SEMINA
Ciências Agrárias

DOI: 10.5433/1679-0359.2021v42n5p2685

Biochemical changes in black oat plants in response to water deficit under different temperatures

Mudanças bioquímicas em plantas de aveia preta em resposta ao déficit hídrico sob diferentes temperaturas

Altamara Viviane de Souza Sartori[1]*; Carolina Maria Gaspar de Oliveira[2]; Claudemir Zucareli[3]

Highlights

Black oat increase protein content in response to water deficit and temperature rise.
There was no increase in catalase and ascorbate peroxidase with water deficit.
The temperature rise aggravates the damage to cell membranes caused by water deficit.

Abstract

The black oat (Avena strigosa Schreb.) stands out as a forage of great importance in Brazilian agriculture. However, the productivity and quality of this forage can be affected by abiotic factors, such as temperature and water availability, which affect the physiological processes and facilitate the accumulation of free radicals (reactive oxygen species - ROS). Thus, the objective of this study was to understand the biochemical changes in black oat plants subjected to water deficit at different temperatures. Experiments were conducted in a greenhouse in two experimental periods, which presented an average temperature of 20 °C and 24 °C, respectively. Black oat seeds, of the variety IAPAR 61, were sown in pots and the plants were irrigated for 60 days. After which, the pots were covered with plastic bags and the irrigation was suspended. The analyses were carried out in five periods of evaluation - M1: plants before the suspension of irrigation, M2: plants at the first wilting point, M3: three days after plastic removal and irrigation return, M4: four days after M3 and before the second suspension of irrigation, and M5: the second wilting point. The levels of total protein and malondialdehyde (MDA), and the activity of the enzymes catalase (CAT) and ascorbate peroxidase (APX), were analyzed. The experimental design was completely randomized, with six replications, in a factorial scheme of average temperature × water management × periods of evaluation, and the means were compared by Tukey's test at 5%. In response to water deficiency and temperature increase, black oat plants increased their levels of total soluble proteins, and there was greater lipid peroxidation due to the increase in malondialdehyde content. There was no change in the activity of the enzymes catalase

[1] Master in Agronomy, Instituto de Desenvolvimento Rural do Paraná, IAPAR-EMATER, IDR-Paraná, Londrina, PR, Brazil. E-mail: altamarasartori@gmail.com
[2] Researcher, PhD, IAPAR-EMATER, IDR-Paraná, Londrina, PR, Brazil. E-mail: carolina@idr.pr.gov.br
[3] Prof. Dr., Department of Agronomy, Universidade Estadual de Londrina, UEL, Londrina, PR, Brazil. E-mail: claudemircca@uel.br
* Author for correspondence

Received: Sept. 17, 2020 - Approved: May 30, 2021

and ascorbate peroxidase under water deficit, and these activities decreased with increasing temperature.
Key words: *Avena strigosa* Schreb. Antioxidant enzymes. Catalase. Ascorbate peroxidase. Malondialdehyde.

Resumo

A aveia-preta (Avena strigosa Schreb.) destaca-se como uma forragem de grande importância na agricultura brasileira. Porém, a produtividade e a qualidade dessa forragem podem ser afetadas por fatores abióticos, como temperatura e disponibilidade de água, que afetam os processos fisiológicos e facilitam o acúmulo de radicais livres (espécies reativas de oxigênio - ROS). Assim, o objetivo deste estudo foi compreender as alterações bioquímicas em plantas de aveia preta submetidas ao déficit hídrico em diferentes temperaturas. Os experimentos foram conduzidos em casa de vegetação em dois períodos experimentais, os quais apresentaram temperatura média de 20 °C e 24 °C, respectivamente. Sementes de aveia preta, variedade IAPAR 61, foram semeadas em vasos e as plantas irrigadas por 60 dias. Depois disso, os vasos foram cobertos com sacos plásticos e a irrigação foi suspensa. As análises foram realizadas em cinco períodos de avaliação - M1: plantas antes da suspensão da irrigação, M2: plantas no primeiro ponto de murcha, M3: três dias após a retirada do plástico e retorno da irrigação, M4: quatro dias após M3 e antes do segundo suspensão da irrigação e M5: o segundo ponto de murcha. Foram analisados os níveis de proteína total e malondialdeído (MDA) e a atividade das enzimas catalase (CAT) e ascorbato peroxidase (APX). O delineamento experimental foi inteiramente casualizado, com seis repetições, em esquema fatorial temperatura média × manejo da água × períodos de avaliação, e as médias foram comparadas pelo teste de Tukey a 5%. Em resposta à deficiência hídrica e ao aumento da temperatura, as plantas de aveia preta aumentaram seus níveis de proteínas solúveis totais e houve maior peroxidação lipídica devido ao aumento do teor de malondialdeído. Não houve alteração na atividade das enzimas catalase e ascorbato peroxidase sob déficit hídrico, sendo que essas atividades diminuíram com o aumento da temperatura.
Palavras-chave: *Avena strigosa* Schreb. Enzimas antioxidantes. Catalase. Ascorbato peroxidase. Malondialdeído.

Introduction

Black oat (*Avena strigosa* Schreb.) is a winter grass of the *Poaceae* family, with its center of origin in the Middle East; from there, it spread to Europe and later to the Americas. It is a cespitose grass, with erect cylindrical stalks, glabrous or slightly hairy leaves, and fasciculated or hairy roots. The inflorescence is a panicle with ridged glumes, and the grain is an indehiscent caryopsis covered by lemmas and paleas. This species is considered to be more rustic, and has a greater tillering capacity than white and yellow oats. In addition, it is more resistant to drought and has less demanding requirements regarding fertility (Manetti, Oliveira, Caramori, Nagashima, & Hernandez, 2018).

The black oat is considered a prominent forage species and is of great importance in Brazilian agriculture. Its production is used to feed animals in bovine milk and meat production systems, in the crop-pasture system, and in crop rotation practices, especially in the southern region of Brazil (Stringari, Sacomori, Roters, Oliveira, & Almeida, 2019). In addition, it is possible to produce conserved forage, such as silage and hay, and it is used as a green cover to

protect and improve the physical, chemical, and biological properties of the soil, thereby, contributing to the sustainability of the no-tillage system (Tonato, Carneiro, Pedreira, & Pequeno, 2014).

Environmental factors modify the physiological functions of plants by modifying their growth, development, and, hence, their agricultural production (Araújo et al., 2019; Ozturk et al., 2020). Thus, considering that according to climate forecasts of increased global temperature and greater occurrences of extreme events, especially water deficits, it can be concluded that severe damage is expected to impact the productive development of agricultural crops (Jiménez-Muñoz et al., 2016; Wang et al., 2018; Habermann et al., 2019).

Water restriction stands out as an agent that reduces crop yields worldwide and can cause damage even greater than that of the other stress factors combined (Ozturk et al., 2020). Thus, the stress caused by water deficiency can impair crop cycles, biomass production, and nutrient absorption (Coelho et al., 2013). This occurs because with the reduction in water availability, plants develop significant morphological and metabolic changes, many of which are believed to be climatic responses to water stress (Hessini et al., 2019; Meena & Kaur, 2019). Closing the stomata is one of the first responses to a lack of water and it is also one of the most common responses. This is a protective mechanism against water loss through transpiration, which results in a decrease in the concentration of intracellular CO_2, thereby, decreasing photosynthesis and increasing photorespiration (Habermann et al., 2019).

High temperatures can also limit the performance of agronomic plants, including the black oat, which is winter forage. This stress is commonly associated with water deficit and even temperatures that are not considered extreme may prove harmful, as there is no water to perform transpiration to cool the plant, resulting in thermal stress. Consequently, in principle, there is a reduction in photosynthesis rates and with increased damage there is also a reduction in membrane stability, as well as protein denaturation and a loss of enzymatic activity (Atkinson & Urwin, 2012; Bita & Gerats, 2013; Habermann et al., 2019).

The most common effects of water scarcity and temperature increase on plant growth are well known, but understanding the molecular and biochemical changes that occur under combined stresses is still incipient for some species. Thus, new approaches must be introduced in studies with the aim of identifying the response of plants to combined stresses of dry conditions and high temperatures. In this way, the development of strategies to improve crop tolerance to stress may be enabled (Atkinson & Urwin, 2012; Ozturk et al., 2020). Several studies indicate that plants respond to a specific combination of stresses in a non-additive way, producing responses that cannot be predicted when the stresses are studied individually (Atkinson & Urwin, 2012). For example, Rizhsky et al. (2004) in *Arapidopsis* verified that with the aim of performing osmotic adjustment, plants accumulate proline in response to drought, whereas with the combination of heat and drought stresses, they accumulate sucrose. With these results, the authors demonstrate the plasticity of the plant genome in response to the varied environmental conditions that occur in the field.

When subjected to any stress, whether biotic or abiotic, plants trigger the production of reactive oxygen species (ROS), and those levels are controlled by the production and consumption of the ROS by antioxidative systems (Atkinson & Urwin, 2012; Nunes et al., 2017). Several enzymes and proteins act to protect the plant from the action of ROS; among them, the catalase (CAT) and ascorbate peroxidase (APX) enzymes stand out.

CAT is one of the main enzymes involved in the elimination of H_2O_2 generated during photorespiration and the beta-oxidation of fatty acids acting in peroxisomes and glyoxysome. It can also be found in mitochondria, which convert two molecules of H_2O_2 to water (H_2O) and molecular oxygen (O^-) (Barbosa, Silva, Willadino, Ulisses, & Camara, 2014). APX is a class I heme protein of the peroxidase superfamily. It is diversely regulated with diverse isoenzymatic forms. APX may be found in the cytosol, mitochondria, peroxisomes, chloroplasts, and cell walls. This enzyme has a high affinity for H_2O_2, enabling the elimination of this peroxide even at low concentrations (Caverzan et al., 2012). In studies of biochemical descriptors, it was found that the increase in the activity of these enzymes may indicate a certain tolerance of species, strains, or cultivars to abiotic stress, which may serve as a basis for studies with markers to indicate tolerance to stresses (Barbosa et al., 2014; Alves et al., 2016). For example, Pereira et al. (2012) observed that the expressive response of CAT in drought-tolerant peanut genotypes is evidence of its adoption as a biochemical descriptor in studies on the selection of genotypes for tolerance to water stress.

Another biochemical plant response to water stress is osmotic adjustment, which is the synthesis and accumulation of osmolytes, with the aim of increasing the osmotic potential of the cells. The main compounds synthesized are carbohydrates, proteins, amino acids, glycine, betaine, and proline (Turner, 2018; Ozturk et al., 2020). Thus, a temperature increase of approximately 5-10 °C, can induce the production and concentration of heat shock proteins (*Heat shock proteins - HSPs*), which prevent the adverse effects of stress in plants (Z. Khan & Shahwar, 2020).

A better understanding of the mechanisms that allow plants to adapt to or tolerate drought abiotic stresses and temperatures above the ideal, and still maintain their growth, development, and yield performance during periods of stress, can help in identifying resistant plants (Bianchi, Germino, & Silva, 2016). However, few studies have evaluated the biochemical changes in the black oat under the abiotic stress conditions of temperature increase and water stress, as well as the plant's recovery from these stresses.

Therefore, this study aims to understand the changes in the biochemistry of black oat plants subjected to water stress at different temperatures.

Material and Methods

The experiments were carried out under greenhouse conditions located at the Research Station of the Instituto de Desenvolvimento Rural do Paraná (IDR-Paraná) in Londrina, PR. The work was done in two experimental periods within the year, with average temperatures of 24 and 20 °C. The first experiment was conducted from June 20th to September 27th and the second from April 17th to August 14th. The maximum and minimum temperatures observed during the experimental period for each evaluation are listed in Table 1.

Table 1
Values of temperature observed inside the greenhouse at the corresponding experimental period, with the average temperatures of 24 °C and 20 °C, for each period of evaluation

Periods of evaluation	Date	T Max (°C)	T Min (°C)
Temperature of 24 °C			
M1	13/09/2016	36	21
M2	16/09/2016	37	16
M3	20/09/2016	33	14
M4	23/09/2016	33	16
M5	27/09/2016	39	16
Temperature of 20 °C			
M1	26/07/2017	29	19
M2	31/07/2017	29	17
M3	03/08/2017	21	15
M4	07/08/2017	29	12
M5	12/08/2017	32	16

*Periods of evaluation: M1 - before the suspension of irrigation; M2 - first wilting point; M3 - three days after plastic removal and the return of irrigation; M4 - four days after M3 and before the second suspension of irrigation; M5 - second wilting point. Date: day/month/year.

In both periods, the black oat variety, IAPAR 61 was used. The seeds were sown in pots with a capacity of 3 liters, containing substrate comprising two part soil, one part tanned barnyard manure, and 1 kg of NPK fertilizer, which had a formulation of 4-30-10 per cubic meter. Two plants were kept per pot and were continuously irrigated. At the end of the vegetative period, 60 days after sowing, the pots were separated into two water management treatments: with and without irrigation, with six replicates of six pots each, totaling 36 pots for each treatment. The pots were randomly arranged inside the greenhouse, resulting in a completely randomized experimental design.

The pots in the treatment without irrigation were covered with a plastic bag to prevent the evaporation of water from the soil (Figure 1), allowing only plant transpiration and irrigation was suspended, simulating the dry season. Once the plants reached their wilting point, reaching a water potential below -2.0 MPa, they were irrigated until recovery, that is, when its water potential became greater than -0.8 MPa. Thereafter, the pots were again covered with plastic bags and irrigation was suspended, subjecting the plants to another cycle of water deficit. The plants of the treatment with irrigation remained constantly under irrigation.

Figure 1. Pot with black oat plant covered with plastic bag in treatment without irrigation.

The plant water potential was determined using two leaf discs, of 1.0 cm^2 each, with the use of psychrometers (model C-30, Wescor, Inc.) connected to a data logger (model CR-7, Campbell Scientific, Inc.). The data logger was programmed so that readings were taken every 10 min until the balance of the vapor pressure in the chamber was checked. The microvoltage supplied by the system was converted to water potential (MPa) based on the previous calibration of the sensors with solutions of sodium chloride, thereby obtaining the total water potential in the leaf.

Periods of sample evaluation

For each of the two experimental periods (with average temperatures of 20 °C and 24 °C) and for the two treatments (with and without irrigation), collections of the plant material were performed at five different periods to evaluate its biochemical characteristics. The collection periods were defined according to the response of the plants to the treatment without irrigation. The first collection was made before the suspension of irrigation (M1); the second collection was carried out when the plants experienced the first wilting point (M2); the third collection was performed three days after withdrawal and the return of irrigation (M3); the fourth collection was carried out four days after M3 and before the second cycle of suspension of irrigation (M4); and the fifth collection was when the plants experienced the second wilting point (M5) (Table 1).

In all periods, approximately 2 g of completely developed and photosynthetically active leaf sheets portions in each pot were collected. The plant material samples were enclosed in a paper bag and stored in a freezer at -80 °C until analysis to preserve the integrity of the protein molecules.

Quantification of malondialdehyde (MDA)

The methodology for quantifying MDA was based on the protocol described by Heath and Packer (1968). To obtain the extract, approximately 100 mg of leaf tissue was macerated in liquid nitrogen and homogenized in 6.5 mL of 80% ethanol (v/v). After which, the solution was centrifuged at 5200 rpm for 10 min. From this extract, 2 mL was collected and transferred to another 15 mL tube containing 2 mL of 0.65% thiobarbituric acid (TBA) (w/v) in 20% (w/v) trichloroacetic acid (TCA). The samples were incubated at 95 °C for 25 min, transferred to ice, and centrifuged again to be read by a spectrophotometer at wavelengths of 532 nm and 600 nm.

Quantification of total proteins and enzymatic activity

To obtain the crude extracts of the samples, 0.250 g of leaf tissue was weighed and macerated in 5 mL of 50 mM potassium phosphate buffer (pH 7.0) and 4% PVP (polyvinylpyrrolidone) (w/v), which was previously cooled to 4 °C. After centrifugation for 10 min at 4 °C and at 785.4 rad/s, the supernatant was transferred to 2 mL Eppendorfs microtubes and stored in a freezer at -14 °C until analysis.

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, using bovine serum albumin (BSA, 0-15 μg μL^{-1}) as a standard. The total protein concentration was calculated by comparing the sample readings with those obtained from the standard curve and expressed as milligram (mg) of fresh matter $protein^{-1}$ (MF).

Catalase activity (CAT) was determined based on the consumption of H_2O_2, monitored by spectrophotometry at 240 nm, considering the molar extinction coefficient of H2O2, which is 36.0 M^{-1} cm^{-1} (Peixoto, Cambraia, Sant'Anna, Mosquim, & Moreira, 1999). The reaction solution consisted of 50 mM potassium phosphate buffer (pH 7.0) and 15 mM H_2O_2. The assay began with the addition of 200 μL of the diluted enzyme extract in a 3 mL quartz cuvette. Readings were taken at 240 nm immediately after the addition of the extract and then every 30 s for a total reaction time of 4 min. The difference in absorbance (ΔA240) was multiplied by the molar extinction coefficient of H_2O_2 and the activity of the enzyme was expressed in millimoles of H_2O_2 consumed per minute per milligram of protein (mmol H_2O_2 min^{-1} mg $protein^{-1}$).

The ascorbate peroxidase (APX) activity was determined following the methodology described by Peixoto et al. (1999) with some modifications. The reaction solution consisted of 50 mM potassium phosphate buffer (pH 7), 1 mM H_2O_2, 0.5 mM ascorbate, and enzymatic extract. The assay started with the addition of 200 μL of the diluted enzyme extract in a 3 mL quartz cuvette. Readings were taken at 290 nm immediately after the addition of the extract and then every 15 s for a total reaction time of 2 min. The activity of the enzyme was calculated using the molar extinction coefficient of 2.8 mM^{-1} cm^{-1}. The values were expressed in units of activity per minute per milligram of fresh matter (UAmin^{-1} mgMF^{-1}), which represents the amount of enzyme that catalyzed the oxidation of 1 μmol^{-1} of ascorbate.

The experimental design was completely randomized in a 2 × 2 × 5 factorial scheme (average temperatures × water management treatments × periods of evaluation), with six replicates. Data were subjected to normality and homogeneity evaluations and, taking into account statistical assumptions, were subjected to analysis of variance. Treatment means were compared using Tukey's test at 5% probability.

Results and Discussion

The triple factorial analysis of variance indicated no statistically significant interaction among the three factors of variation. However, it is not possible to state that the abiotic stress of water restriction (without irrigation) was not modified by the temperature factor, because within each period of evaluation, there were times when the plants were without water restriction (M1, M3, and M4); while in others, the restriction was imposed (M2 and M5).

Malondialdehyde (MDA)

The damage caused by abiotic stress to cell membranes due to lipid peroxidation is demonstrated by the increase in malondialdehyde (MDA) levels (Table 2). Therefore, it is possible to observe a significant interaction only between the factors of average temperature and periods of evaluation, and the factors of water management treatments and periods of evaluation. The other interactions were not statistically significant for this characteristic

Table 2
Malondialdehyde (MDA; nmol.gMF-1) content of black oat leaves under different average temperatures, water management treatments, and periods of evaluation

Treatments	Periods of evaluation				
	M1	M2	M3	M4	M5
Temperature (°C)					
20	73.22 A ab*	77.18 B a	78.39 A a	48.81 B b	67.12 B ab
24	60.73 A c	109.95 A ab	84.70 A bc	91.96 A b	124.25 A a
Water management					
Without Irrigation	66.97 A c	126.66 A a	88.52 A bc	73.28 A bc	97.13 A b
With Irrigation	66.97 A b	60.46 B b	74.58 A ab	67.50 A b	94.24 A a
Temperature (°C)					
Water management	20		24		Mean
Without Irrigation	80.61		100.41		90.51 A
With Irrigation	57.28		88.23		72.75 B
Mean	68.95 b		94.32 a		
Coefficient of Variation: 28.56 %					

*In each separate interaction, means followed by uppercase letters in the column and lowercase letters in the row do not differ by Tukey's test at 5% probability. Periods of evaluation: M1 - before the suspension of irrigation; M2 - first wilting point; M3 - three days after plastic removal and the return of irrigation; M4 - four days after M3 and before the second suspension of irrigation; M5 - second wilting point.

It was verified that in the interaction between temperature and evaluation periods, there was no increase in the MDA contents at 20 °C between M1 (the beginning) with M2 and M5 (periods of water restriction). At 24 °C there was a higher MDA content in M2 and M5, indicating that the association of higher temperature and water restriction results in greater damage to cell membranes. Comparing the average temperatures between each period of evaluation, it can be observed that the black oat plants showed differences among the MDA levels in the evaluations of M2, M4, and M5, which were always higher than those at the temperature of 24 °C.

In the interaction of the water management factor and evaluation periods, from comparing the two treatments of water management, it was observed that there was a higher content of MDA in the non-irrigated group until the period of M2, or in other words, in those plants that have suffered water restriction. From analyzing each evaluation period, it was observed, in both treatments of water, that with irrigation the period of M5 showed the highest value. This was followed by M3, which did not differ from the others. This suggests that peroxidation increases as the plant develops regardless of water limitation. In the treatment without irrigation, the highest MDA content was observed in M2, followed by M5, which did not differ from M3 and M4, and finally M1, coinciding with the moments of lower and higher water availability.

In the treatment without irrigation, the black oat plant showed greater damage to cell membranes in the M2 period, that is, when the first cycle of water restriction occurred. In M5, the plant was also under water restriction, however, the MDA content was lower than that in M2, and it did not differ from the value observed in the plants of the irrigation treatment. These results indicate that the plant may have developed a memory of the procedures to overcome the first incidence of stress, which led to the activation of the metabolism and mechanisms to overcome the second cycle of water stress.

For the isolated factors, in general, there was a higher MDA content at a temperature of 24 °C and in the treatment without irrigation. This indicates the damage caused by the water deficit, associated with a temperature above the ideal, in cell membranes, as also reported by M. H. Khan and Panda (2008).

According to Yang and Miao (2010), lipid peroxidation is an indicator of the free radical reactions in tissues, where the uptake of oxygen charge in tissues generates reactive oxygen species (ROS), particularly H_2O_2, which is produced at elevated rates by glycolate oxidase in peroxisomes during photorespiration. Thus, this increase in MDA content in black oat plants may be associated with excess production and accumulation of ROS, which mainly causes oxidative damage to cell membranes, aggravating the destabilization of ionic homeostasis (Golldack, Li, Mohan, & Probst, 2014).

The increase in MDA content in response to water restriction, as observed in black oats, has also been reported in other species. In their work with maize plants under water deficit stress, Hendges et al. (2015) found that six days of stress induced an increase in MDA content. Corroborating these results, Hasheminasab, Assad, Aliakbari and Sahhafi (2012) observed higher MDA values in wheat plants under water stress, and with the particularity that more stress-tolerant

genotypes had lower MDA contents than the more susceptible ones. In oat plants, Islam et al. (2011) found that drought stress led to the production of free radicals, which increased lipid peroxidation and oxidative stress.

Total proteins

The total protein content of black oat plants showed statistically significant interaction between the factors, but only temperature and evaluation periods, and water management and evaluation periods (Table 3). The other interactions were not significant.

Table 3
Total soluble protein content (mg protein.gMF^{-1}) of black oat leaves under different average temperatures, water management treatments, and periods of evaluation

Treatments	Periods of evaluation				
	M1	M2	M3	M4	M5
Temperature (°C)					
20	2.45 B c*	6.23 B a	4.05 B b	4.66 A b	3.55 B bc
24	5.74 A b	7.54 A a	5.80 A b	5.20 A b	6.02 A ab
Water management					
Without Irrigation	4.10 A c	8.14 A a	4.70 A bc	5.14 A bc	5.72 A b
With Irrigation	4.10 A ab	5.63 B a	5.15 A ab	4.71 A ab	3.86 B b
Temperature (°C)					
Water management	20		24		Mean
Without Irrigation	4.46		6.66		5.56 A
With Irrigation	3.91		5.47		4.69 B
Mean	4.19 b		6.06 a		
Coefficient of Variation: 26.96 %					

*In each separate interaction, means followed by uppercase letters in the column and lowercase letters in the row do not differ by Tukey's test at 5% probability. Periods of evaluation: M1 - before the suspension of irrigation; M2 - first wilting point; M3 - three days after plastic removal and the return of irrigation; M4 - four days after M3 and before the second suspension of irrigation; M5 - second wilting point.

From the interaction of average temperature and period of evaluation, it was possible to verify that the black oat plants presented higher values of total soluble proteins at 24 °C than at 20 °C for all periods of evaluation, with the exception of M4. There were statiscally significant differences among the evaluation periods, and for the temperature of 20 °C the protein content was higher in M2, followed by M4 and M3, which did not differ from M5, and the lowest content was at M1. At 24 °C the highest content was in M2, with no difference from M5, which did not differ from the others. Thus, it was found that an increase in temperature resulted in a higher content of total soluble proteins. The water restriction period of M2 showed a higher value than the others, indicating that the abiotic

stress caused by the combination of high temperature and water restrictions resulted in higher protein contents.

These changes are indicative of the occurrence of osmoregulation in oat plants (Ozturk et al., 2020), because in a stressful situation plants can increase their protein content to reduce the water potential of the cell, thereby, avoiding loss of water (Carvalho et al., 2015). Thus, it promotes the stabilization of the membranes, protecting against extreme temperatures, drought, salt, and oxidative damage (Ozturk et al., 2020). This osmoregulation, also known as osmotic adjustment, is one of the main physiological adaptation mechanisms that plants use to deal with stress, including salinity and water stress (Turner, 2018; Marín de la Rosa de la Rosa, 2019). Thus, in this situation, plant cells need to actively accumulate a total solute concentration that is higher than that of the external solution to attract water into the cells (Bita & Gerats, 2013; Hessini et al., 2019).

After observing the results of the interaction between periods of evaluation and water management, it was discovered that there is a higher protein content for plants without irrigation on the periods of assessment, M2 and M5, or for those that passed through cycles of water restriction. For the periods of evaluation in the treatment without irrigation, the highest protein content was observed in M2, followed by M5, M4, and M3, which did not differ from M1. With irrigation, there was a difference only between M2 and M5 with higher and lower values, respectively.

The results of water management with irrigation indicate a normal change in protein content in leaves as the plant develops, regardless of stress. In other words, in M5 the plant was already in the pre-flowering stage and naturally decreased its leaf protein content. However, the stresses caused by water restriction, added to the stress of temperature increase, resulted in a higher protein content in response to these treatments. Several authors, working with other species, also observed an increase in protein content after water restriction. These include Sousa et al. (2015) working with cowpea plants and Nemati et al. (2019) with wheat plants.

Protein content increases do not always occur in response to water deficit and some studies have shown that, instead of protein, cells can accumulate proline. For example, an assay was performed with six peanut genotypes subjected to drought stress, in which it was found that the total soluble protein content in the leaves increased only in two genotypes, while the proline content increased in all of them (Alves et al., 2016). Similarly, in a study done on sunflowers, Kosar et al. (2020) observed that the total soluble protein content decreased under water stress, while proline, glycine betaine, and the activity of antioxidant enzymes increased.

Some results contrasting with those found for black oats can be found in the literature regarding the protein content in the leaves of plants under water-deficient conditions. Alves et al. (2016) observed that some peanut genotypes subjected to water stress could not be differentiated by analyzing the concentration of total proteins. In addition, Mafakheri, Siosemardeh, Bahramnejad, Struik and Sohrabi (2011), in studying chickpeas found a decrease in the protein content of plants under stress, suggesting that protein degradation may be a result of increased protease activity, or may be caused by the fragmentation of the proteins due to the toxic effect of ROS.

Catalase activity (CAT)

In evaluating the activity of the enzyme catalase (CAT), it was discovered that there was a significant interaction observed only between the temperature and the evaluation periods (Table 4). In this interaction an increase in the activity of the CAT enzyme, at a temperature of 20 °C, was observed in all periods of evaluation. Among the periods, at 20 °C the activity of CAT was higher at M1, followed by the others, which did not differ among themselves. At 24 °C, the greatest activity was observed in M4, followed by the others, which did not differ from each other.

It is noteworthy to mention that there was a significant effect on water management when this factor was isolated. Among the treatments, there were higher levels of CAT activity in black oat plants in the irrigation treatment.

In this way, although CAT is one of the main enzymes involved in the elimination of H_2O_2 generated during photorespiration (Barbosa et al., 2014), it was not activated by black oat plants as a response mechanism to abiotic stress. According to Nxele, Klein and Ndimba (2017), there are some cases in which the activity of the antioxidant system does not increase or is not sufficient to neutralize the large amount of reactive species generated, affecting the level of cellular damage. Thus, the inability of CAT to eliminate H_2O_2 in black oat plants favored the accumulation of ROS, leaving the plant susceptible to membrane lipid peroxidation and oxidative damage from stress (Table 2).

Table 4
Catalase (CAT) activity (mmol H_2O_2.min^{-1}.mg protein^{-1}) of black oat leaves under different average temperatures, water management treatments, and periods of evaluation

Treatments	Periods of evaluation					
	M1	M2	M3	M4	M5	
Temperature (°C)						
20	0.25 A a*	0.14 A b	0.16 A b	0.15 A b	0.17 A b	
24	0.05 B b	0.06 B ab	0.07 B ab	0.10 B a	0.06 B b	
Water management						Mean
Without Irrigation	0.15	0.09	0.12	0.12	0.10	0.11 B
With Irrigation	0.15	0.11	0.12	0.13	0.13	0.13 A
Temperature (°C)						
Water management	20			24		
Without Irrigation	0.17			0.06		
With Irrigation	0.18			0.08		
Mean	0.17 a			0.07 b		
Coefficient of Variation: 28.35 %						

*In each separate interaction, means followed by uppercase letters in the column and lowercase letters in the row do not differ by Tukey's test at 5% probability. Periods of evaluation: M1 - before the suspension of irrigation; M2 - first wilting point; M3 - three days after plastic removal and the return of irrigation; M4 - four days after M3 and before the second suspension of irrigation; M5 - second wilting point.

Corroborating these results, Alves et al. (2016) found that, under water stress, all the peanut genotypes studied showed a reduction in catalase activity. They also found that CAT activity varied depending on the duration and intensity of the stress. In situations of moderate stress, activity increased; however, when stress was more severe, activity decreased. Sousa et al. (2015) found no difference in CAT enzyme activity in cowpea plants under stress. However, in contrast to the results of this study, an increase in the activity of the enzyme catalase after water stress was observed in other species. Coelho et al. (2013) and Hendges et al. (2015) studied maize subjected to water stress and observed an increase in catalase values after stress. Similarly, Pereira et al. (2012) and Anjum et al. (2017) observed an increase in the activity of the enzymes catalase and superoxide dismutase when bean and maize plants, respectively, were subjected to water deficit stress. Hasheminasab et al. (2012) pointed out that CAT enzyme activity increased significantly in wheat plants under water stress, and the enzyme activity in the most tolerant genotypes showed its effectiveness in eliminating free radicals, in the case of hydrogen peroxide, when compared to other less tolerant genotypes.

Ascorbate peroxidase (APX) activity

From evaluating the activity of the enzyme, it was observed that ascorbate peroxidase (APX) showed a significant interaction only between the temperature and the evaluation period (Table 5). At 20 °C the APX activity values were higher than those at 24 °C for all evaluation periods. After comparing the periods, it was found that at 20 °C the highest APX activity was observed in M1, followed by M5 and M2, and finally M4 and M3. At 24 °C there was no statistically significant difference among the periods of evaluation.

Table 5
Ascorbate peroxidase (APX) activity (UA.min^{-1}.mg protein^{-1}) in black oat leaves under different average temperatures, water management treatments, and periods of evaluation

Treatments	Periods of evaluation					
	M1	M2	M3	M4	M5	
Temperature (°C)						
20	1.64 A a	0.77 A bc	0.46 A c	0.60 A bc	0.82 A b	
24	0.37 B a	0.46 B a	0.39 A a	0.34 B a	0.20 B a	
Water management						Mean
Without Irrigation	1.00	0.68	0.41	0.40	0.49	0.61
With Irrigation	1.00	0.55	0.43	0.55	0.53	0.59
Temperature (°C)						
Water management	20			24		
Without Irrigation	0.86			0.32		
With Irrigation	0.85			0.38		
Mean	0.86 a			0.35 b		
Coefficient of Variation: 46.37 %						

*In each separate interaction, means followed by uppercase letters in the column and lowercase letters in the row do not differ by Tukey's test at 5% probability. Periods of evaluation: M1 - before the suspension of irrigation; M2 - first wilting point; M3 - three days after plastic removal and the return of irrigation; M4 - four days after M3 and before the second suspension of irrigation; M5 - second wilting point.

In general, a decrease in APX activity with an increase in drought and temperature was observed. Considering these results, it can be stated that in the case of black oats, this enzyme does not act as an enzymatic defense mechanism and does not have the capacity to neutralize the action of all reactive oxygen species (Barbosa et al., 2014). This was concluded because the highest value observed was in plants before the suspension of irrigation (M1) and at a temperature of 20 °C, which is considered the least stressful, mainly because this is a winter species (Manetti et al., 2018). As in this study, Hendges et al. (2015) found no ascorbate peroxidase activity in maize plants after drought, concluding that CAT is the enzyme responsible for removing the ROS. Similarly, Sousa et al. (2015) found a decrease in APX enzyme activity in cowpea plants under stress.

Differently from the results of this study with black oat, Alves et al. (2016) verified, in peanut plants, an increase in the enzymatic activity of ascorbate peroxidase, associated with a reduction in the effects of oxidative stress, through the removal of H2O2 after water stress. After studying the antioxidant defense mechanisms against oxidative stress caused by water deficiency in *Geranium sanguineum* L., Avramova et al. (2016) concluded that the increase in the activity of enzymes, such as superoxide dismutase (SOD), CAT, and peroxidase (POD) is related to the maintenance of the oxidative balance under stress conditions. Thus, diverse studies have indicated that the activity of one or more antioxidant enzymes was observed in plants exposed to stress conditions, and increased activity levels may be related to increased stress tolerance (Nunes et al., 2017). Atkinson and Urwin (2012) stated that the APX enzyme is crucial for the response to multiple stresses. Koussevitzky et al. (2008) found an accumulation of the APX1 enzyme in *Arabidopsis* in the combined heat and drought response, but not in the response to these stresses individually.

According to the systematization of the information regarding the effect of drought and temperature increases on the black oat plant, the studies that have been carried out with the main focus on physiological aspects are still very scarce, with little attention paid to the biochemical aspects. According to the results of this study, the black oat plant is sensitive to the effects of drought and temperature increase with changes in its biochemical characteristics. These can be exploited in the development of genotypes and management practices to increase tolerance and reduce the damage caused by abiotic stresses.

Conclusions

In response to water deficit and temperature above optimal levels, black oat plants increased the levels of total soluble proteins, and there was greater lipid peroxidation in the leaves.

Furthermore, the activities of catalase and ascorbate peroxidase enzymes decreased with increasing temperature, but they were not altered by water deficit.

References

Alves, G., Rodrigues, M., Pereira, J. W. D., Luz, L. N. D., Lima, L., & Santos, R. C. D. (2016). Genotypic variability of peanut lines in response to water stress,

based on biochemical descriptors. *Revista Caatinga, 29*(3), 528-536. doi: 10.1590/1983-21252016v29n302rc

Anjum, S. A., Ashraf, U., Tanveer, M., Khan, I., Hussain, S., Shahzad, B.,... Wang, L. C. (2017). Drought induced changes in growth, osmolyte accumulation and antioxidant metabolism of three maize hybrids. *Frontiers in Plant Science, 8*(69), 1-12. doi: 10.3389/fpls.2017.00069

Araújo, G. D. N., Jr., Gomes, F. T., Silva, M. J. da, Rosa Jardim, A. M. F. da, Simões, V. J. L. P., Izidro, J. L. P. S.,... Silva, T. G. F. da. (2019). Estresse hídrico em plantas forrageiras: uma revisão. *Pubvet, 13*(1), 1-10. doi: 10.31533/pubvet.v13n01a241.1-10

Atkinson, N. J., & Urwin, P. E. (2012). The interaction of plant biotic and abiotic stresses: from genes to the field. *Journal of Experimental Botany, 63*(10), 3523-3543. doi: 10.1093/jxb/ers100

Avramova, V., Nagel, K. A., Abdelgawad, H., Bustos, D., Duplessis, M., Fiorani, F., & Beemster, G. T. (2016). Screening for drought tolerance of maize hybrids by multi-scale analysis of root and shoot traits at the seedling stage. *Journal of Experimental Botany, 67*(8), 2453-2466. doi: 10.1093/jxb/erw055

Barbosa, M. R., Silva, M. M. A., Willadino, L., Ulisses, C., & Camara, T. R. (2014). Plant generation and enzymatic detoxification of reactive oxygen species. *Ciência Rural, 44*(3), 453-460. doi: 10.1590/S0103-84782014000300011

Bianchi, L., Germino, G. H., & Silva, M. A. (2016). Adaptação das plantas ao déficit hídrico. *Acta Iguazu, 5*(4), 15-32. doi: 10.48075/actaiguaz.v5i4.16006

Bita, C., & Gerats, T. (2013). Plant tolerance to high temperature in a changing environment: scientific fundamentals and production of heat stress-tolerant crops. *Frontiers in Plant Science, 4*(273), 1-18. doi: 10.3389/fpls.2013.00273

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, 72*(1/2), 248-254. doi: 10.1016/0 003-2697(76)90527-3

Carvalho, C. G. P., Ozawa, E. K. M., Amabile, R. F., Godinho, V., Gonçalves, S. L., Ribeiro, J. L., & Seifert, A. L. (2015). Adaptabilidade e estabilidade de genótipos de girassol resistentes a imidazolinonas em cultivos de segunda safra. *Revista Brasileira de Ciências Agrárias, 10*(1), 1-7. doi: 10.5039/agraria.v10i1a3804

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, 35*(4), 1011-1019. doi: 10.1590/s1415-47572012000600016.

Coelho, C. C. R., Neves, M. G., Oliveira, L. M., Conceição, A. G. C., Okumura, R. S., & Oliveira, C. F., Neto. (2013). Biometria em plantas de milho submetidas ao alagamento. *Revista Agroecossistemas, 5*(1), 32-38. doi: 10.18542/ragros.v5i1.1408

Golldack, D., Li, C., Mohan, H., & Probst, N. (2014). Tolerance to drought and salt stress in plants: unraveling the signaling networks. *Frontiers in Plant Science, 5*(151), 1-10. doi: 10.3389/fpls.2014.00151

Habermann, E., Dias de Oliveira, E. A., Contin, D. R., Delvecchio, G., Viciedo, D. O., Moraes, M. A. de,... Martinez, C. A. (2019). Warming and water deficit impact leaf photosynthesis and decrease forage quality and digestibility of a C4 tropical grass. *Physiologia Plantarum, 165*(2), 383-402. doi: 10.1111/ppl.12891

Hasheminasab, H., Assad, M. T., Aliakbari, A., & Sahhafi, S. R. (2012). Influence of drought stress on oxidative damage and antioxidant defense systems in tolerant and susceptible wheat genotypes. *Journal of Agricultural Science, 4*(8), 20-30. doi: 10.5539/jas.v4n8p20

Heath, R. L., & Packer, L. (1968). Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. *Archives of Biochemistry and Biophysics, 125*(1), 189-198. doi: 10.1016/0003-9861(68)90654-1

Hendges, F. B., Rambo, C. R., Alcassa, L. P., Liebl, J., Vendruscolo, E. C. G., & Costa, A. (2015). Avaliação enzimática e fisiológica de plântulas de milho submetidas à seca. *Revista Brasileira de Energias Renováveis, 4*(2), 52-63. doi: 10.5380/rber.v4i2.42287

Hessini, K., Issaoui, K., Ferchichi, S., Saif, T., Abdelly, C., Siddique, K. H., & Cruz, C. (2019). Interactive effects of salinity and nitrogen forms on plant growth, photosynthesis and osmotic adjustment in maize. *Plant Physiology and Biochemistry, 139*, 171-178. doi: 10.1016/j.plaphy.2019.03.005

Islam, M. R., Xue, X., Mao, S., Ren, C., Eneji, A. E., & Hu, Y. (2011). Effects of water saving superabsorbent polymer on antioxidant enzyme activities and lipid peroxidation in oat (Avena sativa L.) under drought stress. *Journal of the Science of Food and Agriculture, 91*(4), 680-686. doi: 10.1002/jsfa.4234

Jiménez-Muñoz, J. C., Mattar, C., Barichivich, J., Santamaría-Artigas, A., Takahashi, K., Malhi, Y., & Van Der Schrier, G. (2016). Record-breaking warming and extreme drought in the Amazon rainforest during the course of El Niño 2015–2016. *Scientific Reports, 6*(1), 1-7. doi: 10.1038/srep33130

Khan, M. H., & Panda, S. K. (2008). Alterations in root lipid peroxidation and antioxidative responses in two rice cultivars under NaCl-salinity stress. *Acta Physiologiae Plantarum, 30*(1), 81-89. doi: 10.1007/s11738-007-0093-7

Khan, Z., & Shahwar, D. (2020). Role of Heat Shock Proteins (HSPs) and heat stress tolerance in crop plants. In *Sustainable agriculture in the era of climate change* (pp. 211-234). Cham: Springer. doi: 10.1007/978-3-030-45669-6

Kosar, F., Akram, N. A., Ashraf, M., Ahmad, A., Alyemeni, M. N., & Ahmad, P. (2020). Impact of exogenously applied trehalose on leaf biochemistry, achene yield and oil composition of sunflower under drought stress. *Physiologia Plantarum, 172*(2), 1-17. doi: 10.1111/ppl.13155

Koussevitzky, S., Suzuki, N., Huntington, S., Armijo, L., Sha, W., Cortes, D.,... Mittler, R. (2008). Ascorbate peroxidase 1 plays a key role in the response of Arabidopsis thaliana to stress combination. *Journal of Biological Chemistry, 283*(49), 34197-34203. doi: 10.1074/jbc.M806337200

Mafakheri, A., Siosemardeh, A., Bahramnejad, B., Struik, P. C., & Sohrabi, Y. (2011). Effect of drought stress and subsequent

recovery on protein, carbohydrate contents, catalase and peroxidase activities in three chickpea ('Cicer arietinum') cultivars. *Australian Journal of Crop Science, 5*(10), 1255-1260. doi: 10.3316/informit.746357591676684.

Manetti, J., Fº., Oliveira, C. M. G., Caramori, P. H., Nagashima, G. T., & Hernandez, F. B. T. (2018). Cold tolerance of forage plant species. Semina: *Ciências Agrárias, 39*(4), 1469-1476. doi: 10.5433/1679-0359.2018v39n4p1469

Marín de la Rosa, N., Lin, C. W., Kang, Y. J., Dhondt, S., Gonzalez, N., Inzé, D., & Falter Braun, P. (2019). Drought resistance is mediated by divergent strategies in closely related Brassicaceae. *New Phytologist, 223*(2), 783-797. doi: 10.1111/nph.15841

Meena, Y. K., & Kaur, N. (2019). Towards an understanding of physiological and biochemical mechanisms of drought tolerance in plant. *Annual Research & Review in Biology, 31*(2), 1-13. doi: 10.9734/ARRB/ 2019/v31i230042

Nemati, M., Piro, A., Norouzi, M., Vahed, M. M., Nisticò, D. M., & Mazzuca, S. (2019). Comparative physiological and leaf proteomic analyses revealed the tolerant and sensitive traits to drought stress in two wheat parental lines and their F6 progenies. *Environmental and Experimental Botany, 158,* 223-237. doi: 10.1016/j.envexpbot.2018.10.024

Nunes, F. H., Jr., Freitas, V. S., Mesquita, R. O., Braga, B. B., Barbosa, R. M., Martins, K., & Gondim, F. A. (2017). Effects of supplement with sanitary landfill leachate in gas exchange of sunflower (*Helianthus annuus* L.) seedlings under drought stress. *Environmental Science and Pollution Research, 24*(30), 24002-24010. doi: 10.1007/s11356-017-0047-6

Nxele, X., Klein, A., & Ndimba, B. K. (2017). Drought and salinity stress alters ROS accumulation, water retention, and osmolyte content in sorghum plants. *South African Journal of Botany, 108,* 261-266. doi: 10.1016/j.sajb.2016.11.003

Ozturk, M., Turkyilmaz Unal, B., García Caparrós, P., Khursheed, A., Gul, A., & Hasanuzzaman, M. (2020). Osmoregulation and its actions during the drought stress in plants. *Physiologia Plantarum,* Special Issue Article, 1-15. doi: 10.1111/ppl.13297

Peixoto, P. H. P., Cambraia, J., Sant'Anna, R., Mosquim, P. R., & Moreira, M. A. (1999). Aluminum effects on lipid peroxidation and activities of enzymes of oxidative metabolism in sorghum. *Revista Brasileira de Fisiologia Vegetal, 11*(3), 137-143. Recuperado de 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

Pereira, J. W. D. L., Melo, P. D. A., Fº., Albuquerque, M. B., Nogueira, R. J. M. C., & Santos, R. C. (2012). Mudanças bioquímicas em genótipos de amendoim submetidos a déficit hídrico moderado. *Revista Ciência Agronômica, 43*(4), 766-773. doi: 10.1590/S1806-66902012000400019

Rizhsky, L., Liang, H., Shuman, J., Shulaev, V., Davletova, S., & Mittler, R. (2004). When defense pathways collide. The response of Arabidopsis to a combination of drought and heat stress. *Plant Physiology, 134*(4), 1683-1696. doi: 10.1104/pp.103.033431

Sousa, C., Pedrosa, E. M., Rolim, M. M., Oliveira, R. A. D., Fº., Souza, M. A. de, & Pereira, J. V. F. (2015). Crescimento e respostas enzimáticas do feijoeiro caupi sob estresse hídrico e nematoide de galhas. *Revista Brasileira de Engenharia Agrícola e Ambiental, 19*(2), 113-118. doi: 10.1590/1807-1929/agriambi.v19n2p113-118

Stringari, J. D., Sacomori, W., Roters, D. F., Oliveira, G. F. de, & Almeida, J. (2019). O efeito da aplicação continuada de dejeto líquido suíno na produção de massa seca da aveia preta. *Revista Agroecossistemas, 11*(2), 73-81. doi: 10.18542/ragros.v11i2.7388

Tonato, F., Carneiro, B., Pedreira, C. G. S., & Pequeno, D. N. L. (2014). Aveia preta e azevém anual colhidos por interceptação de luz ou intervalo fixo de tempo em sistemas integrados de agricultura e pecuária no Estado de São Paulo. *Ciência Rural, 44*(1), 104-110. doi: 10.1590/S0103-84782014000100017

Turner, N. C. (2018). Turgor maintenance by osmotic adjustment: 40 years of progress. *Journal of Experimental Botany, 69*(13), 3223-3233. doi: 10.1093/jxb/ery181

Wang, Z., Li, J., Lai, C., Wang, R. Y., Chen, X., & Lian, Y. (2018). Drying tendency dominating the global grain production area. *Global Food Security, 16*, 138-149. doi: 10.1016/j.gfs.2018.02.001

Yang, F., & Miao, L. F. (2010). Adaptive responses to progressive drought stress in two poplar species originating from different altitudes. *Silva Fennica, 44*(1), 23-37. doi: 10.14214/sf.160