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Seed Germination Ecology and Dormancy Release in Some Native and Underutilized Plant Species with Agronomic Potential

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Abstract

Within the context of sustainable exploitation of phytogenetic resources, the present study aimed to develop species-specific seed germination protocols for eighteen native and potentially underutilized plant species originating from northeastern Greece. The taxa were selected based on their antioxidant potential and their provenance to enhance their regional exploitation potential, thus utilizing the species' local adaptation traits. To quantify the maximum germination potential in each case, seed viability was assessed using the tetrazolium (TTZ) test. The pre-treatments applied for seed dormancy release included cold stratification and the application of gibberellic acid (GA_3) and kinetin. Germination tests revealed that 9 of the 18 species exhibited high germination percentages in the control treatment (ranging between 64 and 90%) indicating that after-ripening was sufficient for any seed dormancy release in a significant portion of the seed lot. Furthermore, cold stratification and hormonal treatments significantly enhanced germination in seven species (final seed germination up to 85%), indicating deeper physiological dormancy and confirming the role of cold stratification and phytohormones in dormancy release. Two species showed no germination under any pre-treatment while viable, indicating the presence of more complex dormancy mechanisms. Germination percentages were frequently lower than the corresponding seed viability values, which ranged from 70% to 100%, suggesting that a portion of the seed lot exhibited deeper dormancy throughout. The results showcased species with favorable germination patterns, thus successfully identifying species that can be readily propagated, as well as species that require specific pre-treatments. The study sets the basis for domestication and sustainable use of local antioxidant-rich flora, providing a clear roadmap for the agronomic utilization of the focal species to support the regional bioeconomy.

Keywords: native plants; germination protocols; antioxidant species; seed dormancy; seed viability; GA_3; kinetin; cold stratification; agronomic potential

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1. Introduction

Oxidative stress in humans is a well-recognized factor to aging and the development of a broad range of chronic illnesses including cancer, cardiovascular disease, neurodegenerative disorders, diabetes, and inflammation [1]. The human body has several mechanisms

to maintain the redox balance through either endogenously produced antioxidants or externally supplied through food or supplements [2]. Polyphenols, flavonoids, carotenoids, and vitamins A, C, and E, originated by plants, can effectively regulate oxidative pathways by neutralizing free radicals and sequestering harmful metal ions [3]. Numerous studies have shown that diets rich in these compounds are associated with reduced risks of major chronic diseases. As a result, there is a growing demand for natural and healthier food options with consumers seeking alternatives to synthetic additives in food and cosmetics [4] and plant-based antioxidants to serve as a primary defense against oxidative stress [5]. This trend has intensified as of recently; the use of synthetic antioxidants has raised some concerns due to potential harmful effects on health [6]. Moreover, pharmaceutical expenditures, one of the largest healthcare costs globally, have driven a shift toward preventative strategies that emphasize well-being through diet and lifestyle [7]. Natural antioxidants are central to this shift, offering protective benefits against oxidative stress-related diseases and supporting efforts to reduce long-term dependence on pharmacological treatments [8]. The public perception that plant-based antioxidants are safer and healthier than synthetic ones has motivated the food industry towards replacing synthetic antioxidants with plant-derived ones [9] and meeting clean-label standards [4,10,11]. In addition, regulatory pressure (e.g., [12,13]) further reinforces this trend [14]. As a result, research activity and industrial investment in plant-based bioactive compounds with antioxidant potential have significantly increased.

Consequently, plant diversity is increasingly regarded as a key resource, since native, often neglected, and underutilized plant species are known to contain a reservoir of useful secondary metabolites due to both their adaptation to harsh environments and the development of their complex natural defense systems [15–17]. In general terms, plants possess a diverse set of secondary metabolites (e.g., antioxidants—including enzymatic and non-enzymatic compounds such as flavonoids, polyphenols, carotenoids, and ascorbic acid) the biosynthesis of which has evolved to serve specific ecological functions, such as environmental adaptation to reduce the impact of oxidative damage [15,18,19]. The re-evaluation of these species is part of a broader bio-economic strategy that extends across nutrition, medicine, cosmetics, and functional food markets [4,20,21]. Therefore, preserving and utilizing plant diversity can both be considered an environmental necessity, as well as a practical strategy for discovering novel nutraceutical and therapeutic agents [22].

A systematic and multi-stage process is essential to domesticate and commercialize native and underutilized species (NUS) for agronomic and industrial purposes. The first stage involves the selection of the promising taxa. This is typically based on ethnobotanical knowledge that is followed by preliminary phytochemical investigations to detect the presence of bioactive substances such as antioxidants [23–25]. The next stage is the development of ex situ propagation protocols to ensure sustainable production and preservation of the selected germplasm [26]. Propagation success is the essential first step to facilitate pilot and large-scale cultivation before the antioxidant potential can be reliably assessed in the cultivated material. As such, the next critical stage is the pilot cultivation trials that can result in the evaluation of the species' agronomic performance and its adaptation to different soil and climate conditions [27]. Such trials are usually accompanied by research on biochemical evaluation under field conditions [26,28]. Finally, the ecological adaptability, yield potential, and economic viability of the species is evaluated offering both environmental resilience and opportunities for novel functional products [26,27].

An essential prerequisite for the agronomic and industrial exploitation of NUS is the successful development of species-specific seed germination and propagation protocols [29]. Many wild species exhibit seed dormancy—a trait evolved as an ecological adaptation to ensure survival under unpredictable conditions—which can significantly hinder their

large-scale cultivation [30,31]. Overcoming these dormancy mechanisms is therefore critical to ensure uniform seedling emergence and enable further evaluation of phytochemical traits under controlled conditions. Species that fail to achieve acceptable germination rates or seedling survival are often excluded from domestication efforts, regardless of their phytochemical potential [32]. Thus, early-stage germination studies serve not only as a technical foundation but as a biological filter for determining which species can viably enter any bioeconomic value chains.

The cultivation of medicinal and aromatic NUS is gaining attention in Greece with significant agronomic and economic prospects [33]. Since 2017 the National Strategic Plan has pointed out the need to develop sustainable practices for the cultivation, processing, and marketing of such plants, to achieve competitiveness through higher yields and better quality. However, the lack of Greek-certified seed sources and the reliance on foreign seed suppliers is a serious problem considering that local germplasm can perform well under suitable agronomic management. In parallel, regional development frameworks, such as the 2021–2027 operational program for eastern Macedonia and Thrace, emphasize innovation and competitiveness in agrifood systems, creating opportunities for the integration of antioxidant-rich native species into bioeconomic value chains. This is aligned with the international trade trends of NUS, and their products, that have been continuously growing due to the increasing demand from the pharmaceutical, cosmetic, and food industries [34]. Consequently, the systematic evaluation of locally adapted wild species is considered an essential step towards domestication which can support both sustainable cultivation and regional economic diversification.

The aim of the present study was to develop germination protocols for eighteen native, and potentially underutilized plant species, selected from the literature for their documented potential to contain bioactive compounds, such as antioxidants. Since germination constitutes the initial stage in any domestication and cultivation process, the development of reliable and replicable germination protocols is considered a critical first step to ensure seedling production, field cultivation, and evaluation of their antioxidant capacity in man-made environments. This initial step can set the base for assessing the suitability of these species to be integrated into bio-economic value chains.

2. Materials and Methods

2.1. Species Selection and Seed Collection

The present study focuses on native and potentially underutilized plant species from the European, Mediterranean, Balkan, and Greek flora, all occurring in northeastern Greece; a region characterized by Mediterranean climatic and ecological conditions with continental features. The selection of the species was based not only on their documented antioxidant potential but also on their local provenance considering that the ecological adaptation of species to their cultivation environment can enhance their successful agronomic use [35]. Moreover, each species was sampled from a minimum of three geographically distinct subpopulations, ensuring enough genetic diversity for reliable protocol development. In addition, each species needed to produce an abundant seed supply to ensure sufficient material for experimental treatments. Finally, only species that are neither rare, threatened, nor legally protected were selected, to avoid any negative impact on natural populations. The collected species along with a summarization of their documented antioxidant activity are shown in Table 1.

Table 1. List of the focal plant species of the current study along with a summary of their documented antioxidant activity.

Species	Family	Activity of Interest	Description	References
Achillea crithmifoliaWaldst. and Kit.	Asteraceae	Enhancement of CAT, GPx, SOD enzymes	Higher activity of CAT, GPx, and SOD enzymes in leukocytes compared to other Achillea species	[36–38]
		Reduction in lipid peroxidation (LPO)	High levels of phenolics such as caffeoylquinic acids and sesquiterpene lactones reduce lipid peroxidation, protecting cell membranes	[37,38]
		Rich phenolic profile	Contains caffeoylquinic acid derivatives, flavonoids (such as luteolin, apigenin), and sesquiterpene lactones with strong antioxidant activity	[37,38]
Achillea millefolium L.	Asteraceae	Free radical scavenging, reduces intracellular ROS levels	Strong DPPH/ABTS scavenging, ROS reduction, activation of SOD, CAT, GPx, protection of DNA and membranes	[39,40]
Epilobium angustifolium L.	Onagraceae	ROS scavenging, inhibition of enzymatic systems, high ellagitannin content	Oenothein B and flavonoids scavenge ROS, inhibit xanthine oxidase, DPPH/ABTS scavenging, pectin scavenging	[41–43]
Geranium macrorrhizum L.	Geraniaceae	ROS scavenging	Quercetin glycosides and phenolic acids scavenge free radicals (DPPH, ABTS, superoxide, H_2O_2)	[44]
Geranium sanguineum L.	Geraniaceae	ROS scavenging, cell membrane protection	Standardized Polyphenolic Complex (PC) scavenges ROS ($O_2^{-}\cdot$, H_2O_2), reduces TBARS, enhances SOD and TAA, and protects cell membranes	[45]
Hypericum olympicum L.	Hypericaceae	Acylphloroglucinol metabolites	Strong DPPH/ABTS/FRAP activity from acylphloroglucinols (olympicins), despite low flavonoid content	[46]
Hypericum perforatum L.	Hypericaceae	ROS scavenging, inhibition of lipid peroxidation	ROS scavenging, lipid peroxidation inhibition, strong DPPH and TAC inhibition (~0.52 μg/mL, lipid peroxidation $IC_{50} \approx 0.0079$ μg/mL)	[47,48]
Hypericum maculatum Crantz	Hypericaceae	ROS scavenging, inhibition of lipid peroxidation	Isoquercitrin and other flavonoid glycosides scavenge free radicals (DPPH, ABTS, FRAP) and inhibit lipid peroxidation (LPO)	[49,50]
Potentilla recta L.	Rosaceae	ROS scavenging	Free radical scavenging (DPPH, SO_2-, NO) with compounds such as rutin, caffeic acid, ellagic acid	[51]

Table 1. *Cont.*

Species	Family	Activity of Interest	Description	References
Primula veris L.	Primulaceae	ROS scavenging	Rich in flavonoids and phenolics (quercetin, rutin, isorhamnetin, kaempferol); strong DPPH, ABTS, FRAP activity	[52–55]
Prunella vulgaris L.	Lamiaceae	ROS scavenging	Extract P 60 shows strong scavenging activity (DPPH, ABTS, FRAP), increases SOD, reduces MDA, while polysaccharides scavenge ROS	[56,57]
Pulsatilla helleri (All.) Willd.	Ranunculaceae	ROS scavenging	Rich in phenolics, flavonoids and anthocyanidins	[58]
Saponaria officinalis L.	Caryophyllaceae	ROS scavenging	Phenolics and flavonoids (protocatechuic acid, rutin, apigenin) scavenge ROS (DPPH, ABTS assays), reduce TBARS	[59]
Scutellaria altissima L.	Lamiaceae	ROS scavenging, inhibition of lipid peroxidation	ROS scavenging, AGEs formation inhibition, strong activity in DPPH, FRAP, and lipid peroxidation (LPO) inhibition	[60,61]
Stachys germanica L.	Lamiaceae	ROS scavenging	Free radical (ROS) scavenging, very low IC_{50} in DPPH and FRAP (~688 μM TE/mL). Activity correlates with total phenolic content (TPC) and caffeic acids	[62,63]
Teucrium montanum L.	Lamiaceae	ROS scavenging	Strong scavenging activity (DPPH, FRAP); protection of proteins and lipids; rich in phenolic acids (p-coumaric, caffeic, ellagic, chlorogenic)	[64,65]
Thymus thracicus Velen.	Lamiaceae	ROS scavenging	Strong scavenging activity; increase CAT and SOD activity and the antioxidant capacity of the THP-1 cells; rich in phenolics, thymol, and linalool	[66,67]
Thymus praecox Opiz	Lamiaceae	ROS scavenging	Strong scavenging activity; neutralize DPPH radicals; rich in phenolics, thymol, and linalool	[67,68]

Most of the species were collected from Mount Falakron in northeastern Greece. The climate of Mount Falakron is Mediterranean at low altitudes and becomes continental at higher elevations. The mid-altitude zone represents a transitional area between Mediterranean and continental conditions. Several species were collected from this zone, including *Achillea crithmifolia*, *Achillea millefolium*, *Primula veris*, *Saponaria officinalis*, *Scutellaria altissima*, *Teucrium montanum*, and *Thymus praecox*. Other taxa, such as *Hypericum maculatum*, *Geranium macrorrhizum*, *Geranium sanguineum*, *Prunella vulgaris*, *Pulsatilla halleri*, *Stachys germanica*, and *Thymus thracicus*, were collected from the high-altitude zone of Mount Falakron. Two species, *Hypericum perforatum* and *Potentilla recta*, were collected from the low-altitude zone. Finally, *Epilobium angustifolium* and *Hypericum olympicum* were collected from the high-altitude zone of the Rhodope Mountain range, which is characterized by a fully continental climate. Mature inflorescences were collected from early July to early fall 2023. After collection, the material was dried at room temperature and seeds were extracted, cleaned, and counted.

Seed lots were subsequently stored under ambient laboratory conditions, approximately 20 ± 2 °C, 40–50% relative humidity, and natural dark–light cycles, for about two months until the initiation of germination experiments in late fall.

2.2. Seed Viability Testing

Seed viability level is crucial for developing a reproduction protocol, since it reveals the reproduction potential. Viability was determined using the tetrazolium chloride (TTZ) test, a relatively simple, rapid, and reliable method used prior to the typically lengthy germination test to identify metabolically active tissue, indicating a viable embryo capable of germination [69]. Four random samples (i.e., repetitions) of 25 seeds each were used for the estimation of seed viability for each species. Initially, the seeds were soaked in water for 12 h and then the testa of each seed was abraded with a dissecting needle, and the embryos were removed under a stereoscope. Seeds were grouped into the following two categories during seed dissection: filled (contained an embryo) and empty (without any gametophytic tissue). Empty seeds or seeds with atrophic embryos were considered non-viable. The viability of the extracted intact embryos was determined by staining them with a 1% *w*/*v* tetrazolium chloride solution (abbr. TTZ) [70]. Metabolically active tissue (so-called viable tissues) is stained red in the tetrazolium chloride solution [71]. Consequently, the percentage of non-germinable seeds is the sum of the percentages of empty seeds and those with non-viable embryo.

2.3. Seed Dormancy and Pre-Treatments

To release any potential dormancy, seeds underwent specific pre-treatments that were designed to break both physical and physiological dormancy. The treatments were selected based on both the literature review for the selected taxa (Table 2) but also on their simplicity and feasibility in common nursery settings. These included:

- Cold stratification at 0 ± 1 °C for up to 6 months. CS is a widely used method for species with physiological dormancy. Seeds were put on moist sand and germination was monitored weekly.
- Hormonal treatments: Seeds were soaked for 48 h in either gibberellic acid (GA_3) or kinetin (KIN) solutions at concentrations of 500, 1500, or 2500 ppm. Seeds were imbibed in 5 mL of solution, between Whatman filter papers, in a 9 cm Petri dish. Stock solutions were diluted using HPLC-grade water, and pH was adjusted to near-neutral for kinetin treatments, which required NaOH for dissolution.

Following viability testing and pre-treatments, seeds were sown in Petri dishes filled with sterilized sand. Each treatment was tested with 4 replicates of 50 seeds per species.

Germination tests were conducted in a controlled-environment chamber under alternating temperature: 25 °C/15 °C, 12 h light/12 h dark. The chamber's conditions were selected based on late spring and early summer or early fall normal weather patterns at the species' occurrence sites, reflecting the conditions during the most suitable period for seed germination and seedling establishment [29,72]. Untreated seeds served as controls, under the same experimental design described above, to assess baseline germination potential and to evaluate the effectiveness of each pre-treatment. Germination was recorded every 7 days for 12 weeks. Germination was defined as radicle emergence $\geq$ 2 mm. When the germination test ended, the non-germinated seeds were dissected, and any empty seed left was removed. Germination percentages were corrected based on the total number of full seeds per repetition. Seedlings were transplanted into paper pots for further observation. Once seedlings reached approximately 1 cm in height, they were moved to larger containers and acclimatized outdoors under nursery conditions.

Table 2. Summarization of the available species-specific and/or genus-specific literature information on the documented dormancy types and seed pre-treatments used for germination concerning the focal species of the current study.

Genus	Species	Dormancy Type	Pre-Treatment	References
Achillea	*A.crithmifolia*	Physiological dormancy	Cold stratification	[73]
Achillea	*A. millefolium*	Likely physiological dormancy	Chilling (cold dry storage), light +alternating temperature regimes	[30,74,75]
Epilobium	*E. angustifolium*	Non-deep physiological dormancy	Dry after-ripening	[76,77]
Geranium	*G. macrorrhizum* *G. sanguineum*	Physical dormancy and physiological dormancy	Dry storage, cold stratification	[78,79]
Hypericum	*H. perforatum*	Non-deep physiological dormancy	Cold stratification, GA3 treatments	[80,81]
Potentilla	*P. recta*	Non-deep physiological dormancy	After-ripening, cold stratification	[82,83]
Primula	*P.veris*	Physiological dormancy	Cold-moist stratification	[84,85]
Prunella	*P. vulgaris*	Physiological dormancy	GA_3, cold stratification	[86]
Pulsatilla	*P. halleri*	Morphophysiological dormancy	Warm + cold stratification	[87]
Saponaria	*S. officinalis*	Physiological dormancy	Cold stratification, scarification	[88]
Scutellaria	*S. altissima*	Physiological dormancy	Cold stratification	[89]
Stachys	*S. germanica*	Physiological dormancy	GA_3, Kin, cold-moist stratification	[90,91]
Teucrium	*T. montanum*	Physiological dormancy	Cold stratification	[92,93]
Thymus	*T. praecox* *T. thracicus*	Physiological dormancy	Cold stratification, light	[30]

2.4. Data Analysis

Germination data were checked for normality and homogeneity using Shapiro–Wilk's and Levene's tests, respectively, and found to meet both assumptions. The effects of pre-treatments on seed germination were evaluated via one-way ANOVA, whereas differences in mean values were assessed using Tukey's HSD test at 5% significance level.

3. Results

In the current work, eighteen native plant species from northeastern Greece were subjected to germination tests under eight pre-treatments: control, cold stratification, GA_3 at three concentrations (500 ppm, 1500 ppm, 2500 ppm), and KIN at the same three concentrations. Table 3 presents the mean germination percentages for each treatment per species, along with the results of the post hoc analysis (Tukey's HSD, $p < 0.05$). The embryo viability for each species is also included. The viability test (TTZ) verified high embryo viability in all examined species, with values ranging from 70% (e.g., *Stachys germanica*) to 100% (e.g., *Achillea millefolium*, *Potentilla recta*) (Figure 1).

Geranium macrorrhizum and *G. sanguineum* showed no germination throughout and they are not included in Table 3. Germination percentages varied significantly between treatments and species (Table 3). The control treatment resulted in the highest germination percentage in 9 of the 16 species, namely *Hypericum* sp. (78.5–90%), *Potentilla recta* (81.5%), *Prunella vulgaris* (79.25%), *Scutellaria altissima* (64.75%), *Stachys germanica* (63.75%), *Thymus thracicus* (79.25%), and *T. praecox* (81.75%) (Table 3). However, in most species, the number of germinated seeds was lower than the number of viable seeds recorded in the TTZ test; the germination percentages were generally close to or slightly lower than the percentages recorded for embryo viability (Table 3).

Cold stratification improved germination only in *Achillea crithmifolia* and *A. millefolium* (71.5% and 72.25%, respectively) that were relatively close to the recorded embryo viability. GA_3 on the other hand enhanced dormancy release in species like *Epilobium angustifolium* (43.75%), *Primula veris* where seed germination rates were maximized (85.5%) after seed imbibition in 500 ppm GA_3, and *Teucrium montanum* which reached 62.75% seed germination at 1500 ppm GA_3 (Table 3, $p < 0.05$). Highly significant differences among GA_3 treatments were recorded in *Stachys germanica*, *Scutellaria altissima*, and *Achillea millefolium* (Table 3, $p < 0.05$).

Kinetin treatments had a generally lower impact, with *Pulsatilla helleri* and *Saponaria officinalis* being the only two species that showed increased seed germination (68.5% and 32%, respectively) with a more profound effect on *P. helleri* (Table 3, $p < 0.05$).

Based on the above results, specific seed pre-treatments are recommended, tailored for each species according to the suspected dormancy type (Table 4).

Table 3. Seed germination (±SD) and viability percentages of the tested species under the applied treatments (GA_3 and Kin at 500–2500 ppm, cold stratification-CS and control). Germination conditions in the growth chamber entailed alternating temperature of 25 °C/15 °C D/N, and photoperiod of 12 h light/12 h dark.

Species	$GA_3$500	$GA_3$1500	$GA_3$2500	Kin500	Kin1500	Kin2500	CS	Control	Viab.
Achillea crithmifolia	18.50 ± 1.658 f	25.75 ± 2.287 ef	38.50 ± 2.398 cd	32.00 ± 1.155 ed	62.00 ± 0.707 b	41.25 ± 1.702 c	**71.50 ± 1.190 c**	41.75 ± 1.109 d	80
Achillea millefolium	15.75 ± 1.887 e	28.75 ± 2.562 d	41.50 ± 3.797 c	35.50 ± 2.533 cd	58.00 ± 2.799 b	39.00 ± 0.913 bc	**72.25 ± 1.750 a**	42.50 ± 1.848 c	100
Epilobium angustifolium	24.00 ± 2.415 c	**43.75 ± 2.213 a**	21.50 ± 2.102 c	36.00 ± 0.707 ab	5.25 ± 3.966 d	2.00 ± 0.408 d	18.00 ± 2.828 c	25.25 ± 1.652 bc	100
Hypericum maculatum	67.25 ± 0.946 b	40.25 ± 1.109 b	3.00 ± 1.080 e	21.00 ± 2.041 d	21.75 ± 1.652 d	14.75 ± 2.955 d	52.75 ± 1.031 c	**84.50 ± 2.598 a**	100
Hypericum olympicum	74.75 ± 0.854 b	38.75 ± 0.479 d	4.75 ± 0.854 f	18.75 ± 0.750 e	21.00 ± 0.707 e	13.00 ± 4.564 ef	51.00 ± 1.683 c	**90.00 ± 1.225 a**	100
Hypericum perforatum	70.00 ± 1.780 b	42.50 ± 1.708 d	1.00 ± 0.408 f	18.75 ± 1.974 e	23.75 ± 1.652 e	17.75 ± 2.869 e	51.50 ± 0.866 c	**78.50 ± 1.848 a**	80
Potentilla recta	13.25 ± 1.702 bc	18.75 ± 1.887 bc	10.50 ± 1.658 c	22.00 ± 3.162 b	18.50 ± 1.708 bc	14.50 ± 1.555 bc	20.50 ± 2.062 b	**81.50 ± 1.708 a**	100
Primula veris	**85.50 ± 1.708 a**	53.00 ± 1.683 bc	47.00 ± 1.581 bc	43.50 ± 1.041 c	42.00 ± 0.707 b	19.25 ± 1.315 d	19.00 ± 2.646 d	47.00 ± 1.581 bc	90
Prunella vulgaris	49.50 ± 0.645 b	29.75 ± 1.250 c	32.00 ± 1.414 c	7.00 ± 1.225 e	4.50 ± 2.630 e	9.00 ± 0.707 e	17.00 ± 1.780 d	**79.25 ± 2.428 a**	90
Pulsatilla halleri	53.50 ± 1.323 b	35.50 ± 1.041 c	34.25 ± 1.109 c	**68.50 ± 2.327 a**	1.50 ± 0.866 e	8.50 ± 1.500 d	4.25 ± 1.652 de	49.25 ± 1.601 b	80
Saponaria officinalis	0.00	1.75 ± 0.479 c	1.50 ± 0.289 c	**32.00 ± 3.317 a**	12.00 ± 3.367 b	12.25 ± 0.250 b	2.25 ± 1.601 c	0.00	80
Scutellaria altissima	34.50 ± 1.258 b	0.00	0.00	22.50 ± 0.289 c	11.50 ± 2.533 d	0.00	31.25 ± 1.109 b	**64.75 ± 1.702 a**	70
Stachys germanica	0.00	62.25 ± 1.797 a	56.25 ± 1.652 ab	11.75 ± 1.652 c	46.75 ± 3.425 b	49.50 ± 1.190 b	15.00 ± 3.342 c	**63.75 ± 1.601 a**	70
Teucrium montanum	35.25 ± 2.562 b	**62.75 ± 1.652 a**	60.25 ± 3.092 a	17.00 ± 2.121 c	20.25 ± 1.702 c	34.75 ± 1.377 b	18.00 ± 3.240 c	2.50 ± 0.289 d	100
Thymus thracicus	16.50 ± 1.555 cd	14.50 ± 1.708 d	13.50 ± 2.062 d	29.75 ± 1.377 b	22.75 ± 1.493 bc	29.25 ± 1.250 b	10.00 ± 1.958 d	**79.25 ± 1.250 a**	100
Thymus praecox	15.75 ± 0.479 e	16.25 ± 0.854 de	11.50 ± 1.756 e	36.25 ± 1.031 b	21.75 ± 1.109 d	30.50 ± 0.957 c	11.25 ± 1.652 e	**81.75 ± 1.250 a**	100

Values within each row followed by the same letter are not significantly different ($p < 0.05$). Cells in bold indicate the cases where the highest germination percentages observed for each focal species.

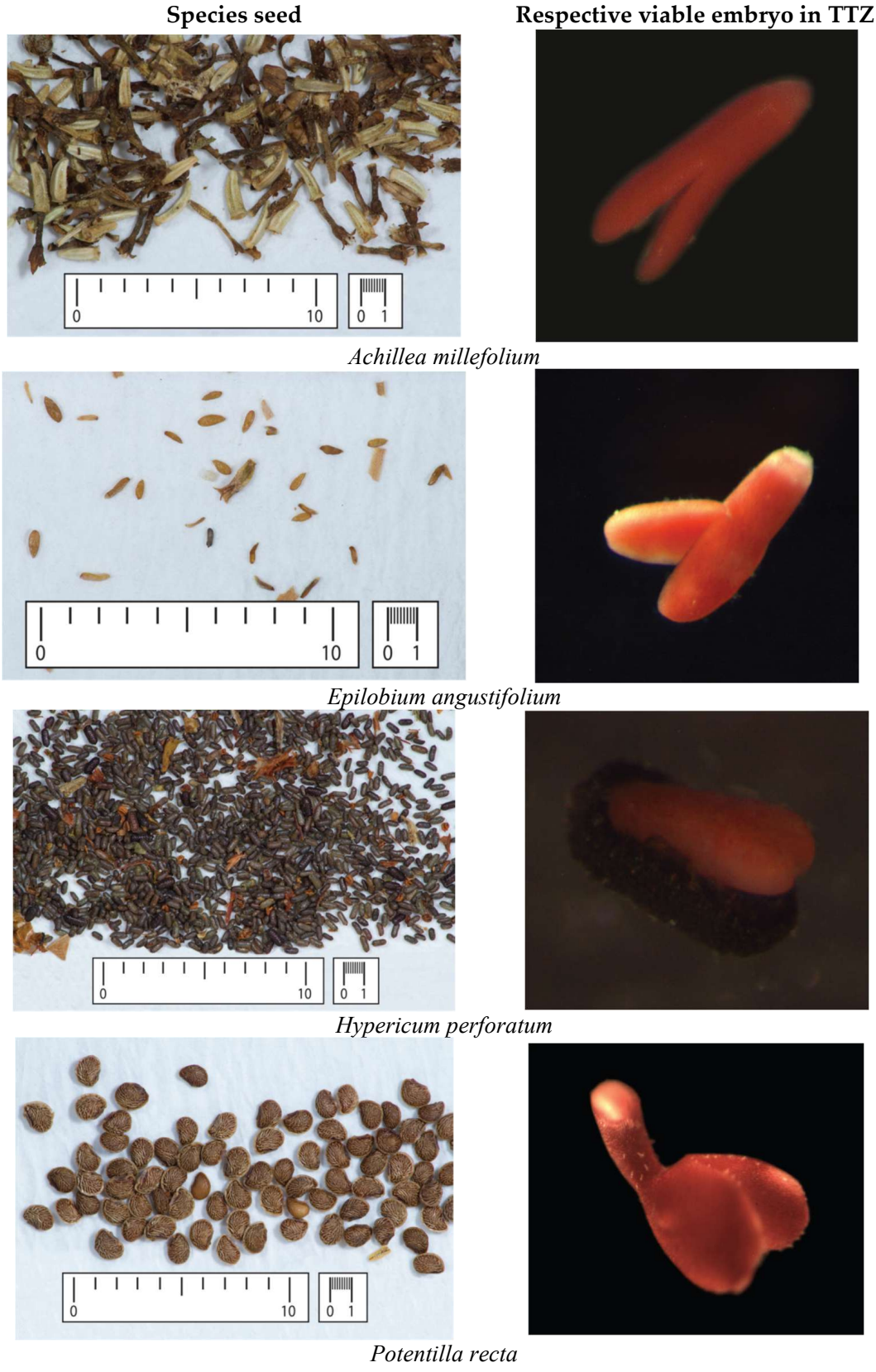


Figure 1. *Cont.*

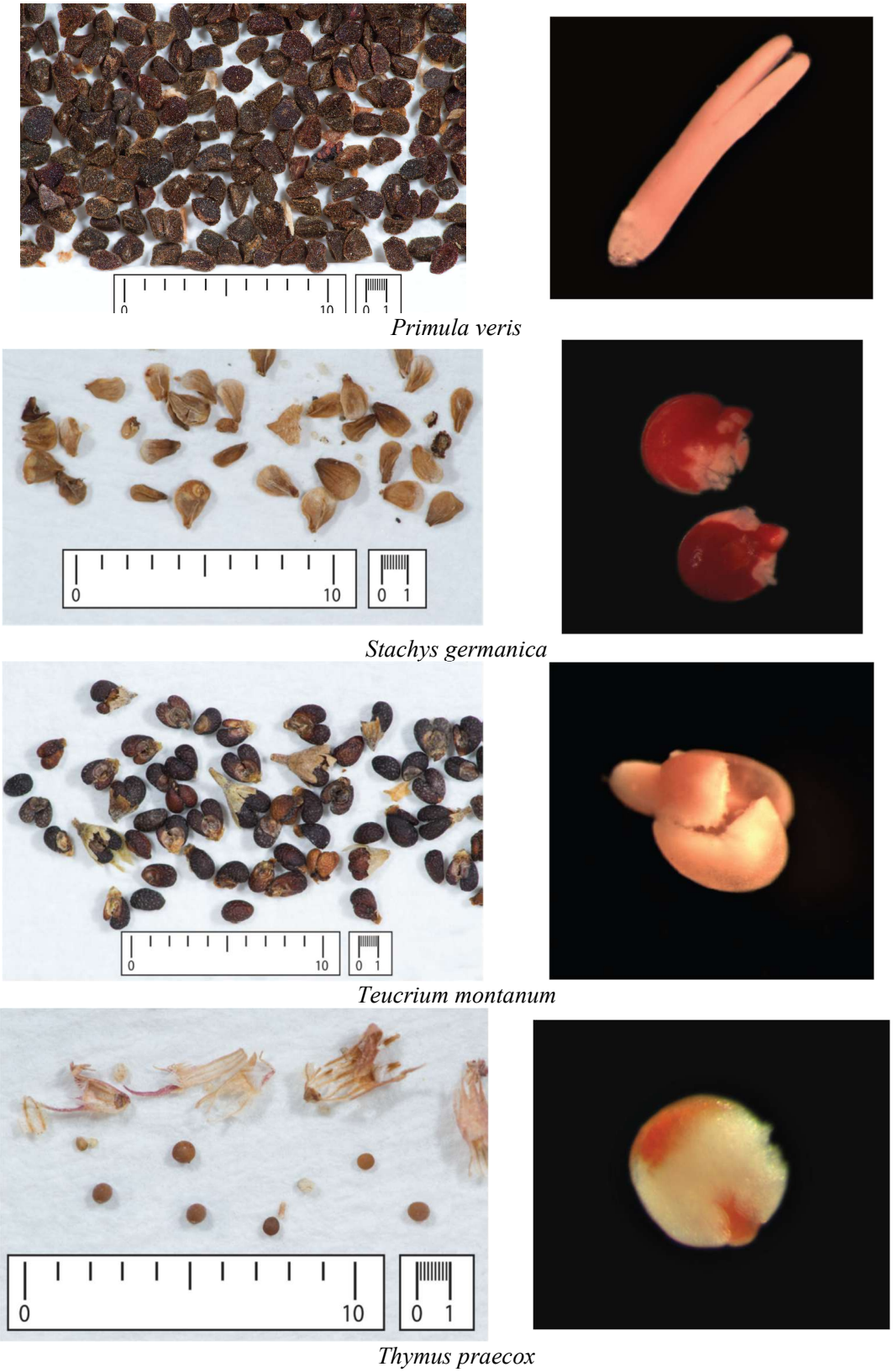


Figure 1. Indicative stereoscope photos of species' cleaned seeds (**left**) with their respective cases of viable embryos stained with 1% *w*/*v* tetrazolium chloride (**right**) for eight focal species. Embryos that are stained red are viable, whereas embryos not stained are considered non-viable. The species presented are (from top to bottom) *Achillea millefolium*, *Epilobium angustifolium*, *Hypericum perforatum*, *Potentilla recta*, *Primula veris*, *Stachys germanica*, *Teucrium montanum*, and *Thymus praecox*.

Table 4. Summarization of seed dormancy types observed in the current study and recommended pre-treatments based on the germination results for each of the focal species.

Species	Dormancy Type	Pretreatment
Achillea crithmifolia	Physiological	Stratification
Achillea millefolium	Physiological	Stratification
Epilobium angustifolium	Physiological	GA3_1500
Hypericum maculatum	Physiological	After-ripening
Hypericum olympicum	Physiological	After-ripening
Hypericum perforatum	Physiological	After-ripening
Potentilla recta	Physiological	After-ripening
Primula veris	Physiological	GA3_500
Prunella vulgaris	Physiological	After-ripening
Pulsatilla halleri	Physiological	GA3_500
Saponaria officinalis	Physiological	Kin_500
Scutellaria altissima	Physiological	After-ripening
Stachys germanica	Physiological	After-ripening
Teucrium montanum	Physiological	GA3_1500
Thymus thracicus	Physiological	After-ripening
Thymus praecox	Physiological	After-ripening

4. Discussion

Provenance plays a significant role in plant establishment and performance, particularly in stress-prone environments such as those of semi-arid Mediterranean climates. Locally adapted genotypes often present higher fitness in conditions like their occurrence range as they often possess adaptive traits [94]. The use of such locally adapted planting material can improve establishment success, reduce crop failures, and minimize the need for intensive management inputs [95]. Therefore, they are usually more suitable for cultivation and bio-economic applications close to their native range [96].

Dormancy provides multiple benefits to seeds [30]. The employed strategy by many plant species is that dormancy release and seed germination occur with the onset of the season during which the risks for seedling death are lowest, conditions for seedling growth are optimal, and therefore seedling establishment is maximized [30]. Numerous studies conducted at different scales, from microhabitats to biomes, have demonstrated that changes from unfavorable to favorable environments for germination can trigger dormancy release [97].

Physiological dormancy is the most common type of dormancy, especially in relatively drier climates and with an increasing range of seasonal temperature variation [98], such as the Mediterranean climate. This type of climate is characterized by high seasonality with hot and dry summers and cold-wet winters. On the other hand, plant reproduction must occur during the period with favorable conditions. Therefore, germination of low-altitude species occurs in the wet season, in fall, and the growing season ends just before the onset of summer. For species within this ecological context, dry after-ripening may be considered a favorable seed treatment for successful germination ex situ [99].

On the other hand, Mediterranean mountain weather presents winter frost at high elevations. However, its duration is usually short. Plants of high-altitude environments usually produce seeds that need overwintering for dormancy release to germinate in the spring following seed dispersal [100]. In these conditions, the favorable period for regeneration is soon after snowmelt in spring/early summer [101]. However, seeds of this origin may germinate readily ex situ, without any treatment, as they seem to be physiologically prepared for rapid germination [99]. A modified germination strategy is

also apparent in high-altitude Mediterranean plants which are generally positively affected by a cold-wet stratification pre-treatment [101].

The sixteen native and potentially underutilized plant species that were used in the present experiment presented clear differences in seed dormancy types. For most species, the highest germination percentages were recorded in the control, suggesting that a period of after-ripening was sufficient to overcome dormancy. After-ripening, the dry storage of seeds at room temperature, is a well-documented mechanism for breaking non-deep physiological dormancy. After-ripening usually causes physiological and biochemical changes by reducing embryo-growth inhibitors, such as abscisic acid (ABA), which gradually release dormancy [30,31]. In accordance with these ecological patterns, the species studied exhibited dormancy-breaking requirements that closely reflected their altitudinal origin. Low-altitude species, such as *Hypericum perforatum* and *Potentilla recta*, presented high germination percentages after simple after-ripening, indicating non-deep physiological dormancy as referred by Picciau et al. [99]. Seeds of species collected in mid-altitude also presented physiological dormancy which, according to the results, may have been deeper in some cases. *Scutellaria altissima* and *Thymus praecox* presented high germination percentages without any treatment, implying that after-ripening was sufficient to overcome dormancy. On the other hand, *Achillea crithmifolia* and *A. millefolium* demanded cold stratification to promote germination. In addition, some species required hormonal treatment for dormancy release (*Primula veris*, *Teucrium montanum*, and *Saponaria officinalis*). Seeds of species of higher altitudes, such as *Hypericum maculatum*, *Prunella vulgaris*, *Stachys germanica*, and *Thymus thracicus* also germinated successfully following after-ripening as referred by Rosbakh et al. [98]. However, *Epilobium angustifolium* and *Pulsatilla halleri* required hormonal treatment to break dormancy, indicating that their seeds have deeper physiological dormancy.

The current results suggest that in most of the species studied herein, dormancy release can be achieved without complex pre-treatments, which is a rather useful trait for agronomic exploitation. For example, several species in our study such as *Prunella vulgaris*, *Potentila recta*, and *Scutellaria altissima*, that have been previously described as possessing seed physiological dormancy [82,86], showed their highest germination in control treatment. Thus, according to the current results, seed dormancy in *P. vulgaris*, *P. recta*, and *S. altissima* was non-deep as simple after-ripening was sufficient to overcome it, making them particularly suitable for agronomic exploitation as they do not require costly or complex pre-treatments. *Hypericum maculatum H. olympicum*, *H. perforatum*, *Stachys germanica*, *Thymus thracicus*, and *T. praecox* also presented their highest germination in the control, in contrast with earlier reports describing physiological dormancy [80,81,90]. This response highlights the suitability of the above species for domestication, since they can be propagated easily, after short-time storage, and without additional pre-germination treatments.

On the contrary, *Achillea crithmifolia* and *A. millefolium* required cold stratification to maximize germination. Cold stratification, which simulates winter conditions, is effective for species with intermediate or deep-seed physiological dormancy. When seeds remain in low temperatures, ABA content is reduced while gibberellin biosynthesis is increased, triggering germination [31,102,103]. The *Achillea* species studied herein showed strong response to stratification, which reinforces their classification into deeper physiological dormancy classes and highlights the necessity of simulating winter conditions to achieve germination. This concurs with published reports, where physiological dormancy and responsiveness to stratification are well documented [73,74].

The use of various hormones as a pre-treatment has been adopted as a method of replacing the time-consuming stratification often required to initiate the germination of

physiologically dormant seeds. The hormones are naturally present in seeds and appear to be crucial for dormancy breaking and initiating germination [103]. It is commonly thought that dormancy release is due to an increase in levels of cytokinin (KIN) or gibberellic acid (GA_3) or both, although their precise mode of action is still unclear [104]. Gibberellins promote embryo growth potential [102] while cytokinins stimulate germination, in certain species by modulating ABA sensitivity and enhancing cell division [105]. However, imbibing the seeds in a hormone solution seems to have a positive effect on dormancy removal in several species, while, at the same time, germination is more uniform. It has been observed that cytokinins, in some cases, break seed dormancy and stimulate germination by counteracting the inhibitory effects of other hormones like ABA, which promotes dormancy [105]. This was particularly evident in species such as *Primula veris*, *Pulsatilla halleri* and *Teucrium montanum*, where GA_3 treatment significantly improved germination compared to the control and stratification treatments, although not always close to the observed frequencies of viable embryos. These results are consistent with deep physiological dormancy reported for these species [84,85,92,106–108], that cannot always be released effectively. On the contrary, although *Epilobium angustifolium* is referred to present non-deep physiological dormancy that can be released through after-ripening [76,77], in the current study it required GA_3 to achieve a relatively medium germination percentage compared to the embryo viability percentage.

On the other hand, *Geranium macrorrhizum* and *G. sanguineum* that presented zero germination in all treatments, clearly require more complex or yet unidentified dormancy-breaking treatments. This observation matches reports on the genus, where complex physical dormancy and physiological dormancy have been described [78]. Physical dormancy in seeds is typically broken by heat exposure, physical scarification (such as scratching or abrasion), or chemical scarification, which degrades the impermeable seed coat and allow water and gases to penetrate, initiating germination. Physiological dormancy, on the other hand, may require extended or yet undefined periods of cold stratification to be effectively released [30]. Similarly, *Saponaria officinalis* presented a relatively low germination percentage after immersion in 500 ppm KIN [88]. The reason for this is unknown as, to our knowledge, no published data are available on the germination requirements of *S. officinalis*. Further investigation is required to determine whether the species exhibits physical dormancy or a combination of physical and physiological dormancy. In such cases, pretreatments involving scarification (mechanical or chemical, or heat) to weaken the seed coat, should be tested prior to the application of cold stratification or hormonal treatments aimed at releasing both types of dormancy. Such species should be further investigated to be proposed for agronomic use, as their propagation remains challenging.

Although many species reached high germination frequencies, these values often remained lower than the viability percentages measured with the tetrazolium (TTZ) test (e.g., *Thymus* spp.) This indicates that a fraction of seeds may possess deeper levels of dormancy that was not released by the tested treatments. Seeds with such dormancy types can persist in the soil and contribute to the persistent seed bank, a strategy that spreads germination over the years [109].

From an agronomic point of view, species exhibiting high germination without any treatment can be considered strong candidates for cultivation, as they can be propagated efficiently without specialized infrastructure (nine species herein that reached high seed germination rates in the control are shown in Table 3). Similarly, species responding to GA_3 imbibition or stratification (e.g., *A. crithmifolia*) also have potential for agronomic use as the pre-treatments are easy, relatively cheap, not time consuming, and do not demand any specific infrastructure. Species whose seeds demand cold stratification need some simple equipment (fridge) but can also over-winter in the open.

The results of the present study demonstrate that several native and underutilized species from northeastern Greece present high germination potential and can therefore be prospective candidates for domestication and agronomic exploitation. This will enhance the aim of strengthening innovation and sustainability in the agri-food sector. Species that present high germination percentages in our trials can be prioritized for pilot cultivation and gradual integration into agri-food value chains. The use of locally adapted germplasm is of particular importance, as it enhances adaptation under the climatic conditions of a given region, such as eastern Macedonia and Thrace, northern Greece, in the current study. These species can contribute to regional development and competitiveness as well as to the locally adapted agronomic systems.

5. Conclusions

The present study reports the germination capacity of eighteen native plant species from northeastern Greece within the context of domestication and sustainable utilization of local phytogenetic resources. The integration of such species into cultivation systems, as locally adapted resources, could provide new opportunities to produce antioxidant-rich products, enhance regional agricultural diversification and support local economies.

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Abbreviations

The following abbreviations are used in this manuscript:

GA_3	Gibberellin
TTZ	Tetrazolium chloride
KIN	Kinetin/cytokinin
CS	Cold stratification
ABA	Abscisic acid

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