SYSTEM DEFENSE OF FORAGE SPECIES SUBMITTED TO LOW TEMPERATURES

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ABSTRACT

This work aims to evaluate the enzymatic system defense of forages exposed to low temperatures. The forage plants alfalfa, sorghum, black oat, marandu grass, pearl millet, mombasa grass, and Bermudagrass were subjected to temperatures of 0.2; -0.9; -1.8; -2.7; -4.1; -4.6 and -6.2 oC for 1 h in a growth chamber in a completely randomized design with six replicates. From the crude leaf extracts were evaluated the enzymes ascorbate peroxidase (APX), catalase (CAT), and superoxide dismutase (SOD) activities. Regression analyses compared the treatments. The biochemical responses of forage species to cold stress are different. The enzymes APX and SOD would play an important role in the response to oxidative stress in the forage species black oat and mombasa. In marandu and sorghum, SOD was the main cell protector. Pearl millet had a positive stimulus in CAT, Bermudagrass and alfafa increase the protein content and respectively SOD and APX activity.

Keywords: *Medicago sativa, Sorghum bicolor, Avena strigosa, Urochloa brizantha, Pennisetum americanum, Megathyrsus maximus, Cynodon spp.*

INTRODUCTION

Climate is a key driver for all ecological and economic systems (STEINER et al., 2018), and for forage species, it is a determinant for plant adaptation and growth throughout the year. Climatic conditions in the cold season make it extremely difficult for animals to perform, as forage plants stop their growth mainly due to low temperatures and a lower incidence of solar radiation. Added to these difficult situations, frosts in autumn-winter may destroy the available forage and consequently cause weight loss in animals (CANTO et al., 2010).

Cold-tolerant species have several mechanisms that protect cells from membrane degradation and dehydration. Among the defense systems, the presence of sugars and proteins that have cryoprotective action stands out. They are induced by cold and lower the freezing point of the cell solution, preventing the formation of ice crystals and the solidification of membrane lipids (TAIZ & ZEIGER, 2017).

Together, there may be an increase in the activity of antioxidant enzymes, such as catalase (CAT), peroxidases (POX), and superoxide dismutase (SOD), which have the function of eliminating or neutralizing reactive oxygen species (ROS) accumulated during stress (CAVERZAN et al., 2012; GUY, 1990; PERL-TREVES & PERL, 2001). These species mainly include O_2^-, OH^-, and H_2O_2, which affect cellular metabolism through the oxidation of lipid membranes, proteins, and nucleic acids, in addition to inhibiting physiological processes such as photosynthesis and respiration (SCANDALIOS, 2005).

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Thus, the antioxidant system is quite complex, and the antioxidant capacity is dependent on the severity of the stress, as well as the species and its stage of development (HASANUZZAMAN & FUJITA, 2022). These enzymes are present in practically all subcellular compartments. Usually, an organelle has more than one enzyme to scavenge a single ROS (SCANDALIOS, 2005).

Each enzyme will act differently, SOD has been proposed to be important in plant stress tolerance and provides the first line of defense against the toxic effects of elevated levels of ROS. The SODs remove O_2^- by catalyzing its dismutation, one O_2^- being reduced to H_2O_2 and another oxidized to O_2. It removes O_2^- and hence decreases the risk of OH^- formation via the metal-catalyzed Haber-Weiss type reaction. The upregulation of SODs is implicated in combating oxidative stress caused by biotic and abiotic stress and has a critical role in the survival of plants under environmental stresses (GILL & TUTEJA, 2010).

The H_2O_2 present in cells, arising from the dismutation of SOD, is constantly used as a substrate by the enzyme catalase, which can degrade H_2O_2 into H_2O and O_2 quickly; therefore, it is very important in the antioxidant system (GARG & MANCHANDA, 2009).

Ascorbate peroxidase (APX) plays the most essential role in scavenging ROS and protecting cells. APX has a higher affinity for H_2O_2 than CAT and peroxidase (POD), and it may have a more crucial role in the management of ROS during stress. Enhanced expression of APX in plants has been demonstrated during different stress conditions (GILL & TUTEJA, 2010). APX is a key enzyme that is the main hydrogen peroxide detoxification system in plant chloroplasts and uses ascorbate acid as a specific electron donor to reduce H_2O_2 to water (CAVERZAN et al., 2012).

Several studies have demonstrated changes in the activities of plant antioxidant enzymes in response to low-temperature stress. Caverzan et al. (2012) verified the relevance of studying ROS scavenging enzymes to further understand the biological process dealing with oxidative stress responses in plants.

The acquisition of new knowledge through research on oxidative stress, abiotic stress, and biotic stress tolerance in plants will aid us in applying stress-responsive determinants and engineering plants with enhanced stress tolerance. Numerous studies have established a strong correlation between the enhancement of antioxidant defense and stress tolerance. However, in the case of forage species, information regarding enzymatic activity under stress conditions is limited. Therefore, this study aimed to evaluate the enzymatic defense system of forages exposed to low temperatures.

MATERIAL AND METHODS

The experiment was conducted at Instituto de Desenvolvimento Rural do Paraná – IAPAR-EMATER (IDR-Paraná) in Londrina, Paraná, Brazil, in a completely randomized experimental design, with four replicates, with the forage species alfalfa (*Medicago sativa*), black oat (*Avena strigosa*), marandu grass (*Urochloa brizantha*), Mombasa grass (*Megathyrsus maximus*), millet (*Pennisetum americanum*), sorghum (*Sorghum bicolor*) and Bermudagrass 'Tifton 85' (*Cynodon spp*).

The Bermudagrass 'Tifton 85' (*Cynodon spp*) was propagated from branches with approximately 20 cm. In contrast, the others were propagated by seeds in 1000 mL pots that contained a 2:1 mixture of soil and manure, as well as 1 kg 4-30-10 (N-P_2O_5-K_2O) fertilizer per m^3.

The plants were grown in a greenhouse for two months, and then four pots of each species were placed inside a growth chamber (S. S. Scientific, Londrina-PR) for 24 hours, followed by exposition to low-temperature stress for one hour and return to ambient temperature. The low-temperature treatments were 0.2, -0.9, -1.8, -2.7, -4.1, -4.6, or -6.2 °C.

After each treatment, the plants were removed from the chamber and immediately it was collected leaves samples of approximately 2 g from each pot, which were packed in paper bags, frozen in liquid nitrogen and stored in an ultra-freezer at -80 °C until analysis.

Subsequently, 0.250 g of plant material was macerated in 5 mL of 50 mM potassium phosphate buffer (pH 7.0) and 4% PVP (polyvinylpolypyrrolidone), previously cooled, and centrifuged for 10 min at 785.4 rad sec^{-1} at 4 °C. The supernatant was transferred to 2 mL Eppendorf's microtubes and kept in a freezer at –14 °C until analysis in an Evolution 300 UV-VIS spectrophotometer to read the reactions.

The crude extracts were subjected to quantification of total proteins, performed using the method of Bradford (1976), which is based on the color change in Coomassie Brilliant Blue G-250 reagent when bound to the protein. For this purpose, the calibration curve of the reagent was used, with bovine serum albumin (BSA, 0-15 µg μL^{-1}) as a standard. The determination of the total protein content was carried out in triplicate, and the amount of extract varied depending on the species so that the value read on the spectrophotometer remained within the previously established standard curve. Thus, for the alfalfa, black oat, and Bermudagrass species, 30 µL was used; for sorghum 90 µL, and marandu grass, pearl millet, and Mombasa grass 150 µL of leaf extract. The total protein concentration was calculated by comparing the sample readings with those obtained from the standard curve and expressed as milligrams (mg) of fresh matter $protein^{-1}$ (MF).

The activity of the enzyme ascorbate peroxidase (APX) was based on the Peixoto et al. (1999) methodology with some modifications for the species. The reaction solution consisted of 50 mM potassium phosphate buffer (pH 7.0), 1 mM H_2O_2, 0.5 mM ascorbate, and 200 µL of the plant crude extract. Furthermore, the readings were performed in a spectrophotometer at 290 nm, after the addition of H_2O_2 in the buffer containing ascorbate and the enzymatic extract, every 15 s for 2 min of the reaction. The activity of the enzyme was calculated using a molar extinction coefficient of 2.8 mM cm^{-1}. These values were expressed in units of activity per minute per milligram of fresh matter (UA min^{-1}. g FM^{-1}).

The activity of catalase (CAT) was determined according to the methodology proposed by Peixoto et al. (1999) with modifications. The assay began with the addition of 200 µL of the enzyme extract homogenized with 2.8 mL of 50 mM potassium phosphate buffer (pH 7.0+ 0.1 mM EDTA), containing 15 mM oxygen peroxide (0.5 M). Specific CAT activity was determined by following the decrease in absorbance at 240 nm in Kinect mode with eight cycles of 30 s, using an extinction coefficient of H_2O_2 of 36 M^{-1} cm^{-1}. The enzyme activity was expressed in mmol H_2O_2 min^{-1} mg $protein^{-1}$.

The superoxide dismutase (SOD) was determined by monitoring the inhibition of photochemical reduction of nitroblue tetrazolium (NBT) according to the method of Giannopolitis and Ries (1977) and modified by Del Longo et al. (1993). In test tubes covered with aluminum foil, 2.8 ml of the reaction solution, composed of 50 mM sodium phosphate buffer (pH 7.8 + 0.1 mM EDTA), 13 mM methionine, 75 µM NBT, and 2 µM

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riboflavin, were added with 200 µL of enzyme extract or extraction buffer (for blanks). The tubes were uncovered (except the blank for the dark) and transferred to a chamber illuminated by fluorescent lamps ref. Commercial Osran Duluxstar 15W/865, 110-130V and 246 mA. After 5 min of illumination, the reaction was completed by turning off the lamps, and the samples were read in a spectrophotometer at 560 nm. The values were expressed in units of activity per minute per milligram of protein ($UA.min^{-1}.mg\ protein^{-1}$), with one unit of activity corresponding to 50% of the inhibition of NBT reduction.

Analysis of variance was used to assess the effects of the cold-stress treatments, and when significant differences were detected, regression analysis was carried out (first and second degrees) ($P \leq 0.05$) using the SISVAR program, for each species separately.

RESULTS

The low-temperatures tested caused different responses in the species studied (Table 1), for the protein content and activity of the enzymes catalase (CAT), ascorbate peroxidase (APX), and superoxide dismutase (SOD). This was already expected, since different plant species may vary widely in regulating antioxidant pathways to resist cold stress (LI et al., 2023).

In Alfalfa, there was no statistically significant difference for SOD (Table 1). The regression analysis was adjusted to the quadratic model for the other evaluations (Figure 1), however for APX the activity decreased until -1.8 °C and then increased again until the temperature of -4.6 °C, with values close to the lowest temperature. There was an increase in protein content and a decrease in CAT activity with decreasing temperature, with highly significant r^2 values for these factors, indicating that there were no outliers (GRAÇA MARTINS, 2019).

Table 1. Mean square values (MS) from analysis of variance and estimation of coefficients of variation (CV) for the variables: Protein content (mg protein. g of FM^{-1}) and activity of catalase - CAT (mmol H_2O_2. $min^{-1}.mg\ protein^{-1}$), ascorbate peroxidase - APX ($UA.min^{-1}.g\ FM^{-1}$) and superoxide dismutase - SOD ($UA.min^{-1}.mg\ protein^{-1}$) from plants of forage species subjected to low-temperature stress (T).

Forage species	Alfalfa	Black oat	Marandu grass	Mombasa grass	Pearl millet	Sorghum	Bermudagrass
Total protein							
MS - T	7.277*	0.228^{ns}	0.104*	0.277*	0.054^{ns}	0.261*	1.652*
Mean	4.106	3.477	1.330	1.052	1.362	1.288	1.678
CV(%)	15.95	24.80	12.69	21.39	30.42	23.16	31.25
APX							
MS - T	0.072*	0.100*	0.375*	0.696*	0.040*	0.091*	0.056*
Mean	0.594	0.599	0.690	0.966	0.386	0.401	0.395
CV(%)	25.56	29.40	26.05	34.29	23.63	33.90	28.84
CAT							
MS - T	0.0008*	0.0002^{ns}	0.0002*	0.0001^{ns}	0.0005*	0.0003*	0.0007*
Mean	0.0257	0.0611	0.0296	0.0208	0.0178	0.0174	0.0223
CV(%)	49.36	24.91	29.45	50.26	29.91	50.19	51.03
SOD							
MS - T	9.77^{ns}	163.07^{ns}	3474.1*	26650.8*	1070.5^{ns}	3663.6*	1919.7^{ns}
Mean	12.964	30.228	62.961	98.944	48.766	69.275	59.666
CV(%)	47.13	40.55	34.94	39.84	44.01	46.32	48.52

* significant at 5 % of probability by F test; ns: no significant; Degrees of freedom of temperature: 6; CV= Coefficient of variation.

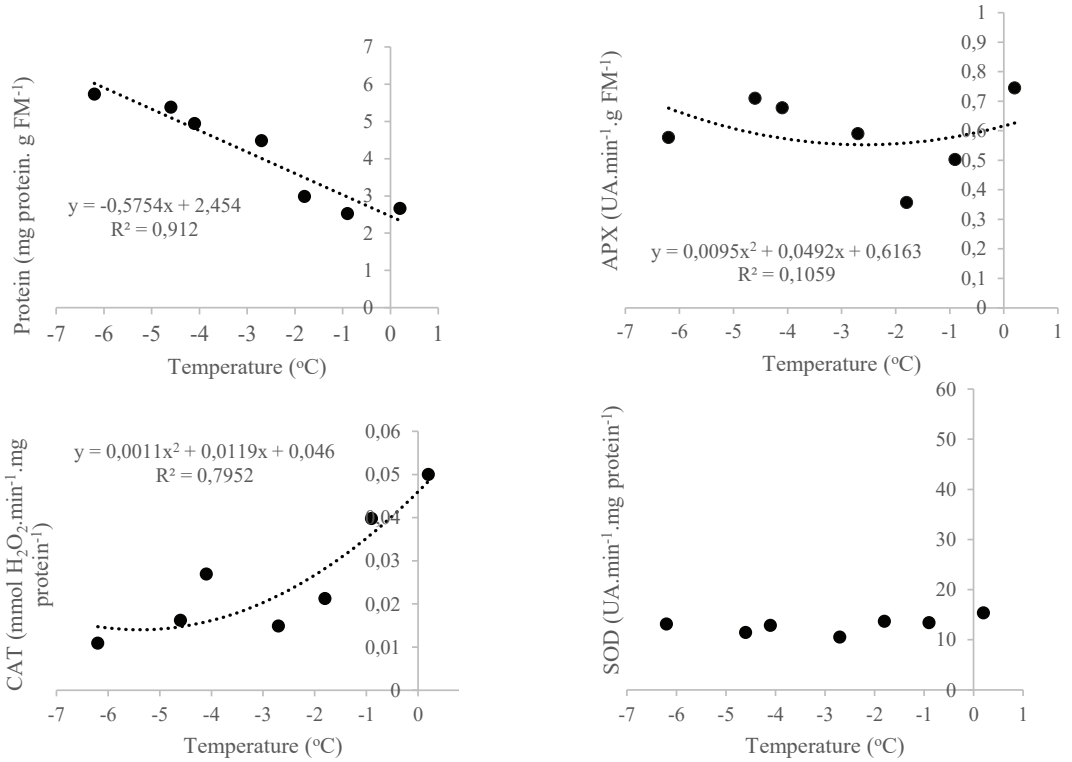

Figure 1.
Total protein (mg protein. g FM^{-1}) and enzyme activity of catalase - CAT (mmol H_2O_2.min^{-1}. mg $protein^{-1}$), ascorbate peroxidase - APX (UA.min^{-1}. g FM^{-1}) and superoxide dismutase - SOD (UA.min^{-1}. mg $protein^{-1}$) of Alfalfa plants submitted to cold stress.

Black oats only showed significant statistical differences for the enzymatic activity of APX (Table 1), with a quadratic model adjustment, decreasing to -1.8 ºC and increasing to -4.1 ºC, with a value close to 0.2 ºC, when it returned decreasing (Figure 2).

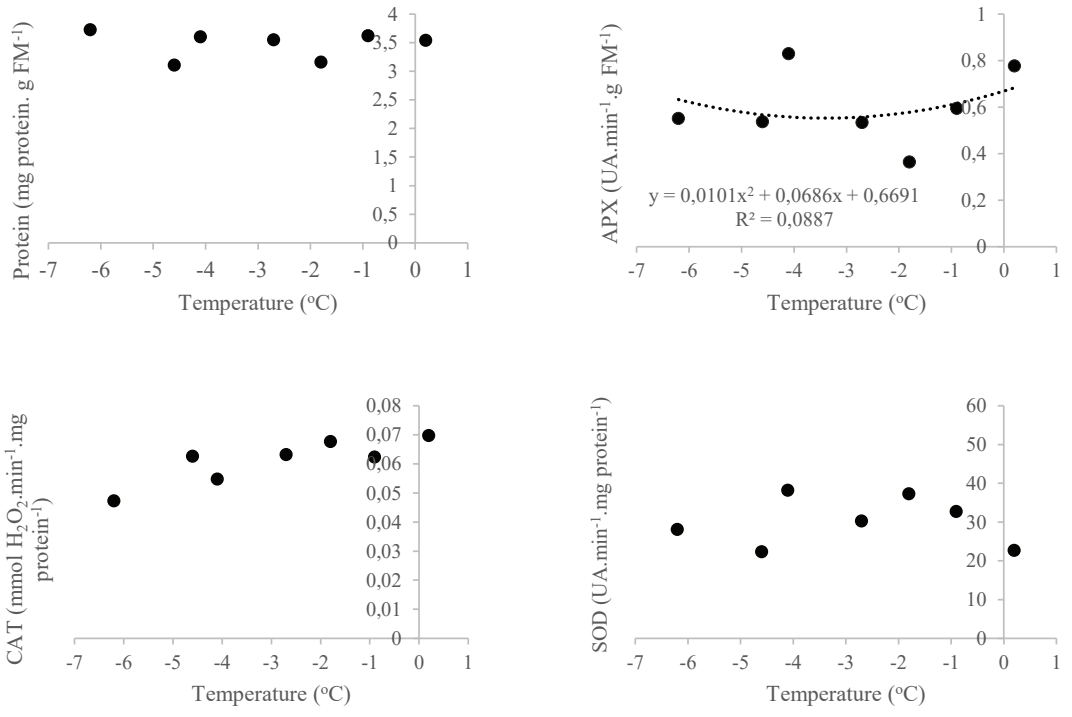

Figure 2.
Total protein (mg protein. g FM^{-1}) and enzyme activity of catalase - CAT (mmol H_2O_2.min^{-1}. mg $protein^{-1}$), ascorbate peroxidase - APX (UA.min^{-1}. g FM^{-1}) and superoxide dismutase - SOD (UA.min^{-1}. mg $protein^{-1}$) of Black oat plants submitted to cold stress.

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In marandu grass, there was a statistically significant difference between temperatures for all evaluated parameters (Table 1). The data on soluble protein content and SOD activity were adjusted to the linear regression model, with a tendency for values to decrease with decreasing temperature. However, for SOD, marandu plants had a significant increase in activity at a temperature of -2.7 °C, being reduced at a temperature of -4.1 °C (Figure 3). The activity of the CAT and APX enzymes fit the quadratic model, with CAT activity being greater at -4.1 °C than at -1.8 °C, but without differing from the others. As for APX, there was a reduction in its activity with the decrease in temperature, with a highly significant r^2 value for this factor.

In Mombasa grass there was no statistically significant difference for CAT (Table 1). The regression analysis was adjusted to the quadratic model for the other evaluations (Figure 4), with tendency of increase in the protein content with the decrease in temperature, while the activity of APX and SOD enzymes were stimulated under stress conditions up to a temperature of -1.8°C, and then reduced with decreasing temperature.

In pearl millet, a significant difference can only be observed for CAT and APX (Table 1), and for both, there was an adjustment to the quadratic model in the regression, with higher CAT activity at two points, at temperatures -2.7 and - 4.1°C, followed by a rapid decline. APX showed a reduction in activity with decreasing temperature, starting from -0.9 °C, with a highly significant r^2 value for this factor (Figure 5).

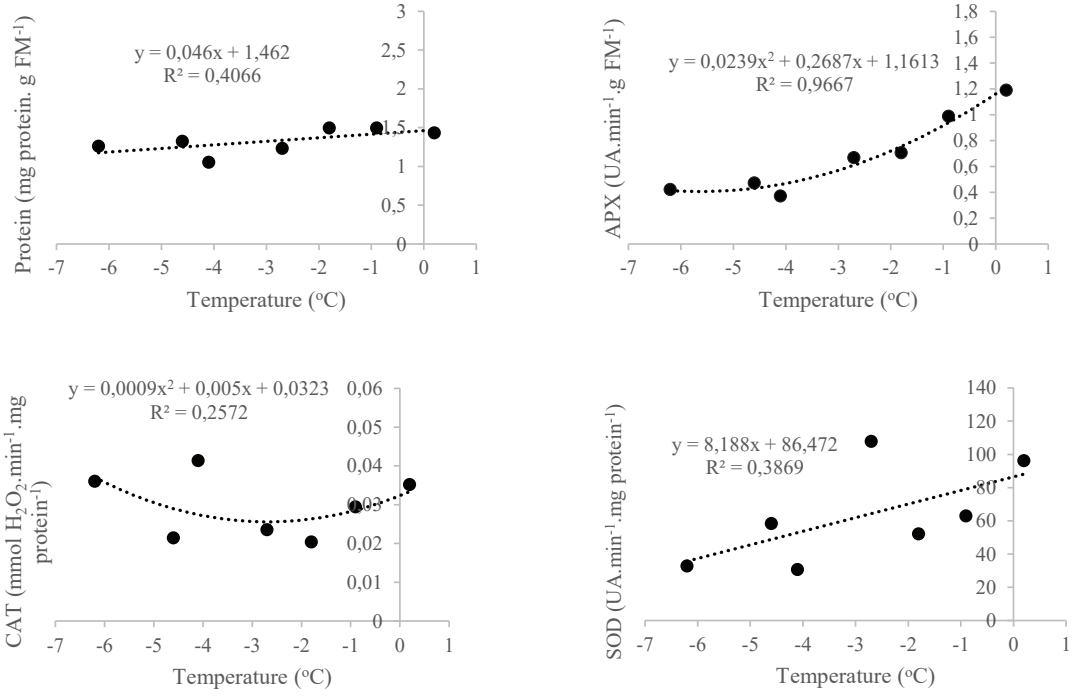

Figure 3. Total protein (mg protein. g FM^{-1}) and enzyme activity of catalase - CAT (mmol H_2O_2.min^{-1}. mg $protein^{-1}$), ascorbate peroxidase - APX (UA.min^{-1}. g FM^{-1}), and superoxide dismutase - SOD (UA.min^{-1}. mg $protein^{-1}$) of Marandu grass plants submitted to cold stress.

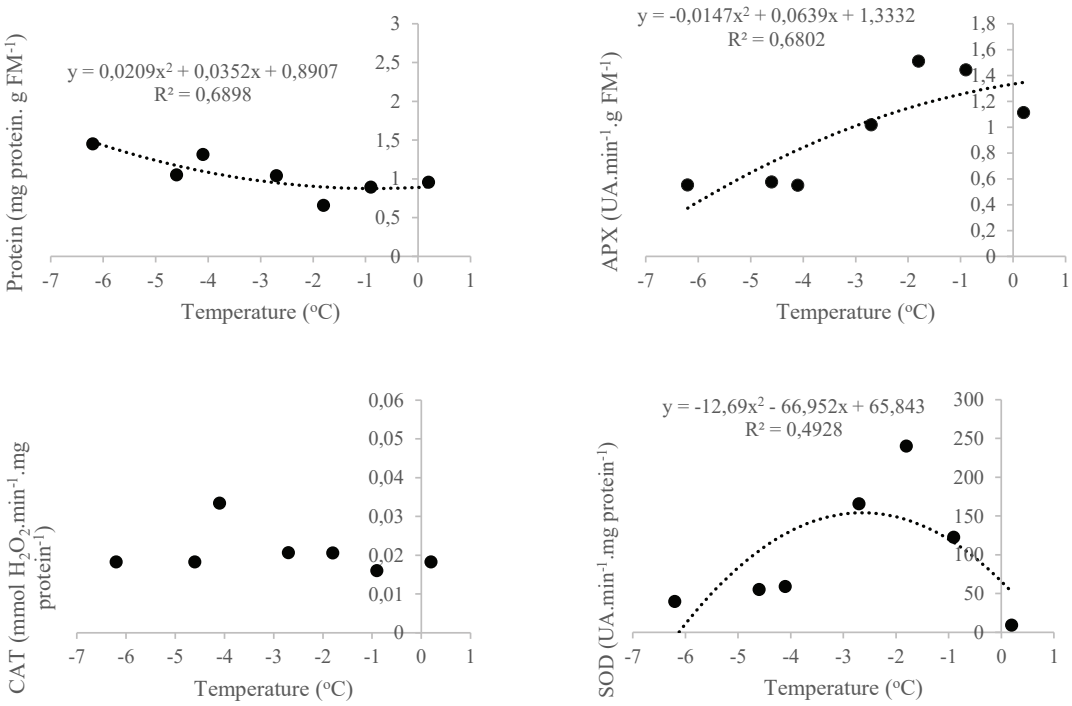

Figure 4.
Total protein (mg protein. g FM^{-1}) and enzyme activity of catalase - CAT (mmol H_2O_2.min^{-1}. mg $protein^{-1}$), ascorbate peroxidase - APX ($UA.min^{-1}$.g FM^{-1}), and superoxide dismutase - SOD ($UA.min^{-1}$.mg $protein^{-1}$) of Mombasa grass plants submitted to cold stress.

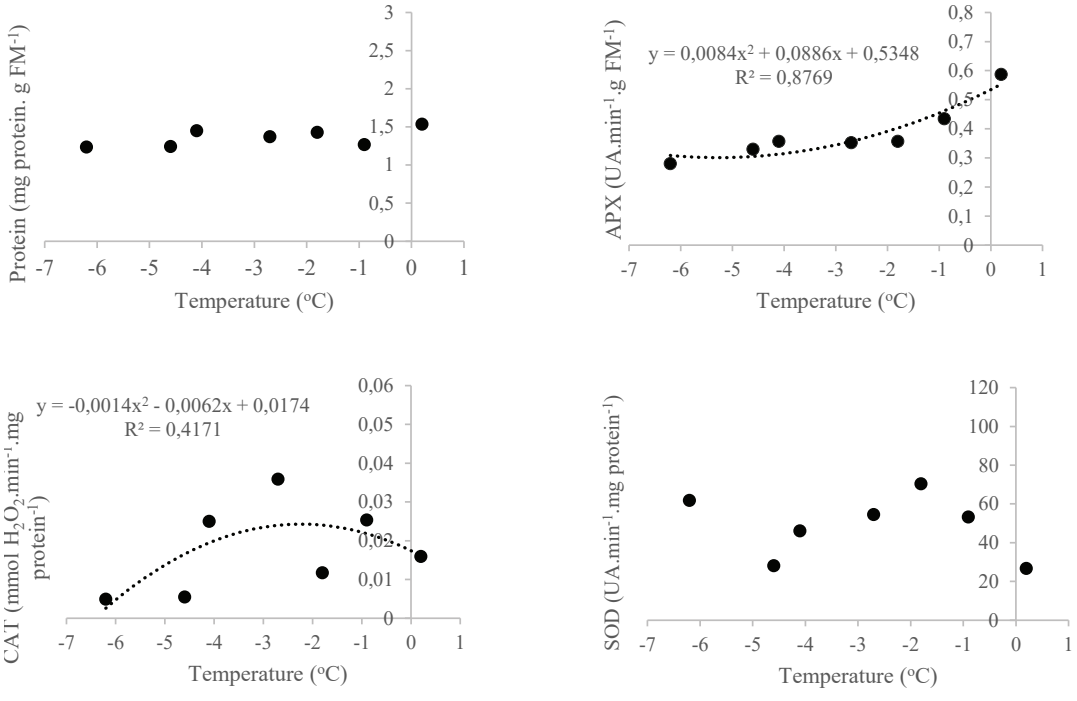

Figure 5. Total protein (mg protein. g FM^{-1}) and enzyme activity of catalase - CAT (mmol H_2O_2.min^{-1}. mg $protein^{-1}$), ascorbate peroxidase - APX ($UA.min^{-1}$. g FM^{-1}), and superoxide dismutase - SOD ($UA.min^{-1}$. mg $protein^{-1}$) of Pearl millet plants submitted to cold stress.

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In sorghum, there was a statistically significant difference between temperatures for all parameters evaluated (Table 1). The protein content and activity of the enzymes APX and SOD were adjusted to the quadratic model, while CAT was to the linear model by regression analysis, with a highly significant r^2 value for APX (Figure 6).

In terms of protein content, there was a higher value at temperatures of -2.7 °C, and at other temperatures values close to the curve. The CAT and APX enzymes had a decrease in their activity at low temperatures. In SOD there was an increase in activity up to a temperature of -1.8 °C, and after this temperature, there was a decrease.

In Bermuda grass, there was no statistically significant difference for SOD (Table 1), and the regression analysis adjusted to the quadratic model for the other evaluations (Figure 7), with the protein content tending to increase with decreasing temperature, with the highest value at -6.2 °C. The activities of the CAT and APX enzymes tended to decrease in activity with the reduction in temperature.

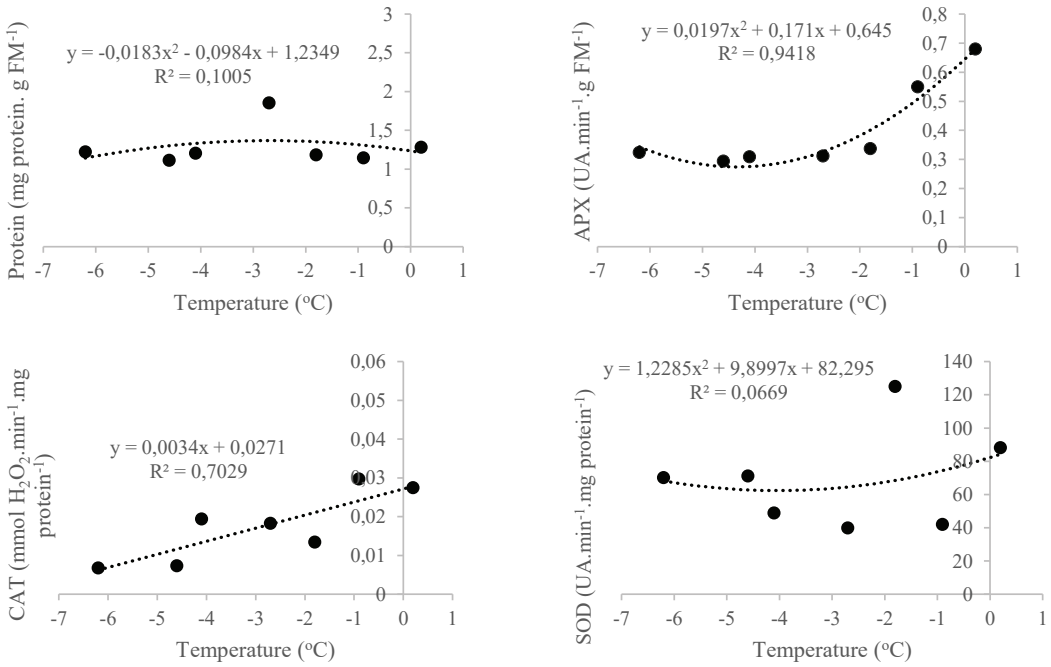

Figure 6. Total protein (mg protein. g FM^{-1}) and enzyme activity of catalase - CAT (mmol H_2O_2.min^{-1}. mg $protein^{-1}$), ascorbate peroxidase - APX (UA.min^{-1}. g FM^{-1}), and superoxide dismutase - SOD (UA.min^{-1}. mg $protein^{-1}$) of Sorghum plants submitted to cold stress.

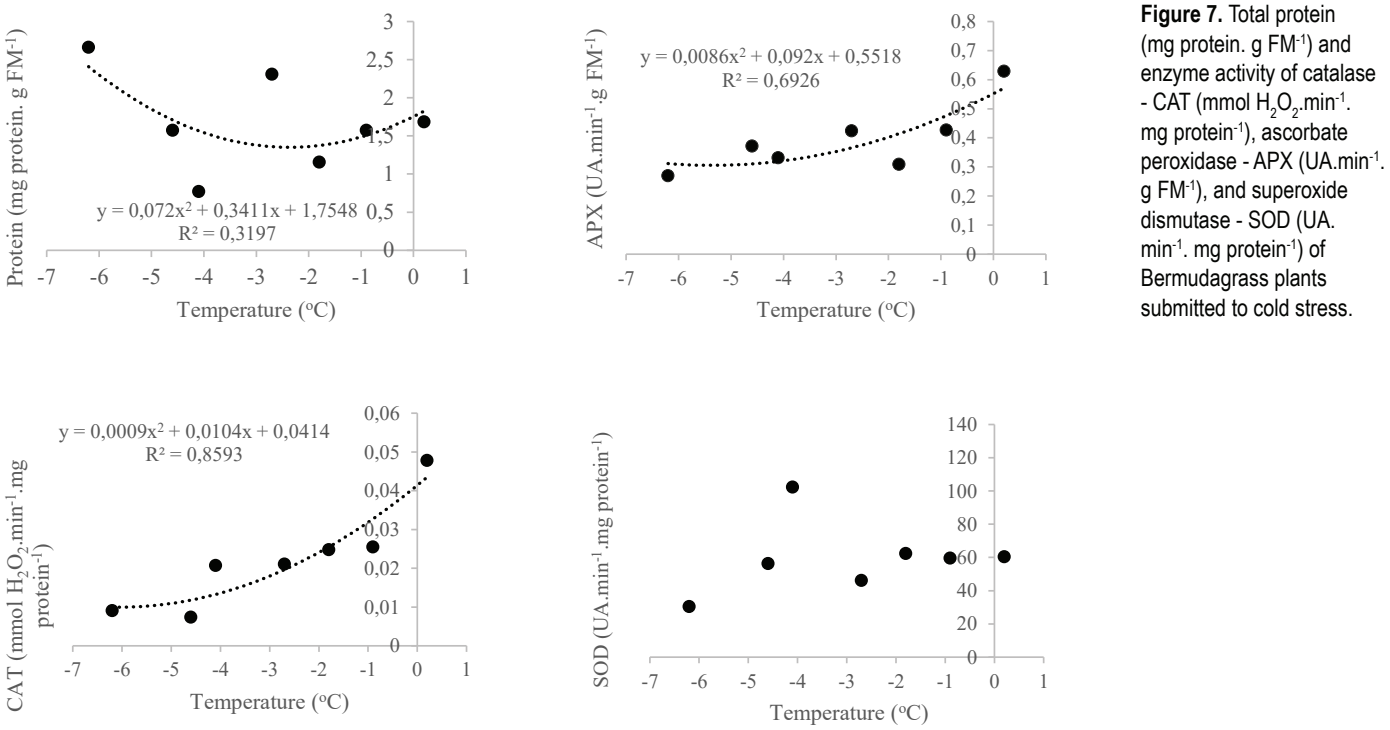

Figure 7. Total protein (mg protein. g FM^{-1}) and enzyme activity of catalase - CAT (mmol H_2O_2.min^{-1}. mg $protein^{-1}$), ascorbate peroxidase - APX (UA.min^{-1}. g FM^{-1}), and superoxide dismutase - SOD (UA. min^{-1}. mg $protein^{-1}$) of Bermudagrass plants submitted to cold stress.

DISCUSSION

The forages that increased protein content as a response to cold stress were alfalfa, Mombasa grass, and Bermuda grass. In these species, this may be a cold tolerance mechanism (RITONGA & CHEN, 2020), as by increasing the protein content of the cells, the osmotic potential of the cytoplasm increases, reducing the freezing temperature and protecting it against the harmful effects of the cold (LI et al, 2023; TAIZ & ZEIGER, 2017), and the repair of denatured proteins as molecular chaperones (BASHIR et al., 2020).

In these species, the protein content was higher at a temperature of -6.2 ºC, showing that plants can tolerate low temperatures without affecting the normal functioning of the cell since only an undamaged cell would be capable of synthesizing proteins (TAIZ & ZEIGER, 2017), as verified by Chen et al. (2018) in rice and Ihtisham et al. (2023) in Bermuda grass fertilized with nitrogen.

There are specific proteins that are activated by cold-responsive (COR) genes, which are produced after an induction signal given by low temperature. These genes are specific to each species and will act on the expression of other proteins and bring necessary biochemical changes (ADHIKARI et al., 2022). For this reason, in some species, such as black oats, marandu, millet, and sorghum, there is no increase in protein content with a decrease in temperature as other biochemical pathways are activated. However, one cannot ignore the fact that stress caused by low temperatures can result in the formation of ROS, which can cause cellular damage due to, among other factors, protein oxidation (HASANUZZAMAN & FUJITA, 2022).

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Thus, antioxidant enzymes play an important role in eliminating ROS and reducing oxidized effects. Among the enzymes studied, it can be observed that there was a decrease in the activity of CAT with lowering temperature for the species alfalfa, sorghum, and Bermuda grass. According to Hasanuzzaman and Fujita (2022), although this enzyme is involved in the elimination of H_2O_2, its accumulation is continuous, and thus the amount of the enzyme can be quickly reduced in any stress condition.

The reduction in CAT activity caused by the effect of low temperature was also observed in Bermuda grass by Fan et al. (2014) and Li et al. (2023), in cultivars different from those used in this research. On the contrary, in alfalfa, Bafeel and Ibrahim (2008), found an increase in CAT activity, in response to the accumulation of H_2O_2 promoted by low temperature, and stated that this is a form of plant protection so that they can quickly recover from the stress.

Unlike marandu grass, which had an outlier point at -4.1 °C, for millet, despite the tendency of the CAT curve to decrease, there was an increase in activity up to -2.7 °C and then a drop, indicating that there was an increase in activity with stress, and consequently a reduction in the accumulation of ROS (HASANUZZAMAN & FUJITA, 2022)

In species in which CAT activity did not change with decreasing temperature, such as black oat and mombasa, it can be stated that this enzyme does not play a major role in preventing oxidative stress caused by cold (RITONGA & CHEN, 2020). In black oat submitted to drought stress, Sartori et al. (2021) also observed that CAT was not activated as a response mechanism to abiotic stress.

For APX, the increase in enzymatic activity with a decrease in temperature prevents H_2O_2 from accumulating and causing cellular damage, suggesting that this enzyme would have an important role in the response to oxidative stress resulting from a decrease in temperature. In black oats, it was observed that APX acted as a protective mechanism against damage caused by cold, but there was no further enzyme production after a temperature of -4.1°C, probably due to the rupture of cell membranes or due to the death of the plant. In rice, Sato et al. (2011) showed that transgenic rice plants overexpressing a cytosolic APX 1 gene (OsAPXa) which exhibited higher APX activity in spikelets than in wild-type (WT) plants, sustained higher levels of APX activity under cold stress, resulting in enhanced cold tolerance at the booting stage.

Alfalfa activated the defense system against cold stress by increasing the production of the APX enzyme up to -4.6°C. In studies with Alfalfa, Krasnuk et al. (1976) observed that at low temperatures there is an increase in the activity of dehydrogenase enzymes, in parallel with the increase in soluble protein content, suggesting that the increase in activity may be the result of an increase in the amount of protein.

The highest CAT value at -4.1 °C in marandu grass was not enough to protect the plant from low temperature. According to Omran (1980) a drop in CAT, with unmodified APX activity, can lead to an increase in the accumulation of H_2O_2, since there was no increase in APX activity to compensate for the slow removal of H_2O_2 by CAT. This author also observed this phenomenon in pumpkin seedlings exposed to cold stress.

In Mombasa grass, the activity of APX and SOD was stimulated under stress conditions up to a temperature of -1.8 °C, indicating an association action of these enzymes for plant defense, with a decrease in activity at temperature -4.1 °C. These results agree with MacRae and Ferguson (1985) who observed the APX pathway as an alternative enzyme system to prevent the accumulation of H_2O_2.

In sorghum, an increase in SOD activity was noted at -1.8 °C, while in Marandu grass this increase was at -2.7 °C, and in Tifton SOD activity increased at -4.1 °C. These are evidences that in these species this enzyme was effective in protecting the plant against the accumulation of ROS at low temperatures, mainly because at these critical temperatures the plants had the lowest protein production, therefore SOD was necessary to eliminate the ROS produced in excess. Superoxide dismutase (SOD) is responsible for catalyzing the dismutation of O_2- to oxygen with the formation of H_2O_2, and the increase in the activity of this enzyme is generally related to high levels of free radicals (BLOKHINA et al., 2003).

No significant differences were observed for SOD activity in alfalfa and millet, indicating that the activity of this enzyme was not inhibited by low temperature in these species.

The enzymatic response of forages to stress varied inconsistently with decreasing temperature. This occurs because stress alters the balance between the production of free radicals and enzyme defense reactions (MÄKINEN et al., 2015) and according to Kratsch and Wise (2000) some plants, by nature, are more resistant to chilling stress than others.

Thus, alfalfa increased protein content and APX activity. In black oat and Mombasa there was a joint action of APX and SOD up to -4.1°C and -1.8°C respectively, these being the most active enzymes in the elimination of ROS. Marandu grass increased SOD activity and millet increased CAT activity, both up to -2.7 °C, to eliminate ROS. Sorghum increased SOD activity down to -1.8°C. Bermudagrass increased the protein content to protect cells against the cold, but as an enzymatic defense, there was also an increase in SOD activity.

CONCLUSION

The biochemical responses of forage species to cold stress are different.

Alfalfa increases protein content and APX activity.

Black oats and Mombasa increase APX and SOD activity.

Marandu grass and sorghum increase SOD activity

Pearl millet increases CAT activity.

Bermudagrass increases protein content and SOD activity.

REFERENCES

ADHIKARI, L.; BARAL, R.; PAUDEL, D.; MIN,D.; MAKAJU, S. O.; POUDEL, H. P.; ACHARYA, J.P.; MISSAOUI, A. M. 2022. Cold stress in plants: Strategies to improve cold tolerance in forage species. **Plant Stress**, v. 4, n. 100081, p. 1-14. Available at: <https://doi.org/10.1016/j.stress.2022.100081>

BAFEEL, S.O.; IBRAHIM, M. M. 2008 Antioxidants and accumulation of α-tocopherol induce chilling tolerance in *Medicago sativa.* **International Journal of Agriculture and Biology,** v. 10, n. 6, p. 593-598. Available at: <https://doi.org/07–438/MFA/2008/10–6–593–598>

BASHIR, M. A.; SILVESTRI, C.; AHMAD, T.; HAFIZ, I. A.; ABBASI, N. A.; MANZOOR, A.; RUGINI, E. 2020. Osmotin: a cationic protein that leads to improve biotic and abiotic stress tolerance in plants. **Plants**, v. 9, n. 8, p. 992. Available at: <https://doi.org /10.3390/plants9080992>

BRADFORD, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. **Analytical Biochemistry**, v. 72, n. 1/2, p. 248-254. Available at: <https://doi.org/10.1016/0003-2697(76)90527-3>

DOI: 10.37856/bja.v99i1.4339

BLOKHINA, O.; VIROLAINEN, E.; FAGERSTEDT, K.V. 2003. Antioxidants, oxidative damage and oxygen deprivation stress: a review. **Annals of botany**, v. 91, n. 2, p. 179-194. Available at: <https://doi.org/10.1093/aob/mcf118>

CANTO, M. W.; JOBIM, C. C.; PAGLIARINI, M. S.2 PANCERA JÚNIOR, E. J.; BARTH, NETO, A.; INTROVINI, E. P.; ZANFOLIN, P. R. L.; FERREIRA, C. W.; MATIVI, T. M.; ALMEIDA, G. M., VIZZOTTO, B. 2010. A pecuária de corte no Paraná–desenvolvimento, caracterização e o papel das pastagens. **Scientia Agraria Paranaensis**, v. 9, n. 3, p.5-21. Available at: <https://doi.org/10.18188/sap.v9i3.5257>

CAVERZAN, A.; PASSAIA, G.; ROSA, S. B.; RIBEIRO, C. W.; LAZZAROTTO, F.; MARGIS-PINHEIRO, M. (2012). Plant responses to stresses: role of ascorbate peroxidase in the antioxidant protection. **Genetics and Molecular Biology**, v. 35, n. 4, p. 1011-1019. Available at: <https://doi.org/10.1590/s1415-47572012000600016>

CHEN, L.; ZHAO, Y.; XU, S.; ZHANG, Z.; XU, Y.; ZHANG, J.; CHONG, K. 2018. Os MADS 57 together with Os TB 1 coordinates transcription of its target Os WRKY 94 and D14 to switch its organogenesis to defense for cold adaptation in rice. **New Phytologist**, v. 218, n. 1, p. 219-231. Available at: <https://doi.org/10.1111/nph.14977>

FAN, J.; REN, J.; ZHU, W.; AMOMBO, E.; FU, J.; CHEN, L. 2014. Antioxidant responses and gene expression in bermudagrass under cold stress. **Journal of the American Society for Horticultural Science**, v. 139, n. 6, p. 699-705. Available at: <https://doi.org/10.21273/JASHS.139.6.699>

GARG, N.; MANCHANDA, G. 2009 ROS generation in plants: Boon or bane?, **Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology**, v. 143, n. 1, p. 81-96. Available at: <https://doi.org/10.1080/11263500802633626>

GIANNOPOLITIS, C.N.; RIES, S. K.1977. Superoxide dismutases: I. Occurrence in higher plants. **Plant Physiology**, v. 59, n. 2, p. 309-314. Available at: <https://doi.org/10.1104/pp.59.2.309>

GILL, S. S.; TUTEJA, N. 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. **Plant Physiology and Biochemistry**, v. 48, n. 12, p. 909-930. Available at: <https://doi.org/10.1016/j.plaphy.2010.08.016>

GRAÇA MARTINS, M. E. (2019). Regressão linear simples. **Revista de Ciência Elementar**, v. 7, n. 03, p. 1-3. Available at: <https://doi.org/10.24927/rce2019.045>

GUY, C. L. 1990. Cold acclimation and freezing stress tolerance: role of protein metabolism. **Annual review of plant biology**, v. 41, n. 1, p. 187-223. Available at:GUY, C. L. 1990. Cold acclimation and freezing stress tolerance: role of protein metabolism. **Annual review of plant biology**, v. 41, n. 1, p. 187-223. Available at: <https://www.annualreviews.org/doi/pdf/10.1146/annurev.pp.41.060190.001155?casa_token=3n9IpC4goOUAAAAA%3A9IqfV_HXdB8tceVBpA7H38rtBIQVoYD8Y2YGoOL_7p09gbnHZ29d_DOt NwOj7xm_FgdivkSYfUSSIjs_> Accessed on: Fev. 1, 2024.

HASANUZZAMAN, M.; EL-SAPPAH, A. H.; ZAMAN, F.; KHAN, N.; RAZA, A.; SARRAF, M.; KHAN, S.; ABBAS, M.; HASSAN, M.J.; LI, J.; ZHAO, X.; ZHAO, X. 2023. Primary plant nutrients modulate the reactive oxygen species metabolism and mitigate the impact of cold stress in overseeded perennial ryegrass. **Frontiers in Plant Science**, v. 14, n. 1149832, p. 1-13. Available at: <https://doi.org/10.3389/fpls.2023.1149832>

IHTISHAM, M.; HASANUZZAMAN, M.; EL-SAPPAH, A. H.; ZAMAN, F.; KHAN, N.; RAZA, A.; SARRAF, M.; KHAN, S.; ABBAS, M.; HASSAN, M.J.; LI, J.; ZHAO, X.; ZHAO, X. 2023. Primary plant nutrients modulate the reactive oxygen

species metabolism and mitigate the impact of cold stress in overseeded perennial ryegrass. **Frontiers in Plant Science**, v. 14, n. 1149832, p. 1-13. Available at: <https://doi.org/10.3389/fpls.2023.1149832>

KRASNUK, M.; JUNG, G. A.; WITHAM, F. H. 1976. Electrophoretic studies of several dehydrogenases in relation to the cold tolerance of alfalfa. **Cryobiology**, v. 13, p. 375–393. Available at: <https://doi.org/10.1016/0011-2240(76)90121-8>

KRATSCH, H. A.; WISE, R. R. 2000. The ultrastructure of chilling stress. **Plant, Cell & Environment**, v. 23, n. 4, p. 337-350. Available at: <https://doi.org/10.1046/j.1365-3040.2000.00560.x>

LI, Z.; HUANG, C.; HAN, L. 2023. Differential regulations of antioxidant metabolism and cold-responsive genes in three bermudagrass genotypes under chilling and freezing stress. **International Journal of Molecular Sciences**, v. 24, n. 14070, p. 1-17. Available at: <https://doi.org/10.3390/ijms241814070>

MAC RAE, E.A.; FERGUSON, J.B. 1985. Changes in catalase activity and hydrogen peroxide concentration in plants in response to low temperature. **Physiologia Plantarum**, v.65, n.1, p. 51-56. Available at: <https://doi.org/10.1111/j.1399-3054.1985.tb02358.x>

MÄKINEN, H.; KASEVA, J.; VIRKAJÄRVI, P.; KAHILUOTO, H. 2015.Managing resilience of forage crops to climate change through response diversity. **Field Crops Research**, v. 183, p. 23-30. Available at: <https://doi.org/10.1016/j.fcr.2015.07.006>

OMRAN, R. G. 1980. Peroxide levels and the activities of catalase, peroxidase, and indoleacetic acid oxidase during and after chilling cucumber seedlings. **Plant Physiology**, v. 65, n. 2, p. 407-408. Available at: < https://doi.org/10.1104/pp.65.2.407>

PEIXOTO, P. H. P. CAMBRAIA, J.; SANT'ANNA, R.; MOSQUIM, P. R.; MOREIRA, M. A. 1999. Aluminum effects on lipid peroxidation and on the activities of enzymes of oxidative metabolism in sorghum. **Revista Brasileira de Fisiologia Vegetal**, v. 11, n. 3, p. 137-143. Available at: <https://www.researchgate.net/profile/Paulo_Peixoto4/publication/267338431_ALUMINUM_EFFECTS_ON_LIPID_PEROXIDATION_AND_ON_THE_ACTIVITIES_OF_ENZYMES_OF_OXIDATIVE_METABOLISM_IN_SORGHUM/links/544db4960cf2d6347f45cb09.pdf> Accessed on: Jun. 10, 2020.

PERL-TREVES, R.; PERL, A. 2001. Oxidative stress: an introduction. In: INZE, D.; VAN MONTAGU, M. (Eds.). 2001. **Oxidative stress in plants**, London: CRC Press, 1st ed. 336p. Available at: <https://doi.org/10.1201/b12593>

RITONGA, F. N.; CHEN, S. 2020. Physiological and molecular mechanism involved in cold stress tolerance in plants. Plants, v. 9, n. 560, p. 1-13. Available at: <https://doi.org/10.3390/plants9050560>

SARTORI, A.V. de S.; OLIVEIRA, C. M. G. de; ZUCARELI, C. 2021. Biochemical changes in black oat plants in response to water deficit under different temperatures. **Semina Ciências Agrárias**, v.42, n.5, p. 2685-2702. Available at: <https://doi.org/10.5433/1679-0359.2021v42n5p2685>

SATO, Y.; MASUTA, Y.; SAITO, K.; MURAYAMA, S.; OZAWA, K. 2011. Enhanced chilling tolerance at the booting stage in rice by transgenic overexpression of the ascorbate peroxidase gene, OsAPXa. **Plant cell reports**, v.30, p. 399-406. Available at: <https://doi.org/10.1007/s00299-010-0985-7>

SCANDALIOS, J. G. 2005. Oxidative stress: molecular perception and transduction of signals triggering antioxidant gene defenses. **Brazilian Journal of Medical and Biological Research**, v. 38, p. 995-1014. Available at: <https://doi.org/10.1590/S0100-879X2005000700003>

STEINER, J. L.; BRISKE, D. D.; BROWN, D. P.; ROTTLER, C. M. 2018. Vulnerability of Southern Plains agriculture to climate change. **Climatic change**, v. 146, n. 1-2, p. 201-218. Available at: <https://doi.org/10.1007/s10584-017-1965-5>

TAIZ, L.; ZEIGER, E.; MØLLER, I. M.; MURPHY, A. 2017. **Fisiologia vegetal**, Porto Alegre: Artmed, 6ª ed. 759 p.

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