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ARTICLE

Temperature, saline stress, sowing method and water availability in the germination of bahiagrass seeds

Temperatura, estresse salino, forma de semeadura e disponibilidade de água na germinação de sementes de grama-batatais

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Abstract: Bahiagrass (*Paspalum notatum* Flüggé), native to Brazil, was once widely used in landscaping and is currently more commonly found along roadsides and in large urban areas with old plantings, such as squares, parks, public and industrial areas, among others. Brazil has been developing research into the selection of varieties of this grass, and it is important to know about seed germination in this process and also in commercial multiplication, aiming to form high-quality lawns, quickly and sustainably. Thus, the objective of this research was to evaluate factors that affect the germination of bahiagrass seeds, such as temperature, saline stress, sowing method and water availability in the substrate. Five temperature conditions were studied (room temperature, 25 °C, 30 °C, 20-30 °C, and 25-35 °C); in the salt stress study, five treatments were applied, including a control (absence) and four NaCl concentrations (25, 50, 75, and 100 mM). Two forms of sowing (on and between sand) were also studied, combined with four water contents (25%, 50%, 75%, and 100%) of the water retention capacity in sand. The germination percentage and the germination speed index (GSI) of the seeds were evaluated. The germination of *P. notatum* seeds was more effective at a temperature of 20-30 °C, in the absence of NaCl, and with a 25% water retention capacity in sand. Sowing in sand, with a water retention capacity of 100%, impairs the germination of bahiagrass seeds.
Keywords: grass, *Paspalum notatum*, Poaceae, salinity, seed technology.

Resumo
A grama-batatais (*Paspalum notatum* Flüggé), nativa do Brasil, já foi muito utilizada no paisagismo e atualmente é encontrada com maior frequência nas margens de rodovias e áreas extensas no meio urbano, de plantio antigo, como praças, parques, áreas públicas e industriais, entre outras. O Brasil vem desenvolvendo pesquisas de seleção de variedades dessa grama sendo importante o conhecimento sobre a germinação de sementes neste processo e, também, na multiplicação comercial, visando a formação de gramados de qualidade, de forma rápida e sustentável. Assim, o objetivo desta pesquisa foi avaliar fatores que interferem na germinação de sementes de grama-batatais como temperatura, estresse salino, forma de semeadura e disponibilidade de água no substrato. Foram estudadas cinco condições de temperatura (ambiente, 25 °C, 30 °C, 20-30 °C e 25-35 °C); no estudo do estresse salino, foram cinco tratamentos sendo, ausência e quatro concentrações de NaCl (25, 50, 75 e 100 mM). Estudou-se ainda, duas formas de semeadura (sobre e entre areia), combinadas com quatro teores de água (25%, 50%, 75% e 100%) da capacidade de retenção de água em areia. Avaliou-se a porcentagem de germinação e o Índice de Velocidade de Germinação (IVG) das sementes. A germinação das sementes de *P. notatum* foi mais efetiva na temperatura de 20-30 °C, na ausência de NaCl e na capacidade de retenção de água em areia de 25%, sobre areia. A semeadura em areia, com capacidade de retenção de água de 100%, prejudica a germinação de sementes de grama-batatais.
Palavras-chave: *Paspalum notatum*, gramínea, Poaceae, salinidade, tecnologia de sementes.

Introduction

Popularly known as Batatais grass and bahiagrass, *Paspalum Notatum* Flüggé, belongs to the Poaceae family and is native to Brazil, non-endemic (Valls et al., 2024). It has broad leaves, exposed rhizomes and long inflorescences, which greatly affect its appearance; as it is native, it develops very well in tropical conditions and is tolerant of low-fertility soils, has high tolerance to drought, but low tolerance to cold, shade and salinity; it can be used on ornamental lawns and functional and sports lawns (Emmons and Rossi, 2016; Villas Bôas et al., 2020).

This grass was part of Brazilian lawns for many decades and was even the official grass of stadiums in the 50s and 60s; however, in Brazil, there are very few registered producers who produce this species, with the majority coming from extractivism (Santos and Carribeiro, 2023).

Commercially available bahiagrass is a mixture of biotypes and ecotypes of the Notatum variety, resulting in heterogeneous lawns. Planting is typically done using seedlings (vegetative propagules) collected in the form of irregular sods from degraded pastures, where it appears as an invasive plant. The commercial availability of high-quality seeds for this grass in Brazil is scarce. The use of seeds, however, facilitates transportation, storage, and planting, and helps reduce the costs of lawn establishment (Souza et al., 2020). Therefore, it is necessary to deepen and update knowledge about the factors that affect the germination of this grass's seeds in order to support research and commercial lawn production using seeds.

There are several factors that affect seed germination, with one of the main ones being temperature as it acts and influences on the speed of water absorption and on the biochemical reactions that determine the entire process, consequently it affects both the speed and uniformity of germination and total germination rate (Carvalho and Nakagawa, 2012).

Abiotic stresses such as salinity also affect seed germination. Excess salts can inhibit the entry of water into the seed or cause structural damage, altering its germination response (Almeida et al., 2020), thus, the presence of salts during the germination process can reduce the percentage and the speed of germination (Silva et al., 2019), increase the average germination time (Adilu and Gebre, 2021), delay or completely prevent the process, in addition to being able to lead to the embryo death (Jacob et al., 2020; Santos et al., 2020). However, the effects will depend on the types of salts, salinity concentrations, the period of exposure to salt stress, crop management practices and species (Arif et al., 2020).

The factors that affect seed germination vary depending on the species; Thus, the Rules for Seed Analysis – RSA indicates, for several species, how the germination test should be carried out, in order to determine the maximum germination potential of a lot of seeds, which can be used to compare the quality of different lots and also estimate the

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value for sowing in the field (Brazil, 2009). *For P. notatum,* the RAS indicates that germination tests should be carried out on paper, between sand and on sand and related to the water retention capacity of sand, it explains that when an appropriate amount of water is added to the sand particles, the sand must have sufficient retention capacity to continuously meet the water needs of seeds and seedlings. In addition, the sand must allow adequate aeration to enable germination and root growth and that sufficient humidity for optimal development depends on the species tested, with general recommendation of 50% of water retention capacity for grasses, in general. Consequently, it is important to evaluate the best specific condition for *P. notatum*.

Therefore, this study aimed to evaluate the effect of temperature, salinity concentration, sowing method, and water availability on the germination of *P. notatum* seeds.

Material and Methods

The work was carried out at the Vegetable Seed Laboratory of the Department of Agricultural Production of São Paulo State University (Unesp), School of Agricultural and Veterinarian Sciences, Jaboticabal, SP. The seeds of *P. notatum* were obtained from the market. Three experiments were carried out.

1) Effect of temperature on the germination of bahiagrass seeds:

In this trial, the experimental design used was completely randomized with five treatments, corresponding to five temperatures (room temperature - average temperature of 21.5 °C, 25 °C, 30 °C, and alternating temperatures of 20-30 °C and 20-35 °C) with four replications of 100 seeds, totaling 2000 seeds. The seeds were sown on sand in "gerbox" boxes and placed in B.O.D. germinators, with a photoperiod of 8 hours of light and 16 hours of darkness (Brasil, 2009) and temperatures according to each treatment.

At room temperature, the "gerbox" boxes were kept in a laboratory environment, with temperature and relative humidity (RH) monitored daily, with the average maximum temperature of 32.8 °C, the average minimum temperature of 19 °C and the average relative humidity of 65%.

2) Effect of salinity on the germination of bahiagrass seeds:

In this trial, the experimental design used was completely randomized with five treatments, with five concentrations of sodium chloride (NaCl) – 0 (distilled water), 25, 50, 75, and 100 mM, corresponding to electrical conductivities of 0.65; 2.15; 4.31; 6.32; and 8.0 dS m^{-1}, respectively, with four repetitions of 100 seeds, totaling 2,000 seeds. Electrical conductivity was measured using a conductivity meter for the five concentrations of the NaCl solution.

The seeds were sown on sand in "gerbox" boxes and placed in a B.O.D. germinator. at an alternating temperature of 20-30 °C with a photoperiod of 8 hours of light and 16 hours of darkness (Brasil, 2009).

For experiments 1 and 2, water was replaced in the sand by weighing, maintaining 50% of the sand's water retention capacity (Brasil, 2009).

3) Effect of sowing method and availability of water in the substrate on the germination of bahiagrass seeds:

In this trial, the experimental design used was completely randomized; there were eight treatments, in a 2x4 factorial design; the first factor corresponds to two forms of sowing (on and between sand) and the second factor corresponds to four water contents in the substrate (25%, 50%, 75%, and 100%) of the sand's water retention capacity. Four replications of 100 seeds were used, totaling 3,200 seeds.

Sowing was carried out on and between sand in "gerbox" boxes and placed in a B.O.D. germinator at an alternating temperature of 20-30 °C with a photoperiod of 8 hours of light and 16 hours of darkness (Brasil, 2009).

Water replacement in the sand was carried out whenever necessary by weighing, maintaining the water retention capacity of the sand, initially calculated for each treatment.

For the three experiments, the "gerbox" boxes were placed inside low-density polyethylene bags, tied at the end with covered wire to prevent moisture loss.

Assessment and statistical analysis

The evaluations were carried out daily for the three experiments, for 28 days (Brasil, 2009), calculating the percentage of normal seedlings that presented an aerial part with a size greater than or equal to 5 mm and the Germination Speed Index (GVI), calculated according to the formula proposed by Maguire in 1962 and described in Ferreira et al. (2021).

In the temperature experiment, the data were subjected to analysis of variance and the means were compared with each other using the Tukey test at 5% probability. In the experiments on salinity and water availability in the substrate, the data were subjected to polynomial regression analysis. All analyzes were carried out using the SAS program.

Results and Discussion

Effect of temperature on the germination of bahiagrass seeds

There was a significant difference between the temperatures for the percentage of germination and Germination Speed Index of *P. notatum* seeds (Table 1). The highest percentage of germination (34.5%) happened at the alternating temperature of 20-30 °C and the seeds germinated faster in the temperature conditions of 30 °C (3.086), 20-30 °C (2.720) and 20-35°C (2.720). According to Brasil (2009), alternating temperatures of 20-30 °C and 20-35 °C and, also, 30-35 °C are recommended for this species. Therefore, the alternating temperature of 20-30 °C was the one that provided the highest percentage and speed of germination of *P. notatum* seeds. Some species require daily temperature fluctuations for proper germination. This need may be related to seed dormancy, which requires a thermal stimulus for the germination process to occur (Braccini, 2011). In this way, it is suggested that the characteristic of germinating under varying thermal amplitudes is beneficial to the specie *P. notatum*, as it may facilitate its dispersal across a greater diversity of environments.

Table 1. Germination percentage (%) and Germination Speed Index (GSI) in *Paspalum* seeds at different temperatures.

Temperatures	Germination (%)	GSI
Room temperature	**12.0 d**	**1.074 b**
25 °C	19.5 bc	1.862 b
30 °C	16.0 cd	3.086 a
20-30 °C	34. 5 a	2.720 a
20-35 °C	20.5 b	2.720 a
CV (%)	9.51	15.96

Means followed by the same letter in the column do not differ significantly from each other according to the Tukey test at a 5% probability level.

The best germination performance of grass seeds at alternating temperatures has also been verified in other research. Zanon et al. (2020) also found that seeds of the grass *Zoysia japonica* had a higher percentage of germination at temperatures of 20-30 °C and 20-35 °C and germinated more quickly at alternating temperatures of 20-35 °C, not differing from 20-30 °C. Fernandes et al. (2021) also found that the temperature alternation of 20-30 °C together with 20-25 °C were the most appropriate for the germination performance of *P. virgatum* seeds.

The seeds of numerous cultivated and wild plant species show a positive response to alternating temperatures (Carvalho and Nakagawa, 2012; Brasil, 2009), most likely because they simulate natural conditions, with lower nighttime and higher daytime temperatures; under high temperatures, the speed of water absorption and enzymatic activities become higher, causing the seeds to germinate quickly (Carvalho and Nakagawa, 2012).

Effect of salinity on the germination of bahiagrass seeds

There was a significant difference between the salinity concentrations tested for the germination percentage and Germination Speed Index of *P. notatum* seeds, with negative linear regression adjustment (Fig. 1).

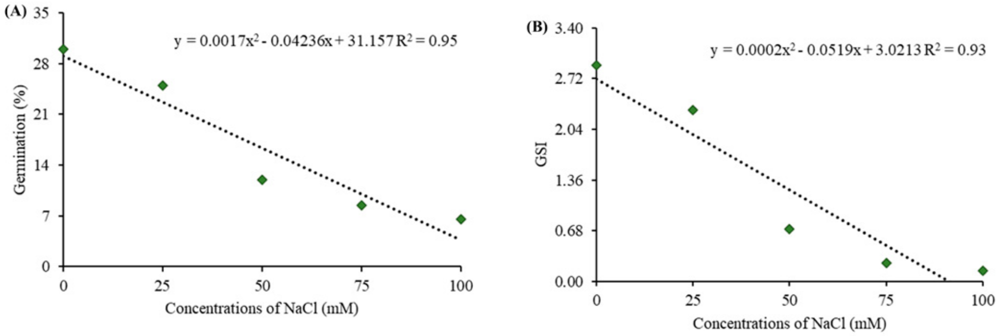

Fig. 1. Germination percentage (A) and Germination Speed Index (GSI) (B) of *Paspalum notatum* seeds in response to different salinity concentrations.

The percentage of seed germination of this grass decreased as the salinity concentration increased (Fig. 1A). The highest germination percentage (29.75%) was observed at zero NaCl concentration; in turn, at the highest saline concentrations, 75 and 100 mM, the germination achieved was relatively very low, in the order of 8.5% and 6.25%, respectively.

The germination of *P. notatum* seeds was negatively affected by salinity, therefore, in environments under saline conditions, direct sowing cannot be recommended in places that present this characteristic, such as coastal regions and areas where sustainable irrigation is carried out with reused water that have high electrical conductivity. Other grasses, however, are tolerant and germinate under these conditions, as verified by Coan et al. (2008) for the species *Cynodon dactylon* 'Mirage' and *Lolium perene*.

The species *P. notatum* was, therefore, not tolerant of salinity during the seed germination process. This grass is considered by Emmons and Rossi (2016) to have low tolerance to salinity. Also, Liu et al. (2023), in an extensive review on the tolerance of grasses to saline stress, considers this species close to salinity intolerance, on a scale that goes from halophyte (highly tolerant), tolerant, moderately tolerant, slightly tolerant and intolerant. In this way, the results obtained in the present study reinforce those found in the literature.

One of the possible reasons may be due to the increase in saline concentration having reduced the osmotic potential of the solution, resulting in a decrease in the absorption and translocation of water from the soil to the plant, affecting plant growth and metabolism, as recommended by Skider et al. (2020), thus compromising the physiological processes during the seed germination stage of this grass.

Effect of sowing method and availability of water in the substrate on the germination of bahiagrass seeds

The interaction between sowing methods and water content in the substrate was significant, both for germination percentage and for the Germination Speed Index (GSI) of *P. notatum* seeds (Fig. 2).

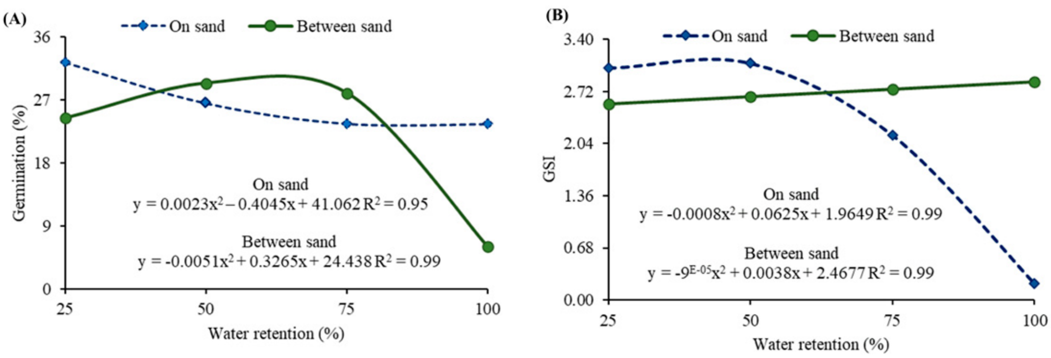

Fig. 2. Germination of seeds (A) and Germination Speed Index (GSI) (B) of *Paspalum notatum* seeds in relation to different sowing methods and water retention capacities of the substrate.

It was observed that when sown in sand, the germination percentage of *P. notatum* seeds decreased as the water retention capacity of the substrate increased (Fig. 2A). Conversely, when the seeds were sown in a sand mix, there was an initial increase in germination followed by a subsequent reduction as the substrate's water retention capacity increased.

The highest percentage of germination (32.39%) of seeds of this grass was observed on sand with a substrate water content of 25%. When the seeds were placed on sand, the other water contents of 50%, 75%, and 100% showed a germination percentage of 26.59%; 23.66%; and 23.61%, respectively.

When the seeds were sown in a sand mix, the highest germination percentage (29.41%) occurred at a substrate water content of 50%, followed by a water content of 75%, with a germination rate of 28.01%. The substrate water content of 100% showed the lowest seed germination percentage, with a value of only 6.08% (Fig. 2A).

The GSI of the seeds decreased as the water retention capacity of the substrate increased when sown in sand, with the most pronounced drop occurring when the seeds were placed at 100% water retention capacity (Fig. 2A). Excess water in the seeds under these conditions must have prevented the penetration of oxygen, making breathing difficult, reducing the activities of the metabolic process, in addition to influencing other factors, which reduce the viability of seeds (Silva et al., 2020).

Overall, the germination percentage of *P. notatum* seeds in this study was very low, not exceeding 35% in any of the tests conducted. The RAS (Brazil, 2009) recommends scarifying the seeds with H_2SO_4 and then sowing in a substrate moistened with KNO_3, however, it was stated on the packaging purchased on the market that the seeds had previously been treated with KNO_3. Zanon et al. (2020) observed significant differences in the percentage and speed of germination of emerald grass seeds, however, studying scarification processes in *P. notatum* seeds, Silva et al. (2021) observed that the treatments that provided the highest percentage of germination, on average 61.2%, were those where thermal, mechanical and chemical scarifications were carried out in the two shortest immersion times, however, the percentage of emergence in the field, in all treatments that presented the highest averages were 33%.

Conclusions

The germination of *P. notatum* seeds is favored by alternating temperatures with a range of 20-30 °C. The presence of NaCl is not beneficial for the germination of *P. notatum* seeds. A water retention capacity of 25% in the substrate (sand) is favorable for the germination of *P. notatum* seeds. Sowing in sand, with a water retention capacity of 100%, impairs the germination of *P. notatum* seeds.

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Author contribution

AMBS: conceptualization, data curation, software, investigation, visualization, writing – original draft. **MVF:** data curation, methodology, software, validation writing – review & editing. **ACB:** data curation, formal analysis, investigation. **MMS:** data curation, formal analysis, investigation. **GLQ:** formal analysis, methodology, software, validation. **TSC:** conceptualization, investigation, methodology, visualization, supervision. **KFLP:** conceptualization, resources, methodology, project administration, validation, visualization, writing – review & editing.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability Statement

Data will be made available on request.

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