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# The Concepts of Seed Germination Rate and Germinability: A Re-Evaluation for Cool-Season Grasses

**Wagdi Ghaleb [1,2], Lina Q. Ahmed [1,3], Marie-Hélène Wagner [4], Annie Eprinchard-Ciesla [1], Wendy E. Olivares-Rodríguez [1], Cédric Perrot [1], Karine Chenu [5], Mark Norton [6] and Abraham J. Escobar-Gutiérrez [1,*]**

1 INRAE, UR4 P3F, Unité Pluridisciplinaire Pairies et Plantes Fourragères, Le Chêne-BP 6, F-86600 Lusignan, France; wagdi.ghaleb@inrae.fr (W.G.); ahmedlin.inra16@gmail.com (L.Q.A.); annie.eprinchard@inrae.fr (A.E.-C.); wndy10@hotmail.com (W.E.O.-R.); cedric.perrot@inrae.fr (C.P.)
2 Biotechnology Research Centre, Tripoli P.O. Box 30313, Tajoura, Libya
3 Department of Field Crops, College of Agriculture, University of Salhaddin, Kirkuk Road, Erbil 44001, Iraq
4 GEVES, Station Nationale d'Essais de Semences, 49071 Beaucouzé, France; marie-helene.wagner@geves.fr
5 Queensland Alliance for Agriculture and Food Innovation (QAAFI), The University of Queensland, 203 Tor Street, P.O. Box 102, Toowoomba, QLD 4350, Australia; karine.chenu@uq.edu.au
6 Agricultural Institute, New South Wales Department of Primary Industries, Pine Gully Road, Wagga Wagga, NSW 2650, Australia; mark.norton@dpi.nsw.gov.au
* Correspondence: abraham.escobar-gutierrez@inrae.fr; Tel.: +33-5-4955-6184; Fax: +33-5-4955-6068

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**Abstract:** Temperature is one the most influential environmental factors for the germination and establishment of grass species. The specific objective of this study was to determine the effects of low constant temperature on the time needed to express the full germination capacity of nondormant seedlots. Fifteen accessions, comprising seven of *Lolium perenne* L., three of *Festuca arundinacea* Schreb., three of *Dactylis glomerata* L. and two of *Triticum aestivum* L., were evaluated at constant temperatures of 5 and 21 °C. As expected, the germination rates were faster at 21 °C than at 5 °C. Indeed, at 5 °C seeds needed up to twenty-one times longer to reach the maximum germination than when tested at 21 °C. The genotypic variability found for the ratio of germination rates between the two temperatures (i.e., germination rate at 5 °C/germination rate at 21 °C) was much more variable than what is found in the literature for perennial cool-season grasses. On the other hand, in most cases, no significant differences were observed in the germinability (the capacity to germinate) response to 5 °C and 21 °C. Within the four species, twelve of the fifteen studied accessions expressed the same germinability at 5 °C and 21 °C, when given enough time. Only three accessions had final germination percentages higher at 21 °C than at 5 °C. Our results suggest that, in general, nondormant seeds at low temperatures germinate as well as nondormant seeds at near-optimal temperatures, provided they have enough time to express their germination capacity. These findings cast doubts on the validity of conclusions drawn in many studies where germination experiments were performed for a period insufficient to obtain full germination at low temperatures. Another major finding in this work concerns the risk of wrongly estimating germinability at low temperatures.

**Keywords:** cocksfoot; orchardgrass; perennial ryegrass; tall fescue; wheat

## 1. Introduction

Germination is a critical stage in the life cycle of plants. It starts with the uptake of water by the dry seed (imbibition), continues with biochemical preparative processes and the elongation of the embryonic axis, and usually terminates with the protrusion of the radicle out of the seed [1]. The effects of environmental factors on seed germination are important for plant establishment, which is relevant in both ecology and agriculture. Optimal germination is a critical factor leading to the successful establishment and growth of plants and crops.

The germination test of a nondormant seed determines the germination potential or viability of that seed. When the seed germinates, the time between the beginning of imbibition and radicle protrusion out of the seed reflects the rate at which bio-physical and physiological processes occur in the embryo and the seed as a whole [1,2]. On the other hand, the germination test of a seedlot aims to determine the germination capacity (germinability), the rate of germination and the homogeneity of this seedlot [1].

From an agronomic point of view, the germinability of a particular seedlot is central to the calculation of optimum seeding rates, as well as helping to determine whether a particular seedlot has the potential to produce a good crop, or should not be used at all. For this reason, seed testing standards, such as those of the International Seed Testing Authority (ISTA, Rules for Seed Testing) [3], aim to provide testing methods for seeds intended for trade transactions. Nevertheless, it has often been noted that the concepts and methods used by seed technologists and testers are not fully relevant for the characterisation needs of seed physiologists, plant ecologists and seed bank curators [1].

There are several reasons why seeds may not germinate under a set of favourable environmental conditions where adequate levels of water, oxygen, light and temperature are present. In fact, under adequate water and oxygen availability, temperature is the most important environmental factor regulating both the timing and rate of seed germination. Indeed, temperature not only affects the physiological status of seed, through effects on seed deterioration and release of dormancy, but also the rates of many bio-physical processes underpinning germination itself [1,2,4–6].

Many studies have investigated the effects of temperature on germination, exploring the responses of several hundred species from different biomes [2]. Germination of many higher plants, including grasses, is affected by temperature in two ways: (i) the speed of the process (also called germination rate, expressed as the relative number of seeds that germinate per unit of time), and (ii) the total fraction of seeds in a lot that germinate (germinability, expressed as percentage) [5,7,8].

With respect to the germination rate, the effects of temperature can be expressed in terms of cardinal temperatures, namely the minimum, optimum and maximum temperatures [9–12]. The rate of the process is fastest at the optimum temperature, while minimum and maximum temperature determine the range at which nondormant seeds can germinate (i.e., beyond these extremes, no germination can occur) [1,5,7,8]. Several mathematical models have been proposed to describe the effects of cardinal temperatures on germination rates [12].

Seed germination has been studied under both alternating and constant temperature conditions. Alternating diurnal temperatures in germination studies have long been employed as a method to mimic field conditions and have been reported as favouring germinability in many species [2], including grasses [13,14], compared to constant temperatures. Nevertheless, this approach does not allow the estimation of cardinal temperatures nor the germination-rate response curves to temperature [12]. Further, most of these results could be artefacts given that studies under constant temperature did not follow germination for a period long enough to allow maximum germinability at suboptimal temperatures, where germination rates are slow. This artefact is notably observed in papers testing only the responses of seedlots of forage crops to constant temperatures (see references presented in Table 1).

**Table 1.** List of articles testing the germination responses of forage crops' seedlots at constant and alternating temperatures over various durations.

| Reference | Species | Temperature Conditions | Temperature Range (°C) | Reported Germinability (%) | Reported Germination Rate | Following-Up Period (day) |
|---|---|---|---|---|---|---|
| Shen et al., 2008 [13] | Perennial ryegrass | Constant and alternating | 5–40 | Yes | No | 7–14 |
| Lu et al., 2008 [14] | Tall fescue | Constant and alternating | 5–40 | Yes | No | 7–14 |
| Monks et al., 2009 [15] | Annual and perennial temperate pasture species | Constant | 5–35 | Yes | Yes | 19 |
| Moot et al., 2000 [16] | White clover, Perennial ryegrass | Constant | 5–30 | Yes | Yes | 36 |
| Larsen and Bibby, 2005 [17] | Perennial ryegrass, Red fescue, Kentucky bluegrass | Constant | 8–24 | Yes | Yes | No germination for 3 days |
| Sharifiamina et al., 2016 [18] | Tall fescue | Constant | 5–32.5 | Yes | Yes | 50 |
| Nori et al., 2014 [19] | Annual clover species | Constant | 5–30 | Yes | Yes | 35 * |
| Palazzo and Brar, 1997 [20] | *Festuca spp.* | Constant | 10–30 | Yes | Yes | 28 |
| Lonati et al., 2009 [21] | Legume and grass species | Constant | 5–35 | Yes | Yes | 19 |
| Butler et al., 2014 [22] | Forage Legumes | Constant | 5–35 | Yes | Yes | 14 |
| Butler et al., 2017 [23] | Cool-Season forage grasses | Constant | 5–35 | Yes | Yes | 14 |
| Zhang et al., 2013 [24] | Ryegrass, Tall fescue | Constant | 6–43 | Yes | Yes | 44 |

* estimated from figures in the reference.

The objective of this study was to test the working hypothesis that nondormant seeds at constant low temperatures germinate as well as nondormant seeds at constant near-optimal temperatures, provided they have enough time to germinate. To this end, we compared the germination dynamics and the time required to achieve full seedlot germinability at 5 °C and 21 °C of thirteen accessions of wild populations from three cool-season grass species. In order to compare the effect of temperature on germination between wild grass species and domesticated species, seeds of two wheat (*T. aestivum* L.) accessions were included in this study.

## 2. Materials and Methods

### *2.1. Seed Material*

Nondormant seeds of thirteen accessions of cool-season grasses were evaluated in this study (Table 2): three accessions of tall fescue (*F. arundinacea* Schreb.), seven accessions of perennial ryegrass (*L. perenne* L.), and three accessions of cocksfoot (*D. glomerata* L.). Two accessions of wheat (*T. aestivum* L.) were also included for comparative purposes. Seeds of grass species were obtained from the turf and forage species genebank of INRAE, Centre Nouvelle-Aquitaine-Poitiers, Lusignan, France (46°24′15″ N, 0°04′45″ E). Seeds of wheat were obtained from The Plant Breeding Institute, The University of Sydney, Australia. Before the experiments, the seeds were stored in dark conditions at 5 °C and 30% relative humidity.

**Table 2.** Description of the 15 accessions used in this study. The species, population type (variety or wild population), name of accession and country of origin are given. For the wild populations, the geographic coordinates of the collection site are indicated.

| Species | Accession | ID Sample | Type | Harvest Year | Geographic Coordinates | | Country |
|---|---|---|---|---|---|---|---|
| | | | | | Latitude | Longitude | |
| *Festuca arundinacea* | 20 | DSVFa5 | Variety | Unknown | | | France |
| | 46 | 500588B1 | Wild | 1988 | 35.9 | 8.9 | Tunisia |
| | 103 | 492991B2 | Wild | 1991 | 43.58333333 | 5 | France |
| *Lolium perenne* | 30 | LP TGF 5 | Variety | 2015 | | | France |
| | 178 | GR 3477 | Wild | 2004 | 54.32111 | 13.53194 | Germany |
| | 187 | GR 5030 | Wild | 1995 | 50.86944 | 13.73028 | Germany |
| | 237 | GR 6346 | Wild | 2002 | 45.05 | 15.1 | Croatia |
| | 268 | GR 12054 | Wild | 2005 | 51.57611 | −9.23444 | Ireland |
| | 408 | 2690??B1 | Wild | Unknown | 43.57152 | 1.787844 | France |
| | 409 | 2690??B2 | Wild | Unknown | 43.57152 | 1.787844 | France |
| *Dactylis glomerata* | 288 | NGB 2735,1 | Wild | Unknown | 59.839704 | 11.474738 | Norway |
| | 290 | NGB 15465,2 | Wild | Unknown | 56.511235 | 8.687534 | Denmark |
| | 293 | PORTO | variety | Unknown | 41.16 | 8.63 | Portugal |
| *Triticum aestivum* | T312 | PB109C001-BC-DH33 | Variety | Unknown | | | Australia |
| | T313 | PB109C001-BC-DH1 | Variety | Unknown | | | Australia |

### *2.2. Germination Experiment*

Seeds of the fifteen accessions were evaluated at constant temperatures of 5 or 21 °C. For each accession, four replicates of 100 grass seeds or 25 wheat seeds were arranged in a randomised complete block design. The seeds were put in 90 mm diameter Petri dishes containing a sheet of autoclave-sterilized Whatman paper humidified with 5 mL of deionised and autoclave-sterilized water. For each of the four blocks, Petri dishes were placed in a vented plastic box. Temperature and humidity were continuously monitored by inserting two self-contained temperature, humidity and dew point data loggers (EL-USB-2+, Lascar Electronics Ltd., Salisbury, UK) in each box. One data logger recorded values every hour and the other one every minute. Seeds were cold stratified in the dark for 7 days at 5 °C and 30% relative humidity (0.61 kPa of vapour-pressure deficit, VPD) in order to release any residual seed dormancy. After cold stratification, seeds were germinated in the dark at constant temperatures of 5 or 21 °C in 1.5 $m^3$ volume growth chambers (Froids et Mesures, Beaucouzé, France). Temperature measurements from five to six thermocouples placed at different positions within each growth chamber were logged every 20 s. Each growth chamber was regulated so that the temperature (average measurement from the thermocouples) did not exceed ±0.2 °C of the target temperature.

The frequency of sampling for germination depended on temperature and the number of non-germinated seeds within each Petri dish [9]. Seeds were considered as germinated when either the radicle or the coleoptile had protruded out of the seed and was at least 2 mm long. At each sampling, germinated seeds were counted and removed from the Petri dishes. Deionised and autoclave-sterilized water at 5 or 21 °C was added as required to ensure moisture was non-limiting for germination. The maximum period of observation was over 7300 h (10 months) for 5 °C and over 2300 h (3 months) for 21 °C. In the case of the accession for which germinability (the final germination percentage) was higher at 21 °C than at 5 °C, seeds that had not germinated at 5 °C were transferred to germinate at 25 °C to verify that they were still able to germinate.

### 2.3. Analysis of Germination Data

For each temperature treatment and replicate, the cumulative percentage of germination values over time was adjusted using a non-rectangular hyperbola [5] of the form:

$$y = \left(\frac{1}{2\theta}\right) \cdot \left(\alpha \cdot (t - tc) + y_{max} - \sqrt{(\alpha.(t - tc) + y_{max})^2 - (4.\theta.\alpha.(t - tc).y_{max})}\right) \quad (1)$$

where $y$ is the cumulated germination (expressed as proportion of seeds); $\theta$ is a convexity parameter (unitless); $\alpha$ is the maximum germination rate (proportion of seed per hour); $t$ is time (hour); $tc$ is the lag time before germination begins (hour), also called delay of germination; and $y_{max}$ is the maximum germinability

For each replicate, the time needed to reach a proportion $k$ of the maximum number of germinated seeds, $t_k$, was calculated as:

$$t_k = tc + \left[\frac{k.y_{max} - k^2.y_{max}.\theta}{\alpha.(1 - k)}\right] \quad (2)$$

Several parameters that relate germination to time (Equations (1) and (2)) were studied, namely: (1) the time when germination starts ($tc$), (2) the time to 50% of final germination ($t_{50}$), (3) the maximum germination rate ($\alpha$), (4) the time to 95% of final germination ($t_{95\%}$) and (5) the time to germination of the last seed (Table 3). Within each accession, the results at 5 and 21 °C were compared using the Student's $t$ test for all response variables. When appropriate, analyses of variance to compare species responses were performed with the general linear model: $Yij = \mu + Si + eeij$, where $Yij$ is the response of accession $j$ of species $i$, $\mu$ is the overall mean, $Si$ is the species $i$ effect and $eeij$ is the experimental error associated to values of accession $j$ of species $i$. The non-parametric Kruskal-Wallis test was used for interspecies comparisons. All calculations and statistical tests were performed using R software [25].

### 2.4. Typologies of Germination Responses to Temperature

Accessions were grouped based on their responses to temperature in terms of both germinability and rate-related parameters of germination as follows (Table 3). Firstly, accessions were differentiated between those germinating at more ('high') or less ('low' germinability) than 90% at most. Secondly, these two groups were further divided on the basis of their germination rate, depending on whether 95% of germination capacity was reached in less than 240 h (10 days) at 21 °C. Thirdly, differences in the germinability at 5 °C and 21 °C were studied within each of the four groups. Overall, this procedure generates a grid with eight (2 × 2 × 2) types of responses.

**Table 3.** Final germination percentage, germination start time, maximum germination rate and times to 50% and 95% of germination maxima for fifteen grass accessions. Within each accession and column, values with the same letter are not significantly different based on Student's *t*-test ($p > 0.01$).

| Species | Accession | Treatment | Final Germination Percentage (%) | Germination Start Time, *tc* (h) | Maximum Germination Rate, $\alpha$ (Proportion of Seed.h$^{-1}$) | Time to 50% of Final Germination (h) | Time to 95% of Final Germination (h) | Time Last Seed Germinated (h) | Time of Last Count (h) |
|---|---|---|---|---|---|---|---|---|---|
| *Festuca arundinacea* | F020 | 5 °C | 97 a | 509 a | 0.05 a | 1471 a | 2344 a | 6545 | 7365 |
| | | 21 °C | 97 a | 40 b | 1.99 b | 68 b | 110 b | 287 | 932 |
| | F046 | 5 °C | 56 a | 498 a | 0.06 a | 1147 a | 3281 a | 5849 | 7365 |
| | | 21 °C | 64 a | 44 b | 0.44 b | 138 b | 483 b | 930 | 1462 |
| | F103 | 5 °C | 97 a | 587 a | 0.06 a | 1393 a | 2276 a | 5517 | 7365 |
| | | 21 °C | 94 a | 40 b | 0.69 b | 120 b | 295 b | 1097 | 1462 |
| *Lolium perenne* | L030 | 5 °C | 94 a | N A | 0.18 a | 137 a | 489 a | 1001 | 4554 |
| | | 21 °C | 96 a | N A | 1.49 b | 35 b | 73 b | 190 | 932 |
| | L178 | 5 °C | 85 a | 58 a | 0.22 a | 299 a | 932 a | 3687 | 5517 |
| | | 21 °C | 91 a | 23 a | 1.88 b | 53 b | 117 b | 328 | 932 |
| | L187 | 5 °C | 64 b | 265 a | 0.22 a | 514 a | 1166 a | 5346 | 7365 |
| | | 21 °C | 97 a | 35 b | 1.88 b | 62 b | 96 b | 244 | 910 |
| | L237 | 5 °C | 81 b | 42 a | 0.16 a | 329 a | 794 a | 837 | 5850 |
| | | 21 °C | 92 a | 25 a | 1.83 b | 56 b | 106 b | 270 | 1179 |
| | L268 | 5 °C | 92 a | N A | N A | 135 a | 524 a | 793 | 4035 |
| | | 21 °C | 98 a | N A | N A | 43 b | 80 b | 252 | 917 |
| | L408 | 5 °C | 71 b | 74 a | 0.17 a | 350 a | 1261 a | 1844 | 4559 |
| | | 21 °C | 90 a | 44 a | 0.66 b | 126 b | 333 b | 1920 | 2352 |
| | L409 | 5 °C | 77 a | 48 a | 0.28 a | 225 a | 823 a | 1704 | 2184 |
| | | 21 °C | 84 a | 44 a | 1.09 b | 97 b | 273 b | 664 | 1436 |
| *Dactylis glomerata* | D288 | 5 °C | 93 a | 89 a | 0.15 a | 549 a | 1461 a | 357 | 4559 |
| | | 21 °C | 90 a | 36 a | 0.71 b | 114 b | 263 b | 524 | 1170 |
| | D290 | 5 °C | 99 a | 479 a | 0.05 a | 1445 a | 2319 a | 357 | 4559 |
| | | 21 °C | 96 a | 37 b | 1.07 b | 85 b | 156 b | 888 | 932 |
| | D293 | 5 °C | 36 a | 364 a | 0.06 a | 717 a | 1625 a | 448 | 4559 |
| | | 21 °C | 40 a | 30 b | 0.51 b | 79 b | 222 b | 608 | 1462 |
| *Triticum aestivum* | T312 | 5 °C | 99 a | N A | N A | 211 a | 282 a | 409 | 2184 |
| | | 21 °C | 97 a | N A | N A | 32 b | 62 b | 236 | 952 |
| | T313 | 5 °C | 98 a | N A | N A | 209 b | 254 a | 409 | 2184 |
| | | 21 °C | 95 a | N A | N A | 24 b | 63 b | 236 | 952 |

## 3. Results

### 3.1. Effects of Temperature on Germination Time and Germination Rate

Significant differences ($p < 0.01$) between seeds germinating at 5 °C and 21 °C were observed for $\alpha$ and timings of $tc$, $t_{50\%}$ and $t_{95\%}$, in general. However, only one of seven accessions of *L. perenne* showed a significant difference in $tc$ between 5 °C and 21 °C. As expected, $tc$, $t_{50\%}$ and $t_{95\%}$ were longer at 5 °C while $\alpha$ was slower (Table 3).

The ratio of $\alpha$ at 21 °C divided by $\alpha$ at 5 °C (($\alpha$21 °C)/($\alpha$5 °C)) is an indicator of the number of times germination is faster at 21 °C. High variability in this ratio was observed between accessions in general and within species (Table 4), such that a parametric analysis of variance was not applicable due to the heteroscedasticity of the residuals. Subsequently, a Kruskal–Wallis test showed that no significant differences existed between species in ($\alpha$21 °C)/($\alpha$5 °C) ($p$ = 0.42). On the other hand, the ratios (($tc$ 5 °C)/($tc$ 21 °C)) and (($t$50% 5 °C)/($t$50% 21 °C)) revealed significant differences between species. In particular, *L. perenne* had lower ratios than *F. arundinacea* (Table 4). As the ratio ((($t$50% − $tc$)5 °C)/(($t$50% − $tc$)21 °C)) was not significantly different among species, the difference observed for $t_{50}$ (($t$50% 5 °C)/($t$50% 21 °C)) seems to be due to the start of the germination process ($tc$) rather than to the slope of the curve of cumulative germination of the seedlots.

**Table 4.** Ratios of maximum germination rates (α) and key germination times (germination start time, time to 50% and 95% of final germination, total and corrected by start time) at 5 °C and 21 °C for nondormant seedlots of grass species and wheat. Within each column, the means of species with the same letter are not significantly different after a Kruskal-Wallis test ($p > 0.01$). NA means: values were not computable.

| Species | Accession | Ratio of $\frac{\alpha 21\ °C}{\alpha 5\ °C}$ | Ratio of $\frac{tc5\ °C}{tc21\ °C}$ | Ratio of $\frac{(t50\%)5\ °C}{(t50\%)21\ °C}$ | Ratio of $\frac{(t95\%)5\ °C}{(t95\%)21\ °C}$ | Ratio of $\frac{(t_{50}-tc)5\ °C}{(t_{50}-tc)21\ °C}$ |
|---|---|---|---|---|---|---|
| *Festuca arundinacea* | F020 | 39.8 | 12.73 | 21.63 | 21.31 | 27.68 |
| | F046 | 7.3 a | 11.32 a | 8.31 a | 6.79 a | 6.90 a |
| | F103 | 11.5 | 14.7 | 11.61 | 7.72 | 10.08 |
| *Lolium perenne* | L030 | 8.3 | NA | 3.29 | 6.70 | NA |
| | L178 | 8.6 | 2.52 | 5.64 | 7.97 | 8.03 |
| | L187 | 8.6 | 7.57 | 8.29 | 12.15 | 9.22 |
| | L237 | 11.4 a | 1.68 b | 5.88 b | 7.49 a | 9.26 a |
| | L268 | NA | NA | 3.14 | 6.55 | NA |
| | L408 | 3.9 | 1.68 | 2.78 | 3.79 | 3.37 |
| | L409 | 3.9 | 1.1 | 2.32 | 3.01 | 4.32 |
| *Dactylis glomerata* | D288 | 4.7 | 2.47 | 4.82 | 5.56 | 5.90 |
| | D290 | 21.4 a | 12.95 ab | 12.0 ab | 14.87 a | 20.13 a |
| | D293 | 8.5 | 12.13 | 9.08 | 7.32 | 7.20 |
| *Triticum aestivum* | T312 | NA | NA | 6.59 ab | 4.55 a | NA |
| | T313 | NA | NA | 8.71 | 4.03 | NA |

### 3.2. Effects of Temperature on Final Germination Percentage

At the near optimal temperature of 21 °C, significant ($p \leq 0.05$) differences in maximum germination (germinability) were found between accessions (Table 3) but not between species. Similar results were observed for maximum germination at 5 °C. These differences might be due to intrinsic properties of the accessions or possibly to the physiological status of the seedlots.

As one of the aims of this work was to test whether germinability is stable between 5 °C and 21 °C for each accession, pair-wise comparisons (Student's $t$-test) of the maximum germination at 5 °C and 21 °C were performed for each of the fifteen accessions. For 12 of the 15 accessions no significant differences ($p > 0.01$) in germinability were found between 5 °C and 21 °C. The three accessions that exhibited significant differences were all *L. perenne*.

### 3.3. Typologies of Germination Responses to Temperature

A typology of germination dynamics was created based on germinability, germination rate and germinability response to temperature (Table 5). The 15 studied accessions belong to six of the eight possible types described below.

**Table 5.** Classification of the accessions depending on their germinability (high, germination percentage $\geq$ 90%; or low, germinability < 90%), their germination rates (fast, 95% of germination in less than 240 h at 21 °C; or slow otherwise), and their germinability response to temperature (germinability similar or significantly different between 5 °C and 21 °C).

| Germinability (High or Low) | Germination Rate (Fast or Slow) | Temperature-Insensitive Germinability | Germinability Varying between 5 °C and 21 °C |
|---|---|---|---|
| High | Fast | Type 1<br>F020<br>L030<br>L178<br>L268<br>D290<br>T312<br>T313 | Type 5<br>L187<br>L237 |
| | Slow | Type 2<br>F103<br>D288 | Type 6<br>L408 |
| Low | Fast | Type 3<br>D293 | Type 7 |
| | Slow | Type 4<br>F046<br>L409 | Type 8 |

#### 3.3.1. Type 1 Germination Dynamics–High Germinability, Fast Germination, Germinability Stable across Temperatures

The type of germination dynamics most common in this study comprised five accessions belonging to the three forage species and the two accessions of wheat (Figure 1). All these accessions had high final germination percentages (>90%) that did not differ significantly ($p > 0.01$) between 5 °C and 21 °C. Furthermore, this group had fast germination rates at 21 °C, with at least 95% of germinable seed germinating in less than 240 h (10 days). Indeed, we graded fast rates based only on $t_{95}$, knowing that $\alpha$, and $t_{50}$ and $t_{95}$ are related (see Equation (2)). Nevertheless, the values of the $tc$ parameter affect the strength of these relationships.

As expected, germination rates were slower at 5 °C than at 21 °C, despite accessions reaching a similar final germination percentage at both temperatures. Furthermore, non-dormant seeds needed much longer to start germination ($tc$) at 5 °C. For example, at 5 °C, accession F020 had a $tc$ of around 509 h and needed about 1284 h to reach 50% of the final number of germinated seeds. On the other hand, at 21 °C this accession needed only 40 h to start germination and 68 h to express 50% of its germinability (Table 3, Figure 1).

The two accessions of wheat had a high germinability at both temperatures (no significant differences, $p > 0.01$) and germinated much faster than any of the accessions of grass species.

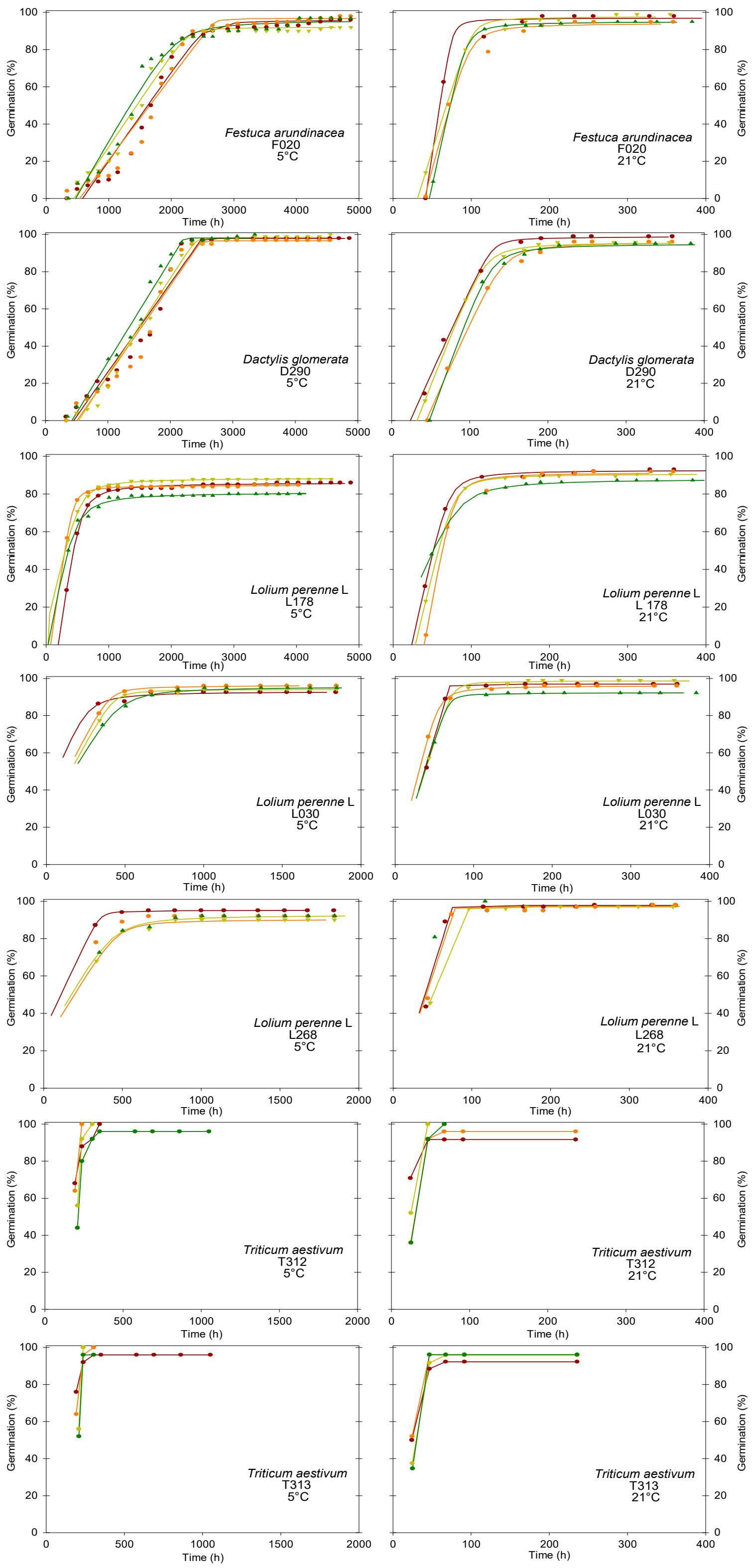


**Figure 1.** Time course of cumulative germination (%) at 5 °C (**left**) and 21 °C (**right**) for Type 1 accessions (i.e., accessions with high germinability that was similar at 5 °C and 21 °C and had a fast germination rate at 21 °C). The results are presented for the five accessions of cool-season grasses (*F. arundinacea* F020, *D. glomerata* D290, *L. perenne* L178, L030, and L268) and two accessions of wheat (*T. aestivum* T312 and T313) that belonged to this group. Each line represents a replicate of 100 nondormant seeds, except for wheat, which had 25. The x-axis scales differ among graphs.

#### 3.3.2. Type 2 Germination Dynamics–High Germinability, Fast Germination (Slower than Type 1), Germinability Stable across Temperatures

*D. glomerata* (D288) and *F. arundinacea* (F103) both had high germinability that did not significantly differ ($p \geq 0.01$) between 5 °C and 21 °C within each accession (Table 3, Figure 2). These accessions had slower germination rates at 21 °C than accessions of Type 1 needing more than 240 h to reach 95% of their germinability. In addition, as expected, germination rates were much slower at 5 °C than at 21 °C, despite accessions reaching similar final germination percentages at these two temperatures (Table 3).

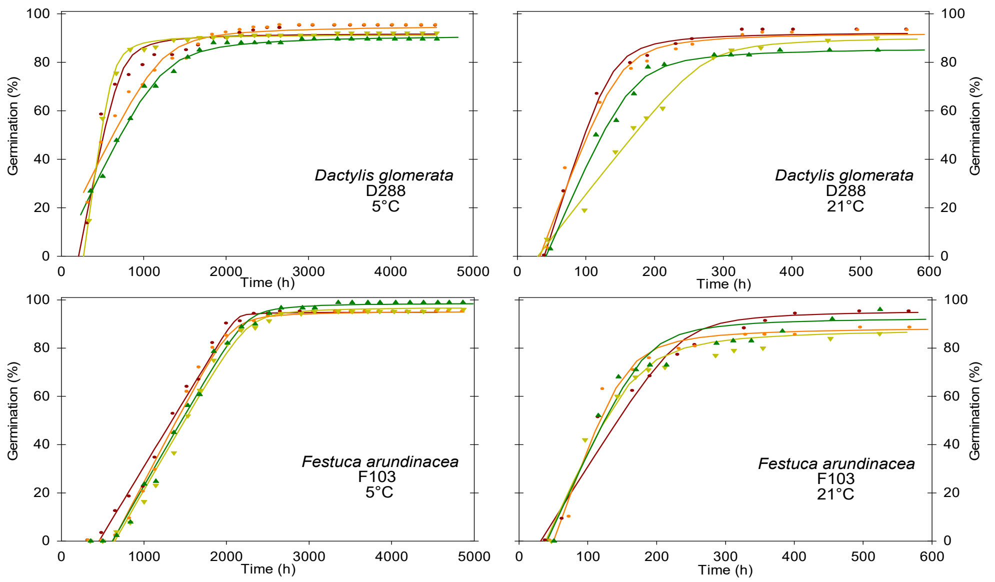


**Figure 2.** Time course of cumulative germination (%) at 5 °C (**left**) and 21 °C (**right**) for Type 2 accessions (i.e., accessions with high germinability that was similar at 5 °C and 21 °C and had a slow germination rate at 21 °C). The results are presented for the two wild-grass accessions (D. glomerata D288 and F. arundinacea F103) that belonged to this group. Each line represents a replicate of 100 nondormant seeds. The x-axis scales differ among graphs.

#### 3.3.3. Type 3 Germination Dynamics–Low Germinability, Fast Germination and Germinability Stable across Temperatures

Accession D293 of *D. glomerata* had low germinability at both temperatures, with a maximum germination percentage of ca. 40%. However, there was no significant difference ($p > 0.01$) in germinability at both 5 °C and 21 °C (Table 3, Figure 3). Despite a low level of germinability, this accession needed only 30 h to start germination at 21 °C and showed a fast germination rate with less than 240 h needed to reach 95% of final germination at 21 °C (Table 3).

#### 3.3.4. Type 4 Germination Dynamics–Low Germinability, Fast Germination and Germinability Stable across Temperatures

In the sample of assessed accessions, two had low germinability in both the 5 °C and 21 °C treatments, and slow germination rates in 21 °C. These Type 4 of germination dynamics included one accession of *F. arundinacea* (F046) and one of *L. perenne* (L409). Both accessions had relatively low final germination percentages (ca. 60 and ca. 80%, respectively) that were not significantly different ($p \geq 0.01$) whether tested at 5 °C or 21 °C (Table 3, Figure 4). These accessions had slower germination rates at 21 °C than accessions of Type 1 and 3, and needed more than 240 h to reach 95% of the final germination percentage at 21 °C. However, accession L409 had a high value of $\alpha$ (1.09 seed per h, Table 3) although it was slow to start germination ($t_c > 56$ h at 21 °C).

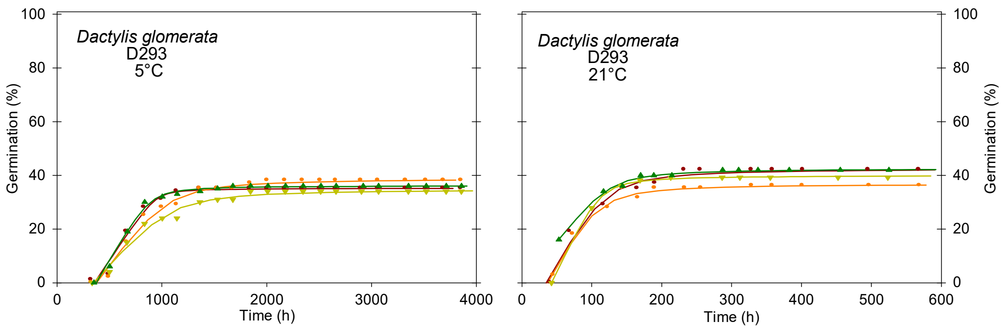


**Figure 3.** Time course of cumulative germination (%) at 5 °C (**left**) and 21 °C (**right**) for Type 3 accessions (i.e., accessions with low germinability that was similar at 5 °C and 21 °C and had a fast germination rate at 21 °C). The results are presented for the only accession (D. glomerata D293) that belonged to this group. Each line represents a replicate of 100 nondormant seeds. The x-axis scales differ among graphs.

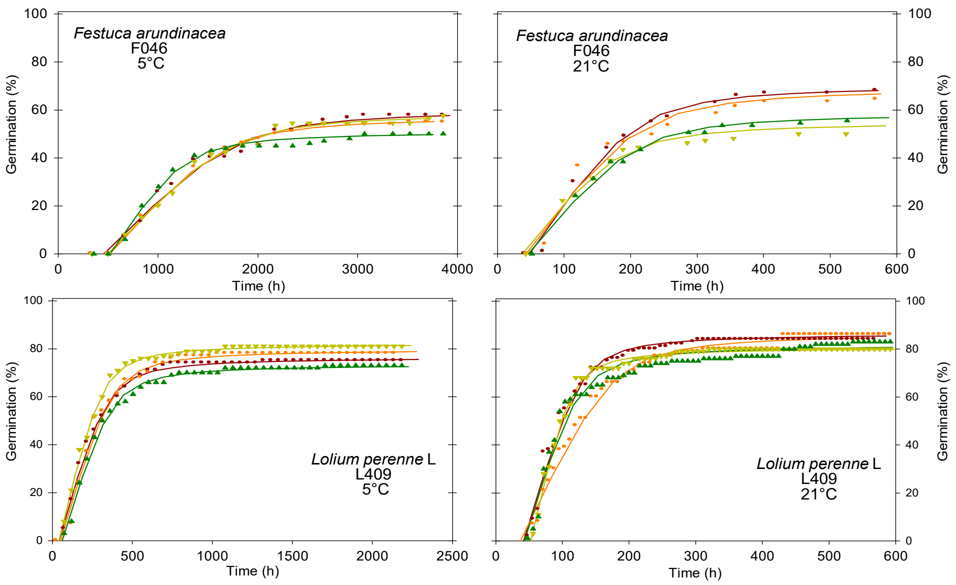


**Figure 4.** Time course of cumulative germination (%) at 5 °C (**left**) and 21 °C (**right**) for Type 4 accessions (i.e., accessions with low germinability that was similar at 5 °C and 21 °C and had a slow germination rate at 21 °C). The results are presented for the two wild-grass accessions (*F. arundinacea* F046 and *L. perenne* L409) that belonged to this group. Each line represents a replicate of 100 seeds. The x-axis scales differ among graphs.

### 3.3.5. Type 5 Germination Dynamics–High Germinability, Fast Germination, Germinability Variable across Temperatures

Differences in germinability between 5 °C and 21 °C were observed in two accessions of *L. perenne* (L187 and L237) that each had high final germination percentages (*ca.* 100 and ca. 90%, respectively) at 21 °C (Figure 5). Furthermore, these accessions had fast germination rates with less than 240 h needed to reach 95% of their final germination at 21 °C. As with the other types, germination rates were lower at 5 °C than at 21 °C (Table 3). Finally, accession L237 started germination early in both treatments (estimated *tc* of 42 h at 5 °C and 25 h at 21 °C). After the last count (more than 5800 h, i.e., 242 days; Table 3), seeds that did not germinate at 5 °C were transferred to germinate at 25 °C. Another 26% (L187) and 6% (L237) of seeds germinated after 500 h, which brought the overall germinability of these accessions to 90% for L187 and 87% for L237.

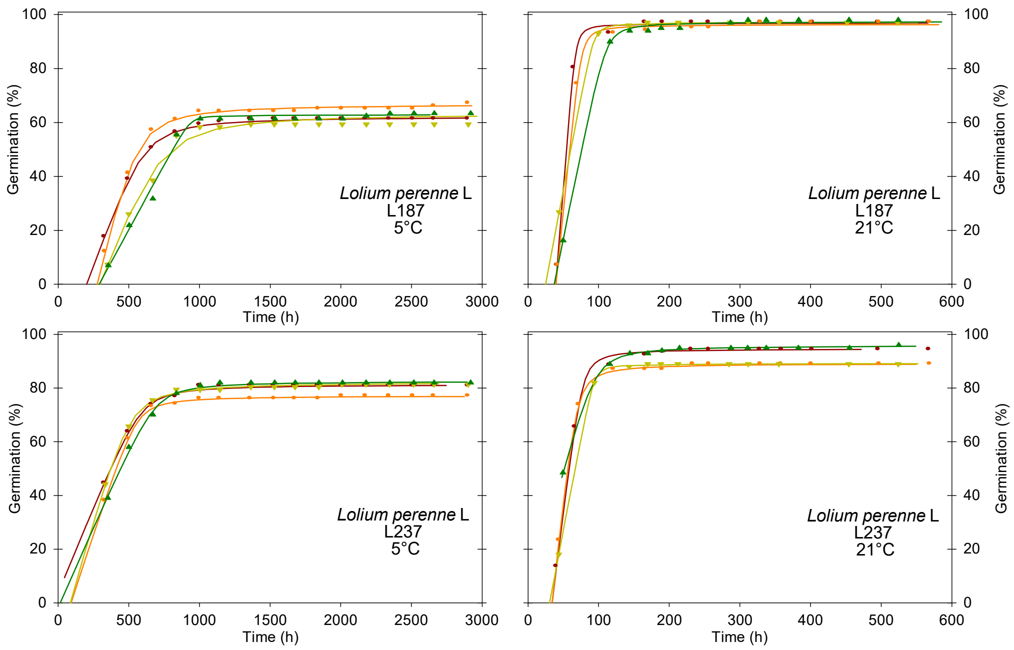


**Figure 5.** Time course of cumulative germination (%) at 5 °C (**left**) and 21 °C (**right**) for Type 5 accessions (i.e., accessions with high germinability that differed between 5 °C and 21 °C and had a fast germination rate at 21 °C). The results are presented for the two wild-grass accessions (L. perenne L187 and L237) that belonged to this group. Each line represents a replicate of 100 nondormant seeds. The x-axis scales differ among graphs.

#### 3.3.6. Type 6 Germination Dynamics–High Germinability, Slow Germination, Germinability Variable across Temperatures

Only one accession of *L. perenne* (L408) exhibited type 6 germination. This accession had relatively high final germination percentage at 21 °C (*ca*. 90%), which decreased (*ca.* 70%) significantly ($p < 0.01$) under the 5 °C temperature treatment (Figure 6). Further, this accession had a slow germinati0on rate at 21 °C with more than 240 h needed to reach 95% of its final germination. As expected, germination at 5 °C was even slower ($\alpha = 0.17$) than at 21 °C ($\alpha = 0.66$) (Table 3). High variability was observed between replicates at 5 °C (Figure 6).

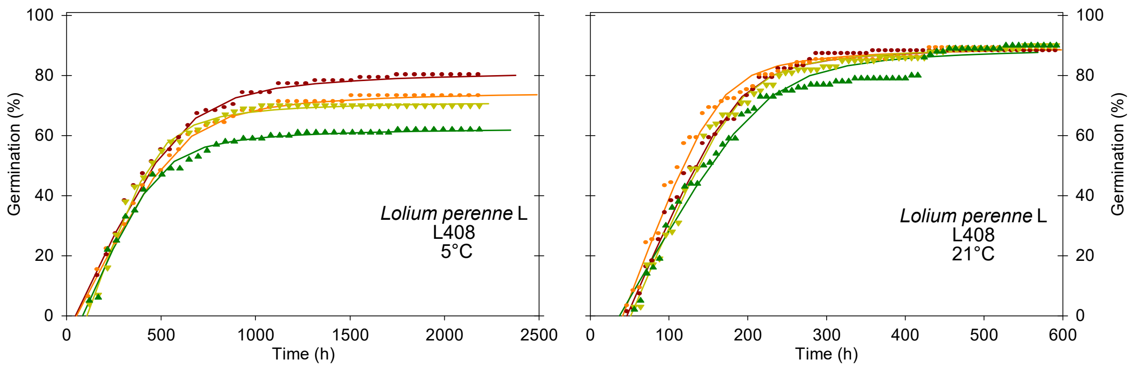


**Figure 6.** Time course of cumulative germination (%) at 5 °C (**left**) and 21 °C (**right**) for the Type 6 accession (i.e., accessions with high germinability that differed between 5 °C and 21 °C and had a slow germination rate at 21 °C). The results are presented for the only wild-grass accession (L. perenne L498) that belonged to this group. Each line represents a replicate of 100 nondormant seeds. The x-axis scales differ among graphs.

### 3.4. Potential Artefacts in Measuring Germinability

One of the major findings in this work concerns the risk of wrongly estimating germinability at low temperatures when nondormant seedlots are not given the time to express their full germination capacity. Indeed, regardless of the species and maximum potential germinability, observing seedlots for only 14 d (336 h) or even for 50 d (1200 h) leads to an underestimation of the germination potential at low temperatures (Figure 7). However, the question arises as to the degree of inaccuracy of such results. In this study, underestimations of germinability at 5 °C ranged from ca. 40% (L030) to 100% in the 14-d test, and from a few points (less than 5%) up to 75% in the 50-d test.

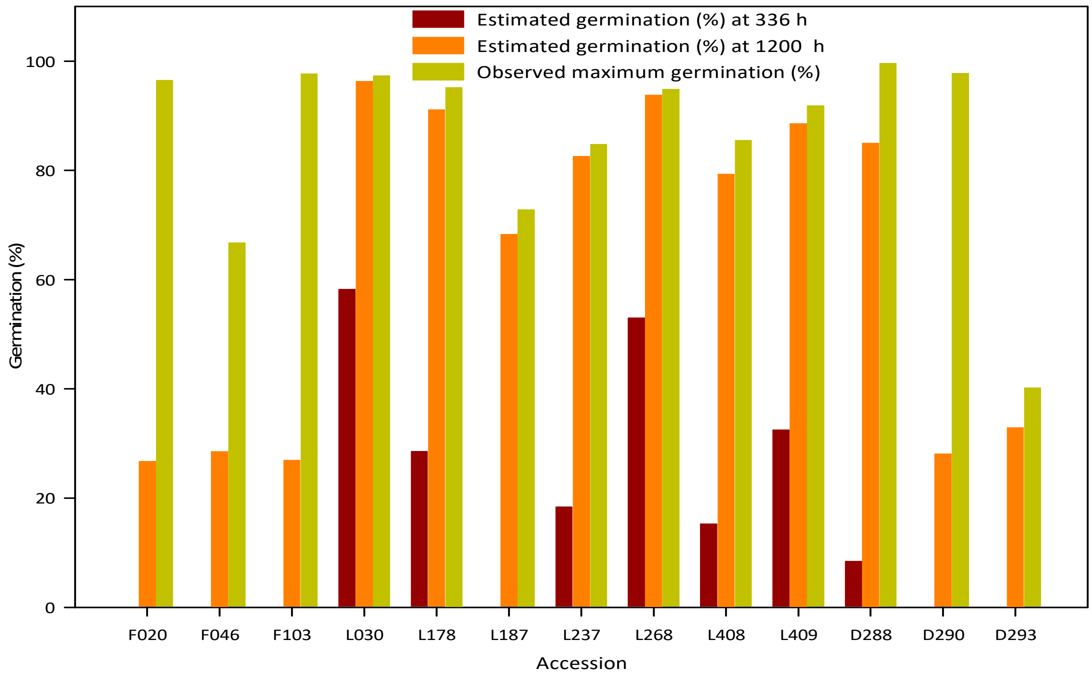


**Figure 7.** Effects of the observation period (336 h, 1200 h and "as long as needed to obtain maximum germination") on estimated germinability at 5 °C of thirteen accessions of cool-season grasses.

## 4. Discussion

The effects of temperature on the germination rate of an individual seed and of lots of seeds have been studied for a long time (e.g., Sachs, [11]), but it remains a major topical issue in agronomy and ecology because of its theoretical and practical implications [2,17,26–28].

The results of this study show that the effects of temperature were greater on germination rates than on final germination percentage. As expected, germination rates were faster at 21 °C than at 5 °C. However, for perennial cool-season grasses, the genotypic variability found for the ratio of germination rates between the two temperatures was higher than what is found in the literature [17,21,23,24]. Furthermore, the range of germination-rate ratios was different between the three perennial grass species, extending from 4 to 11 for *L. perenne*, 5 to 21 for *D. glomerata*, and from 7 to 40 for *F. arundinacea*. These values possibly illustrate part of the range of genetic variability within each species related to the pedo-climatic conditions of their growing habitats [21,24,29,30]. This is a hypothesis that merits testing.

Temperature had a marked effect on the time seeds needed to start germination. Seeds at 5 °C needed longer to start germinating than at 21 °C (Table 3). This is consistent with the Sharifiamina et al. [18] study, where seeds of tall fescue started germination after 30 days at 5 °C while most seeds germinated between 3 and 10 days at 25 °C.

A consequence of the extreme differences in germination rates at 5 °C and 21 °C is that seeds at 5 °C needed much more time to express their maximum germination capacity.

One of our major findings concerns the potential for underestimating germinability at low temperatures when observations are restricted to short periods. Indeed, seeds at 5 °C needed up to 18 times longer to reach the same germination percentage they reached at 21 °C. This fact casts doubts on the validity of conclusions drawn in many studies where germination experiments were performed for a period insufficient to obtain full germination at low temperatures (e.g., Butler et al. [23]). For instance, for the three perennial cool-season grasses we studied at 5 °C, even a 50-d experiment would have yielded misleading results. These results do not apply to wheat, which germinated very rapidly under both cold and optimal conditions (Figure 1), and for which our results are similar to those in the literature [15,16].

Thomson [31] noted, "in ecological studies ... a sound method for comparing germination responses is essential before progress can be made in comparing responses of different species in relation to habitat or distribution". The experimental protocol followed in this study demonstrated no significant differences within the four studied species in the germinability response at 5 °C and 21 °C (Table 5), except for three *L. perenne* accessions (Types 5 and 6; Figures 5 and 6). Overall, twelve of the fifteen studied accessions showed an ability to reach the same final germination percentage at both 5 °C and 21 °C when given enough time. With a few exceptions [16], this is completely different from the conclusions drawn from most of the experimental research on grass germination (Table 1).

Finally, estimates of lower cardinal and base temperatures for thermal time modelling could be negatively impacted by erroneous quantification of germinability and the time course of germination at low temperatures [11,31]. Many of the findings reported in the literature, particularly those concerned with varieties and species comparisons, might therefore be reanalysed bearing in mind that the experimental protocol is of paramount importance, especially in ecology studies [31]. Indeed, the protocols used for testing seedlots aimed at industry are not necessarily appropriate for studies of germination in ecology, physiology or genetics [2].

## 5. Conclusions

The objective of this study was to determine the effects of low constant temperatures on the time needed to express the full germination capacity of seedlots of three species of cool-season grasses and wheat. As expected, germination rates were faster at 21 °C than at 5 °C. Consequently, seeds at 5 °C needed longer (up to 21 times) to reach their maximum germinability. Accessions of wheat, a highly domesticated species, germinated much faster at 21 °C than most of the cool-season grasses, which were wild accessions. One of the major findings in this work concerns the risk of wrongly estimating germinability at low temperatures when observations are restricted to short periods. While the time needed to express full germination capacity varies greatly with temperature, in most cases, no significant differences were observed in seedlot germinability response to temperature. Most accessions expressed 90–99% germinability under both cold (5 °C) and near-optimum (21 °C) temperatures, while a few accessions had a low germinability at both temperatures. On the other hand, only a few accessions exhibited significant differences in their germinability at 5 and 21 °C.

Under the paradigm of cardinal temperatures (e.g., Sachs, [11]), shaping germination rates and, consequently, the appropriateness of experimental protocols and the interpretation of results, the next questions to be answered concern the dynamics of germination at the high end of the temperature spectrum where the process is still possible. In our view, answering these questions for each species is unavoidable prior to any attempt to study the genetic-related diversity both within and between species. The knowledge drawn from studies on germination at temperatures close to the edge of a species' range is also important for seed bank curators and for biodiversity preservation and management.

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