












Seed germination and antioxidant enzyme activity in seedlings of diploid and tetraploid bahiagrass under water restriction

Gabriel Streck Bortolin^{1*}  Alfredo José Salinas-Arcienega²  Ytcelia Carolina Galviz-Fajardo³ 
Luciano do Amarante³  Carlos Eduardo da Silva Pedrosa²  João Carlos Pinto Oliveira⁴ 
Mauricio Marini Köpp⁴  Andrea Bicca Noguez Martins²  Lilian Vanussa Madruga de Tunes² 

¹Programa de Pós-graduação em Ciência & Tecnologia de Sementes, Departamento de Fitotecnia, Faculdade de Agronomia Eliseu Maciel, Universidade Federal de Pelotas (UFPEL), 96050-500, Capão do Leão, RS, Brasil. E-mail: gabrielbortolin91@gmail.com. *Corresponding author.

²Departamento de Fitotecnia, Faculdade de Agronomia Eliseu Maciel, Universidade Federal de Pelotas (UFPEL), Capão do Leão, RS, Brasil.

³Programa de Pós-graduação em Fisiologia Vegetal, Departamento de Botânica, Instituto de Biologia, Universidade Federal de Pelotas (UFPEL), Capão do Leão, RS, Brasil.

⁴Empresa Brasileira de Pesquisa Agropecuária, Centro de Pesquisa de Pecuária dos Campos Sul Brasileiros (Embrapa Pecuária Sul), Bagé, RS, Brasil.

ABSTRACT: *The objective of the following research was to investigate the physiological responses of two bahiagrass genotypes (*Paspalum notatum* Flüggé) submitted to water restriction, induced by polyethylene glycol (PEG₆₀₀₀), during seed germination. Seeds of cv. Pensacola (diploid) and Bagual ecotype (tetraploid) were placed on paper moistened with osmotic solutions at potentials of 0.0; -0.1; -0.2 and -0.3 MPa. Composed of two subsamples of 100 seeds and four experimental units per treatment, the germination test was conducted in a germination chamber under alternating temperatures of 30 °C (8 hours) and 20 °C (16 hours), with presence of light during the higher temperature. Final germination percentage and germination speed index of the seeds, as well as early growth, activity and expression of antioxidant enzymes of seedlings were evaluated. The water restriction induced by PEG₆₀₀₀ reduced the percentage of germination in Pensacola (from 74% to 59%) and Bagual (34% to 10%). For the latter, the evaluation of seedlings showed higher dry mass in relation to cv. Pensacola (1.7 times higher in root and 1.6 times in shoot). In response to moderate water restriction, the genotypes showed increases in root growth (1.7 times higher in -0.1 compared to 0.0 MPa), both in expression and enzymatic activity (superoxide dismutase and catalase).*

Key words: *Paspalum notatum* Flüggé, ecotype, polyethylene glycol, drought stress, oxidative stress.

Germinação de sementes e atividade de enzimas antioxidantes em plântulas de grama forquilha diplóide e tetraploide sob restrição hídrica

RESUMO: *O presente estudo objetivou avaliar as respostas fisiológicas de dois genótipos de grama forquilha (*Paspalum notatum* Flüggé) sob restrição hídrica induzida por polietileno glicol (PEG₆₀₀₀) durante a germinação de sementes. Sementes da cv. Pensacola (diplóide) e do ecótipo Bagual (tetraploide) foram colocadas em papel umedecido com soluções osmóticas nos potenciais de 0,0; -0,1; -0,2 e -0,3 MPa. Composto de duas subamostras de 100 sementes e quatro unidades experimentais por tratamento, o teste de germinação foi conduzido em câmara de germinação sob temperatura alternada de 30-20 °C, com presença de luz durante a maior temperatura. Foram avaliados a porcentagem final e o índice de velocidade de germinação das sementes, assim como o crescimento inicial, a atividade e a expressão de enzimas antioxidantes das plântulas. A restrição hídrica induzida por PEG₆₀₀₀ reduziu o percentual de germinação em Pensacola (de 74 para 59%) e Bagual (34 para 10%). Este último, na avaliação de plântulas, apresentou massa seca superior em relação à cv. Pensacola (1.7 vezes superior em raiz e 1.6 vezes superior em parte aérea). Em resposta à restrição hídrica moderada, os genótipos apresentaram aumentos tanto do crescimento radicular (1.7 vezes superior em -0.1 em comparação com 0.0 MPa), quanto da expressão e da atividade enzimática (superóxido dismutase e catalase).*

Palavras-chave: *Paspalum notatum* Flüggé, ecótipo, polietilenoglicol, estresse por seca, estresse oxidativo.

INTRODUCTION

Bahiagrass - *Paspalum notatum* Flüggé - is, admittedly, one of the ten species with the highest relative cover for the South Brazilian grasslands

(ANDRADE et al., 2019). Rhizomatous plant with prostrate growth habit, bahiagrass is a C4 grass with high persistence, even under conditions of low fertility, flooding, and particularly, severe continuous stocking (GATES et al., 2004). Furthermore,

when introduced in association with favorable environmental conditions and good management practices, its competitive capacity and productivity is significantly improved (PRINCE et al., 2018; MACHADO et al., 2019). In addition, this species can be an alternative for water-constrained regions (SEVER MUTLU et al., 2011). With attributes that confer its recommendation for recovery of degraded areas (STUMPF et al., 2018), also has potential for a greater contribution to long-term C sequestration (BRESCIANO et al., 2019).

In South Brazilian grasslands, different biotypes of bahiagrass are found, depending on the climate or type of soils where they vegetate. Among the promising ecotypes in this region, the Bagual tetraploid ecotype ($2n=4x=40$), originating in the Planalto Médio region of the Rio Grande do Sul State, Brazil, is highlighted for its high potential for forage production. Under experimental conditions still in the first year of evaluations, this ecotype gave a 38% higher dry mass production in comparison to the traditional cv. Pensacola ($2n=2x=20$), a diploid plant (STEINER et al., 2017). In a study conducted for two years, WEILER et al. (2018) reported that Bagual had an accumulated production of dry mass 4.8 times higher than Pensacola cv. In a similar assessment, GRAMINHO et al. (2019) observed higher dry mass production by this ecotype (Bagual) more precisely 2.717, 3.213 and 1.322 kg ha⁻¹ in comparison with cv. Pensacola in three consecutive years of evaluation, respectively.

Despite the high capacity of *P. notatum* to cover the soil and to produce fodder under limiting water conditions, the effect of this condition on seed germination and seedling emergence, a decisive moment in the establishment of a pasture, still needs to be verified. According to MARCOS-FILHO (2015), water deficiency is considered the limiting factor for the germination of non dormant seeds, affecting the percentage, speed and uniformity of germination.

In addition to the effects on seed germination, water limitations soon after this stage can sharply reduce seedling performance. Evidence suggested that water restriction causes oxidative stress in several plant species, in which reactive oxygen species (ROS), such as superoxide radical ($O_2^{\bullet-}$), hydroxyl radical (OH), hydrogen peroxide (H_2O_2) and oxygen singlet (1O_2) are produced, promoting serious changes to normal plant metabolism (KAR, 2011). In order to reduce the effects of oxidative stress, the plants present an efficient antioxidant mechanism, composed in part by enzymatic constituents. Superoxide dismutase (SOD) is recognized for catalyzing the dismutation of the superoxide radical

in H_2O_2 and O_2 . The enzymes catalase (CAT) and ascorbate peroxidase (APX) act on H_2O_2 oxidation, transforming it into $H_2O + O_2$ (CAT) and $H_2O + DHE$ (APX). This set of antioxidants provides an important primary defense against free radicals (GILL & TUTEJA, 2010; DAS & ROYCHOUDHURY, 2014).

The present study aimed to verify the effects of induced water restriction by polyethylene glycol (PEG₆₀₀₀), on seed germination and seedling performance of cv. Pensacola and Bagual ecotype, as well as on the expression and activity of enzymes that act in the antioxidative process of these genotypes.

MATERIALS AND METHODS

The experiment was carried out in the laboratories of Análise de Sementes, Biosementes and Bioquímica Vegetal of the Universidade Federal de Pelotas (UFPEL), Pelotas, RS-Brazil. Seeds of bahiagrass (*Paspalum notatum* Flüggé) cv. Pensacola (viability of 75%) and Bagual ecotype (viability of 65%) were obtained from harvests (held in the first quarter of 2016) in the experimental areas of Embrapa Pecuária Sul and Universidade Federal do Rio Grande do Sul (UFRGS), respectively, and stored in a dry cold room ($12\text{ °C} \pm 1\text{ °C}$). The viability of the seeds was known by the application of the tetrazolium test (BRASIL, 2009) prior to the installation of the tests, in the first quarter of 2018.

Also in the first quarter of 2018, seeds of the two genotypes were placed to germinate in blotting paper moistened with solutions containing the osmotic agent polyethylene glycol (PEG₆₀₀₀). To simulate the water restriction, the osmotic potentials of 0.0; -0.1; -0.2 and -0.3 MPa were evaluated, totaling 8 treatments. The use of these potentials was based on preliminary tests (from 0.0 to -0.8 MPa). The germination test was conducted with 800 seeds per treatment distributed in four replicates (two subsamples of 100 seeds in each replicate), as specified by the Rules for Seed Testing (BRASIL, 2009). After the different treatments were established, the seeds were placed in germination chamber type BOD (Biological Oxygen Demand) with alternating temperatures of 30 °C (8 hours) and 20 °C (16 hours) $\pm 1\text{ °C}$ and 50% relative humidity, with presence of light only during the upper temperature period (BRASIL, 2009).

Final germination percentage (FG) was determined at 28 days from the beginning of the test, by counting the number of normal seedlings (BRASIL, 2009). Seeds with a root extension equal to or greater than 2 mm were considered germinated

for the knowledge of cumulative germination and the germination speed index (GSI), variables determined by the accounting for the germinated seeds percentage every two days. For GSI, the formula proposed by MAGUIRE (1962) was used. At the end of the germination test (28 days), shoot and root lengths were determined in 10 seedlings of each sample, evaluated at random for each treatment. The shoot and root system of these same seedlings were then separated and transferred to a dry oven at $65\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$ until obtaining a constant mass, in order to determine the dry mass of shoot and roots. Root to shoot ratio was determined as the ratio between root and shoot length and, root and shoot dry mass, separately.

To verify the activity of antioxidant enzymes, four samples of 200 mg of complete seedlings were collected at the end of the germination test, macerated with 50% insoluble polyvinyl polypyrrolidone (PVPP), and homogenized in 1.5 mL of the extraction buffer composed of 100 mM potassium phosphate buffer (pH 7.0), 0.1 mM EDTA and 10 mM ascorbic acid. The homogenate was centrifuged at 10,000 g for 20 minutes at $4\text{ }^{\circ}\text{C}$ and the supernatant collected.

The evaluation of the activity of superoxide dismutase (SOD; EC 1.15.1.1) was based on the ability of the enzyme to inhibit the photoreduction of nitro blue tetrazolium (NBT) (GIANNOPOLITIS & RIES, 1977), in a reaction medium containing 100 mM potassium phosphate buffer (pH 7.8), 14 mM methionine, 0.1 μM EDTA, 75 μM NBT and 2 μM riboflavin. The readings were performed at 560 nm. In the activity calculations, one unit of SOD was considered to correspond to the amount of enzyme capable of 50% inhibition of NBT photoreduction under the assay conditions.

The activity of the catalase enzyme (CAT; EC 1.11.1.6) was determined based on the consumption of hydrogen peroxide (H_2O_2). The reaction medium was composed of 100 mM potassium phosphate buffer, 12.5 mM H_2O_2 and enzymatic extract. The decrease in absorbance over a period of 2 min was recorded at a wavelength of 240 nm at $25\text{ }^{\circ}\text{C}$ (AEBI, 1983).

The activity of ascorbate peroxidase (APX; EC 1.11.1.11) was performed according to NAKANO & ASADA (1981), by assessing the ascorbate oxidation rate, recording the absorbance drop in the wavelength of 290 nm in one reaction medium incubated at $28\text{ }^{\circ}\text{C}$ and composed of 100 mM potassium phosphate buffer, pH 7.0, 0.05 mM ascorbic acid, 0.1 mM H_2O_2 and enzyme extract.

The expression of SOD and CAT isoenzymes was determined using the polyacrylamide

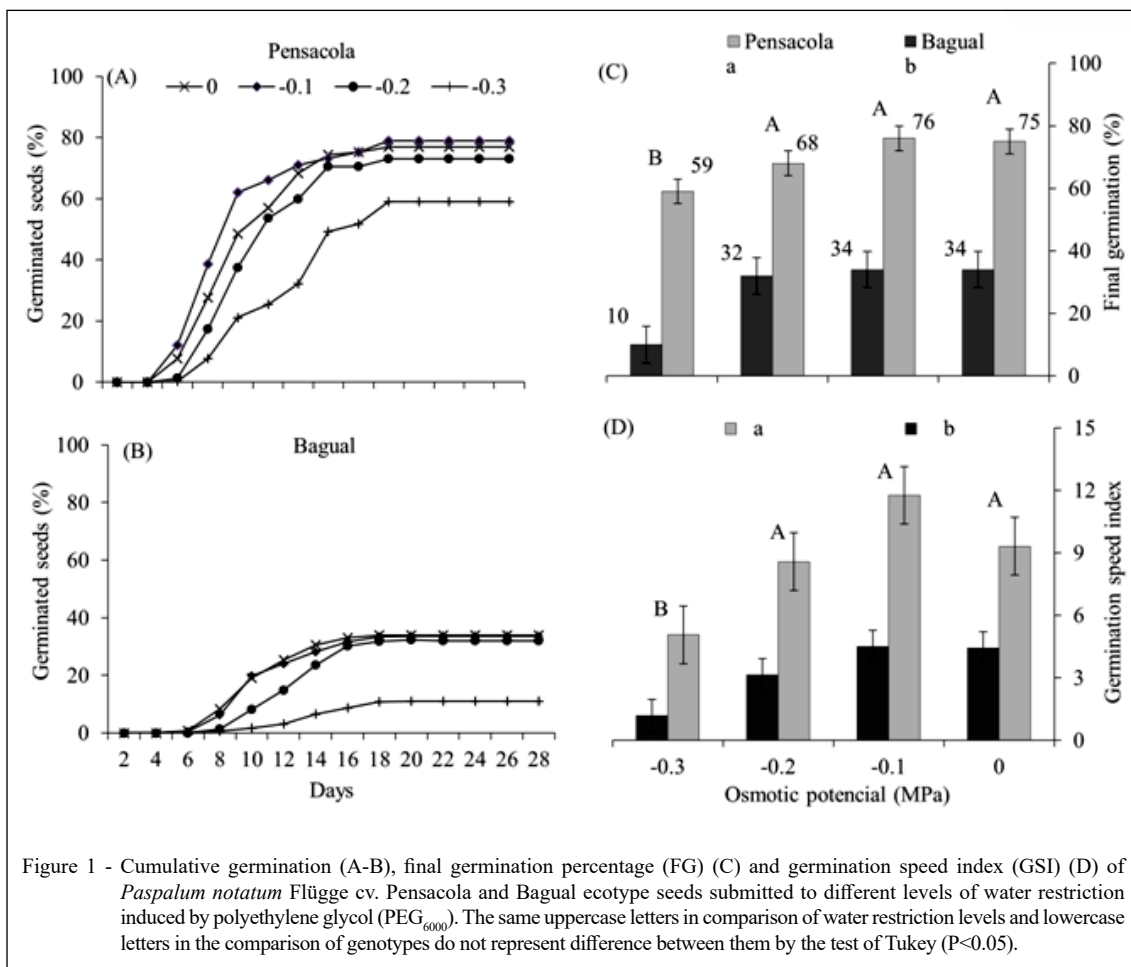
gel vertical electrophoresis system. A total of 200 mg containing complete seedlings were macerated, and placed in a microcentrifuge tube, with the addition of extractive solution composed of gel buffer (200 mM lithium borate, pH 8.3 + 200 mM Tris Citrate at pH 8.3 + 0.15% 2-mercaptoethanol) in the ratio 1:2 (w/v). Electrophoresis was conducted on 7% polyacrylamide gels, with the application of 20 μL of each sample. The coloring systems described by ALFENAS (2006) were used. Interpretation of the results was based on the visual analysis of the electrophoresis gels, taking into account the intensity of expression of each of the bands.

The design was completely randomized and the data were subjected to analysis of variance (ANOVA). Residues of the evaluated variables were distributed normally (SHAPIRO & WILK, 1965). The means of the variables were compared by the Tukey test at 5% probability. Principal component analysis (PCA) was performed to examine relationships between variables and observations using PAST software version 3.2 (HAMMER et al., 2018).

RESULTS

Water restriction affected final germination percentage (FG), germination speed index (GSI) and cumulative germination (Figure 1 A-D). There was no interaction among water restriction and genotypes. However, the individual evaluation showed that the osmotic solution of -0.3 MPa promoted a significant reduction in FG (34%) and GSI (3.1) when compared to the 0.0 MPa (54% and 6.87), -0.1 MPa (55% and 8.13) and -0.2 MPa (50% and 5.8), as well as control treatment. Besides the significant effect generated by the water restriction factor, cv. Pensacola presented higher FG and GSI (Figure 1).

There was progress in the performance of seedlings under moderate water restriction (Figure 2), with the presence of interaction between the factors studied for shoot length and mass of the seedlings (Figure 2 A and C). Water restriction imposed a greater reduction in the length of the Bagual ecotype seedlings. Under severe water restriction (-0.3 MPa), Pensacola seedlings did not differ from the control treatment (0.0 MPa) and showed a higher shoot length (2.96 cm) in relation to Bagual (2.3 cm). The shoot dry mass presented responses, at the water restriction levels, similar to the one observed for the length (Figure 2 C). However, the seedlings of the Bagual ecotype showed higher dry mass at all levels of water restriction, more precisely 5.55 mg compared to the 3.3 mg obtained in Pensacola. There was no interaction between the

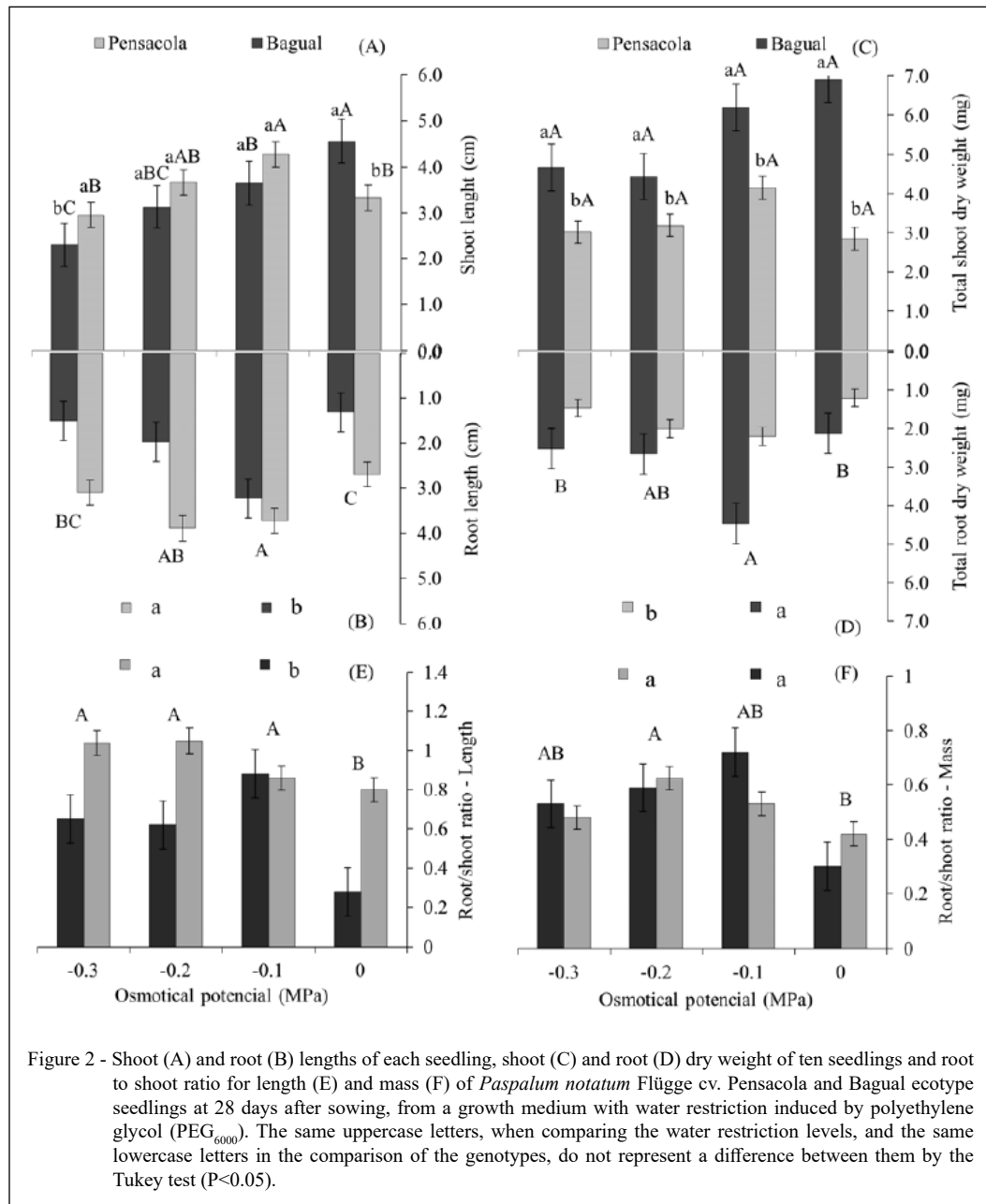


factors studied for the variables root length and mass (Figure 2). Moderate water restriction determined increases in root length (between 40 and 29% at -0.1 and -0.2 MPa) compared to the control (Figure 2 B). In severe water restriction there was a reduction of the main root length in comparison with the higher potentials (2.3 and 3.4 cm in -0.3 and -0.1 MPa, respectively). However, this value did not differ from that obtained for the control treatment.

The cv. Pensacola presented a root growth 40% higher (3.35 cm) than the average value obtained in seedlings of the Bagual ecotype (2 cm). However, seedlings of the Bagual ecotype showed superior root mass (5.5 mg), 41% higher than that obtained for cv. Pensacola (3.3 mg) (Figure 2 D). Water restriction levels affected the root mass. At the -0.1 MPa, a 50% increase of this variable was verified in relation to the control treatment (3.3 and 1.6 mg in -0.1 and 0.0 MPa). Seedling exposure at the -0.3 MPa determined the reduction of root mass in

comparison with the levels -0.1 and -0.2 MPa. However, the mass of the roots under low water availability was not inferior to that verified in the 0.0 MPa.

Absence of interaction is also verified in the root to shoot ratio (Figure 2). There was a significant increase in this ratio with the advance of the water restriction. Significantly higher values of 0.87, 0.83 and 0.84 were verified at the -0.1, -0.2 and -0.3 MPa levels, respectively, in comparison with a value of 0.54 in the control treatment (0.0 MPa) for length (Figure 2 E). In this evaluation, Pensacola presented a 0.33 higher root to shoot ratio than Bagual. For dry mass ratio (Figure 2 F), there was no significance of the genotype factor for this variable. However, the water restriction factor determined significant increases of 0.26, 0.24 and 0.14 in the root to shoot ratio for the -0.1, -0.2 and -0.3 MPa levels in comparison with the control treatment (0.0 MPa).



The expression of the antioxidant isoenzymes superoxide dismutase (SOD) and catalase (CAT) in cv. Pensacola and the Bagual ecotype is shown in figure 3. The highest activity bands of the SOD enzyme were observed at moderate water restriction levels (-0.1 and -0.2 MPa) for the two genotypes (Figure 3 A). Under severe water restriction (-0.3 MPa), both genotypes presented a lower intensity of SOD activity bands, highlighting

the Bagual ecotype. Greater intensity in catalase expression (CAT) occurred at -0.2 and -0.3 MPa for Pensacola and Bagual (Figure 3 B). Bands with lower intensity were observed in Bagual at levels of 0.0 and -0.1 MPa. Bands with higher intensity for the same two levels were observed in Pensacola, where bands with reduced intensity variation were observed in the evaluation of the four proposed water restriction levels.

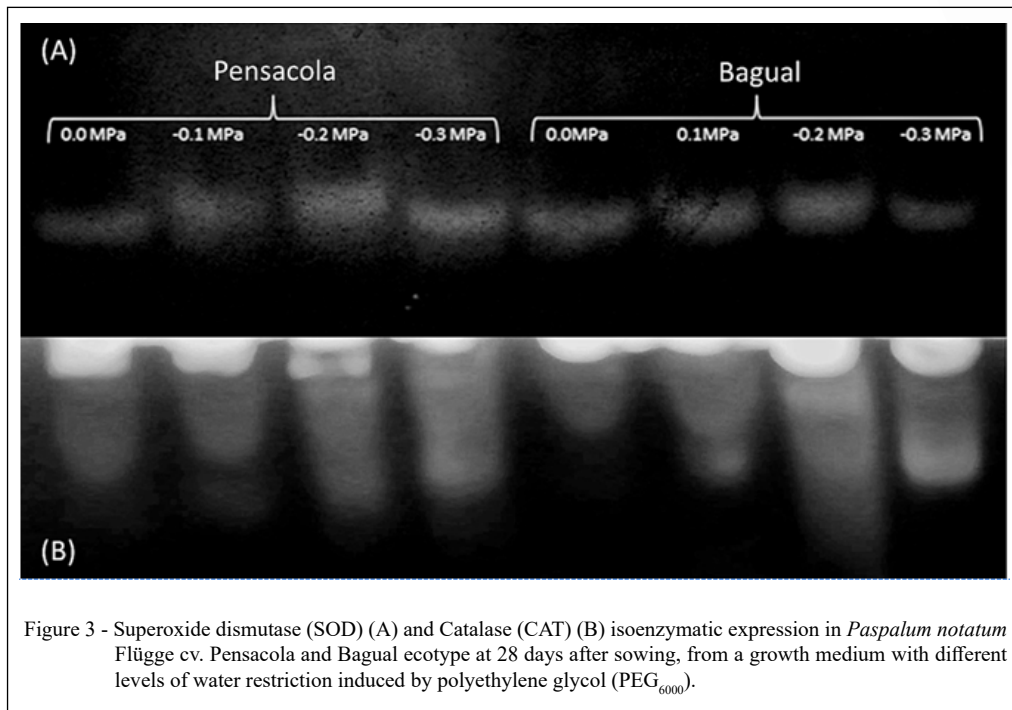


Figure 3 - Superoxide dismutase (SOD) (A) and Catalase (CAT) (B) isoenzymatic expression in *Paspalum notatum* Flügge cv. Pensacola and Bagual ecotype at 28 days after sowing, from a growth medium with different levels of water restriction induced by polyethylene glycol (PEG₆₀₀₀).

Antioxidant activity (Figure 4) indicated in SOD the occurrence of interaction among the factors evaluated (Figure 4 A). Under the osmotic potential of -0.1 MPa, SOD activity in Pensacola showed an increase of 1.69 U mg⁻¹ protein compared to the control (0.0 MPa). There was a 53% higher activity in relation to Bagual at this same level (1.75 U mg⁻¹ protein). This ecotype showed greater activity in seedlings submitted to the -0.2 MPa osmotic treatment, differing significantly in relation to the higher level of water restriction (-0.3 MPa). Under strong water restriction (-0.3 MPa), SOD activity in Pensacola and Bagual did not differ significantly from the activity obtained at each access in the control treatment (0.0 MPa).

Only the water restriction factor acted effectively on CAT (Figure 4 B), promoting a significant increase in the activity of the enzyme. In comparison with the activity obtained in the control (0.0 MPa), increases of 0.00025, 0.00011.5 and 0.00041.5 μmol H₂O₂ min⁻¹ mg⁻¹ protein were verified in the -0.1, -0.2 and -0.3 MPa levels, respectively. In contrast to that observed in SOD and CAT, no interaction or even individual significance was observed in the factors of water restriction and genotype levels for the activity of the enzyme ascorbate peroxidase (APX) (Figure 4 C).

Principal component analysis (PCA) (Figure 5) showed eigenvalues greater than 1, representing 66.28% of the variation in the data. Principal component 1 showed a strong influence of the variables associated with germination and growth. This component accounted for 37.66% of the variation. This allowed for the separation of the biplot at high levels for the variables G, GSI, root length and root to shoot ratio to the right, which are strongly associated with cv. Pensacola.

Shoot and root dry mass was strongly associated with the Bagual ecotype, distributed in left of the y axis. Principal component 2 is largely dependent on enzyme activity (mainly SOD and CAT), shoot length, and root to shoot ratio for dry mass. It can be verified that the treatments that presented higher values for this last variable remained distributed in the lower part of the graph, with emphasis on the level of restriction 0.0 MPa in combination with the two genotypes studied. In the upper part of the graph, the groups where SOD and CAT showed greater activity can be observed.

DISCUSSION

The water restriction levels were effective in verifying differential responses for both cv.

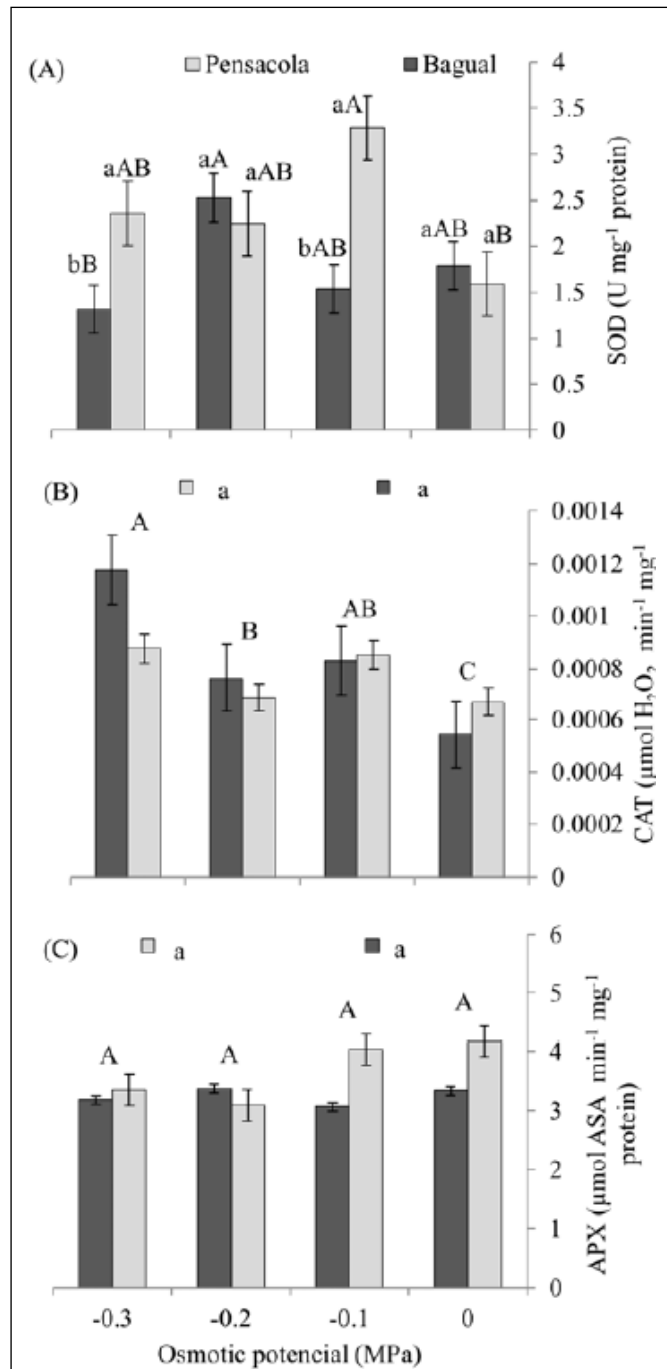


Figure 4 - Activity of antioxidant enzymes Superoxide dismutase (SOD) (A), Catalase (CAT) (B) and Ascorbate peroxidase (APX) (C) in *Paspalum notatum* Flügge cv. Pensacola and Bagual ecotype at 28 days after sowing, from a growth medium with different levels of water restriction induced by polyethylene glycol (PEG₆₀₀₀). The same capital letters, in the comparison of the water restriction levels, and lower case letters, in the comparison of the genotypes, do not represent a difference between them by the Tukey test (P < 0.05).

Pensacola and for the Bagual ecotype, for attributes related to germination. Seed exposure at low water availability (-0.3 MPa) promoted not only the reduction of the percentage of germination, but also the speed of this process (Figure 1).

Under similar restrictions (-0.2 and -0.4), SPRINGER (2005) also observed a reduction in the germination of the native grasses little bluestem (*Schizachyrium scoparium*) and sand bluestem (*Andropogon hallii*). Despite the negative effects, this same author verified the presence of germination in -1.0 MPa, which characterized a lower water requirement for the process in question, in comparison with the genotypes of *P. notatum* used in this study.

This has also been verified in the germination of bermudagrass (*Cynodon dactylon*) and tall fescue (*Schedonorus arundinaceus*) forage seeds; however, under a water restriction level much higher than that studied (-7.0 MPa for bermudagrass and -10.0 MPa for tall fescue) (TUCKER et al., 2017). Reduction of germination under low water availability can be attributed to the low osmotic pressure provided by the solution. When evaluating the exposure of sorghum seeds to reduced osmotic potentials, KADER & JUTZI (2002) observed that seed imbibition occurred more slowly due to osmotic pressure, delaying the activation of seed metabolism, as well as the germination process.

Despite the similarity in viability for the studied genotypes, it is worth noting in figure 1 that the lower percentage of germination presented by Bagual is probably related to a common phenomenon in *P. notatum*: seed dormancy. MAEDA et al. (1997) reported that seeds of this species, under ideal storage conditions, are able to remain dormant for up to three years after harvesting. Despite the same storage period of the seeds, more FG (Figure 1 C) was observed in Pensacola than in Bagual. For the former, it is traditionally known that a seed lot with high dormancy may have its germination increased after one year of storage (WEST & MAROUSKY, 1989), which is still unknown for Bagual. However, it must be emphasized that physical scarification of the seeds is a promising method to overcome this phenomenon, as verified by BERTONCELLI (2018) in seeds of the tetraploid genotype INIA Sepé.

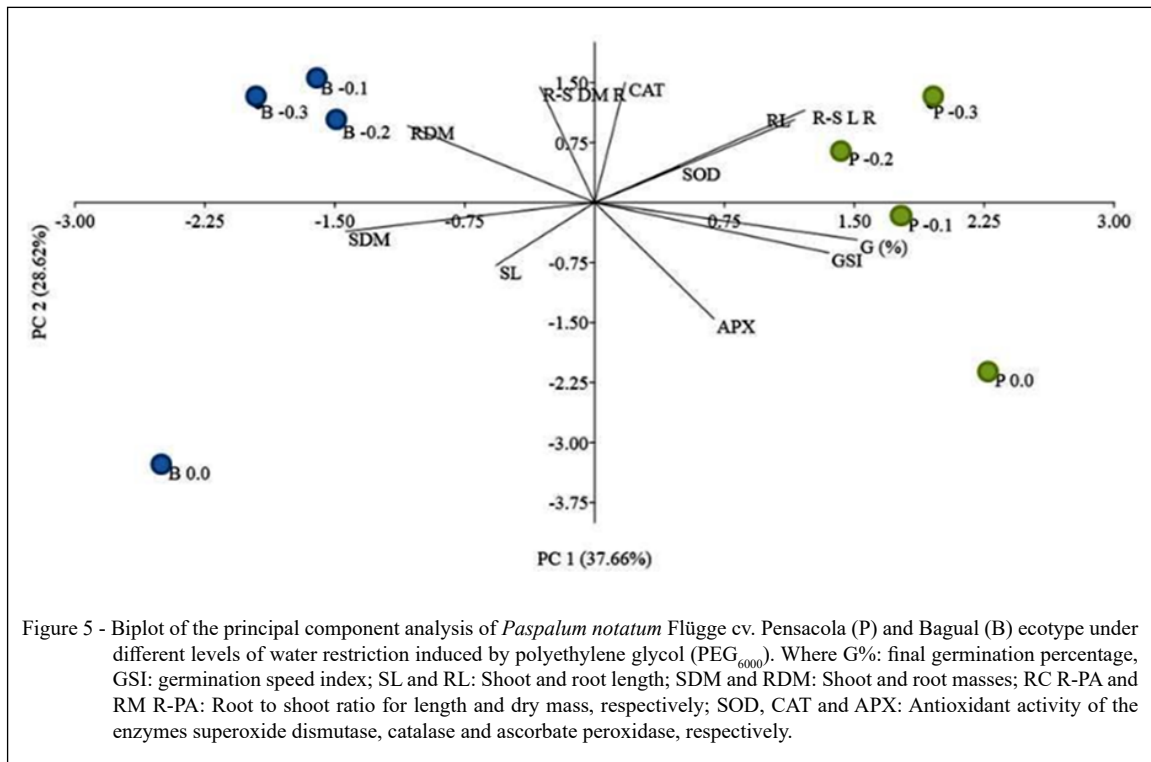
Seedling performance, as well as germination, was affected by the water restriction levels tested (Figure 2). The increase in root dry matter, as verified in Pensacola and Bagual under moderate water restriction, was also determined by XU et al. (2015) in rice seedlings. The authors reported that the higher root to shoot ratio is closely associated with the higher

proportion of dry matter and soluble sugar in roots, and this occurs via an increase in leaf sucrose-phosphate synthase and root invertase activity. The increase of the root to shoot ratio with the water restriction is commonly verified, which may be related to the fact that shoot growth is generally more sensitive to stress than root growth (MAHAJAN & TUTEJA, 2005). Root performance, superior in Bagual (Figure 2 B and D), is an important attribute when conducting work with grasses exposed to water limitation (KARCHER et al., 2008; RICHARDSON et al., 2008).

The strong relationship between seedling mass and Bagual ecotype (Figures 2 and 5), regardless of the level of water restriction, is commonly observed in the comparison between plants that differ in ploidy level. When evaluating the performance of two orchardgrass genotypes (*Dactylis glomerata*), BRETAGNOLLE et al. (1995) reported that tetraploid seedlings presented superior dry mass in comparison with the diploid genotype. Studies also stated that the level of ploidy influences the plant responses to the stress condition. When evaluating the effect of saline stress on rice cultivars, JIANG et al. (2013) reported that duplication of the genome of three cultivars allowed obtaining seedlings with higher mass in the unfavorable condition. These results obtained in seedling performance have affinity with the values verified by GRAMINHO et al. (2019) and WEILER et al. (2018), in which tetraploid genotypes of *P. notatum* presented superior performance (total dry mass) to cv. Pensacola in a field experiment.

Under conditions of stress, the enzymatic components were fundamental in the maintenance of the redox homeostasis of the plant. In detailing the antioxidant attributes, DAS & ROYCHOUDHURY (2014) highlighted the role of SOD in catalyzing the removal of $O_2^{\bullet-}$ dismuting it in O_2 and H_2O_2 , as well as the consequent dismutation of H_2O_2 in H_2O and O_2 by CAT and APX. In the present study, changes in the expression and activity of antioxidant enzymes in cv. Pensacola and the Bagual ecotype suggested that oxidative damage is an important component of water restriction in *P. notatum*. The strong association between the antioxidant activity and the treatments that provided greater performance of seedlings exposed to the water restriction justifies the importance of this apparatus (Figure 5).

Increases of SOD and CAT expression and activity were verified in the reduction of water availability for the two genotypes evaluated in this study (Figures 3 and 4). However, the presence of interaction in the SOD activity coupled with the visible dissimilarity in SOD and CAT expression demonstrated a difference



in the antioxidant metabolism between the genotypes when exposed to water availability levels. This variation of activity was also verified by KURUP et al. (2017) in the evaluation of the effect of salinity on the antioxidant activity of bermudagrass and *Paspalum vagenitum* cultivars, which reported dissimilarity among cultivars evaluated within the same species.

The lower expression and also activity of SOD in Bagual seedlings under high water restriction were verified at the same time as their performance was reduced, a condition possibly linked to an oxidative action. This possible response was confirmed by XU et al. (2013) in the evaluation of the effect of salinity on the antioxidant performance of kentucky bluegrass (*Poa pratensis*) and tall fescue plants. The authors reported that the higher content of malondialdehyde (a product of lipid peroxidation) in kentucky bluegrass implied more severe oxidative damage and that the antioxidant defense mechanisms of this species were probably less effective than those of tall fescue. They further reported that increase in malondialdehyde content appeared to be correlated with a decrease in CAT and APX activity under salt stress.

Addition of oxidative damage may be a possible reason for the reduction of seedling performance at accentuated water restriction levels,

since its increase did not promote higher APX activity (Figure 4 C). A similar result was observed in studies with forage grasses under water restriction, following the example of the study by BIAN & JIANG (2009), which did not verify increased APX activity in kentucky bluegrass roots under water stress. However, the authors also verified that, under stress condition, there was an increase in the activity of this enzyme in leaves of the studied species.

CONCLUSION

The water restriction imposed by PEG₆₀₀₀ (up to -0.3 MPa) affects the germination and seedling performance of *Paspalum notatum* Flügge, cv. Pensacola and the Bagual ecotype. Under the restriction levels evaluated, seedlings of the Bagual ecotype (tetraploid) present superior mass to the seedlings of the cv. Pensacola (diploid), an important characteristic that allowed the recommendation of this tetraploid genotype for introduction in environments with periods of marked water limitation. Changes in the expression and activity of antioxidant enzymes in cv. Pensacola and Bagual ecotype lead to the conclusion that oxidative damage is an important component of water restriction in *P. notatum*.

ACKNOWLEDGMENTS

This study was financed by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

DECLARATION OF CONFLICT OF INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

GSB and AJS-A contribute to conception and design of the experiment. GSB, AJS-A and YCG-F worked in acquisition of data, or analysis and interpretation of data. LA and LVMT allowed the use of their laboratories, with the supply of reagents and contributed in the interpretation of the biochemical analyzes. JCPO and MMK provided the biological material.

CESP and ABNM worked on interpretation of data and review of the manuscript.

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