

## Full Length Research Paper

**Water stress and temperature effects on germination and early seedling growth of *Digitaria eriantha***Brevedan R. E.<sup>1,2</sup>, Busso C. A.<sup>1,2\*</sup>, Fioretti M. N.<sup>1</sup>, Toribio M. B.<sup>1</sup>, Baioni S. S.<sup>1</sup>, Torres Y. A.<sup>1,2</sup>, Fernández O. A.<sup>1,2</sup>, Giorgetti H. D.<sup>3</sup>, Bentivegna D.<sup>2</sup>, Entío J.<sup>4</sup>, Ithurrt L.<sup>1,2</sup>, Montenegro O.<sup>3</sup>, Mujica M. de las M.<sup>4</sup>, Rodríguez G.<sup>3</sup> and Tucac G.<sup>1,2</sup><sup>1</sup>Department of Agronomía, Universidad Nacional del Sur (UNS), 8000 Bahía Blanca, Argentina.<sup>2</sup>CERZOS (CONICET), 8000 Bahía Blanca, Argentina.<sup>3</sup>Chacra Experimental de Patagones, Ministerio de Asuntos Agrarios, (8504) Carmen de Patagones, Argentina.<sup>4</sup>Facultad de Ciencias Agrarias, Universidad Nacional de La Plata, (1900) La Plata, Argentina.

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This study focused on the two major processes critical for plant establishment: Seed germination and seedling survival. We determined the effects of (1) water stress and temperature on the germination, and (2) water stress on early seedling growth of *Digitaria eriantha* cv 'Irene'. Seeds harvested in 2007 were used for temperature studies, and those coming from 2006 and 2007 for water stress studies. In 2009, viability decreased by 65.4% from 2006 to 2007. During the first twenty-four hours, germination was more than 50% at constant (30 or 35°C) than alternating (10/30 or 10/35°C) temperatures, although total germination was about 80% for all temperature treatments. Polyethylene glycol 8000 was used to impose water stress conditions. Germination percentages and coefficients of velocity decreased with decreasing water potentials. Early seedling growth was smaller at lower water potentials. *D. eriantha* cv 'Irene' appeared to germinate within a wide range of temperatures, but it varied greatly in germination response to water potentials. Results suggest that this species could be planted in late spring-early summer, when seedbed temperatures are increasing and soil moisture might still be adequate.

**Key words:** Perennial grasses, rangelands, seeds, forage, arid zones, Argentina.**INTRODUCTION**

In the rangelands of central Argentina, warm-season, native perennial grass genotypes palatable to cattle are scarce (Busso et al., 2004). These genotypes are the major food source for these animals, which reject most of the other monocots and dicots that are part of the plant community. In rangelands at the south of Buenos Aires Province (e.g., the Chacra Experimental de Patagones, Patagones, Argentina (40°39'49,7"S, 62°53'6,4"W; 40 m.a.s.l.), within the Phytogeographical Province of the Monte: Cabrera 1976), *Pappophorum vaginatum* Buckley

is almost the only native, warm-season, C<sub>4</sub>, palatable perennial grass available for livestock grazing (Giorgetti et al., 1997). Need of increasing cattle forage production during the warm season in rangelands of central Argentina is thus critical. This could be achieved via perennial grass species introduction into the region (Anderson, 1980). Success in artificial range seeding requires knowledge of many parameters, including optimum temperature and moisture conditions for germination (Sabo et al., 1979).

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*Digitaria eriantha* (Steudel) subsp. *eriantha* is a C<sub>4</sub>, palatable perennial grass that was introduced in Argentina from South Africa in 1991 (Di Giambattista et al., 2010). Rimieri (1997) bred the cv. 'Irene' from the South African cv. 'Sudafricana'; 'Irene' showed a better adaptation than the subsp. *eriantha* to the soil and climatic conditions of the semiarid regions in Argentina. Besides South Africa (Du Toit, 2000), this species has spread worldwide [USA: Sanderson et al. (1999); Australia: Hacker et al., 1993); China and Europe: [http://www.ehow.com/info\\_8407286\\_genus-species-crabgrass.html](http://www.ehow.com/info_8407286_genus-species-crabgrass.html)]. It characterizes for its resistance to drought, healthiness, high warm-season forage production and adaptability to different soils (Dannhauser, 1988). Lavin and Johnsen (1977) determined that *D. eriantha* might attain a rating of excellent under warm and dry conditions at a site with 330 mm annual precipitation.

Periods of water stress and high temperature are common in rangelands of northwestern Patagonia during late spring and summer (Torres et al., 2011). It means that *D. eriantha* could be a good potential species to introduce in rangelands of central Argentina. Although some research has been conducted on *D. eriantha* in this country (Gargano et al., 2004; Pedranzani et al., 2005), no studies have yet addressed the importance of water potential and temperature on the most sensitive morphological developmental stages to water stress and high temperatures during summers: seed germination and early seedling growth (Brown, 1995).

Optimum temperatures will shorten the time to germination, and they might occur under momentarily favorable moisture conditions (Bonvissuto and Busso, 2007). Fulbright (1988) reported that the warm-season bunchgrass *Sorghastrum nutans* (L.) Nash appeared to germinate within a similar range of temperatures, but it varied in germination responses to water potentials. Several studies showed similar or greater germination percentages at constant than alternating temperatures in various range perennial grasses (Hylton and Bement, 1961; Young and Evans, 1982; Fernández et al., 1991). For example, Brown (1987) reported that alternating temperatures improved overall germination of the perennial bunchgrass *Aristida armata*, but there was little variation in the rate of germination with incubation under constant temperature. Ellern and Tadmor (1966) and Heydecker (1960) stated that speedy germination does not necessarily have to coincide with high germination percentages.

Plants are susceptible to water stress throughout their life-cycle, but are particularly vulnerable during seed germination, and seedling emergence and early growth (Brown, 1995; Almansouri et al., 2001). Seedling development of *S. nutans* cvs. 'Lometa', 'Cheyenne', 'Llano', 'Oto', and 'Tejas', for example, decreased linearly with decreasing water potential (Fulbright, 1988). Another critical factor in successful plant establishment is time to

germination, especially in arid and semiarid environments where favorable conditions of water availability and temperature regimes may only occur over short time periods (Owens and Call, 1985). Germination may be part of the plant life cycle that requires the highest water potentials (Brown, 1995). Temperature also appears to determine the optimal and minimum water potentials for germination in various species (Brown, 1995). This is why studies on the effects of water stress and temperature on seed germination and early seedling growth are so important when thinking in introducing a rangeland species, like *D. eriantha*, in water stress-prone rangelands of northwestern Patagonia, Argentina. This information is actually lacking and essential for trying to increase warm-season forage production for livestock in rangelands of central Argentina. There is also a paucity of information describing the germination ecology of this perennial.

In arid and semiarid areas, the occurrence of optimum conditions for seed germination can vary within and among years (Romo and Eddleman, 1988). The greater the water stress conditions, the lower will be the total germination, and the time to germination (Brown, 1995). Some seeds will not germinate even under favorable conditions because they are dormant; the need for an after-ripening may help to avoid seed germination under unfavorable climate conditions (Brown, 1995).

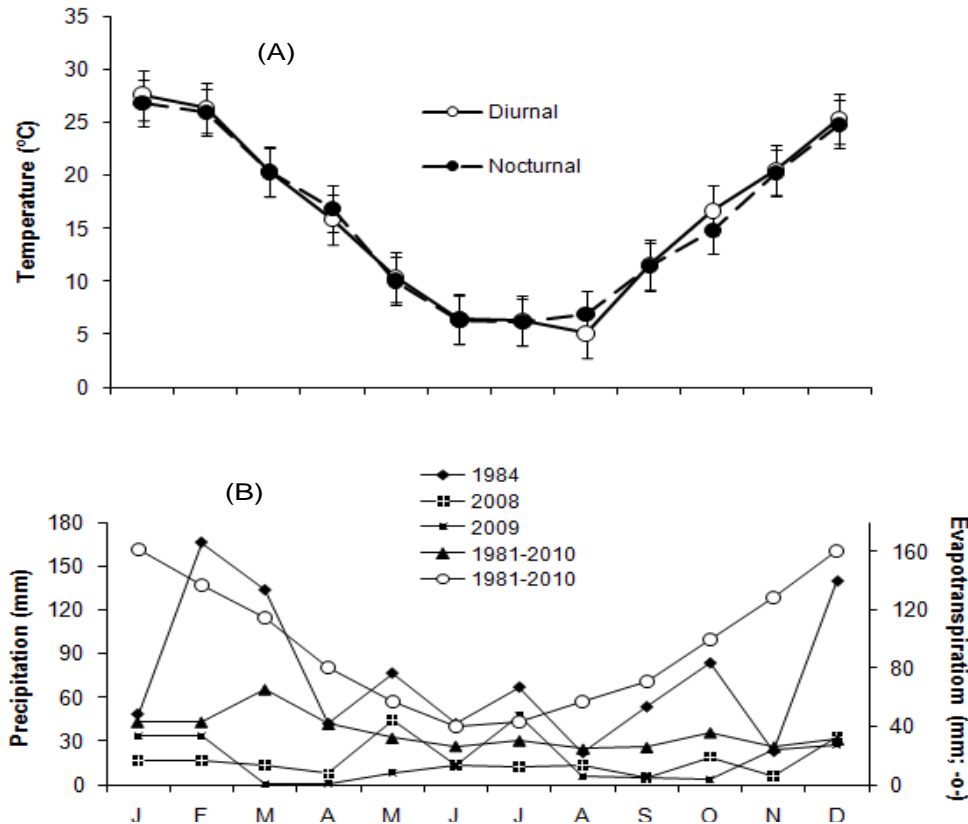
Specific hypotheses were that (1) total germination percentage is similar under constant or alternating temperatures, (2) coefficients of velocity are higher at constant than alternating temperatures, and (3) seedling growth is higher at greater than lower water potentials. Our objectives were to (1) identify germination responses of *D. eriantha* to various temperatures and water potentials, and (2) determine the effect of water stress on its initial seedling growth. This information will assist those charged with developing strategies for reestablishing and managing degraded, central rangelands of northwestern Patagonia, Argentina.

## MATERIALS AND METHODS

### Study site

Characteristics of the study site (the Chacra Experimental de Patagones) where *D. eriantha* could potentially be introduced follow. Climate is temperate semiarid, with higher precipitations during the spring and fall seasons (Giorgetti et al., 2000). Long-term (1981-2010) annual rainfall is 416.7 mm (Figure 1), with a mean annual temperature of 14.6°C, absolute minimum temperature of -7.6°C (August), absolute maximum temperature of 43°C (January), mean annual relative humidity of 60%, and mean annual wind speed of 13 km h<sup>-1</sup>. Long-term (2006-2010) mean monthly diurnal and nocturnal temperatures, precipitations for various years, and long-term (1981-2010) mean monthly evapotranspiration are presented in Figure 1.

Soil is a typical Haplocalcid. Average pH is 7, and depth is not a constraint in the soil profile. The plant community is characterized by an open, shrubby stratum which includes different-quality,



**Figure 1.** (A) Averages during 2006-2010 of mean monthly diurnal and nocturnal soil temperatures at 5 cm soil depth from January to December, and (B) monthly precipitation at various years (black symbols), long-term (1981-2010) mean monthly precipitation (black symbols), and long-term (1981-2010) mean monthly potential evapotranspiration (open symbols), in the Chacra Experimental de Patagones, Patagones, Argentina (40°39'49,7"S, 62°53'6,4"W; 40 m.a.s.l.). Mean annual standard errors for long-term precipitation and evapotranspiration were 29.0 and 38.9 mm, respectively.

herbaceous species for cattle production (Giorgetti et al., 1997). Dominance of a particular grass or shrubby species in the study region is partially dependent on grazing history and fire frequency and intensity (Distel and Bóo, 1996).

#### Storage time and conditions

Seeds of *D. eriantha* cv. 'Irene' used in this study were harvested in 2006 and 2007. Weight of 1000 seeds was 0.339 g in 2006 and 0.329 g in 2007. Seeds were kept in a seed storage room at 20°C until the experiment was conducted in 2009. At this later time, seed viability was tested using the 2,3,5-triphenyl tetrazolium chloride test (TTC; Peters, 2000). Five sets of 50 seeds each were immersed in water in Petri dishes during 24 h. Seeds were then incubated in 20 ml-Petri dishes containing a 0.1% solution of TTC at 35°C under darkness, and embryos were thereafter examined to establish their viability.

#### Temperature effects

A bar of stainless steel (0.95 m length, 0.20 m wide, 0.20 m height) was used to create the thermal gradient. It was achieved submerging one extreme of the bar in cool water (3°C) and the other extreme in hot water (36°C). This extreme was maintained hot

using an electrical resistance. This generated a temperature gradient such as various constant temperatures were obtained throughout the width every 3-increments along the bar length. Thermocouples, and an infrared thermometer, were used to determine these constant temperature conditions. An absorbent paper was placed on the bar that remained saturated with water during the experiment. The stainless steel bar had only one side (at the top) that could be opened throughout its length; this side was a piece of transparent acrylic (5 mm thick) that was in immediate contact with a piece of rubber (5 mm thick; between the acrylic and the stainless steel bar) that prevented water losses via evaporation from the germination chamber. Water vapor loss from germination containers has been shown to change the water potential of PEG solutions: Berkat and Briske (1982). Temperature was measured using a Delta T multi-channel data-logger. Seed germination was tested at different temperatures using seeds from the 2007 harvest. Seeds were placed on top paper on the thermal gradient with a temperature range from 13.9 to 36.9°C. Cumulative germination was recorded by counting germinated seeds every 12 h for six days. A seed was considered germinated once its radicle reached a length of at least 2 mm (Emmerich and Hardegree, 1990).

In another test, seeds were placed on top filter paper in plastic boxes (53 mm length × 59 mm wide × 15 mm height). Distilled water was added, and boxes were placed in a germination cabinet that was set to four temperature regimes: continuous (30 or 35°C), and alternating [30/10°C (14 h light-10 h darkness) or 35/10°C

**Table 1.** Germination percentage and coefficient of velocity (CV) of *D. eriantha* cv. 'Irene' as function of different temperature treatments.

| Temperature (°C) | Time (hours)            |                         |                         |                        |                         | CV                      |
|------------------|-------------------------|-------------------------|-------------------------|------------------------|-------------------------|-------------------------|
|                  | 24                      | 48                      | 72                      | 96                     | Total                   |                         |
| 30               | 48.7 ± 2.1 <sup>b</sup> | 24.7 ± 1.7 <sup>a</sup> | 7.0 ± 1.2 <sup>a</sup>  | 0.3 ± 0.3 <sup>a</sup> | 80.7 ± 1.7 <sup>a</sup> | 67.2 ± 1.3 <sup>c</sup> |
| 30-10            | 11.7 ± 2.8 <sup>a</sup> | 50.3 ± 3.9 <sup>c</sup> | 18.0 ± 1.5 <sup>b</sup> | 0.0 ± 0.0 <sup>a</sup> | 80.0 ± 3.4 <sup>a</sup> | 47.2 ± 1.0 <sup>a</sup> |
| 35               | 54.0 ± 1.6 <sup>b</sup> | 24.3 ± 0.6 <sup>a</sup> | 7.7 ± 1.0 <sup>a</sup>  | 0.3 ± 0.3 <sup>a</sup> | 86.3 ± 3.0 <sup>a</sup> | 68.2 ± 0.9 <sup>c</sup> |
| 35-10            | 23.7 ± 3.1 <sup>b</sup> | 40.0 ± 3.0 <sup>b</sup> | 16.7 ± 1.1 <sup>b</sup> | 0.0 ± 0.0 <sup>a</sup> | 80.3 ± 3.6 <sup>a</sup> | 52.3 ± 1.0 <sup>b</sup> |

Seeds of *D. eriantha* come from the 2007 harvest. Means within a column with the same letter are not significantly different according to the LSD test ( $p=0.05$ ). Values are the mean  $\pm 1$  EE of  $n=6$ .

(14 to 10 h) temperatures. Photosynthetically active radiation within the cabinet was 25 to 45  $\mu\text{mol}/\text{m}^2/\text{s}$ . The study was a completely randomized design, with four temperature treatments. There were six replications (boxes) of 50 seed each. Observations of germinating seeds were carried out every twenty-four hours during four days. After each counting time, boxes from all treatments were randomly distributed within the germination cabinet.

#### Water potential effects

The germination studies under different water potentials were carried out using seeds from the 2006 and 2007 harvests. Solutions were prepared by adding polyethylene glycol 8000 formerly PEG 6000 (Michel, 1983) to distilled water, such as seeds were in direct contact with the PEG 8000 solution, without any support material, in petri dishes. PEG solution-seed contact does not reduce seed germination (Emmerich and Hardegee, 1990). Osmotic potential was determined by a Wescor 5500 osmometer, after calibration with standard KCl solutions. Water potential treatments were 0 (controls), -0.4, -0.6, -0.8, -1.0, -1.2, -1.5 and -2.0 MPa, although no germination was recorded at the lastest two water potentials; six replications (petri dishes) of 50 seeds each were used for each water stress level. Each Petri dish was sealed with Parafilm to prevent water vapor loss, and placed within a Forma Scientific 3770 germination chamber set at  $35\pm 1^\circ\text{C}$ . At each counting time, the petri dishes from all water level treatments were randomly distributed on the chamber shelves. Both the cumulative germination and germination coefficient of velocity were recorded by counting and removing germinated seeds every three hours up to the sixth day when the experiment was ended.

The coefficient of velocity (CV) was calculated as:

$$CV = 100 \left[ \frac{\sum Ni}{\sum NiTi} \right]$$

Where N is the number of seeds which germinated on day i, and T is the number of days from sowing (Scott et al., 1984). One advantage of using the coefficient of velocity as a measure of the vigor index is the simplicity of the formula; CV increases are often obtained as more seeds germinate and with shorter germination time (Scott et al., 1984). Root and shoot lengths of three randomly selected seedlings were measured per Petri dish on each water potential treatment. An average of these measurements was considered to be one replication.

#### Statistical analysis

A completely randomized experimental design with six replications was utilized. Temperature data were analyzed using one-way ANOVA. Percentage germination data were transformed to arc-sin

of the square root to comply with the normality and homoscedasticity assumptions of variance. Within any given study time from imbibition, two-way ANOVA (year x water potential) were used to analyze germination and coefficients of velocity data. When the interaction was significant, each year or water potential was analyzed separately. Growth of young seedlings was analyzed using three-way ANOVA (year x plant part x water potential). Untransformed values are presented in Tables. When F test were significant ( $p<0.05$ ), treatments were compared using LSD. Data were analyzed using the statistical software INFOSTAT version 2009 (Di Rienzo et al., 2009).

## RESULTS

### Storage time

Seeds harvested in 2006 showed 33% viability, while it was 93% for those harvested in 2007.

### Germination percentage

#### Temperature

After one day of study initiation, the lowest ( $p<0.05$ ) germination percentage was reached at the 30-10°C alternating temperature (Table 1). At the same time, percentage germination was about 50% at 30 and 35°C, although percentages at these temperatures were not different ( $p>0.05$ ) than that at the alternating temperature of 35-10°C (Table 1). As a result, germination percentages were greater ( $p<0.05$ ) at the alternating than at the constant temperatures after two and three days from initiation of the study (Table 1). Despite these differences among temperature treatments, total germination was similar ( $p>0.05$ ; aprox. 80%) in all of them after four days of initiating the study (Table 1).

### Water potential

#### Changes in germination percentages with time

Eighteen out of one-hundred and twelve comparisons of all germination percentages at all study water potentials

**Table 2.** Percentage germination of *D. eriantha* seeds in 2006 and 2007 (on an hourly basis from study initiation, and Total, cumulative germination) as a function of water potential. Measurements were conducted at 35±1°C.

| Year 2006             |                            |                            |                           |                            |                            |                          |                            |                          |                            |
|-----------------------|----------------------------|----------------------------|---------------------------|----------------------------|----------------------------|--------------------------|----------------------------|--------------------------|----------------------------|
| Water potential (MPa) | Hours                      |                            |                           |                            |                            |                          |                            |                          |                            |
|                       | 15                         | 18                         | 24                        | 36                         | 48                         | 60                       | 72                         | 84                       | Total                      |
| 0.0                   | 2.5 ± 1.1 <sup>a,bc</sup>  | 4.3 ± 0.8 <sup>a,bc</sup>  | 6.8 ± 0.9 <sup>a,b</sup>  | 5.8 ± 1.2 <sup>a,c</sup>   | 2.0 ± 0.4 <sup>a,bcd</sup> | 1.7 ± 0.6 <sup>a,a</sup> | 0.3 ± 0.2 <sup>a,a</sup>   | 0.3 ± 0.3 <sup>a,a</sup> | 26.0 ± 0.9 <sup>a,b</sup>  |
| -0.2                  | 4.3 ± 1.5 <sup>a,c</sup>   | 5.7 ± 1.7 <sup>a,c</sup>   | 3.7 ± 0.8 <sup>a,b</sup>  | 6.0 ± 1.2 <sup>a,c</sup>   | 1.3 ± 0.7 <sup>a,abc</sup> | 1.3 ± 0.7 <sup>a,a</sup> | 0.0 ± 0.0 <sup>a,a</sup>   | 0.0 ± 0.0 <sup>a,a</sup> | 23.7 ± 1.7 <sup>a,b</sup>  |
| -0.4                  | 1.3 ± 0.7 <sup>a,abc</sup> | 3.7 ± 1.0 <sup>a,abc</sup> | 6.3 ± 1.0 <sup>a,b</sup>  | 3.7 ± 1.0 <sup>a,bc</sup>  | 1.0 ± 0.4 <sup>a,ab</sup>  | 2.0 ± 0.9 <sup>a,a</sup> | 0.3 ± 0.3 <sup>a,a</sup>   | 0.0 ± 0.0 <sup>a,a</sup> | 19.0 ± 1.8 <sup>a,b</sup>  |
| -0.6                  | 1.0 ± 0.7 <sup>a,ab</sup>  | 7.3 ± 1.2 <sup>a,ab</sup>  | 7.7 ± 2.5 <sup>a,b</sup>  | 6.7 ± 1.7 <sup>a,c</sup>   | 0.0 ± 0.0 <sup>a,a</sup>   | 1.0 ± 0.7 <sup>a,a</sup> | 0.0 ± 0.0 <sup>a,a</sup>   | 0.3 ± 0.3 <sup>a,a</sup> | 25.7 ± 3.4 <sup>a,b</sup>  |
| -0.8                  | 0.0 ± 0.0 <sup>a,a</sup>   | 0.0 ± 0.0 <sup>a,a</sup>   | 3.7 ± 1.2 <sup>a,b</sup>  | 5.7 ± 1.6 <sup>a,c</sup>   | 2.3 ± 0.8 <sup>a,bcd</sup> | 1.7 ± 0.6 <sup>a,a</sup> | 1.0 ± 0.7 <sup>a,a</sup>   | 0.7 ± 0.4 <sup>a,a</sup> | 20.3 ± 2.7 <sup>a,b</sup>  |
| -1.0                  | 0.0 ± 0.0 <sup>a,a</sup>   | 0.0 ± 0.0 <sup>a,a</sup>   | 0.3 ± 0.3 <sup>a,a</sup>  | 1.7 ± 1.0 <sup>a,ab</sup>  | 4.0 ± 1.2 <sup>a,d</sup>   | 1.3 ± 0.8 <sup>a,a</sup> | 1.0 ± 0.4 <sup>a,a</sup>   | 1.7 ± 0.6 <sup>a,a</sup> | 12.3 ± 2.8 <sup>a,a</sup>  |
| -1.2                  | 0.0 ± 0.0 <sup>a,a</sup>   | 0.0 ± 0.0 <sup>a,a</sup>   | 0.0 ± 0.0 <sup>a,a</sup>  | 0.0 ± 0.0 <sup>a,a</sup>   | 3.7 ± 1.2 <sup>a,cd</sup>  | 0.0 ± 0.0 <sup>a,a</sup> | 1.7 ± 1.1 <sup>a,a</sup>   | 4.0 ± 1.5 <sup>a,a</sup> | 10.3 ± 1.7 <sup>a,a</sup>  |
| Year 2007             |                            |                            |                           |                            |                            |                          |                            |                          |                            |
| Water potential (MPa) | Hours                      |                            |                           |                            |                            |                          |                            |                          |                            |
|                       | 15                         | 18                         | 24                        | 36                         | 48                         | 60                       | 72                         | 84                       | Total                      |
| 0.0                   | 8.7 ± 1.1 <sup>b,d</sup>   | 10.7 ± 1.5 <sup>b,d</sup>  | 17.7 ± 2.2 <sup>b,c</sup> | 17.7 ± 1.2 <sup>b,c</sup>  | 3.0 ± 1.0 <sup>a,bc</sup>  | 5.7 ± 1.4 <sup>b,b</sup> | 0.0 ± 0.0 <sup>a,a</sup>   | 0.0 ± 0.0 <sup>a,a</sup> | 74.0 ± 2.6 <sup>b,e</sup>  |
| -0.2                  | 7.7 ± 1.2 <sup>a,d</sup>   | 10.3 ± 1.9 <sup>a,d</sup>  | 16.0 ± 2.2 <sup>b,c</sup> | 10.3 ± 2.4 <sup>a,b</sup>  | 0.7 ± 0.4 <sup>a,a</sup>   | 2.7 ± 1.1 <sup>b,b</sup> | 0.3 ± 0.3 <sup>a,ab</sup>  | 0.0 ± 0.0 <sup>a,a</sup> | 52.3 ± 4.1 <sup>b,cd</sup> |
| -0.4                  | 3.0 ± 0.7 <sup>a,c</sup>   | 10.0 ± 2.3 <sup>a,c</sup>  | 26.7 ± 2.5 <sup>b,d</sup> | 13.3 ± 0.9 <sup>b,bc</sup> | 2.0 ± 0.9 <sup>a,ab</sup>  | 3.7 ± 1.3 <sup>b,b</sup> | 0.3 ± 1.0 <sup>a,ab</sup>  | 0.3 ± 0.3 <sup>a,a</sup> | 66.3 ± 6.0 <sup>b,de</sup> |
| -0.6                  | 1.0 ± 0.4 <sup>a,b</sup>   | 9.7 ± 1.9 <sup>a,b</sup>   | 18.0 ± 3.0 <sup>b,c</sup> | 17.3 ± 0.4 <sup>b,c</sup>  | 0.7 ± 0.4 <sup>a,a</sup>   | 5.0 ± 1.2 <sup>b,b</sup> | 1.7 ± 0.7 <sup>a,abc</sup> | 1.7 ± 0.3 <sup>a,a</sup> | 60.7 ± 2.4 <sup>b,de</sup> |
| -0.8                  | 0.0 ± 0.0 <sup>a,a</sup>   | 0.0 ± 0.0 <sup>a,a</sup>   | 5.3 ± 1.4 <sup>a,b</sup>  | 8.3 ± 1.1 <sup>a,b</sup>   | 5.7 ± 1.1 <sup>b,c</sup>   | 6.0 ± 1.9 <sup>b,b</sup> | 2.0 ± 1.3 <sup>a,bc</sup>  | 2.0 ± 1.0 <sup>a,a</sup> | 39.7 ± 7.9 <sup>a,bc</sup> |
| -1.0                  | 0.0 ± 0.0 <sup>a,a</sup>   | 0.0 ± 0.0 <sup>a,a</sup>   | 0.0 ± 0.7 <sup>a,a</sup>  | 1.7 ± 0.7 <sup>a,a</sup>   | 3.3 ± 0.7 <sup>a,bc</sup>  | 6.0 ± 1.5 <sup>b,b</sup> | 7.7 ± 1.3 <sup>b,d</sup>   | 7.7 ± 1.4 <sup>a,a</sup> | 27.7 ± 3.5 <sup>b,b</sup>  |
| -1.2                  | 0.0 ± 0.0 <sup>a,a</sup>   | 0.0 ± 0.0 <sup>a,a</sup>   | 0.0 ± 0.0 <sup>a,a</sup>  | 0.0 ± 0.7 <sup>a,a</sup>   | 1.0 ± 0.7 <sup>a,a</sup>   | 1.7 ± 0.8 <sup>b,b</sup> | 4.0 ± 1.6 <sup>a,c</sup>   | 4.0 ± 1.4 <sup>a,a</sup> | 12.0 ± 1.9 <sup>a,a</sup>  |

Each value is the mean ± EE of n=6. Different letters to the left of the comma indicate significant differences between years, and those to the right of the comma indicate significant differences among water potential treatments.

and times from imbibition in both study years were greater (p<0.05) in 2007 than in 2006 (Table 2). Except at -0.8 and -1.2 MPa, total germination was greater (p<0.05) in 2007 than in 2006 (Table 2). In 2006, germination percentages were greater (p<0.05) at 0 and -0.2 MPa than at -0.4 MPa and lower water potentials after 15 and 18 h from study initiation (Table 2). At 24 and 36 h after initiation of the study in 2006, germination percentages were similar (p>0.05) between 0 and -0.8 MPa, but these germination percentages were greater (p<0.05) than those at -1.0 and -1.2 MPa (Table 2). Differences in germination

percentages among water stress treatments diluted with increasing time from the initiation of the study, and they were similar (p>0.05) between 60 and 84 h from study initiation (Table 2). During 2007, differences in germination percentages among water potential treatments were more marked than in 2006 (Table 2). Until 24 h from study initiation, germination percentages were similar (p>0.05) at 0 than at -0.2 MPa, and decreased as water potentials decreased (Table 2). Twelve hours later, germination percentages were greater at 0 than at -0.2 MPa, and as from the beginning of the study, water potentials between

0 and -0.6 MPa were greater (p<0.05) than those at or below -0.8 MPa.

### Cumulative germination

Cumulative (total) germination percentages were similar (p>0.05) among 0 and -0.8 MPa in 2006 (Table 2). However, these percentages decreased (p<0.05) at lower water potentials in that year (Table 2). During 2007, cumulative germination was greater (p<0.05) at 0 than -0.2 MPa, but values were similar (p>0.05) between -0.2 and -

**Table 3.** Coefficients velocity of germination in the various water potential treatments in 2006 and 2007.

| Water potential (MPa) | Year | Coefficient of velocity    |
|-----------------------|------|----------------------------|
| 0                     | 2006 | 70.4 ± 10.3 <sup>a,c</sup> |
|                       | 2007 | 62.3 ± 1.7 <sup>a,c</sup>  |
| -0.2                  | 2006 | 68.3 ± 3.7 <sup>a,c</sup>  |
|                       | 2007 | 73.9 ± 5.2 <sup>a,c</sup>  |
| -0.4                  | 2006 | 67.2 ± 5.4 <sup>a,c</sup>  |
|                       | 2007 | 68.1 ± 2.1 <sup>a,c</sup>  |
| -0.6                  | 2006 | 70.8 ± 4.1 <sup>a,c</sup>  |
|                       | 2007 | 60.8 ± 2.4 <sup>a,c</sup>  |
| -0.8                  | 2006 | 48.2 ± 2.2 <sup>a,b</sup>  |
|                       | 2007 | 46.6 ± 2.0 <sup>a,b</sup>  |
| -1.0                  | 2006 | 40.9 ± 3.1 <sup>a,a</sup>  |
|                       | 2007 | 35.8 ± 1.0 <sup>a,a</sup>  |
| -1.2                  | 2006 | 34.0 ± 1.7 <sup>a,a</sup>  |
|                       | 2007 | 31.1 ± 1.9 <sup>a,a</sup>  |

Letters to the left of the comma indicate significant differences between years, and those to the right of the comma indicate significant differences among water potential treatments. Each value is the mean ± 1EE of n=6.

0.6 MPa (Table 2). At water potentials lower than -0.6 MPa, germination percentages decreased ( $p < 0.05$ ) as water potentials also decreased (Table 2).

### Patterns in changes of germination percentages

Similar patterns of changes in germination Percentages with time were obtained in both study years (Table 2). Germination percentages followed a similar pattern with time from the initiation of the study in 2006 and 2007 at water potentials from 0 to -0.6 MPa: they increased until after approximately 60 h from incubation before leveling off. At lower water potentials (from -0.8 to -1.2 MPa), germination started to increase later until approximately 84 h from study initiation compared to the other water potential treatments (Table 2). Between -0.8 to -1.2 MPa, time to germination from study initiation delayed as medium water potential decreased (Table 2).

### Coefficients of velocity of germination

No differences ( $p < 0.05$ ) were found between study years in the coefficients of velocity in any of the water potential treatments (Table 3). In both study years, coefficients of velocity were greater ( $p < 0.05$ ) from 0 to -0.6 MPa than at lower water potentials. The lowest ( $p < 0.05$ ) coefficients of velocity were found at -1.0 and -1.2 MPa (Table 3). At

-0.8 MPa, coefficients of velocity were greater ( $p < 0.05$ ) than at lower, but smaller ( $p < 0.05$ ) than at higher, water potentials (Table 3).

### Early seedling growth

Early seedling growth was similar ( $p > 0.05$ ) between years (2006: 6.33 mm,  $n=72$ ; 2007: 5.40 mm,  $n=72$ ), and plant parts (shoots: 5.51,  $n=72$ ; roots: 6.22 mm,  $n=72$ ). It was greater ( $p < 0.05$ ) at 0 than at -0.6 MPa and lower water potentials (Table 4). Also, growth of early seedlings was greater ( $p < 0.05$ ) at -0.4 than at -0.8 MPa and lower water potentials (Table 4).

## DISCUSSION

### Storage time

Maintenance of seed quality in storage from the time of production until the seed is planted is imperative to assure its planting value. There was a marked decrease in seed viability with storage time in *D. eriantha*. This might have been partially the result of the storage conditions. The best alternative to avoid risks associated with storing seeds is to avoid storing seeds. For example, the grass seed industries in Oregon ship the seeds within a few months after harvesting. Another example include

**Table 4.** Early growth of *D. eriantha* seedlings (shoot + root) at various water potentials.

| Water potential (MPa) | Early seedling growth (mm) |
|-----------------------|----------------------------|
| 0                     | 15.09 <sup>a</sup>         |
| -0.4                  | 10.35 <sup>ab</sup>        |
| -0.6                  | 6.48 <sup>bd</sup>         |
| -0.8                  | 1.41 <sup>cd</sup>         |
| -1.0                  | 1.29 <sup>cd</sup>         |
| -1.2                  | 0.56 <sup>cd</sup>         |

Different letters indicate significant differences ( $p < 0.05$ ) among water potentials. Each value is the mean of  $n=24$ .

Bolivia where the wheat seed harvested in the Highlands in April is being planted in May in the Lowlands, or Colombia where rice can be produced twice a year, which decreases the storage period (<http://seedlab.oregonstate.edu/book/export/html/123>).

These strategies are becoming highly desirable not only because they reduce storage, but especially because they make possible to market the seeds and meet financial obligations. However, there are times when seed growers and dealers carry over seed lots from one year to the next due to weak market, to insure an adequate supply the following year, and/or because the production system does not provide choices. Under such circumstances, the question is how to manage the seeds to maintain a high viability. If dry weather prevails during grass seed maturation and harvest, it should be allowed to harvest seeds not only with low moisture but also with high initial viability (Copeland and McDonald, 1995). This should be followed up by placing the seeds in cool and dry warehouses to lower the risks in storage (Copeland and McDonald, 1995). Storage of *D. eriantha* seeds at 20°C most likely caused some loss of seed viability after two years from harvesting. Losses of viability because of storage conditions contribute to explain the lower germination percentages in 2006 than in 2007.

### Temperature effects

Total germination percentages were similar at constant than at alternating temperatures, in agreement with the first hypothesis. Similar to our results Hsu et al. (1985) found that chilled 'IG-2C-F1' *S. nutans* seeds germinated best at constant temperatures of 20 to 35°C. Fifteen-month-old seeds of the perennial, warm-season *Eragrostis curvula* (Schrad.) Nees cvs. 'Don Juan', 'Don Pablo' and 'Don Arturo' showed similar germination percentages when temperatures were (a) constant at 20 or 30°C under darkness, (b) alternate at 4/20, 10/20, 20/30 or 20/35°C under darkness, or (c) alternate as described in (b) but with a 9h-photoperiod at the maximum temperature (Fernández et al., 1991). These authors also reported that the highest germination

percentage in *E. curvula* cv. 'Morpa' occurred at constant temperatures of 30°C, while *E. curvula* cvs. 'Don Eduardo' and 'Tanganyika' were favored by either constant or alternating temperatures higher than 20°C. Young and Evans (1982) found that maximum germination percentages could be obtained under constant temperatures for most of the perennial grasses they studied. Hylton and Bement (1961) also found greatest germination of *Festuca octoflora* Walt. under constant 20°C than under various alternating temperature regimes. Terenti (2004) showed that the best germination (80%) in *D. eriantha* occurred at 30 and 35°C. We showed that high germination percentages occurred in this species at these high, constant temperatures in our study a day after study initiation. When temperatures were outside these values, a significant decrease in germination resulted. From these determinations it is conceivable that large annual differences in *D. eriantha* density could potentially be obtained to the prevailing temperature during moisture availability.

It is important to highlight that cumulative germination percentages found with the thermal gradient were above than 80% between 20.4 and 33.8°C, but these percentages decreased at lower or higher temperatures. There was no germination beyond 36.9°C. These results imply that seeding of *D. eriantha* could be made during late-spring, early-summer (December, January; Figure 1) at the study site, when soil temperatures are at or beyond 20°C, and precipitations keep the soil moist (see long-term precipitation data in Figure 1).

Overall coefficients of velocity supported that, over the short-term, more seeds germinated at constant than alternating temperatures, in agreement with the second hypothesis. These results suggest that 30 to 35°C could be within the optimum temperature for germination in this species. Terenti (2004) also reported the highest total germination for *D. eriantha* at these temperatures, when he only compared constant, but not constant versus alternating temperatures. We need to keep in mind, however, that the mean temperature was lower at the fluctuating temperature than under the constant temperature regimes. Therefore, differences between fluctuating and constant may be due to different mean temperatures rather than to temperatures being constant or fluctuating *per se*. This reasoning, that could help to explain differences between constant versus alternating temperatures, might extend to other germination studies, that have attributed either to constant or alternating temperatures their results on the germination response to temperature in various species.

Our results differ, however, with most studies on perennial grass species, where germination was stimulated most by alternating than constant temperatures. When *Elymus cinereus* cv. Magnar (Scribn. and Merr.) was exposed to different temperature profiles from 0 to 40°C, it showed greater germination percentages after exposure to alternating temperatures between 15 and 25°C (Evans and Young, 1983). A similar response was

obtained with *Achnatherum robustum* (Vasey) Barkworth which optimum germination occurred with 20°C during 8 h, and 15°C during 16 h (Young et al., 2003). *Leymus cinereus* showed the greatest mean germination percentages at alternating temperatures of 15/25°C, and then at 10/20, 20/30 and 5/15°C in decreasing order of magnitude (Meyer et al., 1995).

Results from this study are important since more than 50% germination might occur at constant temperatures within a few hours after seeding, taking advantage of small rainfall events, which are common at the study site (Paez et al., 2005). These authors reported that 61% of the rainfall events were lower than 5 mm after an analysis of 18 years (1983-2000) of rainfall records at the Chacra Experimental de Patagones. After four days of study, however, germination was similar at all temperature treatments. These results suggest that temperature does not appear to place a major restriction on the germination of *D. eriantha*; seeds can be expected to germinate over a wide thermal gradient.

### Water potential effects

Moisture availability imposed severe limitations on seed germination of *D. eriantha*, which has similar germination requirements that many mesophytic crops (Levitt, 1980; Bonvisutto and Busso, 2007): the lower the water potential, the lower the germination percentage and the velocity of germination in this species. The lower coefficients of velocity at lower water potentials are an indication of greater germination times (Scott et al., 1984); in fact, seeds started to germinate later at lower than higher water potentials (Table 2). Plants possessing seeds with exacting requirements for germination can establish more successfully than those with few restrictions (Hegarty, 1978). However, in an environment with changing moisture conditions the opportunities for germination may be reduced for seeds with specific moisture requirements. If moisture stress is low, seeds of *D. eriantha* can germinate over a wide range of temperatures; however, the more severe the water stress, the greater the reduction in germination percentage. This response presumably reflects an adaptive strategy because *D. eriantha* is generally restricted to habitats with moister conditions than those of the Phytogeographical Province of the Monte (Cano, 1988). Such an adaptation protects against germination under conditions of transient or low soil moisture, limiting most germination to periods with protracted conditions of high soil moisture.

Exposure to descending water stress reduced germination. Relatively warm soil temperatures and water stress are usually simultaneous events in the Chacra Experimental de Patagones, within the Phytogeographical Province of the Monte. Providing seed mortality does not occur, no germination induced by the combination of warm temperatures and water stress may act to preserve

a portion of the seedbank for germination at a later date. This lack of germination under unsuitable conditions may also serve to block germination that would otherwise predispose seedlings to temperature and moisture conditions that most likely would not be conducive to their growth and survival.

### Seedling growth

Despite increasing temperatures provide conditions favorable for growth of seedlings (Brown, 1995), water potentials lower than -0.4 MPa greatly reduced seedling growth. These results, in agreement with the third hypothesis, are in accordance with those reported by Brown (1995) in several perennial grasses. Our data showed that *D. eriantha* has the ability to germinate over a broad range of temperatures, but severe restrictions are imposed by reduced moisture availability. Seeding of this species in rangelands of central Argentina (e.g., the Chacra Experimental de Patagones) will most likely fail under water stress conditions (e.g., years 2008 and 2009 in Figure 1). Results suggest that this species should be planted in late-spring, early-summer, when seedbed temperatures are increasing and soil moisture might still be adequate (e.g., year 1984 in Figure 1).

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