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Variability in germination behaviour of *Paspalum dilatatum* Poir. seeds is genotype dependent

N. Glison*, L. Viega*, P. Cornaglia†, L. Gutiérrez‡ and P. Speranza*
*Departamento de Biología Vegetal, Facultad de Agronomía, Universidad de la República, Montevideo, Uruguay, †Cátedra de Forrajicultura, Facultad de Agronomía, Universidad de Buenos Aires, Buenos Aires, Argentina, ‡Departamento de Biometría, Estadística y Computación, Facultad de Agronomía, Universidad de la República, Montevideo, Uruguay

Abstract

Dallisgrass (*Paspalum dilatatum* Poir.) is a warm-season grass, native to South America. Its adoption as a pasture crop has been hindered by low seed germination and slow establishment. However, variability in germination behaviour for this species has never been systematically analysed. For *Paspalum* spp., dry-storage and moist cold or warm pre-conditioning treatments have been reported as effective for the relief or breaking of seed dormancy. In the present work, seed germination responses at 32°C were assayed for sixteen *P. dilatatum* genotypes, representing its known natural genetic variability, to two moist pre-conditioning treatments (at 5 and 20°C) and without pre-conditioning, and three seed storage periods (0, 3 and 6 months). Pre-conditioning at 20°C showed high germination percentages (>80%), with ratios equal to or higher than pre-conditioning at 5°C, suggesting that cold is not required to break dormancy. Longer storage times resulted in increases in germination responses for most of the genotypes, while other biotypes showed no effect. Biotypes Virasoro and Chirú showed a remarkably different behaviour with higher germination percentages of untreated freshly harvested seeds. Our results show that diversity for traits involved in seed germination exists among naturally occurring *P. dilatatum* genotypes and the characterization of these traits should be addressed during ecotype characterization and evaluation of potential domesticates of this species.

Keywords: temperate grassland, dallisgrass, seed dormancy, domestication

Correspondence to: N. Glison, Departamento de Biología Vegetal, Facultad de Agronomía, Universidad de la República, Montevideo, Uruguay.
E-mail: nigli@fagro.edu.uy

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Introduction

Species in the initial stages of domestication frequently show high levels of seed dormancy which prevent uniform and timely seedling establishment (Gepts, 2004). Knowledge of the factors that release seed dormancy could be useful to facilitate the adoption of a new crop (Adkins *et al.*, 2002). Moreover, wild species possess a great amount of variability for this trait (Baskin and Baskin, 2000). This should be taken into account during the initial ecotype selection stage because it may imply that some of them are more suitable for domestication than others. However, research on natural genetic variability of seed germination responses of potential domesticates is not frequently performed during the stages of ecotype characterization and evaluation. Several warm-season grasses that are sown for forage, particularly in the Paniceae tribe, still show seed dormancy levels that are similar to those of wild forms (Adkins *et al.*, 2002).

Dallisgrass (*Paspalum dilatatum* Poir.) is a perennial warm-season grass with a high potential as a summer forage crop in temperate regions. It shows optimal growth at warm temperatures (Pearson and Shah, 1981) and also high levels of frost, drought and flood tolerance (Loreti and Oesterheld, 1996; Campbell *et al.*, 1999), which make it a suitable forage crop for regions where winter conditions are too harsh for the more widely used tropical grasses. However, low seedling establishment in the field is a major constraint for the widespread adoption of *P. dilatatum*. Seedling establishment problems in this species have been attributed, among other factors, to slow and erratic seed germination (Evers and Burson, 2004) which may be partly due to seed dormancy (Schrauf *et al.*, 1995; Cornaglia *et al.*, 2005).

Natural genetic variability has been reported for *P. dilatatum* in its native range (south-eastern South America), including the common apomictic pentaploid biotype (Burson, 1991), sexual tetraploids (Hickenbick,

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1992; Speranza, 2009), apomictic hexaploids and other geographically restricted forms (Burson, 1991; Speranza, 2009). More recently, genetic variability has also been reported within the pentaploid biotype (Machado *et al.*, 2005; Speranza, 2005a, 2009). Morphological and physiological variability has also been reported among *P. dilatatum* biotypes, including tiller and panicle length (García *et al.*, 2007), forage yield and grazing tolerance (Venuto *et al.*, 2003), and response to water stress (Couso *et al.*, 2010). Similarly, differences have been reported in percentage of spikelets with caryopses and germination rate among some biotypes (Souza-Chies and Cavalli-Molina, 1995; Tischler and Burson, 1999; Pitman *et al.*, 2005). Until recently, however, the available cytogenetic, genetic and morphological information has not been considered for the assemblage of representative germplasm collections for agronomic evaluation.

Seed dormancy is modified by environmental factors which interact with the genotype. The initial level of dormancy may change according to harvest date due to environmental conditions during seed development (Fenner, 1991). *Paspalum dilatatum* releases full viable seeds during summer and autumn (Pizarro, 2000), and differences in germination behaviour have been reported among seeds collected on different dates (Tischler and Burson, 1999). Several treatments have been proposed to relieve or break dormancy in *Paspalum* spp., viz. acid and mechanical scarification, light and alternating temperatures (Fulbright and Flenniken, 1988; West and Marousky, 1989; Schrauf *et al.*, 1995). Dry storage increased germination of seeds of *P. notatum* Flüggé (Maeda *et al.*, 1997) and *P. atratum* Swallen (Kalmbacher *et al.*, 1999). Dry storage causes after-ripening in several species (Foley, 2001), which may result in a higher sensitivity to dormancy-breaking or germination-inducing factors (Batlla and Benech-Arnold, 2010). Cold pre-conditioning (pre-chilling) has been proposed as a dormancy-breaking treatment in *P. dilatatum* (Johnston and Miller, 1964; Schrauf *et al.*, 1995), and it has also been recommended for several other Paniceae: *Panicum virgatum* L. (Zarnstroff *et al.*, 1994), *Andropogon gerardi* Vitman (Beckman *et al.*, 1993) and *Sorghastrum nutans* (L.) Nash (Hsu *et al.*, 1985). Seeds of *P. atratum*, however, showed no increase in germination with pre-chilling (Kalmbacher *et al.*, 1999). Moreover, Schrauf *et al.* (1995) reported no differences between cold and warm pre-conditioning treatments. In the present work, we studied whether there is variability in seed germination of selected genotypes of *P. dilatatum* that represent its natural genetic variability. To achieve this, we assayed seed germination of each genotype under the most commonly recommended cold and warm pre-conditioning treatments, compared it to a without pre-conditioning treatment and analysed the germination responses of each treatment to increases in dry-storage times and their interaction with genotypes. Also, we evaluated the effects of different harvest dates on germination behaviour.

Materials and methods

Plant material, seed harvest and dry-storage conditions

Sixteen genotypes of *P. dilatatum*, representing the natural genetic variability reported by Speranza (2005a,b), were obtained from a seed collection from the Germplasm Bank of the College of Agriculture of the University of the Republic, Uruguay. The collection included twelve pentaploid apomictic clones, two hexaploid clones (Uruguaiana and Chirú) and one inbred line from each the sexual ssp. *flavescens* and the Virasoro biotype (Table 1). Five individual plants of each genotype were grown in pots (22 cm diam., 19 cm deep) in a greenhouse without temperature control or supplementary light for three years (2009 to 2012) in Montevideo, Uruguay. Seeds were harvested in March 2011 (autumn 2011) and January 2012 (summer 2012) and cleaned with a seed blower. Further cleaning was conducted by manually screening of the seeds to discard remaining empty spikelets or seeds containing *Claviceps* sclerotia. The remaining seeds were stored in paper envelopes within a closed chamber in the dark at room temperature. Seeds from each lot were evaluated at zero (freshly harvested), three and 6 months of dry storage. The average temperature of the whole storage period was recorded for each seed harvest date, resulting in 21 and 26°C for the autumn 2011 and summer 2012 lots respectively.

Germination assays and treatments

For each harvest date (autumn 2011 and summer 2012) and storage time (freshly harvested, 3 and 6 months of dry storage), randomized complete block experiments with three replications were conducted. Three treatments (cold and warm pre-conditioning, and without pre-conditioning) and the sixteen genotypes were arranged in a split-plot design where the 'main plot' consisted of a tray with all genotypes evaluated for one of the treatments, and 'small plots' consisted of Petri dishes with individual genotypes. Therefore, the experimental units consisted of Petri dishes with thirty seeds of a single genotype exposed to one treatment. The nine trays were carried out at the same time and were grouped into three complete blocks to control for heterogeneity

Table 1 List of selected *Paspalum dilatatum* genotypes with their ploidy levels, reproduction mode and subspecies or biotype identifications. For pentaploid clones, the corresponding genetic group is specified according to Speranza (2005b). The groups a, b and c are recombinant clones, whereas d is mutant variants of a common clone.

Labels	Ploidy	Reproduction	Species/subspecies/biotype	Genetic group
a108	$2n = 5x$	Apomictic	*P. dilatatum* ssp. *dilatatum*	a
a113	$2n = 5x$	Apomictic	*P. dilatatum* ssp. *dilatatum*	a
a62-8	$2n = 5x$	Apomictic	*P. dilatatum* ssp. *dilatatum*	a
b59A	$2n = 5x$	Apomictic	*P. dilatatum* ssp. *dilatatum*	b
b67	$2n = 5x$	Apomictic	*P. dilatatum* ssp. *dilatatum*	b
b73-7	$2n = 5x$	Apomictic	*P. dilatatum* ssp. *dilatatum*	b
c19-1	$2n = 5x$	Apomictic	*P. dilatatum* ssp. *dilatatum*	c
c33-1	$2n = 5x$	Apomictic	*P. dilatatum* ssp. *dilatatum*	c
c44	$2n = 5x$	Apomictic	*P. dilatatum* ssp. *dilatatum*	c
d111	$2n = 5x$	Apomictic	*P. dilatatum* ssp. *dilatatum*	d
d58-2	$2n = 5x$	Apomictic	*P. dilatatum* ssp. *dilatatum*	d
d59C	$2n = 5x$	Apomictic	*P. dilatatum* ssp. *dilatatum*	d
ssp. *flavescens*	$2n = 4x$	Sexual	*P. dilatatum* ssp. *flavescens*	
Virasoro	$2n = 4x$	Sexual	*P. dilatatum* biotype Virasoro	
Chirú	$2n = 6x$	Apomictic	*P. dilatatum* biotype Chirú	
Uruguaiana	$2n = 6x$	Apomictic	*P. dilatatum* biotype Uruguaiana	

due to temperature stratification within the incubation chamber. Germination was carried out on moist filter paper, in 10-cm plastic Petri dishes. The filter paper was moistened with a 0·02 M KNO_3 solution (0·2% w/v) once, and then, irrigation was performed with distilled water when necessary. Cold (5°C) pre-conditioning treatment was carried out by placing the Petri dishes with the moist seeds inside a domestic refrigerator for 7 d in the dark. Warm (20°C) pre-conditioning treatment was performed by placing the Petri dishes with the moist seeds inside a temperature-controlled chamber for 7 d without lighting. After the pre-conditioning treatment time had elapsed, the Petri dishes were transferred into an incubation chamber with fluorescent lighting (15–20 μmol m^{-2} s^{-1}) at 32°C during 14 d, following Maeda *et al.* (1997). The control treatment without pre-conditioning was conducted by placing the Petri dishes directly into the incubation chamber immediately after the seeds were moistened. To incubate all treatments simultaneously, the Petri dishes corresponding to the control treatment were prepared when the pre-conditioning period had elapsed for the other two treatments. Final germination percentages were recorded for each experimental unit. At the end of each germination assay, ungerminated seeds were tested for viability using the tetrazolium test, following Maeda *et al.* (1997), and then, viability percentages were calculated by adding the number of tetrazolium positive seeds to the number of germinated seeds.

To compare the warm pre-conditioning treatment with previously reported optimal germination conditions for this species, such as those suggested by ISTA (1999), freshly harvested seed samples of summer 2012 lots of four divergent pentaploid clones (a62-8, b67, c33-1, d58-2), the Virasoro and Chirú biotypes, and ssp. *flavescens* were placed under alternating temperatures (20/35°C, dark/light) simultaneously to their corresponding warm pre-conditioning germination assays, otherwise following the same experimental procedures.

Statistical analysis

Individual experiments from each harvest date and storage time were analysed within a single model to evaluate the effects of harvest date, storage time, treatment, genotype and their interactions in a full-model as follows:

$$\begin{aligned} Y_{ijklm} = {} & \mu + H_i + S_j + \mathrm{HS}_{ij} + T_k + \mathrm{HT}_{ik} + \mathrm{ST}_{jk} + \mathrm{HST}_{ijk} \\ & + \beta_l + \delta_{ijkl} + G_m + \mathrm{HG}_{im} + \mathrm{SG}_{jm} + \mathrm{TG}_{km} \\ & + \mathrm{HSG}_{ijm} + \mathrm{HTG}_{ikm} + \mathrm{STG}_{jkm} + \mathrm{HSTG}_{ijkm} + \varepsilon_{ijklm} \end{aligned}$$

where μ is the overall mean, H_i the effect of the ith harvest date, S_j the effect of the jth storage time, T_k the kth treatment effect, HS_{ij}, HT_{ik}, ST_{jk}, HST_{ijk} the two- and three-way interactions involving harvest date, storage time and treatment, β_l is the block effect, δ_{ijkl} the error associated with the main plot, G_m the effect of the mth genotype, HG_{im}, SG_{jm}, TG_{km}, HSG_{ijm}, HTG_{ikm}, STG_{jkm} and $HSTG_{ijkm}$ the two-, three-, and four-way interactions with the genotype, and ε_{ijklm} the experimental error. A fixed-effects model was considered with δ_{ijkl} and ε_{ijklm} independent random variables. To study the interactions, analyses by harvest date or storage time were conducted. The data were analysed using the GENMOD procedure in SAS 9.2 (SAS Institute, Cary, NC, USA), with seed germination and seed viability ratios as the response variables, using a binomial distribution and the logit link function. As higher-order interactions were significantly different from zero, orthogonal contrasts were designed to evaluate the effect of storage time on each genotype in each of the treatments and harvest dates. Additionally, orthogonal contrasts were designed to evaluate the effect of harvest date for each genotype on each of the treatments when applied to freshly harvested seeds and 6-month stored seeds.

Results

Treatment effects and treatment*genotype interactions were significant for most genotypes ($P < 0{\cdot}05$) for seeds harvested in autumn 2011 (Figure 1) and also in summer 2012 (Figure 2). High germination percentages (>80%) were obtained with warm pre-conditioning for almost all genotypes and dry-storage times, whereas variable results were observed with cold pre-conditioning. Germination percentages after cold pre-conditioning were lower for freshly harvested than for dry-stored seeds. On the other hand, the lowest germination percentages (<10%) were recorded in freshly harvested seeds without pre-conditioning in pentaploid clones, ssp. *flavescens* and the Uruguaiana biotype, whereas the Virasoro biotype showed 49% average germination under the same treatment. Moreover, the Chirú biotype showed no differences among treatments in any case, and it always showed high germination percentages (>60%).

There were statistical differences among treatments and storage times for seed viability measured at the end of the germination assay period ($P < 0{\cdot}05$, data not shown). Slightly lower viability percentages (ungerminated seeds that were negative to tetrazolium) were observed for seeds germinated after control treatment, compared with seeds of the same lot subjected to either cold or warm pre-conditioning (data not shown), although these differences were never higher than 6%. In spite of this, average seed viability was high in almost all assays (>80%). In a few cases, there were differences among genotypes ($P < 0{\cdot}05$). The sexual and Chirú biotypes frequently showed viability rates higher than 90%, whereas the lowest rates were registered in some pentaploid clones harvested in autumn 2011; i.e. c44, b73-7 and c19-1 (86, 85 and 82% respectively). There was no relationship between germination and viability percentages ($r^2 = 0{\cdot}25$).

Two-way interactions between harvest date and treatment, and harvest date and genotype were significant for freshly harvested seeds ($P < 0{\cdot}05$), but no three-way interaction among harvest date, treatment and genotype was found. Differences in germination responses to the cold pre-conditioning treatment between harvest dates were found for almost all genotypes, except for clones b67, c44, d111 and the Chirú biotype (Table 2), with seed lots harvested in the summer 2012 showing higher germination percentages (Figures 1 and 2). Moreover, after 6 months of storage, all two- and three-way interactions were significant when comparing the proportion of germinated seeds. Differences between harvest dates in all genotypes studied were found in cold pre-conditioning treatment or control treatment, or both (Table 2). In general, seeds harvested in the summer showed higher germination percentages, compared with seeds harvested in the autumn, after 6 months of storage (Figures 1 and 2).

Interactions between dry-storage time and treatment, and dry-storage time and genotype were significant, as well as the three-way interaction among storage time, treatment and genotype interaction for both harvest dates ($P < 0{\cdot}05$). For both harvest seasons, there were increases in germination after cold pre-conditioning for almost all pentaploid clones after the first 3 months of dry storage and no further increases between 3 and 6 months. The behaviour of seeds germinated without pre-conditioning varied with harvest seasons. For seeds harvested in autumn 2011, only a few clones showed increases between 0 and 3, or 3 and 6 months of storage; however, for seeds harvested in the summer 2012, most clones showed increases in germination between 0 and 3 months, and almost all of them showed further significant increases after the following 3 months (Table 3).

Both sexual tetraploid biotypes ssp. *flavescens* and Virasoro showed an increase in germination with cold pre-conditioning in the 0–3 month comparison, but a decrease in the 3–6 month comparison for seeds harvested in autumn 2011. On the other hand, for seeds harvested in summer 2012 with the same treatment, no difference was observed after the first 3 months of storage, but both biotypes showed an increase in the 3–6 month comparison. Increases in germination ratio were observed with the control treatment only between three and 6 months of storage for both sexual biotypes and harvest dates (Table 3). For seeds of

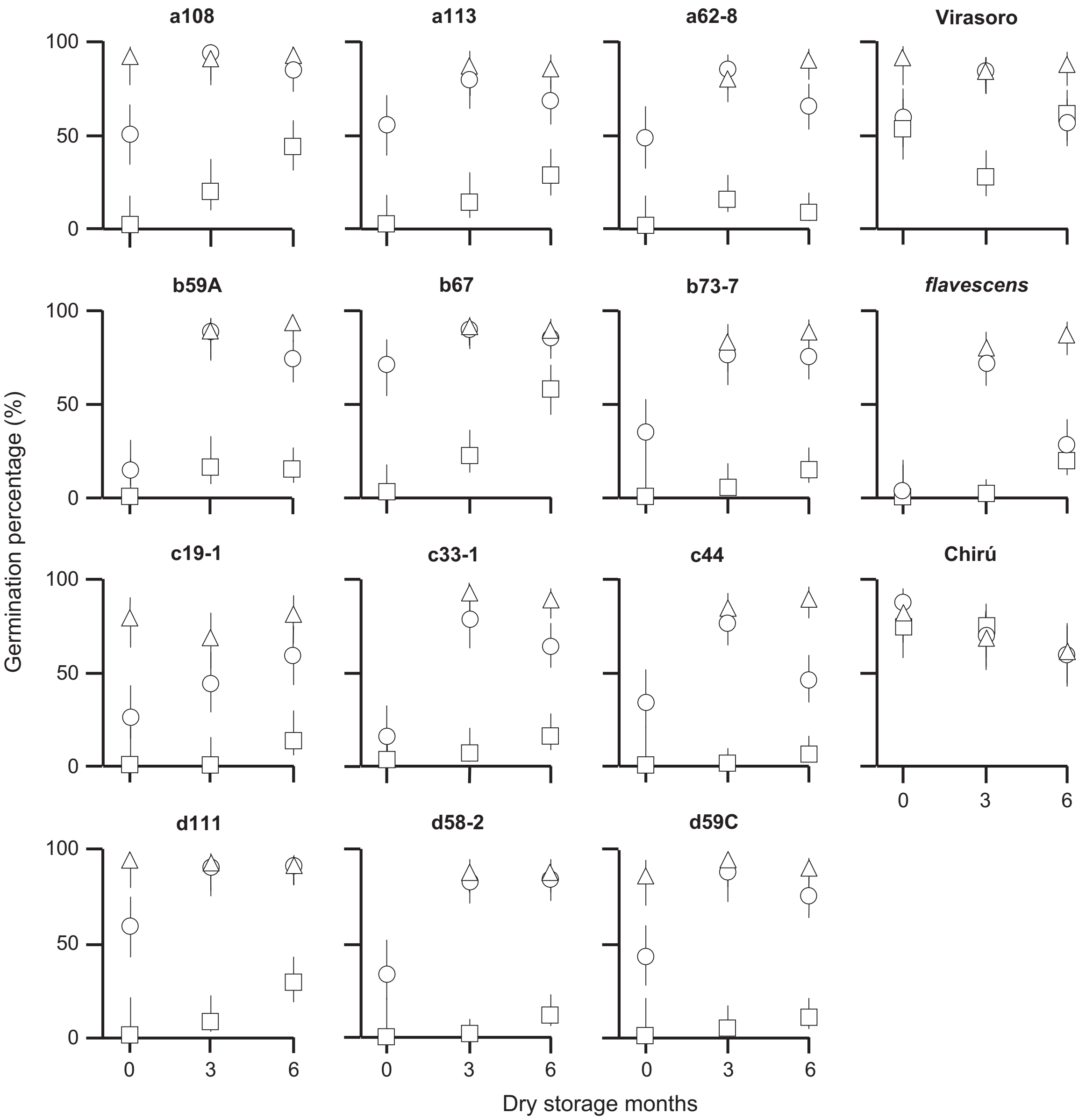

Figure 1 Seed germination percentages (14 d, 32°C, light) for fifteen *Paspalum dilatatum* genotypes, harvested in autumn 2011 at 0, 3 and 6 dry-storage months, subjected to three treatments. Triangles denote warm pre-conditioning (7 d, 20°C), circles denote cold pre-conditioning (7 d, 5°C), squares denote without pre-conditioning. Vertical lines represent confidence limits at 95%. No data were available for the Uruguaiana biotype due to insufficient seed production. Missing symbols were due to assay failures.

the hexaploid biotype Chirú harvested in autumn 2011, no differences were observed with any treatment in the 0–3 and 3–6 month comparisons (Table 3), but a decrease in germination ratio was recorded with cold pre-conditioning when comparing germination between 0 and 6 months of storage ($P < 0{\cdot}05$).

Finally, no significant differences were observed in germination percentages of fresh seeds harvested in the summer of 2012 among the genotypes assayed when comparing alternating temperatures (20/35°C, dark/light) and constant 32°C following the warm pre-conditioning treatment, resulting in mean germination values of 93 and 92% respectively (data not shown).

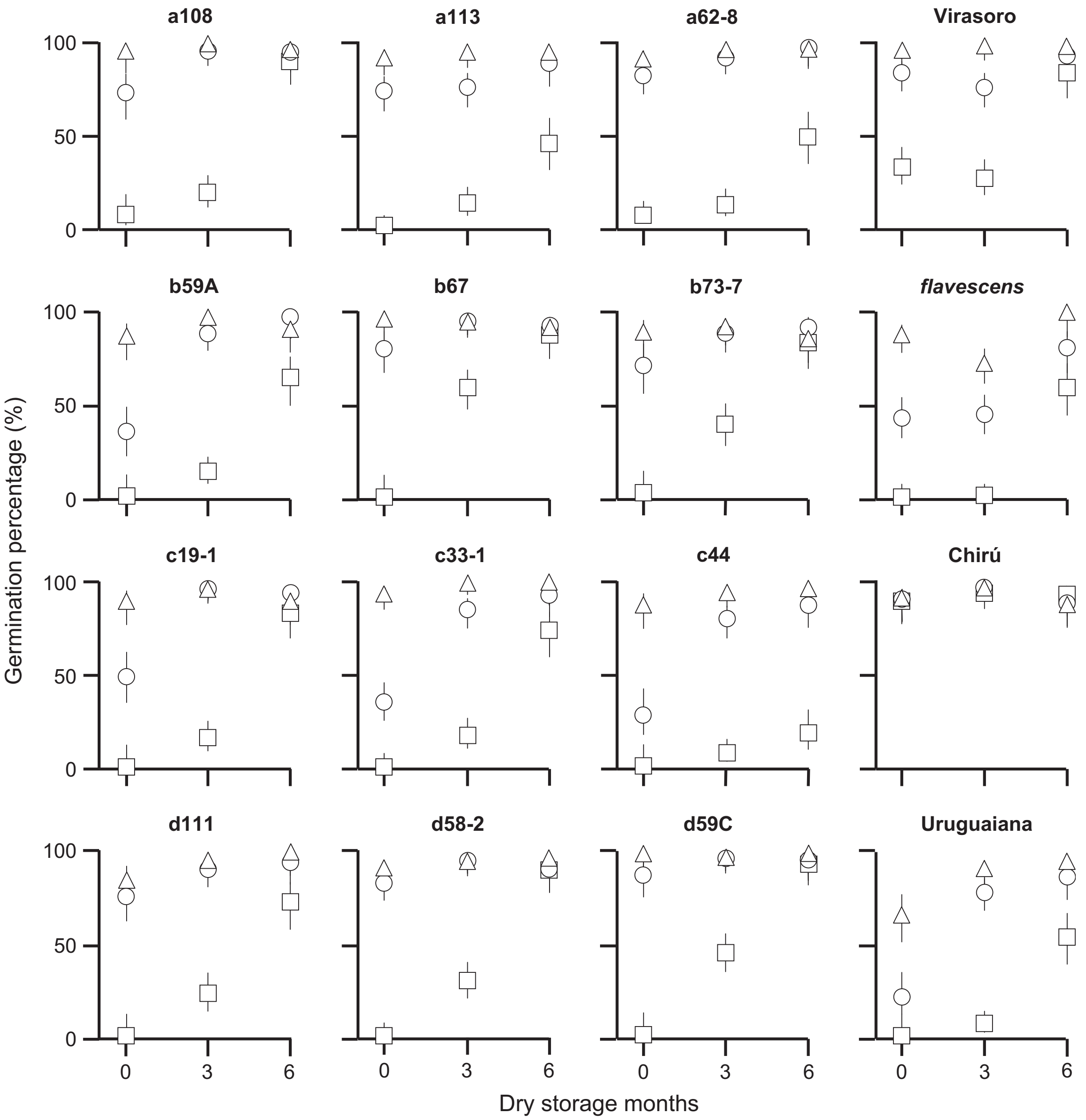

Figure 2 Seed germination percentages (14 d, 32°C, light) for sixteen *Paspalum dilatatum* genotypes, harvested in summer 2012 at 0, 3 and 6 dry-storage months, subjected to three treatments. Triangles denote warm pre-conditioning (7 d, 20°C), circles denote cold pre-conditioning (7 d, 5°C), squares denote without pre-conditioning. Vertical lines represent confidence limits at 95%.

Discussion

Both of the pre-conditioning treatments resulted in higher germination percentages when compared to the control seeds (without pre-conditioning) in almost all genotypes, storage times and harvest dates assayed. This effect could be attributed to a break of seed dormancy due to the exposure of seeds to an earlier initiation of the imbibition period at lower temperatures during pre-conditioning, compared with the control seeds which were incubated directly at 32°C (Schrauf *et al.*, 1995). In spite of the above, the temperature at which the pre-conditioning was carried out produced different germination responses. Cold pre-conditioning, referred to as pre-chilling or moist

Table 2 Orthogonal contrast *P* values between seed harvests (autumn 2011 and summer 2012) for each *Paspalum dilatatum* genotype, considering each treatment of freshly harvested and 6-month-dry-stored seeds.

	Freshly harvested seeds			**Six-month dry-stored seeds**		
Genotype	**WP***	**CP**	**NP**	**WP**	**CP**	**NP**
a108	0·515	**0·015†**	0·168	0·490	0·121	**<0·001**
a113	ND‡	**0·030**	0·381	0·222	**0·026**	0·122
a62-8	ND	**<0·001**	0·155	0·307	**<0·001**	**<0·001**
b59A	ND	**0·013**	0·992	0·550	**0·002**	**<0·001**
b67	ND	0·274	0·402	0·785	0·294	**0·003**
b73-7	ND	**<0·001**	0·559	0·541	0·052	**<0·001**
c19-1	0·167	**0·014**	0·998	0·354	**<0·001**	**<0·001**
c33-1	ND	**0·012**	0·116	**0·013**	**0·002**	**<0·001**
c44	ND	0·528	0·987	0·267	**<0·001**	0·068
d111	0·116	0·058	0·992	0·071	0·648	**<0·001**
d58-2	ND	**<0·001**	0·769	0·184	0·396	**<0·001**
d59C	**0·015**	**<0·001**	0·986	0·122	**0·013**	**<0·001**
ssp. *flavescens*	ND	**<0·001**	0·770	**0·028**	**<0·001**	**<0·001**
Virasoro	0·428	**0·003**	**0·015**	0·102	**<0·001**	**0·030**
Chirú	0·245	0·737	0·058	**0·016**	**0·008**	ND

*Treatments: WP, warm pre-conditioning; CP, cold pre-conditioning; NP, no pre-conditioning.

†Significant contrasts ($P < 0{\cdot}05$) are shown in bold type.

‡ND = not determined. Contrast was not performed due to the absence of some or all germination data.

Table 3 Increases (I) or decreases (D) in seed germination for each *Paspalum dilatatum* genotype, considering each treatment, between 0–3 and 3–6 dry-storage months tested by orthogonal contrasts.

	Autumn 2011 harvest storage comparisons						**Summer 2012 harvest storage comparisons**					
	0 vs. 3			**3 vs. 6**			**0 vs. 3**			**3 vs. 6**		
Genotype	**WP***	**CP**	**NP**	**WP**	**CP**	**NP**	**WP**	**CP**	**NP**	**WP**	**CP**	**NP**
a108	NS†	I	I	NS	NS	I	NS	I	NS	NS	NS	I
a113	ND‡	I	NS	NS	NS	NS	NS	NS	I	NS	NS	I
a62-8	ND	I	I	NS	D	NS	NS	NS	NS	NS	NS	I
b59A	ND	I	I	NS	NS	NS	I	I	I	NS	NS	I
b67	ND	I	I	NS	NS	I	NS	I	I	NS	NS	I
b73-7	ND	I	NS	NS	NS	NS	NS	I	I	NS	NS	I
c19-1	NS	NS	NS	NS	NS	I	NS	I	I	NS	NS	I
c33-1	ND	I	NS	NS	NS	NS	I	I	I	NS	NS	I
c44	ND	I	NS	NS	D	NS	NS	I	NS	NS	NS	NS
d111	NS	I	NS	NS	NS	I	NS	I	I	NS	NS	I
d58-2	ND	I	NS	NS	NS	I	NS	I	I	NS	NS	I
d59C	NS	I	NS	NS	NS	NS	NS	I	I	NS	NS	I
ssp. *flavescens*	ND	I	NS	NS	D	I	D	NS	NS	I	I	I
Virasoro	NS	I	D	NS	D	I	NS	NS	NS	NS	I	I
Chirú	NS	NS	NS	NS	NS	ND	NS	NS	NS	D	NS	NS
Uruguaiana	ND	ND	ND	ND	ND	ND	I	I	NS	NS	NS	I

*Treatments: WP, warm pre-conditioning; CP, cold pre-conditioning; NP, no pre-conditioning.

†I or D = contrast was significant ($P < 0{\cdot}05$); NS = non-significant contrast ($P > 0{\cdot}05$).

‡ND = not determined. Contrast was not performed due to the absence of some or all germination data.

chilling, has been widely used in several species as a dormancy-breaking treatment (Kermode, 2005). In our study, cold pre-conditioning produced variable responses in different genotypes, as well as a generally increased response with longer storage time. However, cold pre-conditioning never resulted in higher germination percentages than the warm pre-conditioning, which was the best-performing evaluated treatment for all genotypes (Figures 1 and 2). These results suggest that cold may not be an essential factor to break dormancy in this species, but rather pre-conditioning itself, similar to what was suggested by Schrauf *et al.* (1995). This response is in agreement with evidence from field assays showing that flooding promotes germination and emergence in *P. dilatatum* (Cornaglia *et al.*, 2009). The comparable efficiency of alternating temperatures and warm pre-conditioning followed by constant germination-promoting temperatures suggests that pre-conditioning at mild temperatures may be enough to break dormancy, and temperature alternation, as reported in other species (Benech-Arnold *et al.*, 2000), is not strictly necessary for *P. dilatatum.*

Longer dry-storage times caused significant increases in germination response to cold pre-conditioning and to no pre-conditioning treatments, probably due to after-ripening, i.e. a lowered seed dormancy level (Batlla and Benech-Arnold, 2010). In spite of this, some genotypes showed a different behaviour, as in the case of Virasoro seeds harvested in autumn 2011, which showed similar germination percentages for all treatments when comparing freshly harvested and 6-month-stored seeds ($P > 0{\cdot}05$ in 0–6 month contrasts), or Chirú seeds of both harvest dates, which did not show increases in germination with storage time (Table 3). Intraspecific variation in response to dry after-ripening has been reported within other wild species such as *Oryza* ssp. subject to similar dry-storage conditions (Veasey *et al.*, 2004). In our work, variability in response to after-ripening was found, even among pentaploid clones (Table 3). Thus, responses to dry-storage time or conditions required for lower dormancy levels may be considered for the selection of agronomically more desirable genotypes.

For most genotypes, fresh seeds harvested in the autumn showed lower germination percentages than seeds harvested in the summer with cold pre-conditioning. This suggests that seeds produced in the summer may have a lower dormancy level. In previous research, differences in germination behaviour were found among harvest dates in *P. dilatatum* (Tischler and Burson, 1999) and also in other species (Andersson and Milberg, 1998). Moreover, when comparing seeds with 6 months of storage, the germination response of seeds harvested in the summer was higher in all genotypes. Temperature has been reported to be a significant factor affecting the after-ripening rate during storage (Meyer and Allen, 2009). The average temperature of the whole dry-storage period was lower for seeds collected in the autumn of 2011 than in the summer of 2012. Thus, higher dry-storage temperatures may have been more effective in releasing dormancy in *P. dilatatum*, as has been reported for *P. atratum* (Kalmbacher *et al.*, 1999) and *Bromus tectorum* L. (Allen *et al.*, 1995). However, our results may also be due to the initial seed dormancy level at harvest, or to a harvest-date by storage-time interaction effect on dormancy. Experimental evidence suggests that more after-ripening may be required when seeds show a higher initial dormancy level at harvest (Veasey *et al.*, 2004). This point should be addressed in future research because of its relevance for commercial seed management.

Different germination responses to constant 32°C conditions (without pre-conditioning) were found between the sexual biotypes, Virasoro and ssp. *flavescens* (Figures 1 and 2). These biotypes, which have been suggested to be genetically related to some of the minor pentaploid clones (Speranza, 2005b), are currently the subject of research to promote their commercial adoption in Uruguay and Argentina, due to their higher seed production and lower susceptibility to *Claviceps* than apomictic clones. In their native range, these biotypes are restricted to geographically distant areas and are genetically isolated (Speranza, 2009), although they can be artificially crossed to produce fertile hybrids (Caponio and Quarin, 1990). Different optimal germination temperatures have been reported among several perennial warm-season grasses (Hsu *et al.*, 1985; Roundy and Biedenbender, 1996) and also among *Panicum virgatum* L. varieties (Seepaul *et al.*, 2011). Therefore, differences in optimal germination temperature between Virasoro and ssp. *flavescens* could be an underlying explanation for our results. Such differences may partly explain their non-overlapping geographical distribution. In our work, we applied only one incubation temperature (32°C). Further work to characterize the optimal germination temperatures of these biotypes may provide additional information that could be used in the domestication and breeding of the species.

The monoclonal Chirú biotype, which has been suggested to be genetically related to Virasoro (Speranza, 2009), often showed high germination percentages and no differences among treatments for any seed lot, resembling the expected behaviour of seeds with little or no dormancy. Evidence of higher germination percentages for this biotype (referred to as 'Uruguayan'), than in the common biotype, has been reported in the literature (Tischler and Burson, 1999; Pitman *et al.*, 2005). On the other hand, Chirú showed a decreasing germination response to longer dry-storage times while maintaining a high level of seed viability. This behaviour suggests that secondary dormancy may have been induced under these conditions (Batlla and Benech-Arnold, 2010). Finally, only slight differences were detected among pentaploid clones in our study, which do not strictly correspond to genetic groups (Table 1). Further work under a wider range of germination conditions is required to assess whether these differences are biologically or agronomically relevant.

Conclusions

Variability in germination behaviour was observed among naturally occurring *P. dilatatum* genotypes under differing treatments. The level of variability in dormancy among genotypes clearly suggests that characterization of seed dormancy levels should be addressed during ecotype evaluation programmes. Furthermore, as sexual biotypes of this group are receiving more attention from researchers, the fact that different sexual biotypes show different germination requirements opens up the possibility of modifying this trait by conventional breeding.

On the other hand, the fact that the highest germination responses were attained after warm preconditioning, even for freshly harvested lots, suggests that *P. dilatatum* seeds have the ability to germinate as soon as they detach from the mother plant, provided that sufficient moisture is available and temperature is adequate. The implications of this behaviour should be studied further, both to understand its dynamics as a highly productive component of natural grasslands and to optimize management practices to improve stand establishment as a forage crop.

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