Research Article

Biochemical parameters and physiological changes in maize plants submitted to water deficiency

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Abstract
This study presents the main changes that occur in the metabolism of corn plants submitted to water deficiency, which can directly affect the development and production of the plants. The aim of this study was to evaluate the biochemical and physiological metabolism responses in maize plants submitted to water deficiency. The experimental design was a randomized block in factorial scheme (3 × 3), being three irrigation depths and three evaluation periods with four replications. The treatments simulated two levels of water deficiency and one control: T1 (control treatment) 10–20 kPa; T2 (moderate water deficiency) 50–60 kPa, and T3 (severe water deficiency) 70–80 kPa. The evaluation periods were E1—45 days after emergence (DAE); E2—52 DAE; and E3—59 DAE. The variables analyzed were relative water content; electrolyte leakage; total soluble proteins; nitrate reductase activity; and activity of the antioxidative response system, namely superoxide dismutase, catalase, and peroxidase. The results showed that stress caused a decrease in the relative water content, reflecting changes in membrane permeability and possible induction of electrolyte losses and an increase in the activities of the enzymes of the antioxidative response system. Thus, corn plants submitted to water deficiency presented interactive responses as a strategy to mitigate the impact of stress.

Keywords Abiotic stress · Water deficiency · Plant metabolism · Antioxidative enzymes

1 Introduction

Maize crops present sensitivity to low soil water availability, especially in the critical period, which starts at flowering and lasts until grain filling. Thus, productivity losses in maize crops in the largest Brazilian producing areas are related to the water availability of each region [1].

Souza et al. [2] explain that the great variability in maize cultures is mainly caused by WD, especially due to the inconstant rainfall regime in the various regions of the country, thus demonstrating the high demand for water by the crop.

Numerous changes occur in the metabolism of plants maintained under abiotic stresses. A stressful environment for crops, such as WD, induces different metabolic events that result in the production of reactive oxygen species (ROS). These cellular components, if not neutralized, can produce several impacts on cellular structures, including cell death.

According to Qi et al. [3], oxidative damage occurs in cell structures when plants are under stress, causing imbalance between antioxidant activity and ROS production. Plants can activate enzymatic and non-enzymatic mechanisms—which can disrupt ROS [4]—in different cell

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compartments, reducing the impact caused by oxidations at the membrane level and other macromolecules.

These enzymes are essential for the proper conservation of all organisms, as they are proteins that catalyze chemical reactions and regulate almost all great diversity of biochemical reactions that compose life [5]. They are found in various plant locations and assist in the ROS balance. Among the antioxidative enzymes are superoxide dismutase (SOD); ascorbate peroxidase (APX, EC 1.11.1.1); glutathione reductase (GR, EC 1.6.4.2); peroxidases (POD, EC 1.11.1.7); catalase (CAT, EC 1.11.1.6), and polyphenol oxidase (PPO, EC 1.14.18.1) [6].

Several studies indicate the sensitivity of maize crops maintained under WD, mainly in the flowering and grain maturation stages. However, few academic researches demonstrate the plant ability to respond to metabolic changes by increasing their tolerance to these stresses.

Therefore, the aim of this study was to evaluate the physiological and biochemical behavior of sweet corn plants submitted to different irrigation depths that simulate moderate and severe WD during the vegetative stage. The main hypothesis is that maize plants produce interactive physiological responses in order to reduce the impact of the treatments applied.

2 Materials and methods

The experiment was conducted at the Institute of Biosciences (IB/UNESP), in the municipality of Botucatu (São Paulo, Brazil), with geographical coordinates of 22° 53′ 33.4″ S and 48° 29′ 36.5″ W and altitude of 840 m above sea level. The local climate is classified as hot and humid temperate, with rainy summer and dry winter, according to Koppen climate classification. The mean annual air temperature is 20.3 °C, and the annual rainfall is 1428.4 mm [7].

2.1 Weather data

In order to monitor the weather conditions during the experiment, temperature and relative humidity measurement were performed with the assistance of an automatic datalogger installed at the central region of the greenhouse and programmed to execute readings at each 30 min. The temperature average during all the period was 27.3 °C, and relative humidity was 66% (Fig. 1a, b).

2.2 Treatments and experimental design

The experimental design was a randomized block in factorial scheme (3x3), being three irrigation depths and three evaluation periods with four replications. Each replication had eight pots. The pots were arranged into subdivided plots, between irrigation depths (plots) and evaluation periods (subplots). The treatments delimited in order to simulate two levels of WD and one control: T1 (control treatment—C)—maintained at a tension from 10 to 20 kPa; T2 (moderate water deficiency—MWD)—maintained at a tension from 50 to 60 kPa; and T3 (severe water deficiency—SWD)—maintained at a tension from 70 to 80 kPa. The evaluation periods were E1—45 days after emergence (DAE); E2—52 DAE; and E3—59 DAE.

All the obtained results were submitted to the analysis of variance (ANOVA), and the means were compared by Tukey's test with $p < 0.05$, using SISVAR 5.5 software.

The experimental units were constituted of polyethylene pots with capacity of 30 L, in dystrophic Red Latosol in sandy loam soil, with the following characteristics: Organic matter = 7 g dm^{-3}; pH ($CaCl_2$) = 4.4; P (resin) = 3.0 mg dm^{-3};

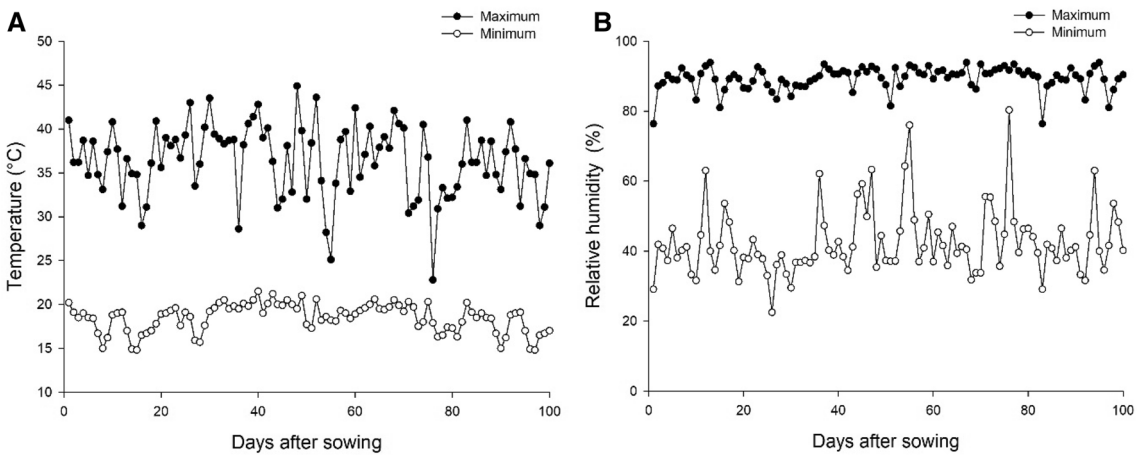

Fig. 1 Average data of the environmental conditions inside the greenhouse, during the experiments. The points represent the daily collections recorded each 30 min

K^+ = 0.34 mmolc dm^{-3}; Ca^{2+} = 6 $mmol_c$ dm^{-3}; Mg^{+2} = 3.0 $mmol_c$ dm^{-3}; H + Al = 23.0 $mmol_c$ dm^{-3}; and CEC (cation exchange capacity = 33.0 $mmol_c$ dm^{-3}; S = 16.0 mg dm^{-3}; B = 0.20 mg dm^{-3}; Cu = 0.6 mg dm^{-3}; Fe = 6.0 mg dm^{-3}; Mn = 1.6 mg dm^{-3}; Zn = 0.2 mg dm^{-3}; Total sand = 77.4%; Clay = 17.7%; Silt = 4.9%; and 30% of saturation bases. Three seeds were sown per pot, and thinning was performed 10 days after sowing, leaving only one plant per pot. The corn cultivar used was *Super sweet*, type Hawaii (Isla Seeds Co). Crop fertilization and topdressing fertilization, performed at V4 and V6 stages, followed the recommendation of Raij et al. [8] for maize crops.

2.3 Crop management and irrigation

The irrigation system was by drip in which self-compensating medium-flow button-type emitters were used (2.0 L h^{-1}) and operation pressure was maintained at 1.0 bar. The distribution uniformity coefficient was calculated and the result found was 97%, classified as excellent according to classification proposed by Bernardo et al. [9].

The soil was maintained in field capacity up to the V7 stage (phenological phase indicating the presence of seven expanded leaves); the plants received 119.6 mm of water, when the treatments proposed for 46 DAE started. After the start of the water treatments until the end of the experiment, 220.6 mm for control plants (10 kPa), 165 mm for plants submitted to MWD and 110.2 mm for plants submitted to SWD was supplied. In total, the plants received 340 mm of water (control), 284 mm for plants submitted to MWD and 229 mm for plants submitted to SWD.

The irrigation management was executed via tensiometer. Four tensiometers were installed per treatment, totaling twelve monitoring points for soil water tension, considering the three irrigation depths studied. For the management via tensiometer, the water tensions corresponding to the depths used in the experiment were determined by means of the characteristic curve of the soil water retention.

The soil water contents for the points corresponding to 10, 30, 50, 100, 300, 500, and 1500 kPa were determined by means of the pressure plate apparatus (Richard's chamber) and modeled in the SWRC v. 3.0 software [10]. In this modeling, the parameters of α, n, m, θr, and θs were generated. The retention curve (Fig. 2) was adjusted considering the model proposed by Van Genuchten [11].

2.4 Physiological and biochemical parameters

The variables analyzed were relative water content (RWC), electrolyte leakage, biochemical analyses, total soluble proteins (TSP), and enzymes of the antioxidative response system, namely superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and nitrate reductase (NR).

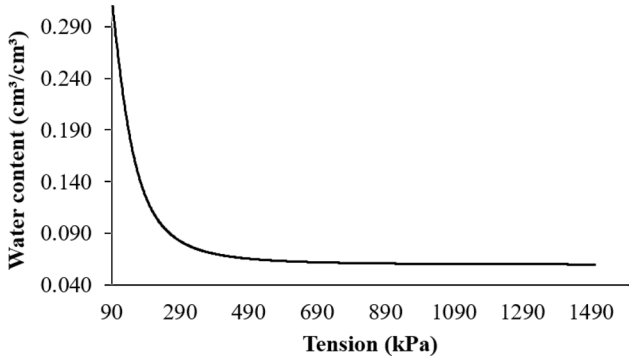

Fig. 2 Soil water retention curve, obtained experimentally using the Richard's Chamber

2.5 Relative water content (RWC) and electrolyte leakage

RWC was determined by the ratio of the mass of the fresh, turgid, and dry vegetable tissues, according to the methodology of Barr et al. [12], and electrolyte leakage was determined using the methodology described by Lafuente et al. [13]. This analysis verifies modifications that may occur in the permeability of cell membranes due to treatments.

2.6 Biochemical analyses

For the biochemical analyses, leaf samples were collected between 8 AM and 9 AM. At the collection time, the leaves were immediately wrapped in aluminum foil envelopes and immersed in liquid nitrogen for rapid freezing. The material was then transferred to a freezer at -80 °C until the analyses were performed.

The extract for the analysis of the protein concentration and enzymatic activity (Superoxide dismutase—SOD, Catalase—CAT, and Peroxidase—POD) was obtained via resuspension of the plant material (300 mg) in 4.0 mL of 0.1 M potassium phosphate buffer, pH 7.8, supplemented with 300 mg of polyvinylpolypyrrolidone (PVPP). After 10 min of centrifugation at 5000×*g*, the supernatant was collected, transferred to Eppendorf, and stored in a freezer at − 80 °C.

2.7 Total soluble proteins (TSP)

TSP content present in the crude extract was determined according to Bradford [14]. For the test, 100 µL of crude extract was mixed with 5 mL of Bradford reagent, and the solution was maintained for 15 min to form the color

complex. The readings were then performed in a spectrophotometer at 595 nm.

2.8 Superoxide dismutase (SOD)

SOD activity was determined following the methodology described by Giannopolitis and Ries [15]. This enzyme detection is based on the prevention of NBT photoreduction in the presence of SOD. Quantitative analysis was performed based on the optical density reading of the blue complex formed (Formazan) at 560 nm. One SOD unit is regarded as the amount of enzyme enough to inhibit 50% of NBT photoreduction. The enzyme activity calculation uses the inhibition percentage obtained, sample volume, and protein concentration in the sample (μg μL^{-1}).

2.9 Catalase (CAT)

CAT activity was determined by the methodology described by Lock [16], by monitoring the absorption of hydrogen peroxide (H_2O_2), considering the absorption interval of 8 to 40 s. The enzyme activity was calculated using the molar extinction coefficient [$\varepsilon = 39.4\ mM^{-1}\ cm^{-1}$]. CAT specific activity (μKat μg $Prot^{-1}$) considered the soluble protein concentration.

2.10 Peroxidase (POD)

POD activity was determined according to the methodology proposed by Lock [16]. The enzyme-specific activity (μKat μg $Prot^{-1}$) was calculated using the $\varepsilon = 2.47\ mM^{-1}\ cm^{-1}$.

2.11 Nitrate reductase (NR)

In order to determine the NR enzyme, leaves from maize plants were collected at 9 AM, preserving the minimum photoperiod of two hours. To perform the enzymatic analysis, 150 mg of leaf tissue was cut and packed into tubes along with 5 mL of the extraction solution, which was composed of phosphate buffer (0.1 M KH_2PO_4, pH 7.5), KNO_3 (0.1 M), and n-propanol (3% v/v). After that, the tubes were incubated in vacuum in three cycles of two minutes each, with an interval of 1 min. NR activity was determined through 1 mL of the sample added 1 mL of sulfanilamide and 1 mL of 0.02% 1-naphthyl ethylenediamine. The reading, in absorbance, was performed in a spectrophotometer at 540 nm, as described by Jaworski [17].

3 Results

Sweet corn plants submitted to WD levels presented physiological changes related to the reduction in water availability. ANOVA results for RWC and electrolyte leakage are given in Table 1.

Considering the irrigation depths and evaluation periods, we could observe that they have differed between the variables analyzed by means of the F test ($p < 0.01$). The variables interacted, thus showing that they are both dependent.

RWC results indicate that there was a significant effect of the interaction between depths and periods (Fig. 3). The highest RWC value was found in plants maintained in field capacity (control), varying 80%, 78%, and 79% in relation to the evaluation periods (with no significant difference between them).

When analyzing the irrigation depths as a stress effect, in the first evaluation period, there was no significant difference. However, under MWD and SWD, the plants showed 70 and 64% RWC in the second season, respectively. In the third evaluation period, the plants showed a decrease of 22 and 26% for MWD and SWD, respectively, when compared to the first evaluation period.

The values for electrolyte leakage are shown in Fig. 4 and indicate a significant difference between the treatments applied. At the beginning of the treatments, when the plants were under field capacity, the electrolyte leakage ranged from 7.09% to 8.74%.

The treatments under SWD in the second and third evaluation periods presented results of damages in the most marked membranes (32% and 36%, respectively).

Table 1 ANOVA for relative content of water (RWC) in leaf tissue and electrolyte leakage (EL) due to different levels of water deficiency (WD) and evaluation periods in sweet corn plants

Sources of variation	Degrees of freedom	F calculated	
		RWC	EL
Blocks	3	0.7151	0.7617
DEP	2	0.0005**	0.0000**
RES (A)	6	–	–
PER	2	0.0000**	0.0000**
DEP*PER	4	0.0000**	0.0000**
Res (B)	18	–	–
Total	35	–	–
CV (%) plot		3.62	2.67
CV (%) subplot		2.40	3.32

CV coefficient of variation, *DEP* irrigation depths, *PER* collection periods

**Significant ($p < 0.01$)

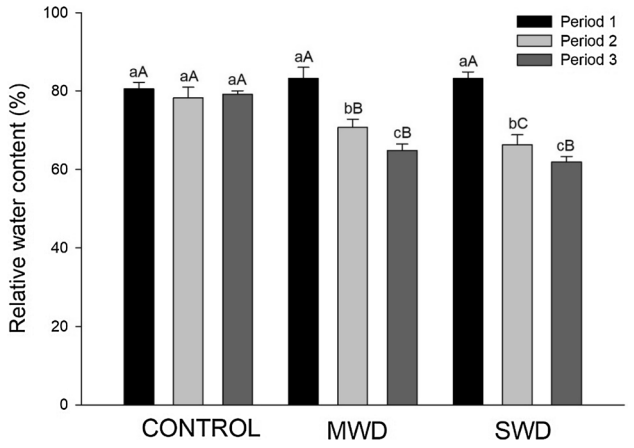

Fig. 3 Relative water content (RWC) in leaves (%) according to the evaluation periods and water treatments (C, MWD, and SWD) in sweet corn plants. Means followed by the same uppercase letter for the depths and lowercase for the periods do not differ by Tukey's test ($p < 0.05$). Slashes indicate the mean standard deviation of four replications. MWD and SWD represent moderate and severe water deficiency, respectively

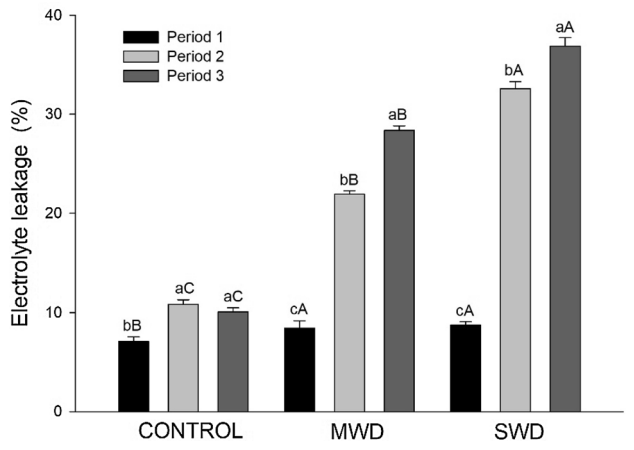

Fig. 4 Electrolyte leakage (%) according to the evaluation period and water treatments (C, MWD, and SWD) in sweet corn plants. Means followed by the same uppercase letter for the depths and lowercase for the periods do not differ by Tukey's test ($p < 0.05$). Slashes indicate the mean standard deviation of four replications. MWD and SWD represent moderate and severe water deficiency, respectively

However, plants submitted to MWD indicated smaller values when compared to plants under SWD: 21% and 28% in the first and second evaluation periods, respectively. Plants submitted to control treatment showed cell membrane integrity throughout the three evaluation periods, with maximum electrolyte leakage of 10.83% in the second evaluation period.

Biochemical parameters are of great importance for understanding the plant ability to respond to WD. In the present study, TSP content and three enzymes related to the antioxidative response system (SOD, CAT, and POD) were evaluated. In addition, NR activity, related to the process of nitrate assimilatory reduction, was monitored.

Irrigation depths and evaluation periods influenced the TSP contents as well as the activity of antioxidant enzymes (SOD, CAT, and POD) in leaf tissues of sweet corn plants (Table 2). Moreover, NR was also induced temporally by physical stress and in function of the stress severity. The factors interacted for all variables analyzed.

At the beginning of the treatments, plants had mean protein contents of 18.1 mg g^{-1} MF, and there was no significant difference between the irrigation depths (Fig. 5a). However, in the second evaluation period, plants under MWD and SWD presented values of 13.9 and 13.7 mg g^{-1} MF, with decrease of 22% and 24%, respectively, when compared to the control treatment. Control plants presented a mean of 18.26 mg g^{-1} MF in the three evaluation periods. Thus, overall, the levels of proteins in plants under MWD and SWD decreased. However, there was no significant difference between the first and second evaluation periods.

SOD activity was similar in the first and third evaluation periods for control treatment, and only the second period differed from the others (Fig. 5b). In the third evaluation period, the plants under MWD showed an activity of 1.46 IU µg protein^{-1}, with an increase of 64% in the enzyme activity in relation to the first season. Likewise, there was an increase in enzyme activity for plants grown under SWD, with activity of 1.95 IU µg protein^{-1}, in the third evaluation period—a significant difference between the evaluation periods.

POD activity in leaves under SWD was 130% higher in the third evaluation period when compared to the control treatment at the same evaluation period (Fig. 5c), presenting activity of 3478.5 µkat µg protein^{-1}. The enzyme activity increased in all water treatments. However, under control treatment, the plants showed values from 1229.79 to 1510.13 µkat µg protein^{-1}, while those under MWD showed activity from 1234.7 to 2452.5 µkat µg protein^{-1}. In the third evaluation period, plants grown under SWD showed an increase in the enzyme activity of 172% in relation to the first period, showing the severity of stress.

During the experimental period, CAT activity in leaves under control treatment had a mean value of 83 µkat µg protein^{-1}, with a significant difference only for the first evaluation period (Fig. 5d). However, plants under MWD and SWD in the third evaluation period showed CAT activity in the of 267.3 and 366.6 µkat µg protein^{-1}, respectively. There was a reduction in the activity of the enzyme nitrate reductase according to the intensity and severity of the stress. Plants under control treatment increased by 10% in the enzyme activity in the third evaluation period when

Table 2 ANOVA for total soluble protein content (TSP) and activity of the enzymes: nitrate reductase (NR), superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), according to the different levels of WD and evaluation periods in leaf tissues of sweet corn plants

Sources of variation	Degrees of freedom	F calculated				
		TSP	NR	SOD	POD	CAT
Blocks	3	0.2281	0.5188	0.2911	0.4169	0.0434
DEP	2	0.0002**	0.0006**	0.0000**	0.0000**	0.0000**
RES (A)	6	–	–	–	–	–
PER	2	0.0000**	0.0000**	0.0000**	0.0000**	0.0000**
DEP*PER	4	0.0001**	0.0000**	0.0000**	0.0000**	0.0000**
Res (B)	18	–	–	–	–	–
Total	35	–	–	–	–	–
CV (%) plot		8.26	10.85	7.87	8.79	9.94
CV (%) subplot		9.04	10.82	5.92	7.06	8.43

CV coefficient of variation, *DEP* irrigation depths, *PER* collection periods

**Significant ($p < 0.01$)

compared to the first one (Fig. 5e). In this treatment, the enzyme activity varied from 2167.7 to 2391.7 nM NO_2 h^{-1} g^{-1} FW. However, moderate stress caused a 39% reduction in enzyme activity for the third evaluation period (1348.8 nM NO_2 h^{-1} g^{-1} FW). Likewise, the severity of stress (SWD) caused a reduction in the activity of the enzyme, with a value of 1000.4 nM NO_2 h^{-1} g^{-1} FW, representing a 56% reduction, when compared to the first evaluation period.

4 Discussion

The results presented indicate the sensitivity of corn plants when subjected to water stress. One of the main indicators of water deficiency in plants is the reduction in the relative water content of leaf tissues, which can induce the loss of electrolytes. Considering the level and intensity of physical or biological stresses, plants can respond to environmental stimulation, showing an increase in the loss of electrolytes due to damage caused to cell membranes. Plants submitted to WD treatments presented significant reductions in the second and third evaluation periods. BASU et al. [18] observed that the RWC in maize leaves was affected by WD, as they noted the RWC value of 60% for plants submitted to WD.

Water stress causes water loss within the plant and therefore a reduction in its relative water content. In this sense, one of the most reliable and widely used indicators to define both sensitivity and tolerance to water stress in plants is the relative leaf water content [19].

As WD intensifies, plants are negatively affected in relation to protoplasm dehydration, causing disturbances in the plant vital processes [20], which explains the results of variable electrolyte leakage, in which the WD applied damaged the cell membranes. Under stress, membranes usually increase the permeability related to electrolyte leakage [21].

The analysis of electrolyte losses in plant tissues can reveal the level of stability of the cell membrane and can be associated with the generation of reactive oxygen species (ROS), which can lead to damage to macromolecules and cell structures [22].Plants submitted to water deficiency present higher production of O_2—and H_2O_2. These compounds are extremely toxic and, in excess, cause lipid peroxidation, damaging cell membranes, and increasing electrolyte leakage [23].

WD decreases the soluble protein concentrations in leaves by increasing the activity of proteolytic enzymes, which degrade proteins, thus decreasing their synthesis [24, 25]. Also, that happens due to the reduction in the protein biosynthesis rate and the increase in protein deterioration in plants under water deficiency conditions. According to Nawaz et al. [26], this reduction in the total soluble protein content may be related to the increase in protease activities, in which its resultant are amino acids essential for osmotic adjustment.

Plants have developed biochemical responses to survive in WD environments. These responses are formed by an antioxidative system and have been studied as indicators/markers of plant stress.

Isolated or combined biotic and abiotic stresses block plant development and production, especially as they cause serious damage to cellular and biochemical physiology due to oxidative stress [27]. Oxidative stress occurs when the production of reactive oxygen species (H_2O_2, $O_2^{\cdot-}$, $OH^{\cdot}$ or O_2) is greater than the activity of the antioxidant response system, thus bringing severe consequences, with damage to lipids, proteins and DNA, culminating in cell death [28]. Plants in turn eliminate these free radicals by antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and other enzymes, which, when associated, cause cellular detoxification [6].

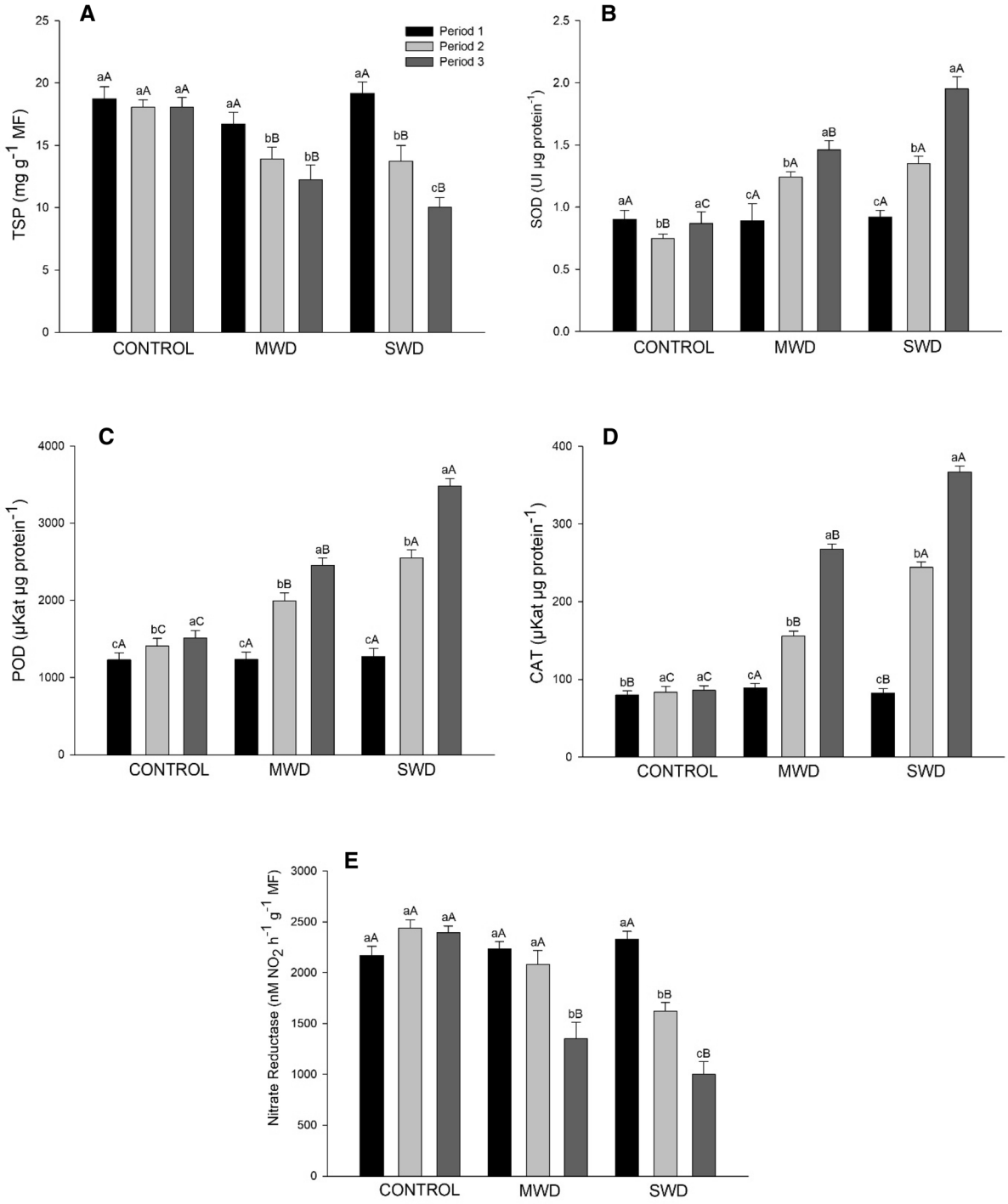

Fig. 5 **a** Total soluble protein content (TSP); **b** superoxide dismutase (SOD) activity; **c** peroxidase (POD) activity; **d** catalase (CAT), and **e** nitrate reductase (NR), according to the different evaluation periods and water treatments (C, MWD, and SWD) in leaf tissues of sweet corn plants. Means followed by the same uppercase letter for the depths and lowercase for the periods do not differ by Tukey's test ($p < 0.05$). Slashes indicate the mean standard deviation of four replications

Anjum et al. [29] observed increased CAT and SOD activity in maize plants under SWD. When studying the antioxidative defense mechanisms against oxidative stress caused by WD, Avramova et al. [30] concluded that the increased enzyme activity SOD, CAT, and POD is related to the maintenance of the oxidative equilibrium under stress conditions.

In the present study, the enzyme activity increased in response to stress intensity and severity. Studies have indicated that the activity of one or more antioxidant enzymes (such as SOD and POD) is observed in plants exposed to stressful conditions, and the increased activity may be related to the increased stress tolerance [31].

The greatest SOD activity under stress conditions promoted greater CAT and POD activities, since SOD is the first enzyme involved in the plant defense system, acting on the superoxide radical dismutation ($^{\cdot}O_2^-$) in hydrogen peroxide (H_2O_2), substrate of peroxidase catalysis [6].

However, among the enzymes evaluated, WD promoted a substantial increase in the CAT activity. Jaleel et al. [32] state that the increased activity of this enzyme occurs because it is the main enzyme to catalyze H_2O_2 elimination. Iqbal et al. [33] infer that CAT is one of the most effective enzymes in the defense against oxidative processes. Thus, high enzyme activities in corn plants under stress conditions represent better acclimatization capacity of the species.

Like the enzymes of the antioxidative response system, the enzyme nitrate reductase was influenced by water deficiency treatments. NR is mainly responsible for the assimilation of nitrogen by plants, which is an important nutrient for plant development. However, this enzyme activity undergoes negative influence from the soil water availability [34].

The relationship between nitrogen and the amount of soil water is of great importance since this nutrient is directly related to the development of plants cultivated in places under water scarcity. NR activity reduction decreases the formation of amino acids, proteins, and chlorophylls, interfering with the plant growth and development [24].

The stress caused by water deficiency induced metabolic changes in corn plants interactively, which were monitored from water relations parameters and enzyme activity related to the antioxidative response system.

5 Conclusions

Corn plants showed high sensitivity to water deficiency. The intensity of the stress changed parameters of water relations, such as relative water content and loss of electrolytes. Enzymes of antioxidative metabolism were induced in response to water stress severity. The integrated responses indicate a strategy to mitigate the impact of stress.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests

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