Effects Of Priming On Seed Germination Of Panicum Coloratum L. Under Saline Stress Conditions

Maira Carabajal, Mónica B. Ruiz, Carlos A. Parera

Abstract: In the present study, we evaluated the effects of osmotic conditioning (priming) on seed germination and emergence rate index (ERI) of Panicum coloratum cv Klein Green, under optimal germination conditions (watered with distilled water) and salt stress (irrigated with different water potentials of NaCl solution). The seeds were primed in 3 osmotic solutions (NaCl, KCl, and K2SO4) during 2 days at 15°C. After the treatments the seed was germinated in 3 levels of salinity/osmotic potential media (12 µscm-1/-0,5 MPa; 30 µscm-1/-1,0 MPa; 43 µscm-1/-1,5 MPa). Salt stress conditions generate a depressive effect on the speed and germination percentage of Panicum coloratum seeds. Priming treatments increase significantly the germination percentage and ERI in prime seeds compared to untreated in non saline condition. At 12 µscm-1/-0,5 MPa salinity levels, the primed seeds showed higher significantly germination velocity (ERI) compared with non primed seeds.

Index Terms: priming, seed emergencen, salt stress, osmoconditioning,forage,germination velocity.

1 Introduction

Panicum coloratum is a perennial Gramineae, with a high forage value and spring-summer growth. Native to Africa, was introduced in Australia and United States and has several subspecies and cultivars with different characteristics (Armando et al., 2013). The importance as a forager lies in the amount of plant mass that produces and palatability (Petruzzi et al., 2003; Stritzler, 2008) as well as the maintenance of its nutritional quality for animal husbandry throughout its development (Petruzzi et al., 2003). Its most common use is as a pasture, but also higher vegetative development cultivars are cut and stored for use as hay (Saha et al, 2008) and the stoloniferous are used for erosion control (Rocco et al., 2016). The main characteristics of this genera are its resistance to late frosts, drought tolerance and fire (Stritzler, 2008) and the absence, so far, of pests and diseases (Petruzzi et al., 2003). A moderate tolerance to low salinity of the soil, which varies among genotypes, emphasizing such features in the variety Makarikariensis has also been reported (Petruzzi et al., 2003). Perennial forages have a slow initial growth, and few reserves in the seed, what makes that the germination period be extended and critic (García-Espil, 1990).

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Dormancy, is also characteristic of summer pastures (Petruzzi et al., 2003), prevents the germination of seeds in a same growing season, hampering development uniform crop. Salinity is one of the most restrictive factors for world food production. The problem extends to over 100 countries and affected soils, surpass the billion hectares (FAO and ITPS, 2015). This problem is increased annually, especially in arid and semi-arid areas, and may worsen with climate change (Rengasamy, 2006). Salinity affects germination and subsequent development of the plant by combination a reduction in the osmotic potential of the soil and by specific ion toxicity (Khajeh-Hosseini et al., 2003). Achieve a rapid germination and crop uniformity in saline soils are factors that determines the crop productivity. The osmotic conditioning of seeds (priming) has been reported as a seed treatment that improves the speed and uniformity of emergence in various species under stress conditions (Parera and Cantliffe, 2010). During priming, a biochemical, and physiological processes are activated that result in a metabolic transformations necessaries for the germination of seeds in the shortest time (Ruiz Torres et al., 2007). It has a positive impact, increasing the germination capacity and stimulating the growth of the root system (Parera and Cantliffe, 2010). Also increases protein synthesis and the processes of repair and synthesis of nucleic acids, along with the repair of damaged cell structures and other macromolecules (Varierl et al., 2010). Osmotic conditioning is based on seeds immersion at low water potential osmotic solution to control the hydration processes (Parera and Cantliffe, 2010). Different osmotic substances such as sugars, polyethylene glycol (PEG), salts and solids (Frett et al., 1991) are used in the processes. The use of salts in osmotic conditioning treatments has been reported by several authors and in different species as a tool to reduce the effects of salinity on the germination (Farhoudi and Lee, 2014). There are contradictory results on the effect of osmotic conditioning in the genus Panicum. Beckman et al. (1993) using the technique of Solid Matrix Priming in Panicum virgatum did not observe a significant difference in germination with respect to the non-treated seeds. On the contrary, Hacisalihoglu (2008) reported that osmotic conditioning with (Microcell) synthetic calcium silicate is a very effective technique to improve the performance of germination in different species of Panicum. The objective of the present study was to assess the effect of osmotic conditioning with salts, on the germination and speed of germination of seeds of Panicum coloratum under salinity conditions.

2. Materials and methods

2.1. Plant Material

The seeds of Panicum coloratum cv Green Klein were obtained from the hotbed of Agricultural Experimental Station INTA San Luis, Argentina. The cv Green Klein is originated in the United States, being the most used in the semi-arid Argentina Pampeana region for its resistance to frost and drought (Petruzzi et al., 2003).

2.2. Osmotic Treatments

One hundred (100) seeds were placed in a 10 cm Petri dish, on a filter paper (Muntkel, 1700) saturated with different solutions and osmotic potential (PO): NaCl (PO: - 3 MPa); KCl (PO: - 1 MPa) and KSO4 (PO: - 2 MPa). The boxes were sealed with plastic wrap and incubated for 2 days at 15° C (12 hours light/12 dark). The solutions were prepared using the formula of Vant ' Hoff and adjusted using an osmometer (Wescor, USA). After the treatment, the seeds were washed with distilled water, treated with Captan 0.5% PM (N-(trichloromethylthio)amixtureofcyclohex-4ene-1, dicarboximida) and dried at 20°C, up to their original moisture.

2.3. Germination Test

Four replicates of 100 seeds were placed for each treatment and control (no primed), in subsamples of 25 seeds in a 5 cm Petri dish, on two layers of Muntkel 1700 germination paper soaked with solutions 3 ml of distilled water (control) and salt solution (NaCl) with the following osmotic potentials and electric conductivity (CE): PO: - 0.5 MPa/EC: 12 µs/cm; PO: MPa - 1 / CE: 30 µs/cm and PO: - 1.5 MPa / CE: 43 µs/cm. Then were incubated in a germination chamber at 25 °C with a photoperiod of 12 h light/12 h dark. Germination was recorded daily for 10 days and counted when the radicle exceeded 2 mm in length. With the results were calculated the percentage of germination and emergence rate index (ERI) (Shmueli and Goldberg, 1971).

2.4. Experimental Design and data analysis

The experiment was conducted as a randomized factorial (osmotic treatment x salinity) with four replications. The data were subjected to analysis of variance (ANOVA). LSD test was used for the separation of means. Linear regression was used where ANOVA p values were statistically significant for the concentration of the solution. Percentage data were arcsine-transformed before analysis of variance was conducted. The data were analyzed with the statistical software Infostat/professional version 1.1.

3. Results

The germination of the seeds of Panicum coloratum cv Klein and speed of germination (ERI) were significantly modified by the type of osmotic treatment, salt concentration and their interaction (table 1). To increased salinity of the substrate, the germination percentage and speed decreased.

Table 1. *Analysis of variance for germination percentage and speed of germination (ERI) after priming.*

	Germination (%)	ERI
Variable	Probability	
Osmotic treatment (T)	0.0244	<0.0001
Salinity (S)	<0.0001	<0.0001
SxT	0.0003	<0.0001

Given the interaction observed between osmotic treatment and the salinity of the germination medium, we explored the effect of treatments in different levels of salinity (0, - 0.5, - 1, and 1.5 MPa). The percentage of germination in distilled water (control) was significantly higher in seeds primed with NaCl and KCl (90% and 81%, respectively) compared with seeds not primed 62% (Table 2), demonstrating the effectiveness of priming treatment on germination. The germination rate (ERI) was significantly higher in osmotically treated seeds with KCl and KSO4 than the non-treated seeds. When the salinity of the substrate increased to - 0.5 MPa, there were not a significant differences in the germination percentage, however all the percentages of osmotic conditioning treatments were greater than the non-treated seeds. The speed of germination (ERI), at these salinity levels (- 0.5 MPa/12 µscm-1) the conditioned seeds showed a significantly greater germination rate compared with the control. At higher levels of salts (- 1 MPa/30 µscm-1) there were no significant differences in the variables evaluated and at - 1.5 MPa/43 µscm-1) germination in all treatments was severely affected.

Table 2. *Germination percentage (%) and rate of emergence (ERI) of primed and no primed seeds at different salinity levels/osmotic potentials.*

	Substrate salinity							
	0 µscm^{-1}= 0 MPa		12 µscm^{-1} = -0,5MPa		30 µscm^{-1}= -1,0 MPa		43 µscm^{-1} = -1,5 MPa	
Osm.	%	ERI	%	ERI	%	ERI	%	ERI
NaCl	90 ^{a}	4.4^{b}	62 ^{a}	4.1^{bc}	48	2.4	0 b	0.0^{b}
KCl	81^{ab}	6.0 ^{a}	72^{a}	4.7^{ab}	35	1.7	1 b	0.0^{b}
KSO_4	73 ^{cb}	5.5 ^{a}	66^{a}	5.5^{a}	41	2.0	5 a	0.1^{a}
Cont.	62^{c}	4.0 ^{b}	61^{a}	3.7^{c}	41	1.9	1 b	0.0^{b}
LSD	12.1	1.0	9.6	0.8	12.7	0.7	3.1	0.1

Means with the same letter between columns indicate no significant difference (LSD p=0,05)

A significant negative relationship between germination percentage and salinity was found in primed and non primed seed, similar results were observed when ERI was evaluated (Table 3, Figures 1 and 2).

Tabla 3. *Regression equation and coefficient of determination (R^2) of the relationship between the percentage of germination, the ERI index and the salinity of the substrate in seeds of Panicum coloratum osmotically-treated and control.*

	Germinatión (%)	
Trat.	Equation	R^2
NaCl	y: (-56,75)x + 95,63	92,95**
KCl	y: (-55,40)x + 88,88	92,01**
KSO_4	y: (-45,00)x + 80,60	88,35**
Control	y: (-40,60)x + 71,70	80,24**
Emergence Rate Index		
NaCl	y: (-2,97)x + 4,94	85,11**
KCl	y: (-4,16)x + 6,23	94,91**
KSO_4	y: (-3,90)x + 6,20	87,31**
Control	y: (-2,81)x + 4,47	87,86**

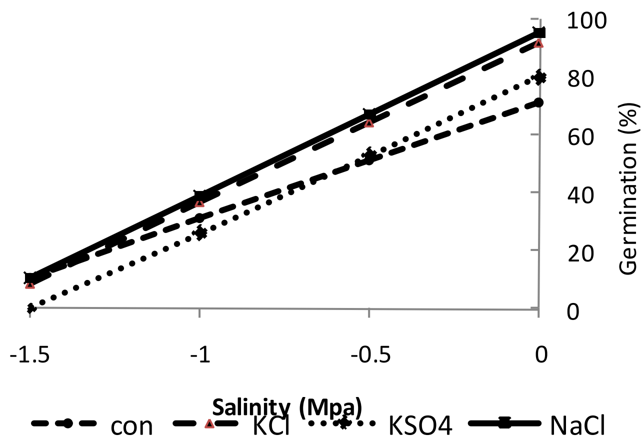

Figure 1. *Linear relationship between the final germination percentage and substrate salinity in osmotically-treated and untreated (con) Panicum coloratum seeds*

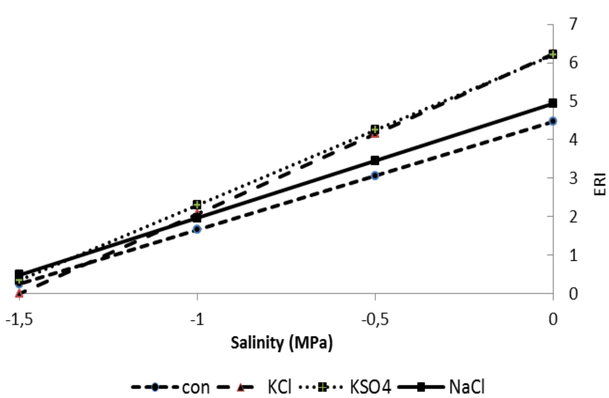

Figure 2. *Linear relationship between the emergence rate index (ERI) and substrate salinity in osmotically-treated and untreated (con) Panicum coloratum seeds.*

4. Conclusión

To evaluate the germination in saline substrates is a legitimate form of knowing the tolerance of plants to that stress condition (Dasgan et al., 2002). In the present trial, the results clearly show the depressive effect of salinity on germination and speed of germination in *Panicum coloratum*, reported in numerous thermic pastures and other plant species (Ma et al., 2014). The negative response of germination to salinity increase was also observed in the priming treatments, however, in non saline conditions or high salinity (0,5 MPa/12 µscm^{-1}) the primed seeds have higher germination percentage and rate compared to non-treated seeds. At extreme values of salinity, it was observed no differences between treatments. The main effects of salinity on germination are a decrease in water availability, given to the low water potential of the soil solution produced by salts (osmotic character), the specific toxicity of the ions and the unbalance of the same (Öztürk et al., 2006). The fact that certain cations and anions can be absorbed during priming treatments, may exercise specific osmotic and Ionic effects (Frett et al., 1991), where ions may accumulate inside the seed, reduce their osmotic potential, and consequently lead to a greater absorption of water at the time of germination (Parera and Cantliffe, 2010). In melon seeds, osmotic conditioning with NaCl, promoted the accumulation of K^+ and Ca^{++} inducing osmoregulation by accumulation of organic solutes (Sivritepe et al., 2003). Also, it has been reported a positive relationship between the concentration of K^+ from the seed, by osmotic conditioning, with the α-amylase activity increasing the amount of sugar in the embryo (Farooq et al., 2006). The soil salinity, affects negatively the *Panicum coloratum* germination, but the introduction of tolerance in the early stages of growth could improve its establishment in the field (Taleisnik et al., 1998). The retrieved results show that the osmotic conditioning of seeds of *Panicum coloratum,* using salts as osmotic substances can significantly improve the germination (percentage and speed) in laboratory conditions, compared to non-treated seeds and could facilitate its implantation in saline soil conditions, however further research must be conducted to determine the effects on field establishment.

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