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RESEARCH ARTICLE

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# Optimal germination conditions for monitoring seed viability in wild populations of fescues

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## Abstract

*Aim of study:* Germination assays are vital in the management of material preserved in germplasm banks. The rules published by the International Seed Testing Association (ISTA) are generally those followed in such assays. In wild species, seed dormancy and inter-population variability increase the difficulty in estimating seed viability. The aim of the present work was to determine the germination requirements of the seeds from different wild populations of pasture grasses species.

*Area of study:* Northwestern Spain

*Material and methods:* Seeds from eight wild populations of different species of *Festuca*, all from northwestern Spain, were studied. Germination assays were performed under constant and alternating temperature conditions. Treatments for removing seed dormancy (cold stratification and gibberellic acid application) were also applied. A full parametric time-to event model was used for data analysis.

*Main results:* In general, the optimum environmental temperature for germination was around 15°C for the populations of *Festuca* group *ovina*, *F.* gr. *rubra* and *F. gigantea*; temperatures of 20-30ºC had a negative effect. All the examined populations, except that of tall fescue (*Lolium arundinaceum*), showed non-deep physiological dormancy at suboptimal germination temperatures, but this was breakable by the application of gibberellic acid and by cold stratification.

*Research highlights:* There are clear inter- and intra-specific differences in germination requirements that might be associated with place of origin. The ISTA germination assay recommendations for wild members of fescues may not be the most appropriate.

**Additional key words:** pasture grasses; genebank; seed dormancy

**Abbreviations used:** CIAM (Centro de Investigaciones Agrarias de Mabegondo); $GA_3$ (gibberellic acid); Gmax (maximum germination percentage); ISTA (International Seed Testing Association); PGRFA (Plant Genetic Resources for Food and Agriculture)

**Authors' contributions:** Conceived and designed the study: IM and JAO. Performed the experiments and the statistical analysis: PV. Interpreted the data and wrote the paper: PV and IM. Revised and improved the manuscript: JAO. All authors read and approved the final manuscript.

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# Introduction

Plant genetic resources for food and agriculture (PGRFA) are essential for maintaining and improving world food security and sustainable development, and play a central role in adapting agriculture and food production to climate change (FAO, 2010). For species producing orthodox seeds that withstand desiccation, seed storage is the preferred *ex situ* PGRFA conservation method; it is usually the most effective and least costly (Rao *et al.*, 2007).

Pasture grasses and forage species are recognised as a food resource since they support livestock. Although these species have not been subjected to the major selection and improvement efforts made with directly consumed crops (*i.e.*, grains, pulses, vegetables, etc.), they are just as important in food security terms. The preservation of their genetic richness is therefore vital (Batello *et al.*, 2007). According to the Second Report on the State of the World's Plant Genetic Resources for Food and Agriculture (FAO, 2010), there are more than 650,000 samples of forage species/pasture grass

held in germplasm banks (accounting for 9% of all samples banked).

The genus *Festuca* encompasses some 450 species of pasture grass. The Iberian Peninsula is one of this genus' main centres of diversification (de Nova *et al.*, 2006). In Spain, the largest collection of pasture grasses is conserved in the germplasm bank at the *Centro de Investigaciones Agrarias de Mabegondo* (CIAM; Mabegondo, Spain); the centre has more than 1300 ecotypes, mostly collected from the Peninsular northwest (López Díaz *et al.*, 2010). A back-up copy of some of this material is held by the *Centro Nacional de Recursos Fitogenéticos* (CRF-INIA; Alcalá de Henares, Spain).

Periodic monitoring of the viability of the seeds stored in germplasm banks is essential for determining when accessions require regeneration. This is usually undertaken via germination assays performed under optimum conditions in an effort to ensure that all viable seeds germinate (Davies *et al.*, 2015). These assays normally follow the standard protocols published by the International Seed Testing Association (ISTA) (FAO, 2014). This body tries to standardise procedures for testing internationally traded seeds, and is responsible for the International Rules for Seed Analysis. However, these rules mainly focus on crops (ISTA, 2017) and therefore on varieties that show a large degree of homogeneity.

Estimating the viability of seeds via germination is more difficult in wild species than in cultivated plants. Seeds of wild plants commonly undergo dormancy, and appreciable variation may exist between populations of the same species in terms of seed germination requirements (Anderson & Milberg, 1998; Baskin & Baskin, 2014; Martín & Guerrero, 2014). Dormancy is a key feature for successful colonization and establishment, enabling seeds to avoid germination during periods that are not favourable for the further development of the plants. In crop species, sowing and successive harvesting drastically change selective pressures from those operating under natural conditions. Thus, throughout domestication process and post-domestication evolution most of the seed dormancy mechanisms are quickly removed (De Wet & Harlan, 1975). Domestication of temperate grasses has mainly been in the past 250 years and systematic selection is little over a century old (Batello *et al.*, 2007); therefore, dormancy phenomena are still present in many cultivars of these plants (Adkins *et al.*, 2002).

Although the literature on germination and dormancy in seeds is ample (Bewley, 1997), many of the processes that determine seed dormancy and germination are not yet fully understood (Chahtane *et al.*, 2017). The two basic dormancy types encountered in seeds of grass species are embryo dormancy and coat-imposed dormancy. Endogenous embryo dormancy is generally considered the result of a delicate balance between inhibitors and promotors (primordially abscisic acid and gibberellins, respectively). Tissues surrounding the embryo may cause a limitation to gas exchange and can also maintain seed dormancy by being either the source of inhibitors or by limiting the escape of inhibitors from the seed. (McDonald *et al.*, 1996; Adkins *et al.*, 2002). Grass seed dormancy is normally overcome or reduced by after-ripening under certain environmental conditions. Many cool season grasses require a warm-dry period after seed shedding, while cool-moist conditions are usually necessary to promote germination of warm-season species (Baskin & Baskin, 1998).

Genetics and mother plant environment during seed maturation are the most important factors controlling the variation in seed dormancy and germination requirements within and between populations (Baskin & Baskin, 2014). This variation must be considered when testing the germination requirements of a wild species (Anderson & Milberg, 1998).

Many of the germination studies involving the genus *Festuca* have been performed on commercial cultivars and selected lines of forage and turf species (Boyce *et al.*, 1976; Danielson & Toole, 1976; Hill *et al.*, 1985; Larsen *et al.,* 2004; Lu *et al.*, 2008; Stanisavljević *et al.*, 2010; Sharifiamina *et al.*, 2016), primarily on tall fescue. Seed germination on wild populations of the genus *Festuca* has been also addressed in several papers, mainly on native species of the American continent (Doescher *et al.*, 1985; Romo *et al.*; 1991; Grilz *et al.*, 1994; Qiu *et al.*, 2010; López *et al.*, 2019). However, only a limited number of studies have investigated fescue seed biology on European populations and none of them has covered southern European areas (Willians, 1983; Chorlton *et al.*, 1997).

Since seed testing is destructive and labour- and resource-intensive, knowledge of the physiological mechanisms underlying germination is essential if efficient procedures are to be developed for monitoring seed viability in genebanks. The aim of the present work was to determine the germination requirements of the seeds from different wild populations of fescues collected in the Iberian Peninsula, and to compare and contrast them with the assay conditions proposed by the ISTA (2017).

# Materials and methods

## Plant material

Seeds from eight wild populations of different species of *Festuca*, all from northwestern Spain, were examined (Table 1). Given the difficulty in identifying the exact species of narrow-leaved fescues, this material was treated at the 'group' level as belonging to either *Festuca* gr. *ovina* or *Festuca* gr. *rubra* (Huff & Palazzo, 1998; Oliveira *et al.*, 2008). Tall fescue (*Lolium arundinaceum* (Schreb) Darbysch syn. *Festuca arundinacea* Schreb and *Schedonorus arundinaceus* (Schreb.) Dumort) can be considered

**Table 1.** Identification codes and origin of the fescue accessions studied.

| Accession n°[1] | Inventory n°[2] | Origin | Latitude | Longitude | Altitude (m) |
|---|---|---|---|---|---|
| ***F.* gr. *ovina*** | | | | | |
| 1300 | NC074812 | Pesoz (Asturias) | 43º 15' N | 6º 52' W | 331 |
| 1518 | NC072091 | Barreiros (Lugo) | 43º 32' N | 7º 15' W | 35 |
| 2732A | NC109946 | Abegondo (A Coruña) | 43º 13' 12" N | 8º 15' 0" W | 33 |
| ***F.* gr. *rubra*** | | | | | |
| 1303 | NC074815 | Puebla de Lillo (León) | 43º 7' N | 5º 14' W | 1490 |
| 1316 | NC074828 | Ribadeo (Lugo) | 43º 31' N | 7º 3' W | 30 |
| ***F. gigantea* (L.) Vill.** | | | | | |
| 1599 | NC109942 | Cuntis (Pontevedra) | 42º 37' 48" N | 8º 34' 48" W | 250 |
| 1652 | NC109943 | Larouco (Ourense) | 42º 22' 48" N | 7º 10' 12" W | 325 |
| ***L. arundinaceum* (Schreb) Darbysch.** | | | | | |
| 2842 | NC109944 | Paderne (A Coruña) | 43º 17' 2" N | 8º 10' 0" W | 178 |

[1] Accession number in the CIAM collection. [2]Internal code used by the Spanish National Inventory of Plant Genetic Resources.

a species complex consisting of three major (continental, mediterranean and rhizomatous) morphotypes (Hand *et al.*, 2010). In this work, the tall fescue population is hereinafter named *Lolium arundinaceum.*

All the accessions studied were multiplied in the same year in isolated micro-greenhouses at the CIAM (López-Díaz & Calvete, 2016). Sowing was performed in October-November 2015, and harvesting performed in June and September of 2016. The harvested seeds were stored in the dark at 5ºC until being used in germination assays.

## Germination assays

Germination assays were performed in quadruplicate in 90 mm Petri dishes at the *Centro de Recursos Fitogenéticos* (CRF-INIA) over January-May 2017; all replicates involved 25 seeds. Seeds were cleaned to leave only the grain, lemma and palea, and care was taken to avoid selecting empty seeds. Seeds were placed on 155 g $m^{-2}$ paper within the dishes, moistened with 2.5 mL of distilled water. The dishes were then incubated in controllable light/temperature chambers, adding water whenever necessary. Chamber light was provided by cool white fluorescent tubes (36W).

Assays were performed under constant temperature conditions of 10ºC, 15ºC and 20ºC, as well as under alternating temperature conditions of 10/20ºC and 20/30ºC, all under a 8/16 h light/dark photoperiod. In these latter assays the higher temperature coincided with the 8 h period of light (ISTA, 2017).

Under the 20/30ºC conditions (those recommended by the ISTA for all species of *Festuca* (ISTA, 2017), germination assays were also performed 1) in total darkness, 2) with cold stratification, and 3) with the application of gibberellic acid ($GA_3$). For the dark germination assays, the Petri dishes were wrapped in aluminium foil, with exposure to light minimised during counting. The last two treatments were intended to break any dormancy. Cold stratification was performed maintaining the seeds at 5ºC on the same kind of paper as above (moistened in the same way) for seven days (ISTA, 2017). $GA_3$ was applied with the first 2.5 mL of water (concentration 500 mg/L).

Counts of germinated seeds were made every 1-3 days. Seeds were deemed to have germinated when the emerged radicle reached ≥3 mm in length. In general, the assays ran for 21 days (ISTA, 2017), although at 10ºC they were extended to 27 days when germination events were observed close to the 21 day. At the end of the assays, non-germinated seeds were gently pressed using forceps, and classified as empty, dead or fresh (ISTA, 2017). Seeds that remained firm and sound were considered viable -fresh- and soft or rotten seeds were accounted as dead seeds. The number of empty seeds (non- true seeds) were excluded from calculation of final germination percentages.

## Data analysis

A full parametric time-to event model for interval-censored data was used to examine the results, employing a three parameter log-logistic function with a zero lower limit to fit the data (Ritz *et al.*, 2013). This method of analysis, which requires no assumptions be made, is well adapted to the present type of data and the object of investigation (Onofri *et al.*, 2010, 2011; McNair *et al.*, 2012). Other methods traditionally used in such studies, including those to check whether assumptions have been respected, are generally inadequate (Scott *et al.*, 1984; Warton & Hui, 2011; Sileshi, 2012).

For each population and treatment, maximum percentages of germination (Gmax) and the time to reach a percentage "n" of germinated seeds ($t_n$) were estimated via the fitted functions; $t_{50}$ was used in the temperature assays and $t_{25}$ for all other treatments in order to compare the largest possible number of curves (Soltani *et al.*, 2015). Significance was set at $p<0.05$ for all pairwise comparisons.

All procedures were undertaken using R (R Core Team, 2017), employing the "drm" function of the "drc" package (Ritz *et al.*, 2015).

# Results

Figures 1 to 4 show the results of the germination assays performed under the different conditions. All populations returned germination percentages of >85% under some set of conditions without additional dormancy breaking treatment. The fraction of dead seeds was small, with an overall average of 5.5%. Tables 2 and 3 show the estimated Gmax and $t_n$ values for the different germination temperatures and treatments respectively. In cases in which the germination rate had not stabilised by the end of the assay (*Festuca* gr. *ovina* 1300 - $GA_3$, darkness; *F.* gr. *ovina* 2732A - 20/30ºC), the Tables 2-3 show the unadjusted values obtained in the assays (in such cases abnormally high germination percentages are predicted when extrapolating beyond the study period).

For *Festuca* gr. *ovina*, the highest Gmax values were achieved under the 10/20ºC and 15ºC conditions (Table 2), with no significant difference between them; nor was any difference seen in this respect between its three representative populations (1518, 2732A and 1300). Similar Gmax values were also achieved at 10ºC, although for population 2732A the difference between the 10ºC and 15ºC conditions (90.3% and 98%, respectively) was significantly different. At 20ºC the Gmax was significantly lower than at 15ºC in populations 1518 and 2732A. The 20/30ºC conditions were clearly unfavourable in all cases; in population 1300 no seeds germinated at all, and in 1518 and 2732A less than 50% did so (Fig. 1, Table 2). In all three *Festuca* gr. *ovina* populations, the $t_{50}$ value fell as the temperature increased up to 15ºC, but rose significantly at 20ºC in *Festuca* gr. *ovina* 1300 and 2732A.

Both populations of *Festuca* gr. *rubra* (1300 and 1316) behaved in a manner very similar to the *Festuca* gr. *ovina* populations (Fig. 2, Table 2), with the highest Gmax values reached under the 10ºC, 10/20ºC and 15ºC conditions. In addition, the $t_{50}$ values were clearly higher at 10ºC. At 20ºC the germination rate was significantly lower than at 15ºC in population 1316, while the 20/30ºC conditions were clearly unfavourable to both populations.

For the *F. gigantea* populations (1599 and 1652) no differences were seen in Gmax at 10ºC, 10/20ºC, 15ºC and 20ºC. Again, the value of $t_{50}$ fell with rising temperature. Under the 20/30ºC conditions, a significant reduction in Gmax was seen, especially for population 1652 (Fig. 3, Table 2).

The single population of *L. arundinaceum* (2842) behaved differently to the other populations, reaching Gmax (96-98%) under all temperatures except the coolest (10ºC). The $t_{50}$ was lowest under the 20ºC and 20/30ºC conditions. At 10ºC, the germination rate was clearly inferior (Fig. 4, Table 2).

The 20/30ºC/dark germination conditions had an inhibitory effect on germination compared to the regular 20/30ºC conditions for the populations of *F.* gr. *ovina* and *F.* gr. *rubra.* Indeed, the germination rate was lower and

**Table 2.** Maximum germination percentages (Gmax) and $t_{50}$ values obtained for the seeds of different fescue accessions at different temperatures. Values were estimated from fitted curves.

| Accession | Gmax (%) | | | | | $t_{50}$ (days) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 10ºC | 10/20ºC | 15ºC | 20ºC | 20/30ºC | 10ºC | 10/20ºC | 15ºC | 20ºC | 20/30ºC |
| ***F.* gr. *ovina*** | | | | | | | | | | |
| 1300 | 78.9 ab | 85.6 a | 74.7 ab | 62.3 b | 0.0 * | 15.1 b | 11.7 a | 11.1 a | 17.4 b | - |
| 1518 | 96.5 ab | 96.0 ab | 99.0 a | 89.5 b | 46.5 c | 12.4 c | 8.5 b | 7.3 a | 7.1 a | - |
| 2732A | 90.3 b | 96.0 ab | 98.0 a | 88.3 b | 25.8 * | 12.8 c | 8.1 b | 6.4 a | 7.6 b | - |
| ***F.* gr. *rubra*** | | | | | | | | | | |
| 1303 | 93.6 a | 93.0 a | 96.0 a | 89.1 a | 38.1 b | 13.4 c | 8.3 b | 7.5 a | 7.1 a | - |
| 1316 | 90.9 a | 96.0 a | 97.0 a | 76.0 b | 23.6 c | 14.3 c | 8.4 b | 7.7 a | 8.5 ab | - |
| ***F. gigantea*** | | | | | | | | | | |
| 1599 | 96.9 a | 99.0 a | 95.0 ab | 93.9 ab | 88.1 b | 10.8 e | 6.6 d | 5.6 c | 4.3 b | 3.5 a |
| 1652 | 84.7 a | 91.0 a | 90.0 a | 86.5 a | 46.4 b | 9.8 d | 7.5 c | 6.8 b | 5.0 a | - |
| ***L. arundinaceum*** | | | | | | | | | | |
| 2842 | 52.3 b | 98.0 a | 98.0 a | 97.0 a | 95.9 a | 17.4 d | 5.8 c | 4.6 b | 3.2 a | 3.5 a |

* Unadjusted value (*i.e.*, as recorded) (see Results). Values with the same letter within accessions are not significantly different ($p>0.05$).

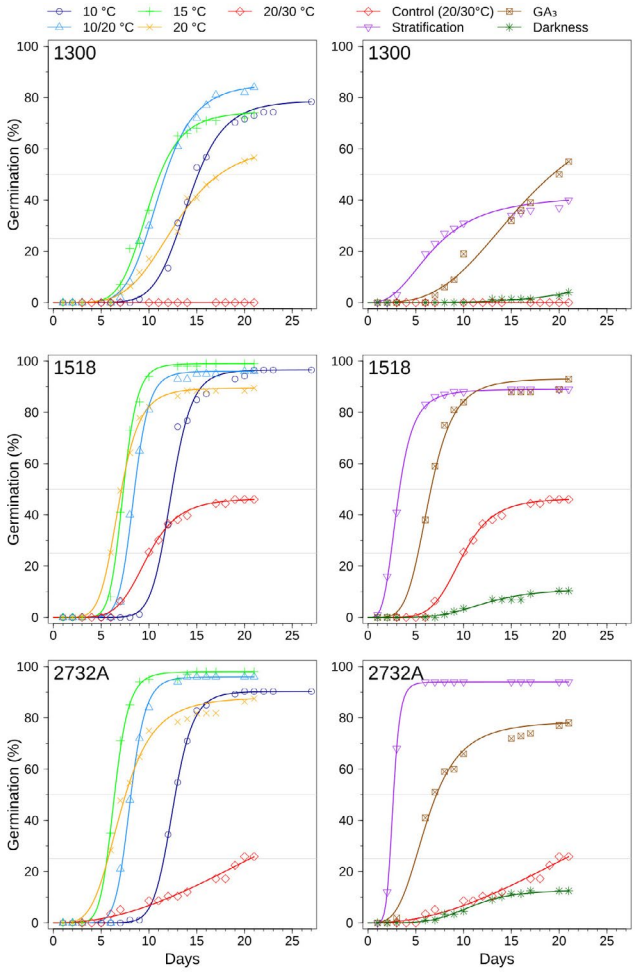


**Figure 1.** Effect of different temperatures (left), darkness, and dormancy breaking treatments (right) on the seed germination rate of three populations of *Festuca* gr. *ovina.*

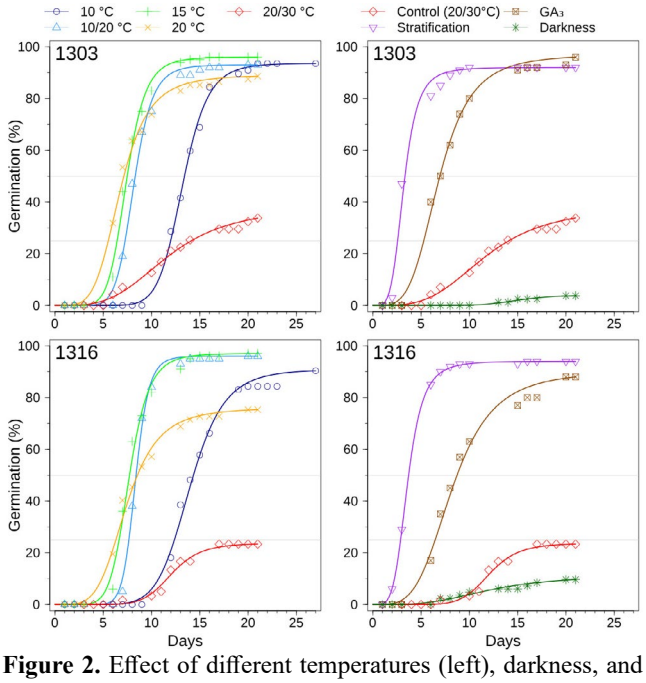


**Figure 2.** Effect of different temperatures (left), darkness, and dormancy breaking treatments (right) on the seed germination rate of two populations of *Festuca* gr. *rubra.*

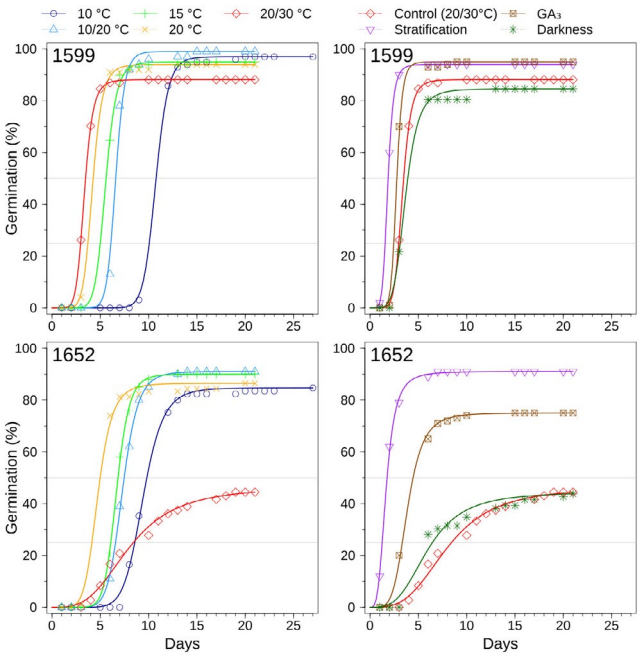


**Figure 3.** Effect of different temperatures (left), darkness, and dormancy breaking treatments (right) on the seed germination rate of two populations of *Festuca gigantea.*

$t_{25}$ not reached until later (Table 3, Figs. 1 and 2). For the *F.* gr. *rubra* populations,the differences did not reach significance (Table 3). This is probably due to an imperfect performance of the parametric time-to event model and a high degree of uncertainty (wide confidence intervals) when germination rates are low. For the populations of *F. gigantea* and *F. arundinacea,* no significant differences were seen between the 20/30ºC/dark and 20/30ºC conditions with respect to Gmax or $t_{25}$ values.

The dormancy-breaking treatments ($GA_3$ and cold stratification) had a marked stimulatory effect on all the examined populations (Figs. 1-3). The population of *L. arundinaceum* was not subjected to these treatments since its germination rate was very high (95.9%) under the standard 20/30ºC conditions. In all cases the germination rate was higher with cold stratification, and the $t_{25}$ values obtained were significantly lower (Table 3). The Gmax was similar under both sets of conditions, with significant differences seen only between *F.* gr. *ovina* 2732A and *F. gigantea* 1652.

# Discussion

The use of adequate germination protocols is essential in germplasm bank management. Only then can viability monitoring be reliably performed. Ideally, all the viable seeds in a test sample should germinate, which demands that the germination conditions be optimum and any dormancy phenomena overcome. Given the large number of assays that have to be performed, and the generally

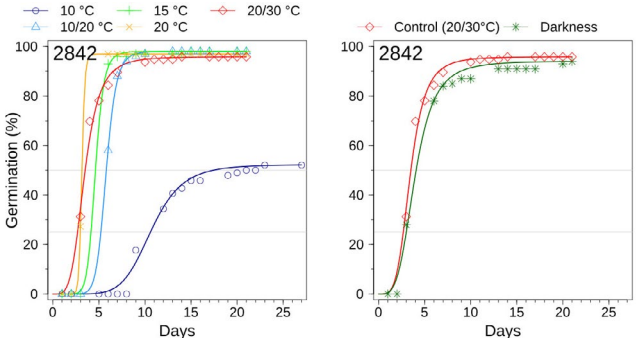


**Figure 4.** Effect of different temperatures (left), darkness, and dormancy breaking treatments (right) on the seed germination rate of a population of *Lolium arundinaceum.*

limited resources of germplasm banks, it is essential that procedures require the least effort possible. Moreover, rapid germination reduces the possibility of contamination by saprophytic microorganisms. Given that the number of seeds preserved is commonly quite small, especially for wild species, material is not wasted if assays doomed to failure can be avoided.

Temperature is one of the most relevant factors that regulate seed germination and dormancy. In the present work, the reduction in germination at 20/30ºC was common for all the narrow-leaved fescues (*F.* gr. *rubra, F.* gr. *ovina*) and for *F. gigantea* This results are similar to those previously reported. Kearns & Toole (1939), and Williams (1983) both obtained higher germination rates for *Festuca rubra* at 10-20ºC than at higher temperatures. The different behaviour of tall fescue (*L. arundinaceum*), which showed a clear fall in germination at 10ºC, also agrees with studies reporting an optimum germination temperature ranging from 20ºC to 30ºC (Hill *et al.*, 1985; Lu *et al.*, 2008; Sharifiamina *et al.*, 2016).

Many cool-season grass seeds have a light requirement for optimum germination. The light-absorbing pigment phytochrome can detect the difference in light quality and maintain seed dormancy under a leafed canopy until gaps in the vegetation are encountered (McDonald *et al.*, 1996). In contrast, in several grass species, *e.g. Festuca hallii,* the prolonged exposure to light can decrease germination rates and ability of seeds to germinate, especially at low water potentials (Mollard & Naeth, 2014). In our study, the presence of light under the standard 20-30ºC conditions appeared to be a positive influence on germination in the narrow-leaved fescues. A stimulatory effect of light is also reported in *F. rubra* and *F. arundinaca* (Danielson & Toole, 1976; Williams, 1983). Therefore, for seed germination testing, photoperiod conditions are advisable for most fescues and related species.

Although high percentages of germination were obtained under some temperature conditions, *F.* gr. *ovina*, *F.* gr. *rubra* and *F. gigantea* showed non-deep physiological dormancy at suboptimal temperatures. The existence of seed dormancy limited to a range of conditions is usually termed conditional or relative dormancy; its function is to prevent germination until conditions are more suited to plant development (Baskin & Baskin, 2004). In the above taxa, $GA_3$ and stratification at 5ºC for 7 days efficiently promoted germination at 20/30ºC. The positive effect of cold stratification on germination has previously been reported for *F. rubra* (Kearns & Toole, 1939), *F. gigantea* (Chorlton *et al.*, 1997) and *F. arundinacea* (Boyce *et al.*, 1976).

**Table 3.** Maximum germination percentages (Gmax) and $t_{25}$ values obtained for the seeds of different fescue accessions germinated at 20-30ºC, with different treatments to break dormancy. Values were estimated from the fitted curves.

| Accession | Gmax (%) | | | | $t_{25}$ (days) | | | |
|---|---|---|---|---|---|---|---|---|
| | Strat. | $GA_3$ | Dark | Control | Strat. | $GA_3$ | Dark | Control |
| ***F.* gr. *ovina*** | | | | | | | | |
| 1300 | 42.2 b | 55.0 * | 4.0 * | 0.0 * | 8.0 a | 13.0 b | - | - |
| 1518 | 89.1 a | 93.2 a | 11.1 c | 46.5 b | 2.4 a | 5.2 b | - | 10.0 c |
| 2732A | 94.0 a | 78.9 b | 13.0 c | 25.9 * | 2.3 a | 5.0 b | - | 20.6 * |
| ***F.* gr. *rubra*** | | | | | | | | |
| 1303 | 92.0 a | 97.0 a | 4.0 b | 38.2 b | 2.6 a | | - | 14.1 c |
| 1316 | 94.0 a | 90.3 a | 11.1 b | 23.6 b | 2.9 a | 6.5 b | - | - |
| ***F. gigantea*** | | | | | | | | |
| 1599 | 94.0 a | 95.0 a | 84.5 b | 88.1 ab | 1.6 a | 2.5 b | 3.1 c | 3.0 c |
| 1652 | 91.0 a | 75.0 b | 44.5 c | 46.4 c | 1.3 a | 3.3 b | 6.5 c | 8.4 c |
| ***L. arundinaceum*** | | | | | | | | |
| 2842 | - | - | 94.2 a | 95.9 a | - | - | 3.0 a | 2.7 a |

* Unadjusted value (recorded directly during the assays (see Results). Values with the same letter within accessions are not significantly different ($p$>0.05). Strat.: cold stratification at 5ºC for 7 days; $GA_3$: exposure to gibberellic acid; Dark: germination without light; Control: no treatment.

The taxa examined in the present work belong to the 'cold season grasses'. The seeds produced by the members of this group commonly show after-ripening or post-harvest dormancy, which prevents them from germinating soon after their dispersal or collection at the start of summer. This dormancy fades over the next few months, allowing germination after the dry season has passed. The exposure of seeds to high after-ripening temperatures favours their exit from this kind of dormancy (Baskin & Baskin, 1998; Steadman *et al.*, 2003). Post-harvest dormancy among members of *Festuca* has been known for decades (Kearns & Toole, 1939). Stanisavljević *et al.* (2010) reported *F. arundinacea, F. pratensis* and *F. rubra* to show high germination percentages three to four months after seed collection. In the present work, the populations of *F.* gr. *ovina* and *F.* gr. *rubra* still showed relatively high percentages of dormant seeds at 20/30ºC even at 4-11 months post-harvest (when the assays were performed); their storage at low temperature could have contributed to the maintenance of dormancy (Chorlton *et al.*, 1997). It should also be noted that in the above work performed by Stanisavljević *et al.* (2010), the seeds were subjected to cold stratification for 5 days, which very probably reduced the intensity of their dormancy. It is not improbable that the seeds of the present population of tall fescue, which showed no reduction in germination at 20/30ºC, experienced a degree of post-harvest dormancy before the assay was performed.

The differences seen between *L. arundinaceum* and the remaining taxa might be associated with the environmental conditions under which natural emergence and germination occur. In temperate climates, the germination of fescues usually takes place in autumn; the seed bank that builds up in the soil during the summer is practically absent by the winter (Thompson & Grime, 1979; Gibson & Newman, 2001). The capacity of *L. arundinaceum* seeds to germinate at higher temperatures than those of the other taxa might be related to its better adaptation to hot, dry conditions (Cross *et al.*, 2013). This might favour earlier emergence and aid in the colonisation of disturbed ground, which is usually warmer.

The present results not only reveal inter-specific variation in terms of germination and dormancy, but also differences between populations of the same species. This might be due to differences in the environmental conditions of the mother plants during seed development or to genetic factors associated with selection pressures at work in their habitats (Lord, 1994; Chorlton *et al.*, 1997; Anderson & Milberg, 1998). However, since all the seeds used in the present work were produced by multiplication in the same place and in the same year, the differences seen between the populations of *F.* gr. *ovina* and *F. gigantea* are more likely to have a genetic explanation. Compared to the other members of their groups, the smaller percentages of germination recorded at high temperatures for *F.* gr. *ovina* 1300 and *F. gigantea* 1652 might be explained by their adaptation to their place of origin (further from the coast and at higher altitude, Table 1). However, the coastal and montane populations of *F.* gr. *rubra* showed very little difference in their germination behaviour.

The rules published by ISTA (2017) for all species of *Festuca* indicate that germination assays be performed at 15/25º or 20/30°C, with cold stratification if needed. However, in the present work, and with the exception of *L. arundinaceum*, the 20/30ºC conditions were those associated with the smallest Gmax values when no cold stratification was provided. Indeed, even when such stratification was provided, only 55% of the *F.* gr. *ovina* 1300 seeds assayed had germinated by day 21. The present Gmax results indicate the optimum temperature for the germination of fescue seeds to be around 15ºC, with no significant differences between the constant 15ºC and the alternating 10/20ºC conditions. In the narrow-leaved fescues, the $t_{50}$ values were generally smaller at 15ºC although similar to those obtained at 20ºC. For *F. gigantea,* germination was slightly earlier under the 20ºC conditions that at 15ºC. Finally, *L, arundinaceum* germinated most rapidly under the 20ºC and 20/30ºC conditions.

The present results could help germplasm banks improve the viability monitoring of their pasture grass seeds. The optimum germination temperature for wild fescue seeds would appear to be 15 or 20ºC depending on the species. However, only a limited number of populations was examined; it may be that greater variation in this and in the degree of dormancy would be seen across a larger number. Cold stratification is recommended when apparently viable but dormant seeds ("fresh seeds") remain at the end of an assay. Indeed, this could be performed a posteriori for the non-germinated seeds, thus avoiding the need to repeat the entire assay.

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