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Seed dormancy and germination in different populations of the Argentinan endemic halophyte grass, *Sporobolus phleoides* (Poaceae: Chloridoideae)

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Abstract. *Sporobolus phleoides* Hack. is an endemic grass of Argentina that is considered an important genetic resource for saline environments. Knowledge of its germination behaviour is an indispensable requirement for the future potential use of this species. Thus, the effects of different factors on germination were evaluated in six representative populations collected from plants cultivated in a uniform environment. In addition, we investigated how the different parts of the seed contributed to dormancy and intraspecific variability. *S. phleoides* has non-deep physiological dormancy that appeared to be related to its testa. Thereby, dormancy was completely removed with puncturing treatments. High levels of germination were also obtained in seeds stratified at 8°C over 100 days in a solution of nitrates. After-ripening treatment did not have a significant effect in breaking dormancy. Similar germination behaviour and dormancy levels were observed in the different populations, especially when they were subjected to cold stratification. Although further work is needed, cold stratification appears to be the major factor in determining the time of seedling establishment in natural environments for this species.

Additional keywords: cold stratification, nitrates, after-ripening, non-deep physiological dormancy, seed coat.

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Introduction

Sporobolus phleoides Hack. is a perennial warm-season grass endemic to the north-central region of Argentina (Zuloaga *et al*. 1994). It grows in saline environments with salt tolerant species from other genera including *Distichlis*, *Atriplex*, *Sarcocornia* and *Allenrolfea* (Ragonese 1967). *S. phleoides* is considered an important genetic resource as a source of germplasm for stress tolerance and rehabilitation of degraded habitats (Aronson 1989; Rogers *et al*. 2005). Its high seed production and predominantly self-pollinating reproductive system (Richard *et al*. 2015) are favourable features for field establishment under such condition. However, its germination behaviour has not been described. The lack of knowledge about seed dormancy and germination in wild species constitutes one of the major impediments to the potential use of its germplasm, for species reintroduction or habitat restoration (Hay and Probert 2013).

The germination in many *Sporobolus* spp. can be promoted by a range of temperatures between 20 and 35°C and different light conditions (Toole 1941; Lodge and Whalley 1981; Martínez *et al*. 1992; Andrews 1995; Khan and Gulzar 2003; Joshi *et al*. 2005; Ferrari 2008; Rana *et al*. 2012). However, the seeds of several species have a primary dormancy period (Toole 1941; Persad 1980; Andrews 1995; Andrews *et al*. 1997; Khan and Ungar 2001; Vogler and Bahnisch 2006; Ferrari 2008).

During dormancy, germination is delayed or completely inhibited, even under favourable conditions (Baskin and Baskin 2014). This impediment or block to germination has evolved differently across plant species, leading to diverse innate mechanisms or combinations (dormancy mechanisms) related to morphological, physical, and/or physiological properties of the seed (Finch-Savage and Leubner-Metzger 2006). In warm-season grass species these mechanisms are associated with the embryo or to its covering structures (Adkins *et al*. 2002). The mechanisms within the covering structures may involve mechanical, permeability and chemical barriers to germination. In contrast, the mechanisms within the embryo may involve the expression of certain genes, levels of certain plant growth regulators, the activity of important

respiratory pathways or the mobilisation and utilisation of food reserves. In addition, some embryos may be too immature to germinate immediately, requiring them to undergo a further growth phase before germination is possible (Adkins *et al.* 2002).

For *Sporobolus* spp., many reports indicate that the dormancy mechanisms are related to their seed coats (Toole 1941; Andrews 1995; Vogler and Bahnisch 2006). Its fruit is a modified caryopsis (utricle), with a thin and mucilaginous pericarp separated from the testa (Brandenburg 2003). In many of its species, including *S. phleoides*, seeds are released from the mother plant without their pericarp, glumes, lemma and palea (Richard *et al.* 2015). These structures, which are often involved in the dormancy of grasses (Simpson 1990), do not affect the germination in *Sporobolus* (Toole 1941; Lodge and Whalley 1981). However, a diversity of treatments has been employed in breaking the dormancy of these species, such as cold stratification, after-ripening, the addition of nitrates and chemical scarification (Toole 1941; Lodge and Whalley 1981; Vogler and Bahnisch 2006; Ferrari 2008; Roemmich *et al.* 2012).

Seed dormancy in wild species may be highly variable, and may be determined by genetic and environmental factors during seed development (Simpson 1990; Finch-Savage and Leubner-Metzger 2006; Baskin and Baskin 2014). This important adaptive trait is likely to vary among populations, especially in self-pollinated species that are prone to show more population differentiation than outcrossed species (Silvertown and Charlesworth 2009; Baskin and Baskin 2014). This should be taken into account because it may imply that some ecotypes are more suitable for domestication than others (Glison *et al.* 2015).

The objectives of this study were to: (i) determinate the type of seed dormancy in *S. phleoides*, (ii) investigate the contribution of different parts of the seed involved in restricting germination, (iii) find methods to achieve a rapid, uniform, and high germination, and (iv) analyse intraspecific variability in seed germination.

Materials and methods

Seed material

Seeds were collected from six experimental populations of *Sporobolus phleoides* Hack. plants, grown in the experimental field at the Facultad de Ciencias Agrarias-Universidad Nacional del Litoral (FCA-UNL), Esperanza, Argentina (31°27′ S, 60°56′ W). The mean annual temperature is 18°C, and the annual average precipitation is 1046 mm, which is mainly concentrated in the summer (GeoINTA 2008; Bianchi and Cravero 2010). Each experimental population consisted of 20 plants arranged in a completely random design. These were originated from seeds collected at different locations of Argentina representing arid (8028D; 7610D; 8393D), and humid regions (8452W; 8415W; 9722W) (Table 1). In this species, flowering begins in the spring (early October) and ends with the first frost in early winter (Richard *et al.* 2015). Seeds harvests for all tests were conducted in April 2012 (autumn). Each population of seeds was represented by seeds harvested from the 20 experimental plants. A fraction of these was stored in paper bags at 20°C for the after-ripen assays. The seeds employed in all germination assays were free of the lemma, palea, glumes and pericarp (Fig. 1*b*), in the same way they are naturally released (Fig. 1*a*).

Viability was determined in freshly harvested seeds and in seeds stored for 12, 18 and 24 months. For this, the seeds were previously soaked in distilled water for 4 h, after which part of the endosperm was removed. The remaining part of the seed containing the embryo was then soaked in 1% tetrazolium phosphate-buffer solution for 10 h at 30°C in darkness (Ferrari 2008). The seeds were considered viable if the embryo is stained red, and non-viable if unstained.

Effect of light and temperatures on germination

This test was conducted using a completely randomised factorial design, consisting of three main factors. For this, freshly harvested seeds from the six populations ($k = 6$) were placed in Petri dishes with three layers of Whatman No. 1 filter paper moistened with distilled water. Seeds were germinated under five temperature regimes ($k = 5$): two constant (20°C, 30°C) and three alternating (15/20°C, 20/30°C and 25/35°C in daily cycles of 16/8 h). In combination, three lighting conditions ($k = 3$) were employed: constant light, 16/8 h light/dark photoperiod each day, and continuous dark. Four replicates of 50 seeds per Petri dishes (experimental unit) were used for each treatment. The full factorial design consisted of 90 treatments, with four replicates per treatment (and hence requiring a total of 360 Petri dishes).

The light conditions were created with three 30 W cool white fluorescent light tubes (delivering 35 µmol m^{-2} s^{-1}), and for darkness, the Petri dishes were wrapped in black plastic sheets. The number of germinated seeds was counted daily for 15 days after sowing. Only the seeds showing at least a 2 mm radicle length were considered germinated. The seeds that did not

Table 1. List of populations of *Sporobolus phleoides* and environmental characteristics of the collection site

Population ID[A]	Phytogeographic province (district)[B]	Annual average temperature (°C)[C]	Annual rainfall (mm)[C]	Latitude	Longitude
8028 D	Chaqueña (dry Chaco)	19–20	500–600	30°38′	65°35′
7610 D	Chaqueña (dry Chaco)	19–20	600–700	29°51′	64°40′
8393 D	Espinal (del Caldén)	15–16	600–700	33°37′	65°26′
8452 W	Chaqueña (humid Chaco)	20–21	900–1000	28°09′	61°44′
8415 W	Espinal (del Algarrobo)	19–20	1000–1100	30°01′	61°16′
9722 W	Espinal (del Algarrobo)	19–20	1000–1100	30°09′	61°00′

[A]Identification number of the specimen collected, present in the herbarium (SF) of the FCA-UNL.
[B]According to work by Cabrera (1994).
[C]According to work by Bianchi and Cravero (2010) and GeoINTA (2008).

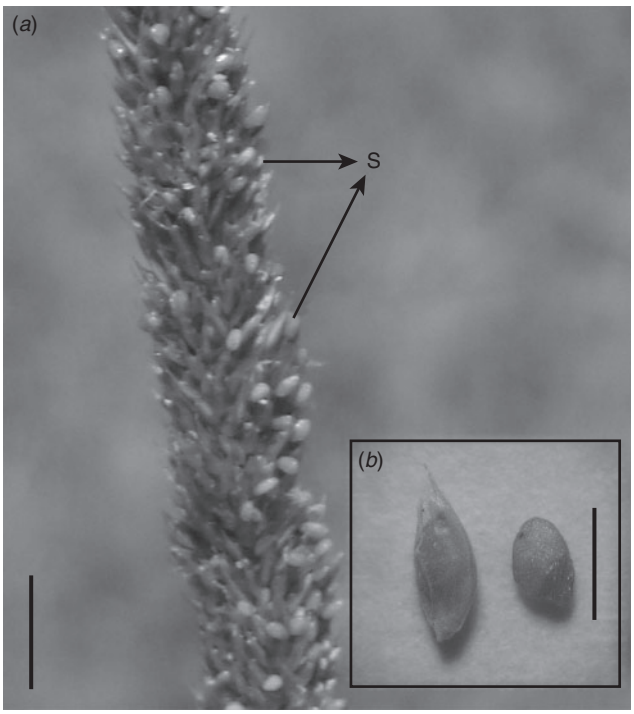

Fig. 1. Seeds (S) of *Sporobolus phleoides* without lemma, palea, glumes and pericarp: (*a*) sector of the inflorescence with naturally released seeds due to ambient moisture: scale bar = 1 cm.; (*b*) details of seeds with (left) and without their lemma, palea, glumes and pericarp (right), scale bar = 1 mm.

germinate were further tested using the tetrazolium test to evaluate their viability. The final percentage of germination (FPG) of the viable seeds and the coefficient of germination (CG) were calculated. CG, also known as 'speed of germination', denotes the number of germinated seeds (seedlings) per day, and was estimated by the Maguire Index (Maguire 1962):

$$CG = (G_1/N_1) + (G_2/N_2) + .. + (G_i/N_i) + .. + (G_n/N_n), \quad (1)$$

where:

N_1, N_2,. . ., N_n: represents the number of days from the initiation of germination test and G_1, G_2,. . ., G_n: represents the number of seedlings at the i-th day.

Effect of different pretreatments on dormancy break

The following germination tests were conducted as completely randomised factorial designs, under similar conditions described in the previous section. The different evaluated treatments are described below.

After-ripening

The seeds of the six populations ($k = 6$) stored at 20°C for 0 (control), 12, 18 and 24 months ($k = 4$) were tested at two constant (20°C and 30°C) and one alternating temperature regime (20/30°C in cycles of 16 h/8 h) ($k = 3$). In addition, three lighting conditions (constant light, 16/8 h light/dark photoperiod each day, and continuous dark) ($k = 3$) were applied. The full factorial design included 216 treatments, with four replicates per treatment (a total of 864 Petri dishes).

Cold stratification and chemical (nitrate) treatments

Freshly harvested seeds of the six populations ($k = 6$) were placed in Petri dishes with three layers of Whatman No. 1 filter paper. A set of seeds were moistened with distilled water, and another set was moistened with a nitrate solution of 0.2% KNO_3 ($k = 2$). These Petri dishes were wrapped in aluminium foil and stored in a refrigerator at 8°C for 0 (control), 30, and 100 days ($k = 3$). Then these seeds were placed to germinate in continuous darkness at 30°C in new plates, keeping them moistened with the same solution (distilled water or nitrate solution) in which they were imbibed during the cold stratification treatment. Four replicates were conducted per each of the 36 treatments (a total of 144 Petri dishes).

Puncturing

The intact (control) and testa-altered seeds ($k = 2$) of the six populations ($k = 6$) were used. The testa was altered by puncturing the middle of the seed with a dissecting needle after the seeds were hydrated with distilled water for 4 h. Then, the seeds that had undergone the two treatments were placed in Petri dishes with three layers of Whatman No. 1 filter paper moistened with distilled water and cultivated in continuous darkness at 30°C. Four replicates were used per each of the 12 treatments (a total of 48 Petri dishes).

Analysis of the contribution of different seed tissues involved in dormancy

Water uptake

These assays were performed with populations 8028D and 8415W ($k = 2$). The water uptake was examined in both the punctured and intact seeds. For this, all the seeds were hydrated in sterile distilled water during 4 h. Then, four replicates of 50 seeds per population were perforated in the middle, and another set of four replicates of 50 seeds per population remained intact ($k = 2$). Then both groups of seeds were dried for 24 h at room temperature (25°C) in the dark before initiating the water uptake study. Each group was weighed (considered weight at time 0), placed in Petri dishes with three layers of Whatman No. 1 filter paper moistened with distilled water, and incubated at room temperature. At different time intervals, the groups of seeds were blotted, re-weighted, and returned to the Petri dishes for further imbibition. Measurements of weight were conducted at 2, 4, 6, 8 and 24 h. The percentage of imbibition was estimated through percentage increase in mass of the seeds.

Embryo germination and presence of inhibitors

A set of 500 seeds was ground and placed in Eppendorf tubes with 2 mL of distilled water for 24 h at room temperature (25°C) in the dark to extract possible chemical inhibitors. One mL of this extract solution was poured onto germination papers of 1 × 1 cm, which was then cut in half and folded in the shape of a triangle. A set of excised and dry embryos from the 8028D and 8415W populations ($k = 2$) were placed on the blotters treated with the extract, and another set was placed on blotters

with distilled water ($k = 2$). Then they were cultivated in a humid chamber under continuous darkness at 30°C. Each treatment consisted of four replicates of 30 embryos for treatment and population following a factorial experiment. The embryos were examined daily for germination for obtaining FPG and CG. This technique was adapted from work by Duclos *et al*. (2013).

Statistical analyses

The data were analysed using the INFOSTAT statistical package (Di Rienzo *et al*. 2011). Before analysis, all variables were tested for normal distribution and equal variances, and values were arcsine transformed when required. However, the untransformed means re reported in the tables and figures. Data on the viability percentages obtained in the seed viability test were subjected to a two-way analysis of variance (ANOVA) to evaluate the combined effects of 'time of storage' and 'populations'. For the 'effect of light and temperatures on germination' experiment, a three-way ANOVA was performed to test the effects on germination (FPG and CG) of 'light', 'temperature', 'population' and their interactions. In the 'after-ripening' experiment, the effects of 'time of storage', 'temperature', 'population' and their interactions on germination (data obtained under darkness condition) were assessed by a three-way ANOVA. For the 'cold stratification and nitrates' experiment, the effects of 'time of cold stratification', 'nitrates', 'population' and their interactions on germination were analysed also by a three-way ANOVA. For the 'puncturing' experiment, the combined effects of 'puncturing' and 'population' on germination were tested by a two-way ANOVA. For the 'water uptake' experiment, a two-way ANOVA was performed to assess the effects of 'puncturing' in combination with 'population' on the percentage of imbibition after the 24 h, and at each time point separately. For the 'embryos germination and presence of inhibitors' experiment, the effects of the 'inhibitor', 'population' and their interaction on germination were examined by a two-way ANOVA. All factors were treated as fixed effects. Differences between mean values were tested for significance using Tukey's test ($P < 0.05$).

Results

Seed viability

No significant differences were registered for the effects of population, time of storage or the interaction in the viability percentage of seeds. The mean viability percentage was 94 ± 5% among seeds with 0, 12, 18 and 24 months of storage from all evaluated populations.

Effect of light and temperatures on germination

In general the evaluated treatments in this experiment were not effective in promoting germination. Because the interaction temperature × light × population had a significant effect on germination (FPG: $F = 1.55$, $P < 0.05$; CG: $F = 1.54$, $P < 0.05$), we next analysed FPG at each temperature level separately, with light, population and interactions as predictors. Significant effects of interaction between these factors were detected only for seeds cultivated at 30°C. However, even in these cases, the mean values remained below the 6% of FPG.

Effect of pretreatments on dormancy break

After-ripening

The three-way interaction time of storage × temperature × population was highly significant (FPG: $F = 2.87$, $P < 0.0001$; CG: $F = 3.47$, $P < 0.0001$). Due to this, we analysed the FPG at each temperature level separately, with time of storage, population and interactions as predictors. However, it was observed that these factors did not have important effects on germination (Fig. 2). With respect to lighting conditions, no differences in the FPG were observed among populations or performed treatments (data not shown). Consequently, light as factor was excluded from the model to simplify the analysis,

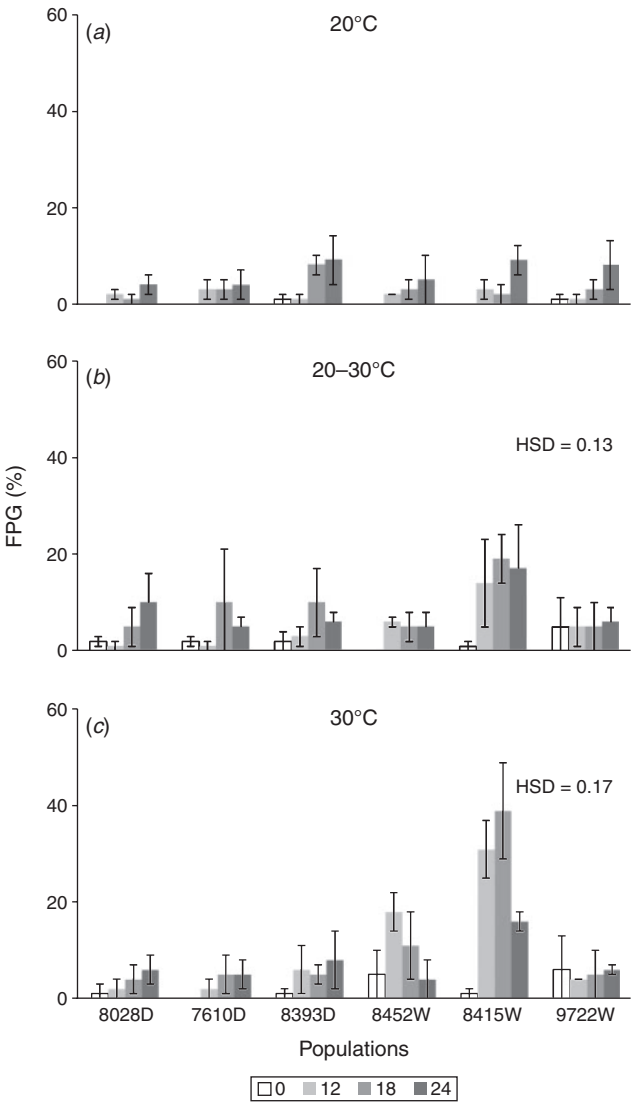

Fig. 2. Means (± s.d.) of the final percentages of germination (FPG) of viable seeds of six populations of *Sporobolus phleoides* with different storage times (0, 12, 18 and 24 months) exposed to different temperature treatments in continuous darkness: (*a*) 20°C; (*b*) 20−30°C; and (*c*) 30°C. Honestly significant differences (HSDs) from Tukey's test are indicated on each graph.

and the results that are shown correspond to those obtained only under continuous darkness.

Slight differences were registered among the FPG of seeds with different time of storage cultivated at 20°C, but these remained below the 10% in all populations (Fig. 2*a*). At higher temperatures, significant effects of the interaction populations × time of storage were registered ($F = 1.88$; $P < 0.05$ at 20–30°C; and $F = 5.07$ $P < 0.0001$ at 30°C). However, germination was promoted only in two populations (8452W and 8415W) with 12 and 18 months of storage grown at 30°C (Fig. 2*c*). Among these, the greatest response was obtained for the 8415W, which reached 31 ± 6% and 39 ± 10% of FPG respectively (Fig. 2*c*). Moreover, the mean values of the remaining populations did not achieve the 10% of FPG for the evaluated treatments (Fig. 2*b*, *c*).

Cold stratification and chemical (nitrate) treatments

Cold stratification had an important influence on the breaking of dormancy of freshly harvested seeds. Significant effects of the three-way interaction population × time of cold stratification × nitrates was registered for the two analysed variables (FPG: $F = 4.08$, $P < 0.05$; CG: $F = 2.96$, $P < 0.05$). Then, germination was analysed for each time of cold stratification level separately, with nitrate, population and interactions as predictors (Fig. 3).

An increase in germination was identified in seeds subjected to 30 days of cold stratification, but in combination with nitrates, germination was greatly increased further, reaching germination values of 44–64% of FPG (Fig. 3*b*). With 100 days of cold stratification, germination responses showed a further increase in all populations reaching between 46–83% of FPG in seeds cultivated in distillate water (control); and 66–86% in seeds cultivated with nitrates (Fig. 3*c*). No differences in FPG and CG values were observed in seeds without cold stratification from all the evaluated populations that were cultivated with or without nitrates (control) (Fig. 3*a*).

Puncturing

S. phleoides seeds overcame dormancy in response to the puncturing treatment. In all the evaluated populations, the seeds with altered testa reached their maximum FPG and CG germination values (95 ± 5% and 16.5 ± 1 seedlings per day respectively) after the third germination day. In contrast, for the intact seeds, FPG values of 2 ± 2% and a CG value of 0.1 ± 0.1 seedlings per day were obtained within 15 germination days.

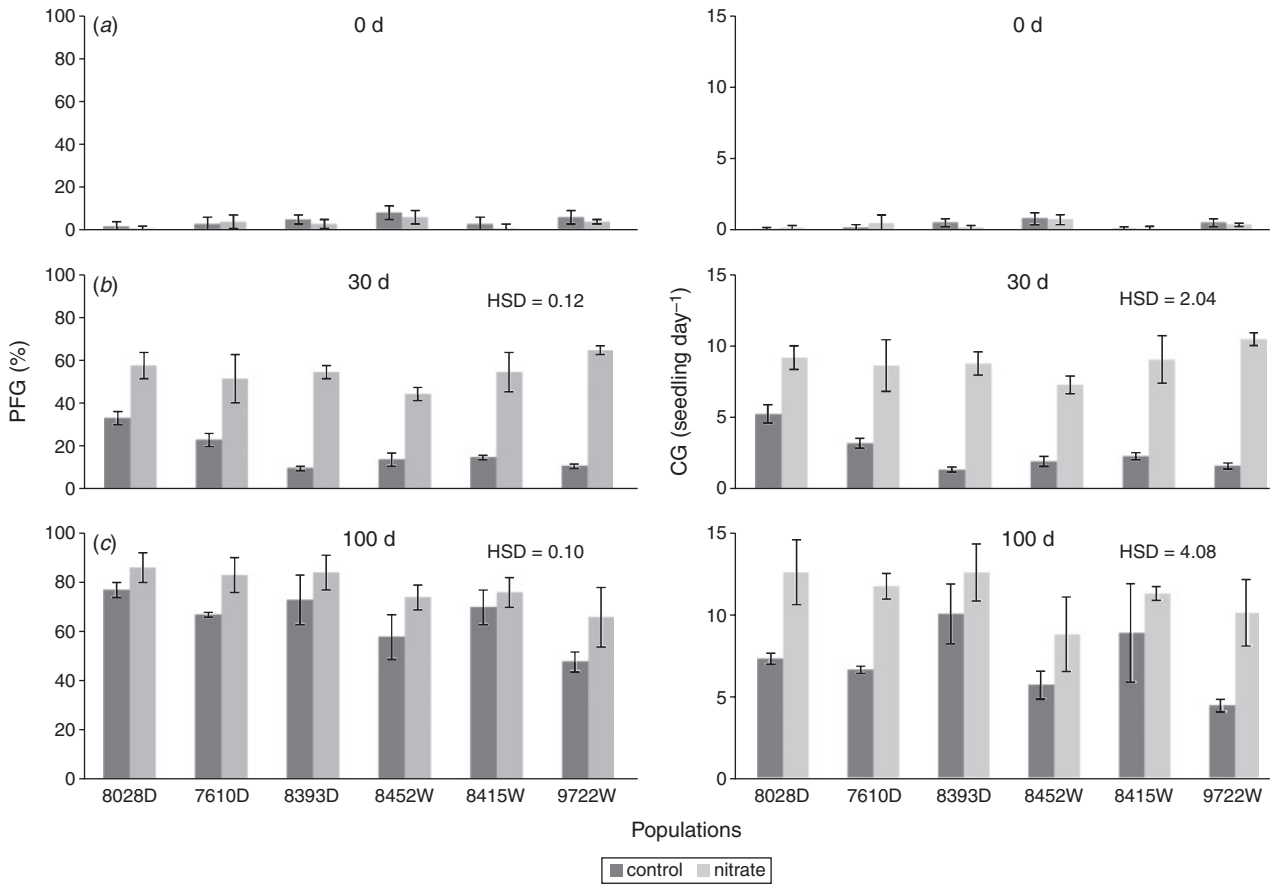

Fig. 3. Means (± s.d.) of the final percentages of germination (FPG) of viable seeds and coefficient of germination (CG) of six populations of *Sporobolus phleoides* cultivated at 30°C in continuous darkness in distilled water (control) or in 0.2% of KNO3 (nitrate) exposed to cold stratification at 8°C during: (*a*) 0 days; (*b*) 30 days; and (*c*) 100 days. Honestly significant differences (HSDs) from Tukey's test are indicated on each graph.

Analysis of the contribution of different seed tissues involved in dormancy

Water uptake

The evaluated factors population and puncturing showed significant effects on the imbibition percentages of seeds after 24 h ($F = 92.5$, $P < 0.0001$; $F = 11.08$, $P < 0.05$ respectively). Population 8415W showed a more imbibition capacity than 8028D, but both seed lots showed similar imbibition curves (Fig. 4). The imbibition of punctured seeds was higher than intact seeds, characterised by a fast imbibition phase during the first 2 h, reaching almost the double of the imbibition percentages than intact seeds. In punctured seeds, radicle growth was noted after the 24 h. For intact seeds, the maximum percentage was obtained after 8 h of imbibition, and no signs of radicular growth were detected. There was no significant difference in the initial dry weight between intact and perforated seeds ($F = 1.97$, $P = 0.18$).

Embryo germination and presence of inhibitors

No significant differences between the treatments were observed. Embryo germination reached 94 ± 6% of FPG. The extracts obtained did not affect the germination of embryos.

Discussion

The results obtained in this work indicate that the seeds of *Sporobolus phleoides* are dispersed with high levels of primary dormancy. This is consistent with findings for other *Sporobolus* spp. (Toole 1941; Persad 1980; Andrews 1995; Andrews *et al*. 1997; Khan and Ungar 2001; Vogler and Bahnisch 2006; Ferrari 2008; Roemmich *et al*. 2012).

The available evidence suggests that there are two possible locations of seed dormancy mechanisms among grass species: in the embryo covering structures, or inside the embryo (Simpson 1990; Adkins *et al*. 2002). In *S. phleoides* the embryo are viable,

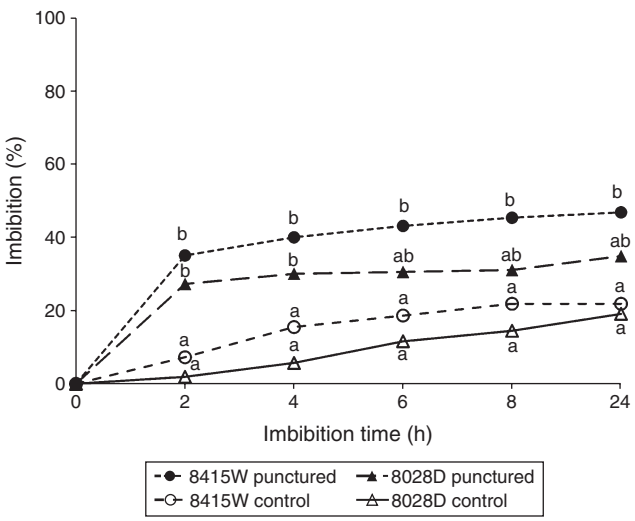

Fig. 4. Means of the percentage of imbibition at different times, in punctured and intact (control) seeds of two populations (8028D and 8415W) of *Sporobolus phleoides*. Letters indicate significant differences between the four combinations of populations and puncture treatments at a given time point ($P < 0.05$) according to Tukey's test.

and almost all of them are able to germinate when the testa is damaged. This indicates that the seed tissue enclosing the embryo is playing a primary role in regulating germination and dormancy. This is a common aspect in some *Sporobolus* whose seeds are able to germinate after the seed coat has been damaged (Toole 1941; Andrews 1995; Vogler and Bahnisch 2006). In these species, the seed coat comes from the development of the inner integument of the ovule, which, after fertilisation, develops into a hard layer capable of blocking germination (Satyamurthy 1983; Astegiano 1989). The pericarp remains as a loose layer that has no effect on germination (Toole 1941; Lodge and Whalley 1981).

The seed coat can exert influence on germination and dormancy of warm season grass species through: (a) permeability barriers preventing water uptake or gaseous exchange; (b) mechanical barriers preventing embryo expansion; or (c) a source of germination inhibitors (Adkins *et al*. 2002). The prevention of water uptake is not so common in grasses because their seed coats lack of the thick palisade layers typical of seeds with physical dormancy (Baskin and Baskin 1998; Adkins *et al*. 2002). In *S. phleoides*, the testa does not prevent the water uptake, which was also observed in many *Sporobolus* spp. (Toole 1941; Andrews 1995; Vogler and Bahnisch 2006). This led some authors to propose the presence of chemical inhibitors in seed of these species (Toole 1941; Vogler and Bahnisch 2006), although this was never proven. For *S. phleoides*, the extracts with possible inhibitors obtained from the testa/endosperm did not affect the embryo germination. The seed coats could be restricting gas exchange like in *Hordeum*, *Triticum* spp., *Setaria faberii* and *Panicum virgatum* (Bewley and Black 1994; Dekker and Hargrove 2002; Vogler and Bahnisch 2006; Duclos *et al*. 2013). However, in the present work, it was observed that water uptake and imbibition rate significantly increases in pierced seeds, thus allowing them to germinate. Toole (1941) also noted that water absorption rate increases in acid-treated and non-dormant seed of some *Sporobolus* spp. It is possible that puncturing the seed coat increases the embryo's access to water. This allows the embryo to generate enough expansive force to break through the seed coat (Baskin *et al*. 2006). Nevertheless, the nature of the restriction was not determined in the current study.

Based on the description of the different types of dormancy and their classifications (Baskin and Baskin 2004; Finch-Savage and Leubner-Metzger 2006), it is suggested that this species has a non-deep physiological dormancy (NDP). This type of dormancy is well known in grasses and is usually broken by periods of cold stratification (Baskin and Baskin 2014). This factor has a significant effect in releasing dormancy of *S. phleoides*. Stratification at 8°C promoted germination in all evaluated populations, with maximum germination occurring after 100 days. However, for other *Sporobolus* spp., a period of 15 to 60 days at 3 8°C was enough to release all the seeds from their dormancy (Toole 1941; Ferrari 2008).

Nitrogen-containing compounds (nitrates, nitric oxide, nitrite, nitrogen dioxide, ammonium, azide, and cyanide), promoted dormancy release and seed germination in many species, including grasses (Adkins *et al*. 2002; Sarath *et al*. 2006; Hu *et al*. 2014). This could be due to its effects on metabolism, oxidation state, or signalling (Arc *et al*. 2013). In *Sporobolus*

indicus, the addition of potassium nitrate unlocks primary dormancy (Ferrari 2008), but its effects on other species were different (Toole 1941). In *S. phleoides*, nitrates produce effects only when in combination with cold stratification. Similar responses were obtained in other grasses (Ferrari 1999; Matus-Cádiz *et al*. 2003). For the evaluated species, rapid, uniform and high seed germination responses were obtained when the seeds were cold stratified for 100 days in combination with nitrate treatments.

Dry storage can slowly unlock dormancy levels due to a ripening process (after-ripening), which results in changes in physiological status that induce seed germination (Graeber *et al*. 2012). However, its effect depends on the moisture and oil content of the seed, the seed coat components, and the storage temperature (Meyer *et al*. 2000; Sharif-Zadeh and Murdoch 2001; Manz *et al*. 2005; Finch-Savage and Leubner-Metzger 2006; Finkelstein *et al*. 2008). In *S. indicus*, 18 months of dry storage at 20°C unlocked dormancy (Ferrari 2008). Toole (1941) shares evidence of the incidence of after-ripening on germination of *Sporobolus cryptandrus*, *Sporobolus airoides*, *Sporobolus wrightii* and *Sporobolus asper*, but not for *Sporobolus flexuosus* and *Sporobolus giganteus*. In *S. phleoides* the after-ripening was not an effective factor to break dormancy. Germination was partially promoted in only two populations with 12 and 18 months of storage, and cultivated at 30°C. But in both cases the germination decreased for seeds stored during a long time, suggesting that secondary dormancy may have been induced (Batlla and Benech-Arnold 2010).

The puncturing treatment can completely remove dormancy on *S. phleoides*, but it is difficult to apply to a large number of seeds. Scarification can simulate the effects of puncturing (Simpson 1990) and is the most widely used methods of breaking the coat imposed dormancy in warm season grass (Adkins *et al*. 2002). Many techniques have been developed to scarify grass seeds, including scarification trough blades, sandpaper, percussion, exposure to dry heat, hot water and chemical scarification using acid or alkalis. Toole (1941) demonstrated that sulfuric acid can break dormancy in some *Sporobolus* spp., but some samples of seeds were injured. In this regard, puncturing represents a more accurate technique for standard germination tests. For *S. phleoides* it is recommended to piercing the pre-hydrated seeds before its cultivation at 30°C under darkness. Under these conditions, we suggest that 3 days is enough for final germination counts. However, an adequate scarification technique should be adjusted for the specie studied in future works.

Seed dormancy is an important adaptive trait that shows intraspecific variability, determined by genetic and environmental factors during seed development (Simpson 1990; Baskin and Baskin 2014). When seeds from different populations are cultivated in a uniform environment, the variability could be mainly attributable to genetic differences (Baskin and Baskin 2014). However, similar dormancy levels were registered for the evaluated populations in this work, especially when they were subjected to cold stratification. These results support the idea that dormancy is highly conditioned by cold stratification in this species. This has important implications to the potential uses of *S. phleoides*.

For many perennial grasses, the requirement for cold stratification constitutes a natural mechanism that ensures germination occurs during spring (Meyer *et al*. 1995; Baskin and Baskin 1998; Huang *et al*. 2004). This may represent advantages to many halophytes as *S. phleoides*, whose seeds remain dormant until the season of high precipitation, when salinity levels are reduced (Gul *et al*. 2013). For this species, the duration of winter could be an important factor that determines seedling establishment after the spring, during a rainy season. Additionally, winter may determine the sensitivity to some others dormancy-relieving factors such as nitrates (Batlla and Benech-Arnold 2010). However, studies in natural conditions are required because the effect of temperature on dormancy release could be modulated by soil moisture conditions and/or different levels of salinity (Batlla and Benech-Arnold 2010; Wang *et al*. 2013).

In conclusion, similar germination behaviour and dormancy levels were observed in the different populations of this native species. *S. phleoides* seed has non-deep physiological dormancy related to its seed coat. Dormancy could be completely removed when the testa is damaged. Thus, the puncturing of seeds is suggested as a pretreatment for standard germination tests. However, cold stratification in combination with nitrates can also reduce dormancy at optimum levels avoiding pre-harvest sprouting. Cold stratification appears to be a natural factor that determines the time of seedling establishment in natural environments. This could indicate that seeds are released from dormancy during winter months, ensuring that germination occurs after the spring, and during rainy season. Nevertheless, further studies are required to better understand its dynamics in natural environments and to optimise management practices to improve stand establishment of this potential resource for rehabilitation of degraded habitats.

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