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Controlled environmental conditions on germination of bermudagrass seeds

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***Cynodon dactylon* (L.) Pers., widely known as bermudagrass, is a cosmopolitan species used to form lawns, what provides aesthetic effects in parks and gardens, but also composes pastures and sports fields, such as golf and football. The use of seeds for the formation of new lawns is a common practice in Europe and in the United States, and is currently considerably expanding also in Brazil. It is important to understand the ideal environmental conditions for seed germination of each species, or cultivar. The aim of this study was to evaluate effects of salinity, temperature, light, substrate water contents, and sowing methods on germination of two bermudagrass cultivars: Princess 77 and Riviera. Three experiments, arranged in factorial schemes, were conducted: Experiment 1. Five salt concentrations (0, 25, 50, 75, and 100 mM) x two salt sources [sodium chloride (NaCl) and potassium chloride (KCl)]; Experiment 2. Three temperatures (constant at 30°C, alternating at 20 to 30°C, and alternating at 20 to 35°C) x presence or absence of light (8 h of light and 16 h of darkness, and total darkness); and Experiment 3. Four substrate water contents (25, 50, 75 and 100% of the substrate water retention capacity) x two sowing methods (in sand, and on sand surface). Germination percentage and germination rate were evaluated. Germination of Princess 77 was more effective in the absence of NaCl and KCl; at 20 to 35°C, either in the light or darkness; and at around 50% of the substrate water retention capacity, sown either in sand or on sand surface. Germination of Riviera seeds was more effective in the absence of NaCl and presence of KCl; at 20 to 35°C, in the light; and at 100% of the substrate water retention capacity, sown on sand surface.**

Key words: Poaceae, *Cynodon dactylon*, Princess 77, Riviera, salinity, temperature, light, substrate water content, sowing method.

INTRODUCTION

Cynodon dactylon (L.) Pers., widely known as bermudagrass or silk grass, is a perennial cosmopolitan species that hybridizes either naturally or artificially, and produces gray-green short blades, usually 2 to 15 cm long, with jagged edges; its erect stems can grow up to 30 cm height (Walker et al., 2001). It may be considered

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a weed, forage, or ornamental (Vilela et al., 2005). Lawns, which are formed by grass species, usually provide aesthetic effects in parks and gardens, but also compose pastures and sports fields, such as golf and football. Furthermore, grasses may act, among others, on slope stabilization and erosion control (Freitas et al., 2012; Raven et al., 2001).

Use of seeds for the formation of new lawns is a common practice in Europe and in the United States, and is currently considerably expanding also in Brazil. Because of sporting events carried out in the country in 2014 and, also, to be accomplished in 2016, there is even greater expectation of increased market due to investments towards the necessary infrastructure (Godoy et al., 2012), what includes the formation of new lawns or renovation of existing ones.

Grasses can self-propagate by seeds, rhizomes, stolons, and tillers. Although plants from vegetative propagation show better initial development, this method has several agricultural limitations, such as higher dissemination of pests and diseases, elevated demand of propagation material from extensive areas, great need of hand labor, and high costs, apart from faster perishability of vegetative materials. The use of seeds, thus, is preferable whenever possible (Carmona et al., 1998; Evers and Parsons, 2010).

Germination usually depends on seed both endogenous and exogenous characteristics (Santos et al., 2004); therefore, it is essential to understand the ideal environmental conditions for each species, that vary according to each region, such as water, temperature, and light which, according to Santos et al. (2004), are considered the most important ones, besides salinity and soil/substrate moisture.

High salt contents in the soil, for instance, especially sodium chloride (NaCl), may inhibit germination mainly due to the osmotic effect. Although some plants are able to osmotically adjust themselves to maintain growth and turgor (Alshammary, 2012), an increase in salt concentrations greatly enhances the percentage of abnormal seedlings, due to the salt toxic action on germinating seeds (Lima et al., 2005), apart from affecting plant establishment and productivity (Ortiz et al., 2014). Furthermore, grasses may differ in their capacity to tolerate salinity at the germination stage (El-Keblawy et al., 2011).

This study aimed to evaluate the effects of salinity, temperature, light, substrate water content, and sowing method on seed germination of two bermudagrass cultivars: Princess 77 and Riviera.

MATERIALS AND METHODS

Three experiments with two bermudagrass cultivars, Princess 77 and Riviera, were conducted from May to June 2012, in the Seed Laboratory of the Department of Crop Production at the College of Agricultural and Veterinary Sciences of the State University of São Paulo (FCAV/UNESP), campus in Jaboticabal, Brazil.

The experimental design for Experiment 1, which tested the salinity effect on seed germination, was entirely randomized, with 10 treatments arranged in a 5 x 2 factorial scheme: five salt concentrations (0, 25, 50, 75 and 100 mM) x two salt sources [sodium chloride (NaCl) and potassium chloride (KCl)]. There were four replications of 100 seeds, totaling 4,000 seeds. The NaCl electrical conductivity of those concentrations was, respectively, 0.59, 2.10, 3.48, 5.23 and 8.00 dS m^{-1}; for KCl concentrations, it was, respectively, 0.59, 2.34, 4.25, 6.20 and 8.20 dS m^{-1}.

The experimental design for Experiment 2, which tested the effect of temperature and light on seed germination, was entirely randomized, with six treatments arranged in a 3 x 2 factorial scheme: three temperatures (constant at 30°C, alternating at 20 to 30°C, and alternating at 20 to 35°C) x two light regimes (8 h of light and 16 h of darkness, and total darkness). There were four replications of 100 seeds, totaling 2,400 seeds.

The experimental design for Experiment 3, which tested the effect of substrate water contents and sowing methods on seed germination, was entirely randomized, with eight treatments arranged in a 4 x 2 factorial scheme: four water contents (25, 50, 75 and 100% of the sand water retention capacity) x two sowing methods (in sand, and on sand surface), with four replications of 100 seeds, totaling 3,200 seeds.

Seeds were sown on sand surface in plastic boxes (11 x 11 x 3.5 cm), with the exception of Experiment 3, which seeds were also sown in sand. The plastic boxes remained in translucent plastic bags of low density polyethylene, and were placed in an incubator under the alternating temperatures of 20 to 35 and 20 to 30°C for Experiment 1 and 3, respectively; a photoperiod of 8 h of light and 16 h of darkness was settled, according to the recommended for *Cynodon dactylon* seeds in the Rules for Seed Analysis (Brasil, 2009). For Experiment 2, besides those mentioned, the temperature of 30°C and light regime of total darkness were also tested, as specified.

Substrate was weighted daily for water replacement, what was performed whenever it showed, for Experiments 1 and 2, 50% of its water retention capacity, which was calculated before the beginning of the experiment. For Experiment 3, water replacement was performed whenever necessary to maintain the sand water content of each treatment (Brasil, 2009). Germination was recorded daily for 28 days. Percentage of normal seedlings that were either equal to or higher than 2 mm were noted. Germination percentage and germination rate were determined as described by Maguire (1962). Germination data were arcsine $(x/100)^{1/2}$ transformed before the variance analysis. Means of the resulting values were compared by the Tukey test at 1 and 5% probability. Polynomial regression analysis was also performed to verify the effect of the tested salinity and substrate water contents on Princess 77 and Riviera bermudagrass seed germination.

RESULTS

We verified, from the results of Experiment 1, that there was a significant interaction among salt concentrations and sources for germination percentage and germination rate for both bermudagrass cultivars (Table 1). However, at the concentrations of 0, 25, and 50 mM, there were no significant differences among salt sources for both germination percentage and germination rate of Princess 77 seeds, but at the concentrations of 75 and 100 mM, there was greater germination percentage and faster germination when seeds were submitted to the KCl treatment in comparison with NaCl. Even with the KCl superior results at higher concentrations when compared

Table 1. Variance analysis of germination percentage (%G) and germination rate (GR) of Princess 77 and Riviera bermudagrass (*C. dactylon*) seeds submitted to different salt concentrations and sources.

Princess 77

Variation sources	DF	%G	GR
Salt concentrations (SC)	4	13.02**	70.83**
Salt sources (SS)	1	6.01*	8.79**
SC x SS	4	7.67**	8.79**
Residue	30		
CV (%)		5.40	8.68

	%G				GR	
Salt concentrations	**NaCl**		**KCl**		**NaCl**	**KCl**
0 mM	(49.90)[1]	58.50[2a]	(49.90)	58.50 a	14.43 a	14.43 a
25 mM	(49.03)	57.00 a	(47.15)	53.75 a	10.77 a	9.85 a
50 mM	(46.72)	53.00 a	(43.99)	48.25 a	8.85 a	9.68 a
75 mM	(42.70)	46.00 b	(44.57)	49.25 a	8.21 b	10.83 a
100 mM	(38.34)	38.50 b	(44.57)	49.25 a	6.51 b	8.12 a

Riviera

Variation sources	DF	%G	GR
Salt concentrations (SC)	4	43.07**	103.45**
Salt sources (SS)	1	438.97**	219.57**
SC x SS	4	65.79**	77.20**
Residue	30		
CV (%)		3.57	5.92

	%G				GR	
Salt concentrations	**NaCl**		**KCl**		**NaCl**	**KCl**
0 mM	(60.44)[1]	75.50[2a]	(62.06)	78.00 a	15.84 a	15.84 a
25 mM	(58.69)	73.00 a	(61.57)	77.25 a	12.60 a	12.03 a
50 mM	(47.86)	55.00 b	(61.53)	77.25 a	10.08 b	11.88 a
75 mM	(46.00)	51.75 b	(65.32)	82.50 a	8.69 b	13.97 a
100 mM	(35.05)	33.00 b	(64.16)	81.00 a	3.97 b	13.93 a

**Significant at 1% probability; *Significant at 5% probability.[1]Data transformed to arcsine $(x/100)^{1/2}$; [2]Non-transformed data. Means followed by the same letter in the line do not differ from each other by the Tukey test at 5% probability.

with NaCl, germination percentage of Princess 77 seeds linearly decreased with increasing salt concentrations of both NaCl and KCl. The highest seed germination percentage found for this cultivar was observed in the absence of NaCl and KCl: 49.9% for both salts (Figure 1). The germination rate also decreased with increasing salt concentrations of both NaCl and KCl.

Similarly to what happened to Princess 77 seeds, there were no significant differences among lower concentrations (0 and 25 mM) of both salt sources for Riviera. However, at the concentrations of 50, 75, and 100 mM, there were greater germination percentage and faster germination when seeds were submitted to KCl in comparison with NaCl (Figure 1).

Germination percentage of Riviera seeds decreased with increasing concentrations of NaCl; the highest percentage, 60.44%, was observed under salt absence. However, there was a little gain (from 78 to 81%) on seed germination of this cultivar with increasing concentrations of KCl (Figure 1). Germination rate of this cultivar also linearly decreased with increasing NaCl concentrations, but under KCl, the rate increased from 50 mM, showing decreasing values under lower levels. Although the statistical analysis has shown that there were no differences for germination percentage and germination rate when Riviera seeds were submitted to KCl concentrations (Table 1), the generated regression equation (Figure 1) indicated that the germination rate had a little gain under the salt absence (15.30) when compared with results from the 100 mM KCl concentration (13.37). However, Figure 1 also shows that these values tend to increase if seeds were submitted to KCl concentrations higher than 100 mM.

Regarding the effect of temperature and light on seed germination (Experiment 2), there was a significant interaction among factors for germination percentage and germination rate for both bermudagrass cultivars (Table 2). The highest germination percentage and fastest

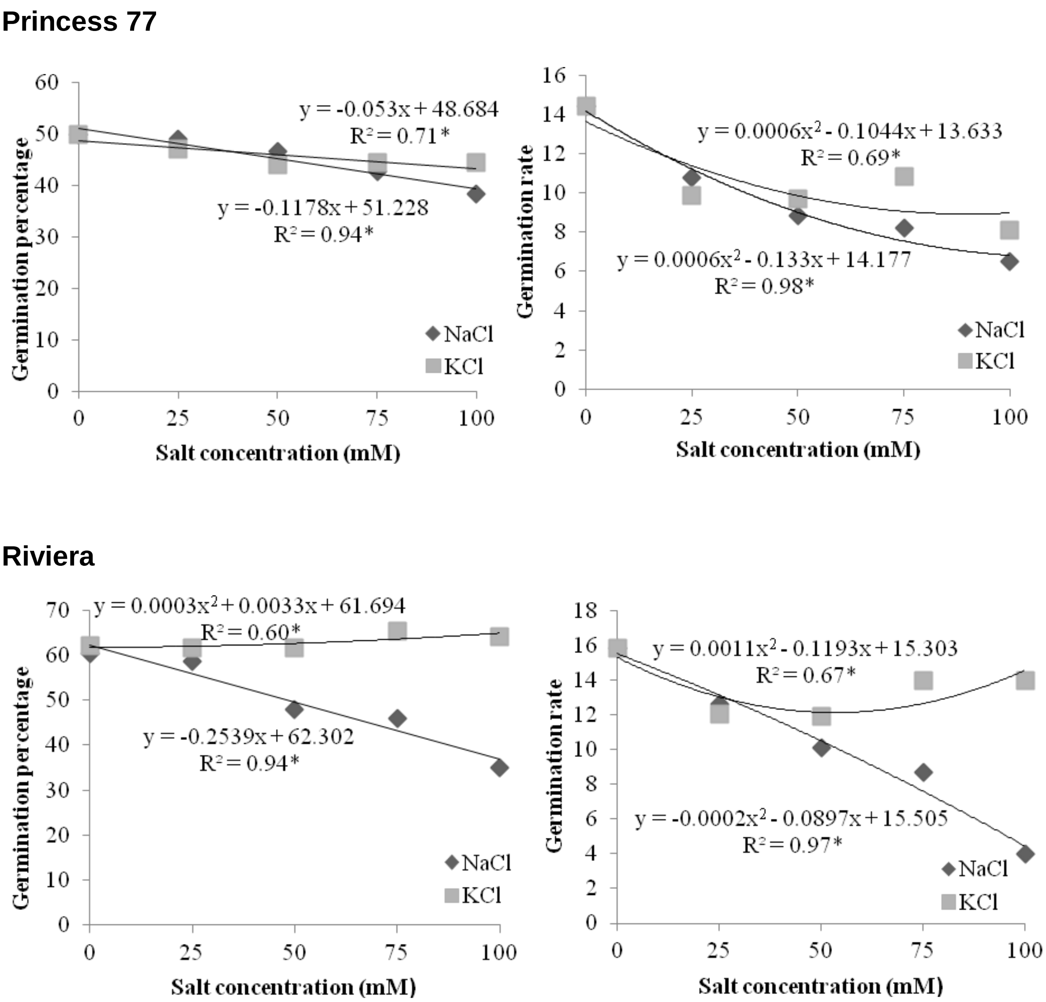

Figure 1. Germination percentage and germination rate of Princess 77 and Riviera bermudagrass (*C. dactylon*) seeds submitted to different salt concentrations and sources. *Significant at 5% probability.

germination of Princess 77 seeds were observed at the alternating temperature of 20 to 35°C, either in the light or darkness. For Riviera, highest germination percentage and fastest germination were also found at the alternating temperature of 20 to 35°C, but only when seeds were submitted to the light regime.

For Experiment 3, there was a significant interaction among substrate water contents and sowing methods for germination percentage and germination rate for both Princess 77 and Riviera bermudagrass cultivars (Table 3).

High germination percentages of Princess 77 seeds occurred: when sown either in sand or on sand surface, at 25 and 50% of the substrate water retention capacity; on sand surface, at 75% of the substrate water retention capacity; and in sand, at 100% of the substrate water retention capacity (Table 3). Regarding the germination rate, seeds presented much faster germination when sown: either in sand or on sand surface, at 25 and 50% of the substrate water retention capacity; and in sand, either at 75 or 100% of the substrate water retention capacity.

Seed germination percentage and germination rate of Princess 77, when sown on sand surface, increased up to 53 and 46% of the substrate water retention capacity, respectively, when it started to decrease (Figure 2). When seeds were sown in sand, germination percentage linearly decreased with increasing substrate water content; the germination rate, on the contrary, increased according to the increment in the substrate water content up to 100% of its retention capacity.

Riviera seeds presented increasing germination percentages and germination rates when sown in sand, up to 55 and 57% of the substrate water retention capacity, respectively (Figure 2). When it reached 100% of its retention capacity, higher germination percentage occurred on sand surface, but there was no difference for the germination rate between sowing methods (Table 3). Seeds sown on sand surface, therefore, showed

Table 2. Variance analysis of the germination percentage (%G) and germination rate (GR) of Princess 77 and Riviera bermudagrass (*C. dactylon*) seeds submitted to different temperatures and light regimes.

Princess 77

Variation sources	DF	%G	GR
Temperature (T)	2	138.02**	90.32**
Light (L)	1	10.47**	8.90**
T x L	2	21.15**	29.12**
Residue	18		
CV (%)		4.44	8.86

	%G				GR	
Temperatures	**Light**		**Dark**		**Light**	**Dark**
30 °C	(30.16)[1]	25.25^{2cB}	(39.37)	40.25^{bA}	4.72^{cB}	9.06^{bA}
20-30 °C	(44.14)	48.50^{bA}	(41.26)	43.50^{bA}	7.86^{bA}	7.33^{cA}
20-35 °C	(49.90)	58.50^{aA}	(51.07)	60.50^{aA}	12.19^{aA}	11.21^{aA}

Riviera

Variation sources	DF	%G	GR
Temperature (T)	2	473.78**	143.99**
Light (L)	1	1,719.71**	581.79**
T x L	2	40.81**	65.93**
Residue	18		
CV (%)		7.14	16.26

	%G				GR	
Temperatures	**Light**		**Dark**		**Light**	**Dark**
30 °C	(28.95)[1]	23.50^{2cA}	(0.00)	0.00^{bB}	4.33^{bA}	0.00^{bB}
20-30 °C	(33.52)	30.50^{bA}	(9.19)	2.75^{bB}	4.79^{bA}	0.58^{bB}
20-35 °C	(62.07)	78.00^{aA}	(21.54)	13.50^{aB}	12.59^{aA}	1.82^{aB}

**Significant at 1% probability.[1]Data transformed to arcsine $(x/100)^{1/2}$; [2]Non-transformed data.Means followed by the same lower case letters in the column and same upper case letters in the line do not differ from each other by the Tukey test at 5% probability.

decreasing germination percentage and germination rate up to 51 and 46% of the substrate water retention capacity, respectively, when started to increase according to the greater increment in moisture up to 100% of the substrate water retention capacity (Figure 2).

DISCUSSION

The NaCl treatment negatively affected germination of both Princess 77 and Riviera cultivars; on the other hand, KCl promoted a positive effect on Riviera seeds, what was not observed for Princess 77. The salt source, therefore, did influence the salinity tolerance behavior of those cultivars as, according to Ortiz et al. (2014), each source has chemical differences that may affect seed germination differently even when the osmotic potentials are similar. Also, Zhou and Xiao (2010), when studying the effects of specific ions on germination of sunflower seeds, concluded that germination is influenced not only by salt concentration (or osmotic potential) but also by ion nature in the salt solution and its interactions.

In accordance with these results, Zapryanova and Atanassova (2009), when studying the salinity tolerance of *Tagetes patula* and *Ageratum mexicanum* cultivated in pots, concluded that substrate salinity did inhibit plant growth; with increasing NaCl concentrations, the flowering period of both species decreased from 54 to 23 days for *T. patula*, and from 71 to 28 days for *A. mexicanum*. The inhibitory effect was best expressed in plants treated with 2% NaCl.

Some plants, however, do benefit from high salinity levels during germination, what provides greater adaptability to salinity during the remainder of their life cycles (Viana et al., 2004). Also, salt effects depend on other factors, such as plant species, cultivar, and phenological stage, apart from salt source, intensity and duration of salt stress, crop management, irrigation, and climatic conditions (Tester and Davénport, 2003). Coan et al. (2008), for instance, found that salinity levels up to 6.0 dS m^{-1} did not restrict seedling emergence of both Mirage bermudagrass and *Lolium perenne* seeds.

Each species, and even cultivar, therefore, responds differently to substrate salinity, so that it is important to correctly select the cultivar or species according to soil or substrate conditions, thus aiming at normal seedling

Table 3. Variance analysis of the germination percentage (%G) and germination rate (GR) of Princess 77 and Riviera bermudagrass (*C. dactylon*) seeds submitted to different substrate water contents (% substrate water retention capacity) and sowing methods.

Princess 77

Variation sources	**DF**	**%G**	**GR**
Water retention capacity (W)	3	12.59**	8.87**
Sowing methods (SM)	1	9.33**	36.18**
W x SM	3	31.41**	24.12**
Residue	24		
CV (%)		5.46	14.88

	%G				**GR**	
Water retention capacity	**On sand**		**In sand**		**On sand**	**In sand**
25%	(40.40)[1]	42.00[2a]	(41.26)	43.50 [a]	5.59 [a]	6.10 [a]
50%	(42.70)	46.00 [a]	(42.42)	45.50 [a]	7.54 [a]	6.38 [a]
75%	(44.57)	49.25 [a]	(38.78)	39.25 [b]	4.38 [b]	6.50 [a]
100%	(28.97)	23.50 [b]	(37.90)	37.75 [a]	1.90 [b]	7.72 [a]

Riviera

Variation sources	DF	%G	GR
Water retention capacity (W)	3	0.75[ns]	21.89**
Sowing methods (SM)	1	182.40**	431.21**
W x SM	3	78.45**	75.71**
Residue	24		
CV (%)		10.33	14.43

	%G				**GR**	
Water retention capacity	**On sand**		**In sand**		**On sand**	**In sand**
25%	(11.70)[1]	4.25[2b]	(23.13)	15.50 [a]	0.62 [b]	2.08 [a]
50%	(9.90)	3.00 [b]	(27.07)	20.75 [a]	0.47 [b]	4.14 [a]
75%	(10.76)	3.50 [b]	(26.55)	20.00 [a]	0.57 [b]	2.72 [a]
100%	(22.71)	15.00 [a]	(14.40)	6.25 [b]	1.57 [a]	1.57 [a]

[ns]Not significant; **Significant at 1% probability.[1]Data transformed to arcsine $(x/100)^{1/2}$; [2]Non-transformed data.
Means followed by the same letters in the line do not differ from each other by the Tukey test at 5% probability.

emergence stand.

The results from the temperature x light experiment endorse the indicated in the Rules for Seed Analysis (Brasil, 2009) that recommends such alternating temperature (20 to 35°C) for the germination of bermudagrass seeds. Evers and Parsons (2010) also found the alternating temperature of 25 to 35°C to be the most appropriate for germination of common bermudagrass, generating better results of both germination percentage and germination rate. On the other hand, the Rules for Seed Analysis (Brasil, 2009) also indicates the alternating temperature of 20 to 30°C, which was not the most effective for the studied cultivars, evidencing the importance of reviewing those tests for new released cultivars.

Seeds of some species show better germination behavior when subjected to alternating temperatures, what mimics the natural fluctuations encountered in the environment; however, according to Lima et al. (1997), there are also species which seed germination is favored when submitted to constant temperatures. Moreover, El-Keblawy et al. (2011) mention that there are species which response to light during germination is linked to temperature, what is confirmed by the interaction among temperatures and light regimes found in this study.

According to Orozco-Segovia and Vasquez-Yanes (1992), seeds may be classified according to their germination behavior to light. They are called positive photoblastic when exposure to light is a condition for germination; negative photoblastic, when germination is inhibited by light; and non-photoblastic, when germination happens regardless light presence. Seeds of Princess 77 bermudagrass are non-photoblastic, as they germinated either in the light or darkness. However, Riviera seeds were considered positive photoblastic, since germination occurred only when submitted to the light regime.

Regarding Experiment 3 and the tested substrate water contents and sowing methods, Evers and Parsons (2010)

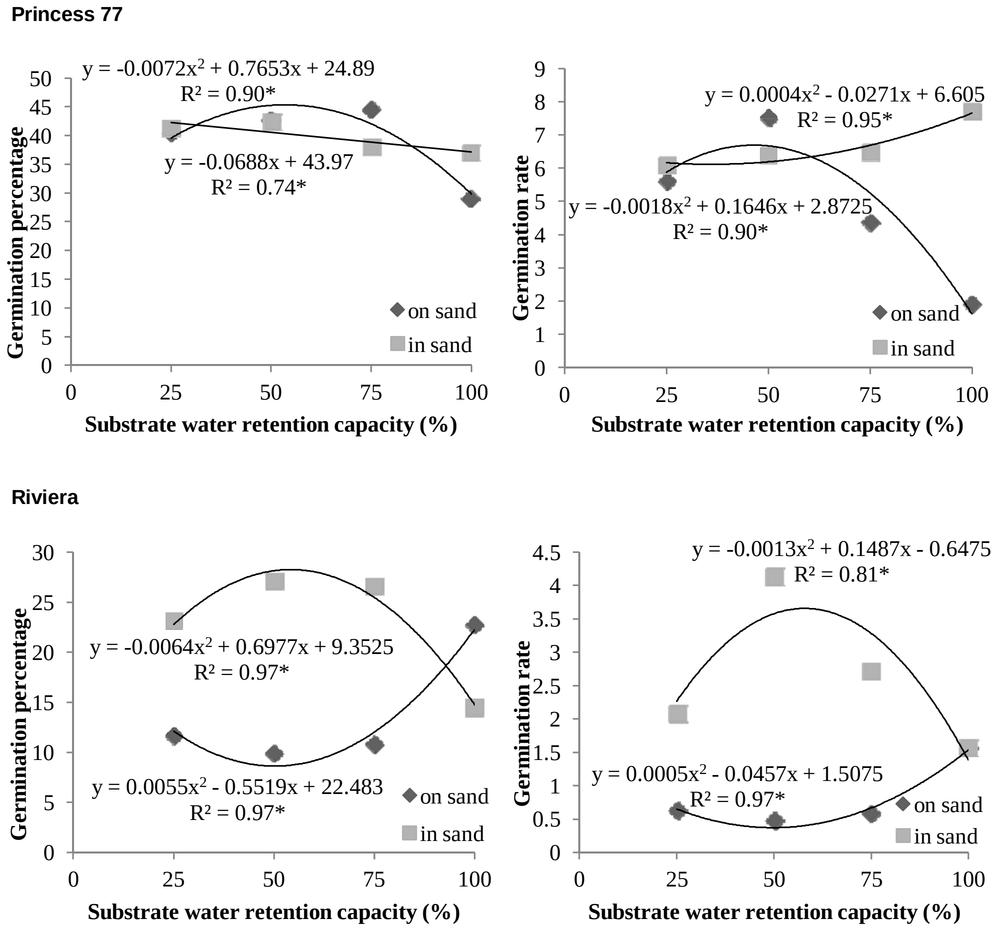

Figure 2. Germination percentage and germination rate of Princess 77 and Riviera bermudagrass (*C. dactylon*) seeds submitted to different substrate water contents (% substrate water retention capacity) and sowing methods. *Significant at 5% probability.

mention that, for best results, bermudagrass seeds should be sown on the substrate surface, so that the seed is not placed too deep to have its germination hampered. However, at least for Princess 77, seed location in relation to the substrate did not seem to interfere much. For this cultivar, the substrate water content was the limiting factor for seed germination.

According to Piana et al. (1994), the substrate water content level that most favors seed germination of many species ranges from 40 to 60% of its water retention capacity. For bermudagrass cultivars, the Rules for Seed Analysis (Brasil, 2009) indicate 50% of the substrate water retention capacity as the ideal moisture level, which falls around the most suitable percentage for both Princess 77 and Riviera seeds.

Conclusion

Seed germination of Princess 77 bermudagrass (*C. dactylon*) was more effective in the absence of NaCl and KCl; at the alternating temperature of 20 to 35 °C, either in the light or darkness; and at around 50% of the substrate water retention capacity, when sown either in sand or on sand surface.

Seed germination of Riviera bermudagrass (*C. dactylon*) was more effective in the absence of NaCl and presence of KCl; at the alternating temperature of 20 to 35°C, in the light; and at 100% of the substrate water retention capacity, when sown on sand surface.

Conflict of Interest

The authors have not declared any conflict of interest.

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