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Germination Kinetics of *Ferula communis* L. Seeds, a Potentially Multipurpose-Use Wild Species

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Abstract: Despite exhibiting intriguing features associated with its multipurpose applications and drought tolerance, *Ferula communis* remains a wild and uncultivated species, with limited experimental research on its biology, starting from seed germination and extending to its ecology. The purpose of this study was to investigate potential germination and kinetics in *F. communis* seeds in response to four cold stratification periods (0, 15, 45, and 90 days at a constant temperature of 5 °C) and four temperatures (5, 10, 15, and 20 °C) under continuous darkness. *F. communis* exhibited a pronounced germination potential exceeding 90%, with the optimal temperature for germination falling within the range of 5 °C to 15 °C, without necessitating cold stratification. A dramatic drop of the germination percentage was observed at 20 °C (<10%), suggesting a form of conditional dormancy attributed to the higher temperature tested.

Keywords: *Ferula communis* L.; germination kinetic; temperature; cold stratification

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1. Introduction

Ferula communis L. (giant fennel) is a perennial wild plant, belonging to the Apiaceae family, that grows naturally on the wasteland of the Mediterranean and Central Asia regions, blossoms in March and April, and goes dormant in early summer in dry climate conditions. It presents deep tap roots, clumps of leaves developing in winter and early spring, and a tall flowering stem (up to 3 m) with numerous yellow flowers clustered in umbels. *Ferula communis* has a fairly abundant production of seeds that varies in size and absolute weight of the seeds depending on the place of growth of the plant. Usually, they measure between 3 and 6 mm in length with an elongated and cylindrical shape, and a color that can vary, but they commonly appear dark brown or black. Seeds of the *Ferula* genus may exhibit dormancy, characterized by a hard seed coat that needs to be scarified or stratified to improve germination rates [1].

Ferula species have a long history of extract application in various medical and therapeutic contexts due to their well-documented significant biological activities. These extracts have been utilized in both human and veterinary practice to address a wide range of ailments, including headaches, digestive disorders, rheumatism, arthritis, and tumoral activity [2–7]. The biological activities of plant extracts from areal parts and roots were investigated in in vitro experiments involving several fungi, demonstrating fungitoxic effects on colony growth [8]. Historical records, dating back to the Roman period, document symbiotic relationships between *Ferula* species and other plants [9], notably with Pleurotus eryngii, a widely appreciated edible mushroom species [10]. Alternatively, some authors reported a toxic effect on animals and humans [11] and prenylcoumarin compounds were thought to be responsible for the toxic effects on sheep, goats, cattle, and horses [12]. Nevertheless, Arnoldi and coauthors [4] and subsequently Rubiolo and coauthors [13] differentiated two different chemotypes of *F. communis*, the 'nonpoisonous' and the 'poisonous', helping in interpreting the opposite effect and use described.

Traditional use of the stems is also reported for small furniture, but limited scientific studies are available on the properties of fibers. *Ferula*-plant-derived fillers have been used as an alternative reinforcing agent in composite materials due to its lightness, biodegradability, low cost, and renewability [14]. Recently, Touil and coauthors [15] investigated the potential integration of *F. communis* fiber into building materials to enhance their thermomechanical properties and reduce weight, thereby minimizing the utilization of landfills dedicated to this waste category. Potential use as an energy source was also proposed in bioethanol production [16].

All the aforementioned issues associated with *F. communis* suggest its potential valorization within the context of the new approach of the European Union's common policy for agriculture and environment aimed at maintaining rural areas and landscapes, in which farmers are protectors and promoters not only of the agro-environment but also of the natural habitat including wild species. Due to its rapid growth, the ability to tolerate drought linked to the deep root system and xerophytic adaptations, the widespread distribution in wasteland characterizing many areas of the Mediterranean basin, and its versatile use, *F. communis* appears as an interesting wild species worthy of study.

Despite the recognition of its multipurpose potential, *F. communis* largely remains an undomesticated and uncultivated species with limited experimental research on its biology starting from seed germination and its ecology. The germination traits of diverse accessions within the *Ferula* genus were examined, revealing complex germination cues characterized by the presence of dormancy mechanisms that inhibit germination until the requirement for cold stratification is fulfilled [17–21].

With this consideration in mind, we conducted an experimental trial on the seed germination of *F. communis* L., specifically focusing on germinability and its kinetics, to evaluate the optimal seed germination temperature and the effects of cold stratification in terms of duration.

2. Materials and Methods

2.1. Seed Material and Collection Site

We identified and collected mature seeds of *F. communis* from Etna Mountain (Pedara, 700 m asl—37°37′25″ N, 15°03′24.19″ E) during the late summer of 2023. The collection area is classified as Csa (warm temperate, dry and hot summer) following the Köppen and Geiger climate classification, with 14.4 °C as the average annual temperature and an annual precipitation of 595 mm.

In the region where the seeds were collected, the physiological maturity of seeds begins in late spring, and their natural dispersal occurs during the summer and early autumn periods, characterized by acropetal kinetics of the composed umbels. In late July 2023, seeds were collected from disseminating flowers, selecting bottom simple umbels. Immediately after collection, seeds were air-dried for 1–2 days and then stored at room temperature (20 ± 2 °C) in paper bags until the start of the germination experiment. Immediately preceding with the germination test, seed surface disinfection was performed by immersing them in a 5% sodium hypochlorite (NaOCl) solution for 5 min, followed by two rinses with demineralized sterile water. No fungicide treatment was performed to avoid possible interference of the active ingredient on germination.

2.2. Germination Test

The experimental treatments were as follows: firstly, seeds were subjected to four different levels of cold stratification (0, 15, 45, and 90 days) at a constant temperature of 5 °C and a relative humidity of 70% (±2%); then, seeds were maintained in a cabinet germinator (KW-Apparecchi Scientifici, Italy) at four distinct temperature levels (5, 10, 15, 20 °C) in a condition of continuous darkness. The experiments were arranged in a completely randomized design with three replications per thesis. Each replication involved thirty seeds arranged in Petri dishes containing double layered Whatman No. 1 filter paper, moistened with 5 mL of distilled water. Sterilized–distilled water (maintained at the same

treatment temperature) was added as required to ensure non-limiting moisture conditions for germination.

Germinated seeds were counted and removed every 24 h for 40 days. Seeds were considered germinated when a primary radicle protrusion 2 mm long was observed, according to the guidelines of the International Seed Testing Association [21].

2.3. Statistical Analysis

At the end of the trial, the final germination percentage (FGP) was computed. A factorial 2-way (cold stratification × temperature) ANOVA model was performed. When ANOVA indicated a significant effect, differences among the combination of treatments were tested with the Tukey multiple comparison test (HSD test) using the CoStat 6.4 statistics software package. Percentage data were transformed in arcsen before the statistical analysis, whereas data included in the tables represent original values.

To study seed germination kinetics, we plotted the cumulated number of germinated seeds over time and fitted the derived data modifying the three-parameter logistic curve proposed by P. F. Verhulst (Verhulst 1838, 1845) as follows:

$$y = a/(1 + \exp(-(x - x0)/b))$$

This function allowed the direct biological interpretation of germination behavior and generates cumulative estimates of seed germination.

$$G_{cum}(t) = \frac{G_{max}}{1 + e^{-(\frac{t-t_{50}}{b})}}$$

where $G_{cum}(t)$ is the cumulative percentage of germination at time t (days); G_{max} is the asymptotic final germination percentage value at $t \to +\infty$; t_{50} is the inflection point where G_{cum} equals half of G_{max}, representing the time, in days, to reach 50% of germinated seeds; and b is the slope, a dimensionless "shape factor," which primarily controls the steepness of the germination curve (Figure 1).

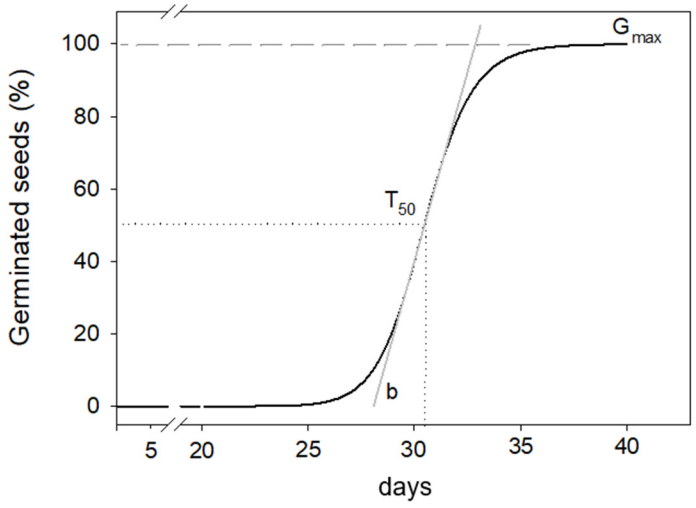

Figure 1. Three parameters' sigmoid curve chosen to interpolate percentage of germinated seeds over time.

The obtained curves were compared, applying the extra sum of the square F test. When the null hypothesis was rejected, the best-fit values of each obtained parameter

(G_{max}, t_{50}, and b) were compared with a t test using GraphPad 6.0 (GraphPad Software Inc., San Diego, CA, USA):

$$t = \frac{|\alpha - \beta|}{\sqrt{SE\alpha^2 + SE\beta^2}}$$

where the numerator is the difference between best-fit values of the target parameter for two specific theses and the denominator is an estimate of the standard error of that difference, computed as the square root of the sum of the squares of the two standard error values.

The three parameters' sigmoid curve chosen for this experiment, and widely adopted for a germination test, is affected by the lack of information on some key phases of the germination process. Based on the experimental dataset, we computed the following intervals: (i) the initial stage encompassing imbibition and the lag phase (IS-LP), extending from the experiment's onset to the date of the first seed germination, and (ii) the interval between the end of the lag phase and the date when the last seed germinated (LP-LSG), indirectly inferred through the dimensionless "shape factor" b. The ANOVA analysis was performed on obtained data.

3. Results

The analysis of variance for the final germination percentages (Table 1) showed significant differences exclusively due to temperature. The comparison of the mean values for the different levels elucidated that the observed result was mainly related to the germination failure observed under the highest temperature (10% of germinated seeds at 20 °C).

Table 1. Result of two-way ANOVA for the effects of cold stratification and temperature.

Source of Variation	FGP		IS-LP		LP-LSG	
	F Values	*p*	*F Values*	*p*	*F Values*	*p*
Cold Stratification (CS)	1.67	ns	n.d.		n.d.	
Temperature (T)	113.14	***	7.22	*	7.22	ns
Interaction (CS × T)	0.87	ns	n.d.		n.d.	

*, *** Significant at the 0.01 and 0.001 probability level, respectively; ns = not significant; n.d. = not detected. FGP: final germination percentage; IS-LP: duration of the initial stage (imbibition and lag phase); LP-LSG: duration of the interval between the date when the last seed germinated and the end of the lag phase.

Conversely, 94% of germinated seeds were counted, averaging the remaining temperature levels (Table 2).

Table 2. Final germination percentage and phase durations computed from experimental dataset of *Ferula communis* seeds.

Temperature	FGP	IS-LP	LP-LSG
	%	days	days
5 °C	96.7 [a]	27.0 [a]	27.0
10 °C	90.0 [a]	18.0 [b]	18.0
15 °C	96.7 [a]	20.0 [b]	20.0
20 °C	10.0 [b]	--	--

Means followed by the different letters in each column are significantly different based on the Tukey test at the 0.05 probability level. FGP: final germination percentage; IS-LP: duration of the initial stage (imbibition and lag phase); LP-LSG: duration of the interval between the date when the last seed germinated and the end of the lag phase.

Considering the final germination percentage (FGP) as the principal variable in a germinability assessment and noting the absence of a discernible response to stratification for FGP, the investigation of germination kinetics has focused exclusively on temperature-related data. Furthermore, the low number of seeds germinated at the highest level of temperature (20 °C) conflicts with the biological interpretation of the sigmoid curve

parameters. Consequently, the curve interpolation was restricted to datasets corresponding to temperatures of 5, 10, and 15 °C.

The extra sum of the square F test showed statistical differences between the interpolated curves for the three temperature levels (Figure 2).

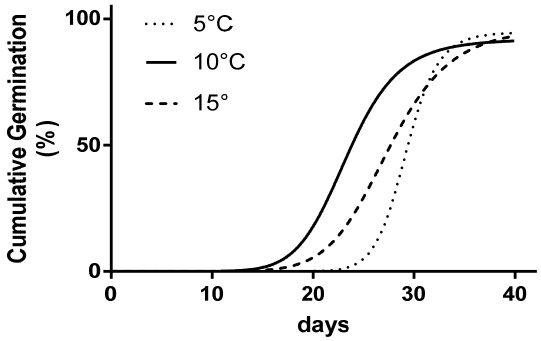

Figure 2. The comparison of three parameters' sigmoid curves fitting the three datasets (5, 10, and 15 °C) where seed germination kinetics has been studied.

From the analysis of the single parameters of each curve (Table 3), no differences emerged comparing the asymptote G_{max} values (94.4%), whereas for T_{50}, the extreme values were achieved at 10 °C (23.4 days) and 5 °C (29.2 days), respectively. The lowest temperature obtained the highest b (18.4), twofold higher when compared to the other levels, and the longest duration of the initial stage.

Table 3. Sigmoid curve parameters (mean ± standard error).

Temperature	G_{max}		T_{50}		b	
	%	s.e	days	s.e		s.e
5 °C	94.7	±1.67	29.2	±0.15 [a]	18.4	±1.46 [a]
10 °C	91.9	±1.98	23.4	±0.26 [b]	9.1	±0.80 [b]
15 °C	96.6	±2.97	27.5	±0.33 [ab]	8.9	±0.73 [b]

G_{max} is the asymptotic final germination percentage value; t_{50} is the inflection point where the cumulative percentage of germination equals half of G_{max}; b is the slope, a dimensionless "shape factor". Means followed by the different letters in each column are significantly different based on the Tukey test at the 0.05 probability level.

4. Discussion and Conclusions

Cold stratification is a vital mechanism for various species in temperate regions, where winters are cold, ensuring the timely cessation of dormancy when favorable conditions for seedling emergence are established after the winter season [22,23]. In the Mediterranean climate condition, characterized by mild/wet winters and hot/dry summers, the temperature plays a critical ecological role, governing the germination dynamics, ensuring that seeds remain dormant during the hot and dry summer months, thereby synchronizing their germination with the cooler and moister conditions, typically experienced during the winter season.

Our results emphasize that the *F. communis* exhibits high germination potential (>90%) within an optimal temperature range of 5 to 15 °C and does not require cold stratification for germination. However, it germinates at significantly lower percentages at 20 °C (10%), indicating a kind of conditional dormancy. These conditionally dormant seeds became nondormant the following winter, when lower temperatures and increased moisture levels signal the ideal condition for germination and seedling emergence [24–26].

Limited researchers addressed the germination of the wild genus of *Ferula*, whereas many authors observed primary seed dormancy in the *Apiaceae* family and reported a positive effect of cold stratification on germination [27–30]. In particular, on the *Ferula* genus, Nikolaeva [1] reported a cold stratification requirement of some species for germination

and the effective temperature for this requirement was 0–3 °C. On the contrary, Aghilian and coauthors [30] stated that pre-chilling had no effect on seeds of *Ferula gummosa* Boiss dormancy breaking. Even fewer researchers focused on *F. communis*, and contrasting results have been obtained. Ari and coauthors [31] observed higher germination in *F. tingitana* L. (60%) than *F. communis* (3%) and hypothesized that *F. communis* might have seed dormancy and need prechilling treatment, but no specific trials have been described in their paper. On the contrary, Sanna and coauthors (2009, cit. by Dettori et al. [32]) reported a high germination percentage of *F. communis* (higher than 80%) in a temperature range of 10–15 °C without prechilling treatment. Our experiment's findings, involving an even lower temperature (5 °C), provided further support for this hypothesis. Additionally, our analysis of germination kinetics revealed a specific impact of the coldest temperature, in particular on the duration of the period when radicle protrusion is observed. The significantly higher value of b calculated at 5 °C, reflecting the interval between the initial and final primary radicle protrusions, suggests a higher synchronicity of the protrusion phase at lower temperature. This result should be stated even though the ANOVA, conducted on the duration of the LP-LSG interval in response to germination temperatures, failed to define this difference (Table 2), likely due to the large variation observed in the different replications of 15 °C treatment. In addition, the dramatic drop in the germination percentage observed at 20 °C represents, as well, a relevant result of our experiment. Assuming the optimum range of temperature usually imposed in germination trials (20–25 °C), the sensitivity stated in our study could explain the very low values reached in many of the studies concerning this species or related genus. In addition, the positive effect on the germination of priming treatment reported by several authors in *Ferula* spp. suggests the opportunity to evaluate if the high percentage of non-germinated seeds observed at 20 °C remains viable in a soil seed bank for the following season.

In summary, our research provides valuable insights into managing *Ferula communis* L. seed germination, crucial for biodiversity conservation under the Mediterranean climatic conditions. However, it is imperative that future studies delve deeper into elucidating the underlying mechanisms governing seed germination activities, as well as the associated physiological processes. Such investigations aim to devise strategies for enhancing and standardizing germination rates.

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