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Germination Conditions for Cebadilla de Agua (*Glyceria multiflora* Steudel), a Native Grass of the Flooding Pampa Rangeland (Argentine)

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Abstract: Mature inflorescences of Cebadilla de Agua (*Glyceria multiflora* Steudel), were obtained from five sites, in the Flooding Pampa, in the Buenos Aires Province, Argentina. A four factor completely randomized design with four replications was applied. Treatments combined original sites, pre chilled at 7°C for 72 h (present or absent), substrate moistened with 0.2% solution of potassium nitrate (KNO_3) (present or absent) and three levels of alternating germination temperatures: 15-10, 20-10 or 20-15°C in 8/16 h light/dark periods. The germination value recorded was percentage of germination of normal seedlings; Afterwards, the germination rate and uniformity were calculated. ANOVA, Tukey tests and correlations were performed. The five origins had a similar performance (93% of the total germination) when the temperature alternating of germination was 20-10 and 20-15°C in 8/16 h light/dark periods with KNO_3 and 20-10°C pre chilled. The KNO_3 solution positively enhanced the germination response. Higher germination rates were obtained with 20-15°C pre chilled + KNO_3 as well as with 20-15°C KNO_3. Pre chilled effect was related to germination temperature. When the total germination, rate and synchronization were considered, 20-15°C with KNO_3 was stated as optimal germination condition of *Glyceria multiflora* for all sites of collection.

Key words: Temperature alternating, pre chilled, temperature alternating, potassium nitrate (KNO_3)

INTRODUCTION

Cebadilla de agua (*Glyceria multiflora* Steudel) is a winter to late spring bunch perennial grass species (Festuceae, Festucoidae). It is a forage adapts to flooded soils, native from Argentine and Chile (South America).

This valuable native grass is distributed in natural grasslands of the Flooding Pampa sub-region (Humid Pampa region, Buenos Aires province, Argentina) to flooding environments avoiding saline soils (Deregibus and Cahuepé, 1983; León *et al*., 1979).

Fernández Grecco and Viviani Rossi (1995) proposed a major spread of the species in flooding environments due to its forage qualities and taste. Digestibility of dry matter at vegetative state in spring is 70% and protein content is 26% (Fernandez Grecco and Viviani Rossi, 1995; Fernandez Grecco, 1991). However, the former authors pointed out the low values of the germination percentage of seed samples (approx. 40%) as a serious limitation for a greater spread of this species. No other references were found for seed quality or germination conditions for *G. multiflora* (Ellis *et al*., 1985; ISTA, 1996).

The factors controlling germination (water, oxygen, light, temperature and chemicals) apply to seeds whose dormancy has been broken, as well as to non dormant seeds (Bewley and Black, 1994). These factors act as pretreatments like moist pre chilling, KNO_3 solution, dry storage or factors such as alternating temperatures or light (ISTA, 1996).

In previous work, Ferrari and Rossi (1997) determined the best germination and pre-treatment conditions for quality tests of *Glyceria multiflora* seeds. Working with only one accession (Brandsen), 20-10 and 20-15°C were the best germination temperatures, followed by 15-10°C. Pre chilling did not enhance germination and KNO_3 had significant effect (Matus-Cádiz *et al*., 2001).

Hardegree and Emmerich (1991) warned against the validity of inferences made when germination is studied from a single seed lot. Kitchen and Monsen (1994) working with a native species: *Pseudoroegneria spicata* (Pursh) A. Löve (bluebunch wheatgrass), recorded germination rate and emergence success and observed considerable variation among seed collections representing a widespread geographic distribution. Haferkamp *et al*. (1994) informed that different locations

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of *Bromus japonicus* Thunb. seed (japanese brome) respond similarly to changing environmental conditions.

The aim of this present experiment was to record the germination patterns of *Glyceria multiflora* from different sites of collection in response to different temperatures and pretreatments to establish the best germination conditions to be used in laboratory tests in order to state its seed quality.

MATERIALS AND METHODS

Mature inflorescences of *Glyceria multiflora* were obtained from several areas of the Flooding Pampa sub-region, Buenos Aires Province, Argentina from February to March, 1998. No mature inflorescences were found before February. The seeds collected were dried in paper bags at room temperature for seven days and then threshed by hand. Collection sites were the towns of Jeppener, Brandsen, Ranchos, Alegre and Oliden.

Seeds were cleaned to obtain five homogeneous pure seed samples which were stored in paper bags in the laboratory at 20°C. Tests were performed with five-six months of after-ripening.

A 4-factor completely randomized design with four replications of 100 seeds was applied. All replications were evaluated at the same time.

Treatments combined five collection sites, pre chilling at 7°C during 72 h: Present (P) or absent, directly at germination temperatures (N); KNO_3, 0.2% solution of potassium nitrate to saturate the germination substrate at the beginning of the tests: Present (K) or absent, replacing it by distilled water (W) and three levels of alternating germination temperatures 15-10, 20-10 or 20-15°C, in 8/16 h light/dark periods across all treatments. Neither pre chilling nor distilled water (NW) were considered as control for pretreatment.

Seeds were placed on top moistured filter paper in trays that were then enclosed in polyethylene bags and clip-sealed. Germination chambers with light and temperature control were used.

Normal seedlings (healthy seedlings, with root system, coleoptile and first leaf) were counted and removed each count day. The final count was done after forty four days of incubation. Two indices were calculated for the seeds of each treatment: Germination rate (Maguire, 1962) and synchronization index Ē (Labouriau, 1983). The lower the E, the more synchronized the germination process is. The germination percentage and synchronization indices were regarded as response variables for this experiment.

Germination values were transformed by arcsine (Little, 1985) for statistical analysis but are reported as germination percentages in figures. ANOVA and Tukey tests were performed to determine differences among treatments and within main factors. Correlations were performed among response variables. Statistical significance was assumed at $p<0.05$.

RESULTS AND DISCUSSION

Germination without pre chilling and without KNO_3 may be considered controls for each temperature whereas some dormant seeds were present at the beginning of the experiment.

ANOVA showed that all main factors (collection site, germination temperature, pre chilling and KNO_3) as well as all interactions had significant effect for germination. So, ANOVA was performed within each site of collection. For the five sites, main factors showed significant effect, except KNO_3 for Jeppener. Double interactions were significant except KNO_3 x pre chilling for Brandsen and Alegre and temperature x pre chilling for Alegre. All triple interactions had significant effect for germination.

Germination percentages of normal seedlings classified by site of collection and temperature regime and pretreatment within each one are given in Fig. 1. Although some similarities were evident, two groups of germination responses could be considered mainly regarding pre chilling effect (PK and PW): Branden-Oliden and Jeppener-Ranchos-Alegre.

Alternating temperatures of 20-10 and 20-15°C resulted in the best performance in opposition to 15-10°C. Our results agreed partially with Ferrari and Rossi (1997) that pre chilling did not enhance germination but KNO_3 did.

Working with 5 origins allowed us to confirm germination temperatures 20-10 and 20-15°C, but pre chilling showed interaction with site of collection and germination temperatures, making its effect no clear.

Germination temperature of 15-10°C was insufficient to overcome lasting dormancy. Although level of dormancy was similar in the five collection sites, their response to pretreatments, mainly pre chilling and pre chilling + KNO_3 showed different patterns. Oliden showed highest values when germination substrate was pre chilling and moistured with KNO_3 (PK) or pre chilling and moistured with distillate water (PW); this can be attributed to interaction between factors. For all sites of collection a low germination percentage was obtained for alternating 15-10°C when no pretreatment was applied (NW).

Alternating temperature 20-10°C evidenced the seed quality of all the origins studied. All sites of collection studied showed a similar trend at 20-10°C temperature, irrespective of the pretreatments applied. High germination values were obtained for pre chilling (PW) and KNO_3 (NK). For this temperature (20-10°C) can we

agree with Haferkamp *et al.* (1994) in the sense that seeds grown in different locations respond similarly to changing environmental conditions. This thermal condition may be compared with that of fall in the studied area. *Glyceria multiflora* seeds germinate in fall when temperature is around 20-10°C and rainfall is abundant. These conditions strengthen an aquatic environment favorable for germination and seedling survival. This strategy (avoiding Winter time or warmer summer temperatures) is well known in other range species that evade germination until rainfall and other climatic conditions are favorable for its survival (Milby and Johnson, 1987).

Temperature 20-15°C showed that pre chilling without KNO_3 (PW) had a negative effect over germination except for Alegre. Pre chilling showed a detrimental effect for Brandsen and Oliden. The moistening of substrate with KNO_3 (NK) reached higher germination percentages for all sites of collection.

Pretreatment with KNO_3 solution assumed dormancy broken at this temperature. The differences in germination could relate to ecological conditions prevailing in their habitats as well as to genetic differences (Pannangpetch and Bean, 1984) although these have not been considered in this study.

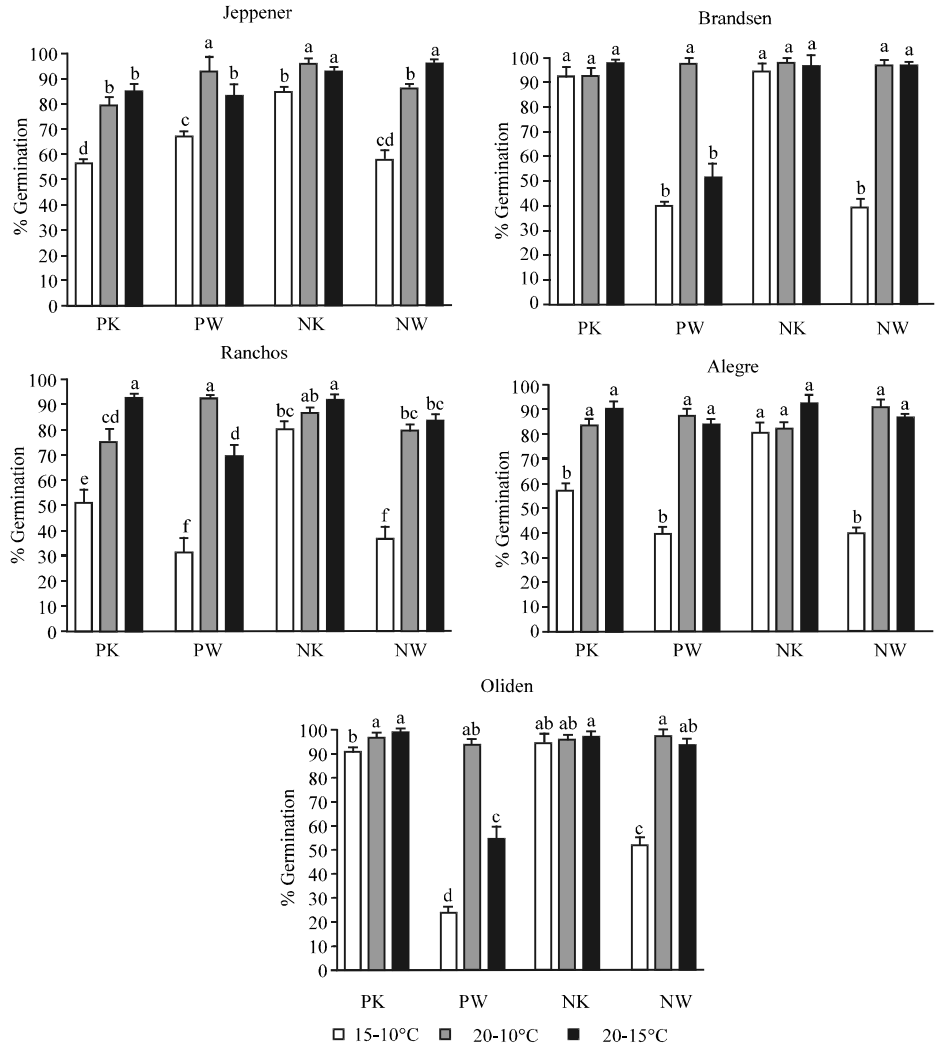

Fig. 1: Germination percentages of normal seedlings classified by site of collection and temperature regime and pretreatment. Bars represent a combination of germination temperature (15-10, 20-10 and 20-15°C) and pretreatment (PK: Pre chilling + KNO3, PW: Pre chilling, NK: KNO3 and NW: Control). Each bar represents the mean of four replicates of 100 seeds each. Bars with the same letter within site of collection are not significantly different ($p<0.05$) by the Tukey test

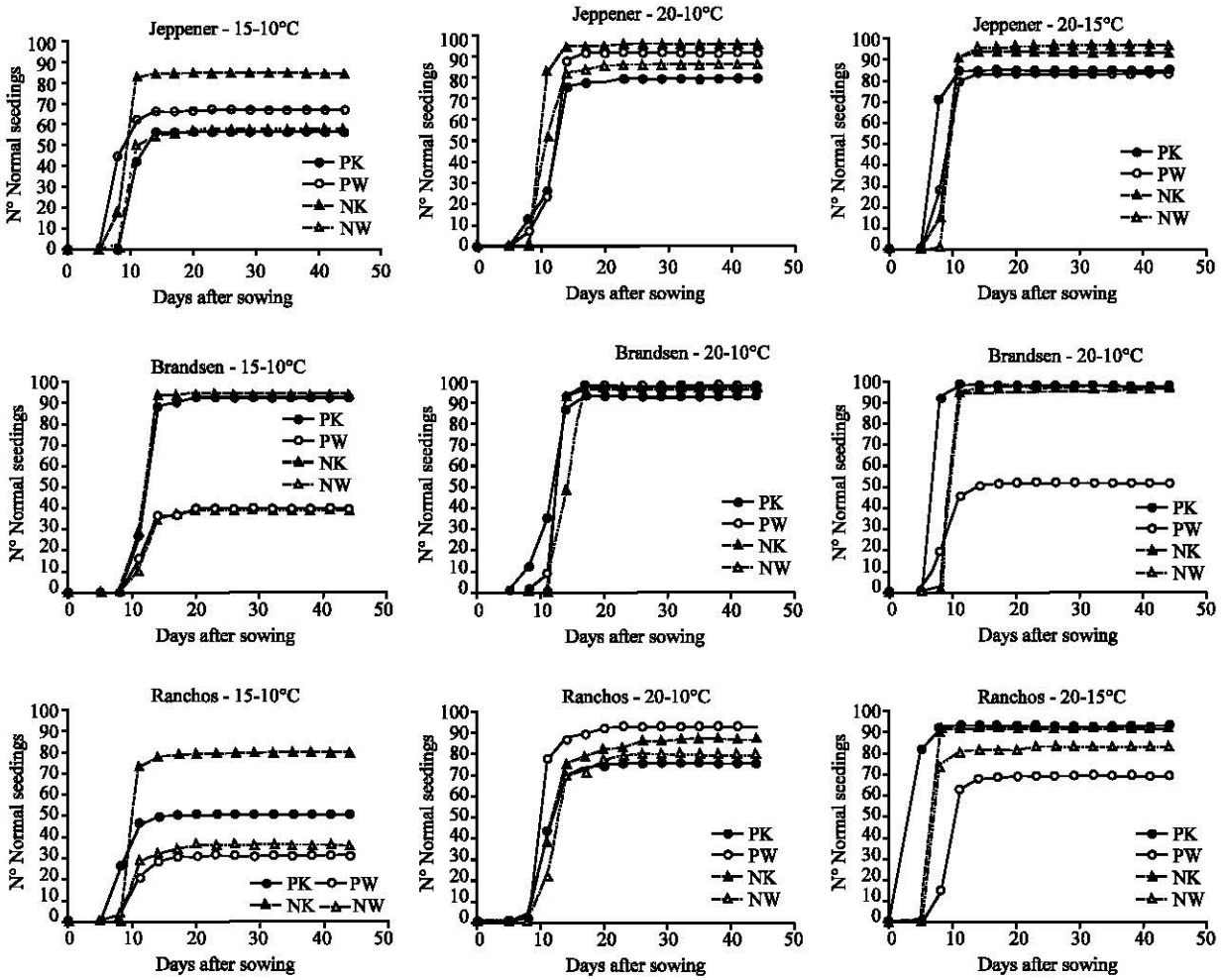

Fig. 2: Germination curves for sites of collection Jeppener, Brandsen and Ranchos. Time axis relates to the days the seeds were under the germination test but does not consider length of pre chilling. Curves represent a combination of germination temperature (15-10, 20-10 and 20-15°C) and pretreatment (PK: Pre chilling + KNO_3, PW: Pre chilling, NK: KNO_3 and NW: Control). Each point represents the mean of four replications of 100 seeds each

The germination values obtained for the seeds with five-six months of after ripening studied in the optimum conditions for this experiment, pointed out the high quality of *Glyceria multiflora* seeds: Jeppener 96%, Brandsen 98%, Ranchos 92%, Alegre 93% and Oliden 99% germination. Perhaps germination percentage smaller than 40% mentioned by Fernandez Grecco (1991) for the species was obtained in seed tests performed under not optimal conditions.

The relationship between germination of normal seedlings and time is shown for each site of collection classified by temperature regime and, within it, by pretreatment is given in Fig. 2 and 3. Germination was advanced for to 20-15°C when KNO_3 was supplied reflecting that not only total germination was stimulated by this treatment.

All seeds studied had some level of relative dormancy when germination substrate was pre chilling (PW) and incubated at alternating 15-10°C (Fig. 1-3) regardless of site of collection. This response was also evident for Brandsen, Oliden and Ranchos when substrate was pre chilling (PW) and germination temperature was 20-15°C. As the pretreatment control (NW) showed, possible primary dormancy was overcome at alternating 20-10° and 20-15°C.

Rate and synchronization of germination were differentially affected by germination temperatures, pre chilling and KNO_3 (Table 1). The highest germination rate was obtained with pre chilling + KNO_3 (PK) with 20-15°C for all sites of collection. KNO_3 (NK) and 20-15°C treatment also stated high values. Rate of germination was consistently the slowest at 15-10°C without pretreatments

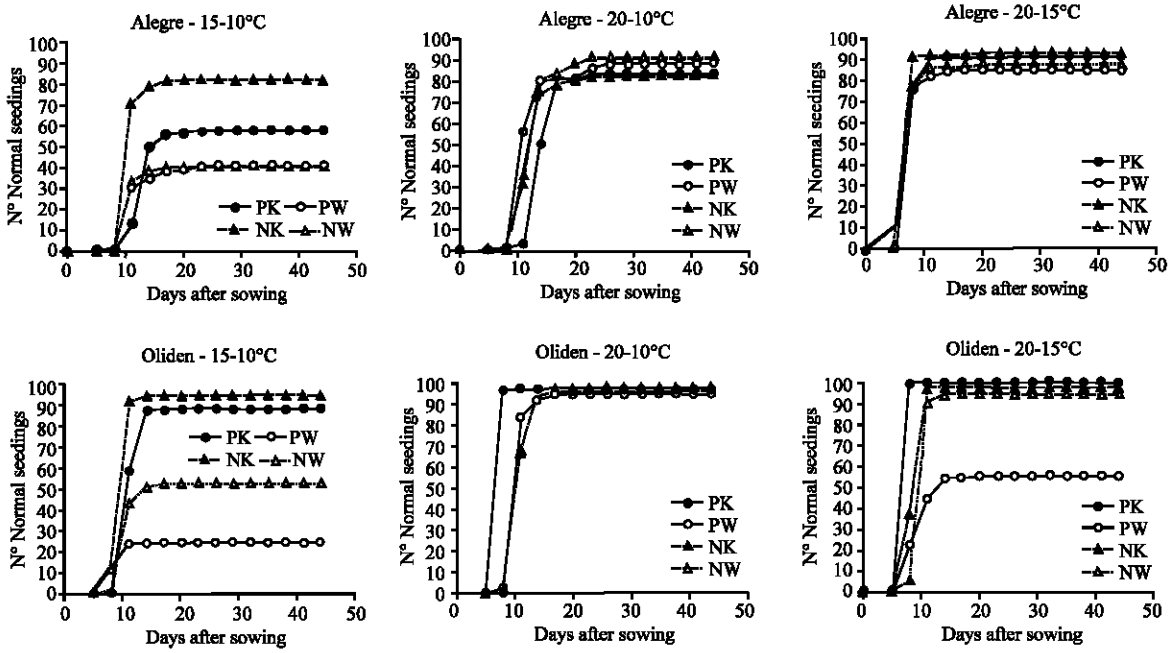

Fig. 3: Germination curves for sites of collection Alegre and Oliden.
Time axis relates to the days the seeds were under germination test but does not consider length of pre chilling. Curves represent a combination of germination temperature (15-10, 20-10 and 20-15°C) and pretreatment (PK: pre chilling + KNO_3, PW: Pre chilling, NK: KNO_3 and NW: Control). Each point represents the mean of four replications of 100 seeds each

Table 1: Germination rate and synchronization index for each site of collection classified by germination temperature (15-10, 20-10 and 20-15°C) and pretreatment (PK: Pre chilling + KNO3, PW: Pre chilling, NK: KNO3 and NW: Control)

Site of collection	Treatments	Germination rate	Treatments	Synchronization index
Jeppener	20-15°C PK	10.057a	20-15°C NK	8.9172ab
	20-15°C NW	8.6422b	20-10°C NK	8.4350bc
	20-15°C PW	8.4220bc	15-10°C NK	8.2660bcd
	15-10°C PW	7.4857cde	20-10°C PW	7.1720de
	20-10°C NW	7.0800e	20-10°C PK	6.4190e
	15-10°C NW	5.1230f	15-10°C PK	4.8360f
	20-10°C PK	1.4500a	20-10°C PW	1.2412ab
	15-10°C PW	1.1907ab	20-15°C PW	1.1570abc
	20-10°C NW	1.1517abc	15-10°C NW	0.9132bcd
	15-10°C PK	0.8075bcd	20-15°C NK	0.7968bcd
	15-10°C NK	0.7860bcd	20-15°C PK	0.6529cd
	20-10°C NK	0.6120d	20-15°C NW	0.4191d
Brandsen	20-15°C PK	11.966a	20-15°C NK	9.4895b
	20-15°C NW	8.8200b	20-10°C PK	7.4720c
	15-10°C NK	7.2672c	20-10°C PW	7.0565cd
	15-10°C PK	6.9795cd	20-10°C NK	6.9175cd
	20-10°C NW	6.2800d	20-15°C PW	5.1625e
	15-10°C PW	3.0628f	15-10°C NW	2.9205f
	20-10°C PK	1.5330a	20-15°C PW	1.4632ab
	15-10°C NW	1.2877abc	15-10°C PW	1.2335abc
	15-10°C PK	1.0550bcd	20-10°C NW	1.0235bcd
	15-10°C NK	0.9430cd	20-15°C NK	0.9153cd
	20-10°C PW	0.7440de	20-15°C PK	0.3500e
	20-15°C NW	0.3348e	20-10°C NK	0.2940e
Ranchos	20-15°C PK	17.207a	20-15°C NK	11.482b
	20-15°C NW	9.9742c	20-10°C PW	7.9205d
	15-10°C NK	7.1190de	20-15°C PW	6.5907ef
	20-10°C NK	6.5878ef	20-10°C PK	6.0420fg
	20-10°C NW	5.8273fg	15-10°C PK	5.3785g
	15-10°C NW	3.0898h	15-10°C PW	2.6768h
	20-10°C NK	1.6275a	20-10°C PK	1.5393ab

Table 1: Continued

Site of collection	Treatments	Germination rate	Treatments	Synchronization index
	20-10°C NW	1.5090ab	15-10°C PW	1.4198ab
	15-10°C PK	1.2717abc	20-15°C PW	1.1883abcd
	15-10°C NW	0.9245bcd	20-10°C PW	0.8950bcde
	20-15°C NW	0.6300cde	15-10°C NK	0.5662de
	20-15°C PK	0.5500de	20-15°C NK	0.2278e
Alegre	20-15°C PK	14.359a	20-15°C PW	12.385a
	20-15°C NK	11.916ab	20-15°C NW	10.602abc
	20-10°C PW	7.1215bcd	20-10°C NW	6.7372cd
	20-10°C NK	6.5805cd	15-10°C NK	6.4295cd
	20-10°C PK	6.1915cd	15-10°C PK	5.1180d
	15-10°C NW	4.3093d	15-10°C PW	3.9450d
	20-10°C NW	1.5442a	20-10°C NK	1.4532ab
	15-10°C PW	1.3542abc	20-10°C PW	1.3295abc
	20-10°C PK	1.3190abc	15-10°C PK	1.1520abcd
	20-15°C PW	1.0115abcd	15-10°C NK	0.8432bcde
	15-10°C NW	0.7252cde	20-15°C PK	0.6983cde
	20-15°C NW	0.5843de	20-15°C NK	0.2170e
Oliden	20-15°C PK	16.936a	20-15°C NK	13.563ab
	20-15°C NW	12.582abc	15-10°C PK	11.302abcd
	20-15°C PW	10.811abcd	20-10°C PK	9.9143abcd
	20-10°C PW	8.7955bcd	20-10°C NW	8.1553bcd
	20-10°C NK	8.1285bcd	15-10°C NK	7.3602bcd
	15-10°C NW	5.5980cd	15-10°C PW	4.4565d
	20-15°C PW	1.5372a	20-10°C NW	1.0682ab
	15-10°C PW	1.0108bc	15-10°C NW	0.9867bc
	20-10°C NK	0.9745bc	20-15°C NK	0.9487bc
	15-10°C PK	0.8978bc	20-10°C PK	0.8708bc
	20-10°C PW	0.7450bc	15-10°C NK	0.7263bc
	20-15°C NW	0.5030cd	20-15°C PK	0.0352d

Each value represents the mean of 4 replicates of 100 seeds each. Values followed by the same letter (s) within site of collection are not significantly different ($p<0.05$) by the Tukey test

Table 2: Correlation coefficients (*statistical significance) among total germination percentage as arcsin and germination rate (GR) and synchronization index (Ē), for all data set (A) and within each temperature regime 15-10°C (B), 20-10°C (C) and 20-15°C (D) for all pretreatments and sites of collection

Complete data set		Total germination (%)	GR
	GR	0.5901	-
	Ē	-0.3832	-0.4312
15-10°C	GR	0.8406*	-
	Ē	-0.2986	-0.2827
20-10°C	GR	0.5131	-
	Ē	-0.6091	-0.4053
20-15°C	GR	0.5533	-
	Ē	-0.6673	-0.4507

(NW) and when prechilling was applied (PW). Heydecker and Coolbear (1977) stated that the prevailing soil temperature determines both the fraction of seeds in a sample which germinates and the rate at which they germinate. In accordance to this, temperature was the major effect affecting germination. Extreme values were recorded for Ranchos with 17.21±0.768 and 2.68±0.417, respectively PK 20-15 and PW 15-10°C. Results from statistical analysis of synchronization of germination showed lower values -that is to say higher synchronization were obtained applying KNO_3. Extreme values were recorded for Oliden with 0.04±0.071 and 1.54±0.174, respectively PK 20-15 and PW 20-15°C.

No significant correlations were detected among response variables when the entire data set was considered. Only when data were classified by temperature (Table 2) total germination was significantly correlated with the germination rate at 15-10°C considering ll pretreatments and origins.

Although total germination, rate and synchronization are affected by temperature, they were independent parameters for thermal conditions 20-10 and 20-15°C. Also Kitchen and Monsen (1994) working with *Pseudoroegneria spicata* stated that germination rate at optimum temperature regime was not significantly correlated with emergence percentage.

Optimal conditions for germination must consider not only total germination percentage but also germination rate and synchronization. If only germination percentage is considered, then 20-10°C pre chilling (PW) may be recommended. But when germination rate and synchronization are included, this treatment is discarded.

CONCLUSIONS

Sites of collection had a significant effect, showing variations within pretreatments and germination temperatures applied. All sites of collection had a similar and high performance when temperature of germination was alternating between 20-10 and 20-15°C in 8/16 h light/dark periods.

Within each site of collection germination temperature was the first main effect. The second main

effect was KNO_3 solution. Pre chilling effect was not clear and evidenced differences within origins behavior in 15-10 and 20-15°C germination temperatures.

In order to estimate physiological seed quality of samples of *Glyceria multiflora* with five-six months of after-ripening the best germination conditions to be used in laboratory tests regarding total germination percentage and germination rate was 20-15°C with KNO_3 solution.

The results informed in this study point out that seed quality must not be regarded as a limitations for the distribution of *Glyceria multiflora*. May be 40% of the germination mentioned in the literature for the species was obtained in seed tests performed under not optimal conditions.

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