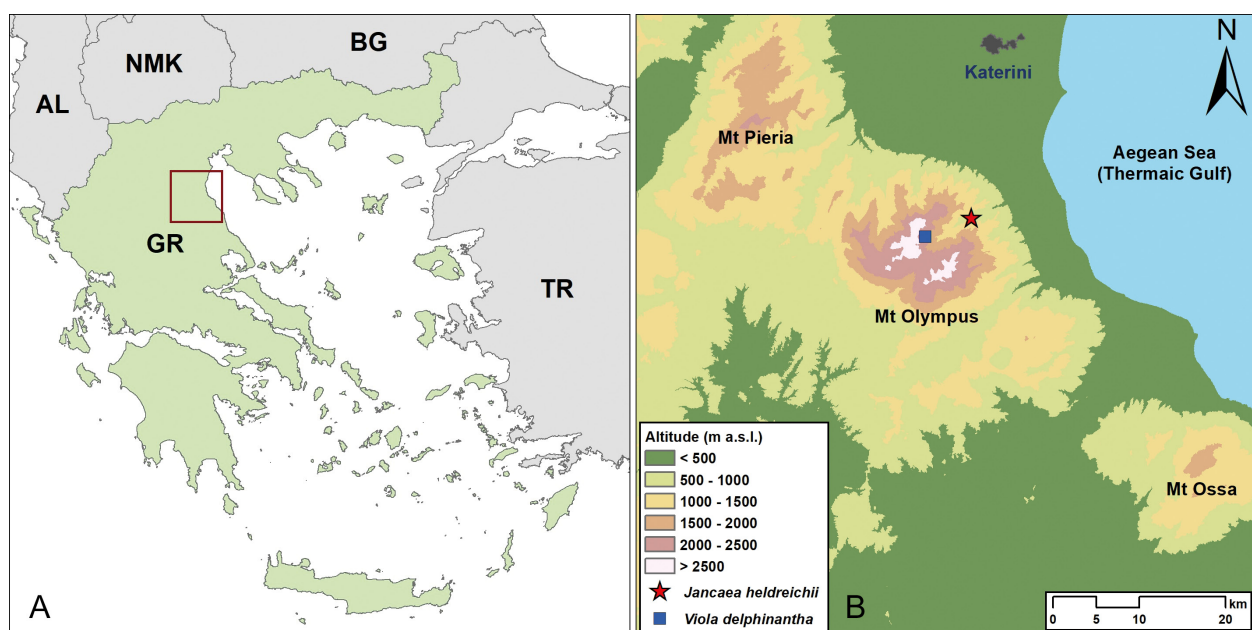


Fig. 1. Maps showing (A) the location of the study area in Greece and (B) the sampled populations of *Jancaea heldreichii* and *Viola delphinantha*.

lack of germination due to dormancy status (Fig. 2A, B) (Zheng & Sun 2008; Bertsekis & al. 2022). Embryos remaining unstained in the TTZ test were placed for staining in EvBl (0.25% w/v; Busso & al. 2015; Gokul & al. 2016), initially for 30 minutes and then examined every 30 minutes for six hours to determine the time of imbibition. There was no difference in the staining patterns of embryos after 30 minutes. An embryo was considered non-viable if it was stained blue, whereas it was considered viable but deeply dormant if it remained either unstained or superficially stained (Busso & al. 2015) (Fig. 2C–E). The percentage of seeds with viable embryos was calculated by summing the percentages of seeds stained in TTZ and those that were unstained in EvBl. Non-viable and dead seeds were considered as separate categories. A non-viable seed had a hard testa with a firm embryo during seed cutting after the end of the germination period, whereas a dead seed had a soft testa without a firm embryo and was mouldy during seed cutting after the end of the germination period.

Differences in mean germination values were checked through a non-parametric Kruskal-Wallis ANOVA using STATISTICA software v.10 (Kołodziejek & al. 2019).

Results

Seeds of both *Jancaea heldreichii* and *Viola delphinantha* exhibited positive responses to hormone treatments, particularly with gibberellic acid, where the highest germination percentages were observed (69.4% and 88.3%, respectively) (Fig. 3, 4). Kinetin treatment also enhanced germination, with a considerable higher increase in *J. heldreichii* compared to *V. delphinantha* (50.1% and 6.4%, respectively). In contrast, cold stratification stimulated germination to a lesser extent for both species (28% and 4.2% for *J. heldreichii* and *V. delphinantha*, respectively) compared to the two hormone treatments. Notably, the control treatment resulted in negligible germination percentages for both species. The differences in germination percentages for *J. heldreichii* were statistically significant ($p < 0.05$) for all comparisons. In contrast, for *V. delphinantha*, only the gibberellic acid treatment showed a significantly higher germination percentage compared to the other treatments.

Seed cutting at the end of the germination period revealed the presence of dead seeds across all applied treatments. In *Jancaea heldreichii*, the highest percentages of

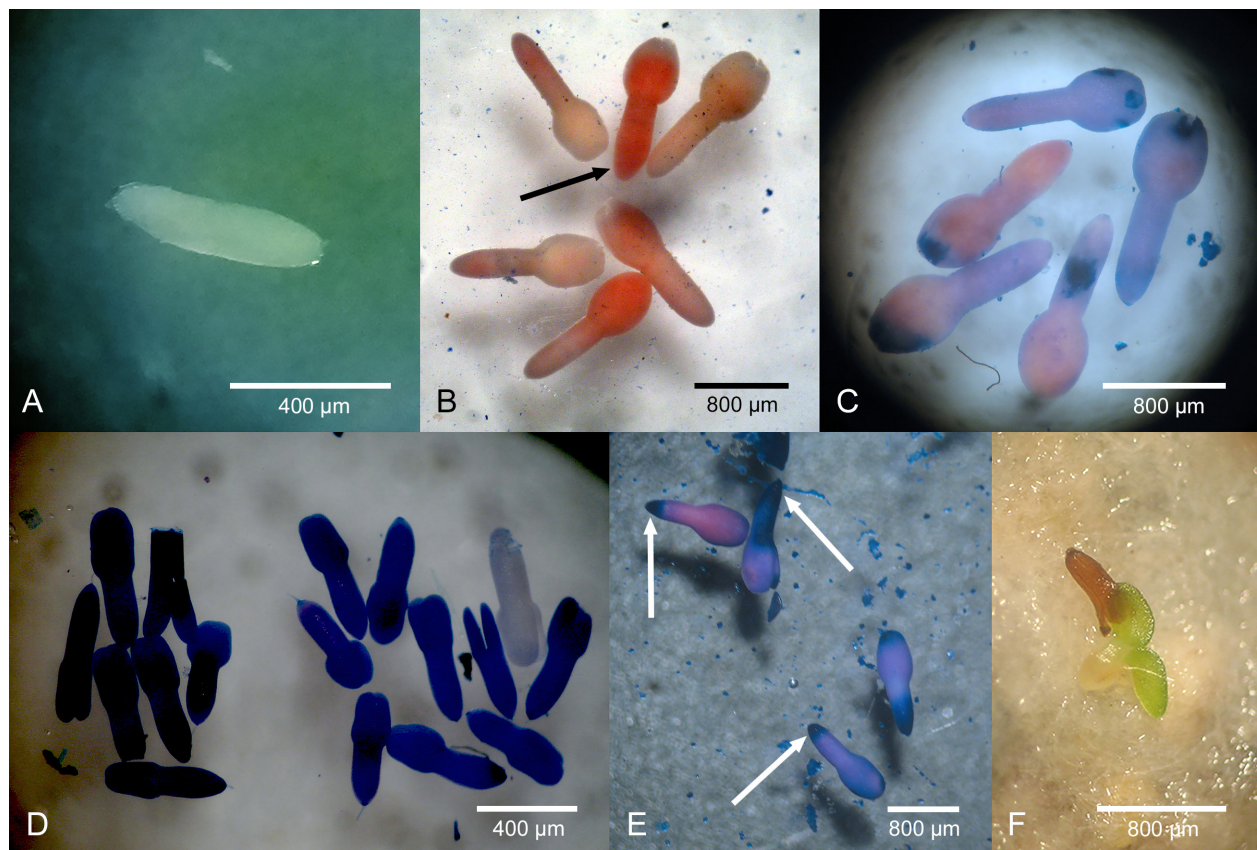


Fig. 2. A: TTZ unstained embryo of *Jancaea heldreichii*; B: embryos of *Viola delphinantha* after staining with TTZ; black arrow indicates viable embryo, whereas the rest considered unstained and therefore potentially non-viable; C: TTZ unstained or partially stained embryos of *V. delphinantha* placed in EvBl; all embryos considered unstained, viable but dormant; D: TTZ unstained embryos of *J. heldreichii* placed in EvBl and processed in photoshop CS5 by attributing mid-tone colours set at -0.1082px ; embryos on right side (light blue or white) considered viable but dormant, whereas those on left side (dark blue) considered non-viable; E: TTZ unstained or partially stained embryos of *V. delphinantha* placed in EvBl; all embryos considered non-viable due to intense blue colour of radicle indicated by white arrows; F: young seedling of *J. heldreichii*.

Table 1. Germination and viability results of *Jancaea heldreichii* and *Viola delphinantha*.

Seed treatment	<i>Jancaea heldreichii</i>				<i>Viola delphinantha</i>			
	Seed category		Seeds with embryo		Seeds with embryo		Seeds with embryo	
	Dead seeds (%) [*] (mean ± SD)	Germinated (%)	Viable (%) (mean ± SD)	Non-Viable (%) (mean ± SD)	Dead seeds (%) [*] (mean ± SD)	Germinated (%)	Viable (%) (mean ± SD)	Non-Viable (%) (mean ± SD)
Cold stratification	63.5 ± 5.0	28.0 ± 8.3	5.8 ± 0.2	2.7 ± 1.6	40.5 ± 2.7	25.8 ± 4.5	29.5 ± 6.7	4.2 ± 1.6
Gibberellic acid	30.6 ± 0.5	69.4 ± 1.1	0	0	11.7 ± 0.1	0	0	88.3 ± 1.7
Kinetin	29.7 ± 3.6	50.1 ± 9.2	10.6 ± 4.4	9.6 ± 3.5	71.6 ± 1.0	0	22.0 ± 3.6	6.4 ± 5.6
Control	100	0	0	0	58.2 ± 5.0	27.5 ± 6.3	14.3 ± 6.7	0

^{*}Seeds with no firm embryo after germination, always mouldy.

dead seeds were observed after cold stratification and the control treatment (63.5% and 100%, respectively) (Table 1). Conversely, in *Viola delphinantha*, while cold stratification and the control treatment exhibited relatively high percentages of dead seeds (40.5% and 58.2%, respectively), the highest percentage was recorded in seeds treated with kinetin (71.6%) (Table 1).

The proportion of viable, ungerminated seed embryos was generally lower in *Jancaea heldreichii* compared to *Viola delphinantha*. In *J. heldreichii*, approximately equal percentages of viable and non-viable embryos were observed in the kinetin treatment (10.6% and 9.6%, respectively), while the percentages in the cold stratification treatment were lower (5.8% viable and 2.7% non-viable) (Table 1). Conversely, in *V. delphinantha*, a notable and similar percentage of viable seed embryos was found in the cold stratification and control treatments (25.8% and 27.5%, respectively), whereas the percentage of non-viable seed embryos was higher in the cold stratification treatment compared to the control (29.5% and 14.3%, respectively). Notably, neither viable nor non-viable ungerminated seed embryos were detected in both *J. heldreichii* and *V. delphinantha* when treated with gibberellic acid.

Discussion

Chasmophytes represent a unique plant group as they occur in restricted habitats, which in some cases are rather unstable, and therefore conservation, especially of the rarest ones, is a challenging task (Panitsa & Kontopanou 2017). In the present study, the germination potential of *Jancaea heldreichii* and *Viola delphinantha* was investigated under different treatments.

In our study, there was no germination observed in seeds from the control treatment under the applied temperature and light scheme for both species. Contrary to our results, Vokou & al. (1990) germinated *Jancaea heldreichii* seeds without any pretreatment such as hormone imbibition or cold stratification. There are several reasons for the differing results. Firstly, experimental conditions during the germination test are known to influence dormancy breaking (Baskin & Baskin 2001; Varsamis & al. 2014). Both temperature and light conditions differed and are likely to have affected the relevant germination percentage. Additionally, the season of the germination test, which can also affect the results, was not specified. Seed responses to seasonal weather conditions and this adaptation create distinct dormancy cycling patterns (Finch-Savage & Footitt 2017). Therefore, during certain seasons, dormancy tends to be lighter, allowing seeds to respond more readily, resulting in faster and more successful germination (Finch-Savage & Footitt 2015). Our results demonstrated that both *J. heldreichii* and *Viola delphinantha* exhibited a rather deep physiological dormancy, because cold stratification

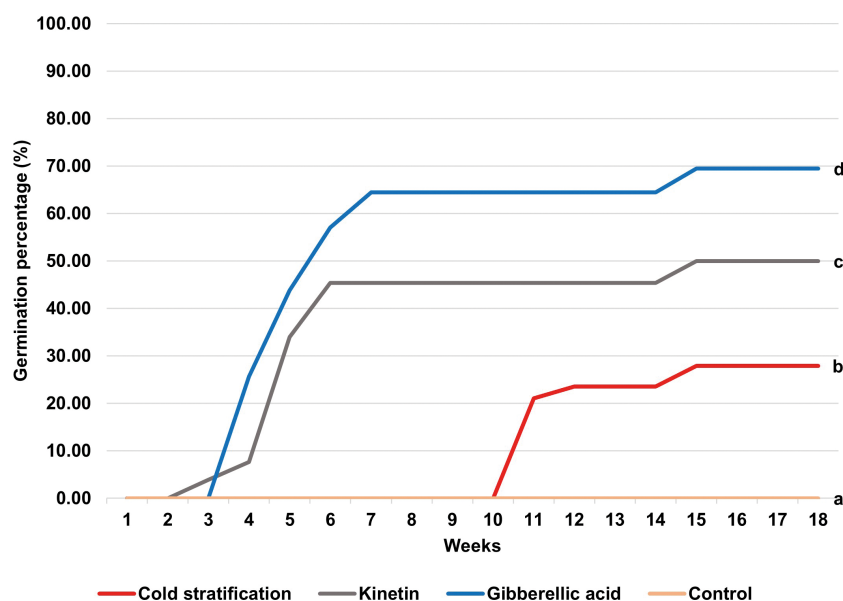


Fig. 3. Germination results of different seed treatments applied in *Jancaea heldreichii*. Germination curves followed by same letter do not differ at 0.05 level of significance.

resulted in low germination percentages (c. 5 and 30 %, respectively). Seed physiological dormancy is the most common type of dormancy in terrestrial plants (Baskin & Baskin 2014), which is usually released by cold, moist stratification or exogenous hormone application (Baskin & Baskin 2022). The presence of physiological dormancy is further supported by the germination percentages of two species within the genus *Ramonda*, *R. serbica* Pančić and *R. nathaliae* Pančić & Petrović, which are apparently closely related to *J. heldreichii*, which Plants of the World Online (POWO 2024) placed in *Ramonda*. These percentages were approximately 10% and 28%, respectively, when the seeds were subjected to soaking with distilled water (Gashi & al. 2012). However, if seeds of *J. heldreichii* are to be placed for germination under the current study conditions (+25°C during the day and +15°C at night with a 12-hour photoperiod), they need gibberellic acid pretreatment to maximize seed germination response.

Typically, seeds with non-deep physiological dormancy are expected to germinate within 8–10 weeks of stratification (Baskin & Baskin 2022). However, the fact that both species exhibited low germination percentages after a 10-week cold stratification suggests that their dormancy could be either intermediate or deep. *Viola kosaninii* (Degen) Hayek, a species endemic to northern Albania and the former Yugoslavia, and apparently one of the closest relatives to *V. delphinantha* (Strid 1986), showed an equally low germination rate (5%) following cold stratification and seed immersion in water (Krigas & al. 2022). Moreover, *V. striis-notata* (J. Wagner) Merxm. & Lippert, a stenoendemic species also found on Mount Olympus, similarly demonstrated a remarkably low germination rate (< 5%) following cold stratification (Varsamis & al. 2023).

The presence of physiological dormancy in both species is reinforced by the positive impact of hormone treatments, particularly gibberellic acid, on germination. Exogenous hormone treatments have been found effective in breaking dormancy in various species (Baskin & Baskin 2014). Gibberellins play a crucial role, counteracting the inhibitory effects of abscisic acid and stimulating endogenous gibberellic acid production, thereby promoting seed germination (Bewley & al. 2013). Moreover, gibberellic acid is known to shorten the after-ripening process in seeds and promote germination (Rahsid & al. 2023; Ge & al. 2023). On the other hand, there is research that supports a species-specific effect of external hormone application such as gibberellic acid (GA3) in seed

germination (Kilgore & al. 2022). Gibberellic acid effectively promoted seed germination in both *Jancaea heldreichii* and *Viola delphinantha* under the applied light and temperature germination conditions, as evidenced by the absence of viable embryos in the seed cutting test of ungerminated seeds, indicating the success of the specific treatment. Therefore, under alternating temperature conditions of +25/+15°C and a 12-hour photoperiod, it appears that gibberellic acid was essential for seed germination. Comparing this differential response to the findings of Vokou & al. (1990), where no pretreatment was applied, we could hypothesize that the seeds entered secondary dormancy (or conditional dormancy) due to the alternating germination conditions, necessitating gibberellic acid application to overcome it.

The role of cytokinins, including kinetin, in various aspects of plant growth, development, and stress responses, including seed germination, has been thoroughly investigated. Research has shown that kinetin can enhance seed germination by promoting cell division and growth in germinating seeds. It can also overcome dormancy in some seeds, thereby facilitating germination. Kinetin achieves these effects by interacting with various signalling pathways and gene expression mechanisms involved in seed germination (Araújo & al. 2019; Samaan & al. 2000; Sawan & al. 2000), particularly when conditions are suboptimal for germination (MoK 1994). In our study, kinetin treatment effect was species specific because it increased germination in *Jancaea heldreichii* but not in *Viola delphinantha*.

In both cold stratification and kinetin treatment, a portion of well-developed but non-viable embryos was recorded after the two-step staining test (i.e. sequential use of TTZ and EvBl) (Table 1). Despite being fully developed, these embryos failed to initiate germination and

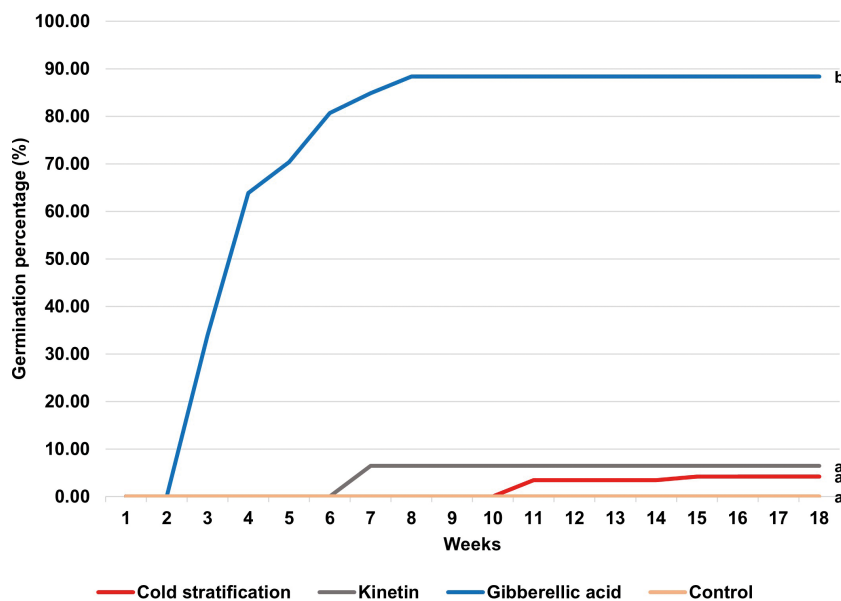


Fig. 4. Germination results of different seed treatments applied in *Viola delphinantha*. Germination curves followed by same letter do not differ at 0.05 level of significance.

produce normal seedlings. This could be attributed either to insufficient dormancy breaking by the specific treatments or to genetic factors related to life-history traits, mating systems or pollinators, which are observed particularly in rare or endangered plant species (Ellstrand & Elam 1993; Delnevo & al. 2021). Those embryos remained unstained in TTZ but turned blue in EvBl. Therefore, it is possible that the TTZ test is less reliable for the specific species to elucidate the success of the treatment by determining the actual germination from the potential germination, compared with the EvBl test, which is non-enzymatic (Busso & al. 2015; Pradhan & al. 2022).

Moreover, in both species, a notable proportion of ungerminated, mouldy seeds, which were classified as dead, were observed across all treatments, including the control, although the specific numbers varied depending on the species and treatment. The exact cause of this outcome remains unclear. However, it can be postulated that these seeds might have absorbed water but lacked the hormonal regulation required for germination, ultimately resulting in decay (see review by Ranganathan & Groot 2023).

The germinated seeds of *Viola delphinantha* were successfully planted, and the seedlings exhibited normal growth (unpublished data). In contrast, the young seedlings of *Jancaea heldreichii* either failed to grow altogether or exhibited extremely slow growth rates, ultimately decaying within two months due to unclear reasons (Fig. 2F). Data regarding *Haberlea rhodopensis* Friv., another relict species of the *Gesneriaceae* found in the Balkan Peninsula, and apparently closely related to *J. heldreichii*, revealed that although seed germination after stratification was relatively fast (occurring within 5–10 days), the young seedlings also failed to grow, with less than 1% surviving after a few months (Gashi & al. 2012; Bogacheva-Milkoteva & al. 2013). The authors hypothesized that this out-

come may be attributed to the absence of symbiotic fungi necessary for the formation of mycorrhiza, which typically aids young seedlings in survival. If a similar scenario exists for *J. heldreichii*, it becomes imperative to thoroughly investigate seedling survival to facilitate the ex situ conservation of this valuable relict species.

In general, germination protocols that facilitate propagation from seeds play a crucial role in plant conservation and habitat restoration efforts (Kildisheva & al. 2020; Varsamis & al. 2023). The results of the current study are highly relevant in developing viability and germination protocols for both in situ and ex situ restoration efforts targeting chasmophytic endemics like *Jancaea heldreichii* and *Viola delphinantha*. These protocols are crucial for effectively restoring and conserving these unique plant species in their natural habitats as well as in controlled environments.

By comparing the results of our study with those of Vokou & al. (1990), we conclude that further research is needed to explore the effects of different pretreatments under various germination temperatures and light conditions in these two highly specialized plant species of Greece.

Author contributions

ST and TM coordinated the study; ST conducted the field survey; GV and KT conducted the experiments; GV prepared the first draft of the manuscript. All authors contributed to the conceptualization of the study, as well as the final revision and editing of the manuscript.

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