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CROP PRODUCTION

Oxidative damage associated with salt stress during germination and initial development of purple corn seedlings

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ABSTRACT. In various parts of the world, agricultural exploitation faces saline soil or water, such that cultivable species tend to be limited regarding the establishment of seedlings, with effects on productivity. The objective of this study was to evaluate the effects of salinity levels associated with different temperatures on the germination, initial development, and oxidative damage indicators of purple corn seedlings. The experiment was completely randomized in a 5 × 2 factorial design (moistening of the germination paper with 0, 25, 50, 75, and 100 mM NaCl solutions at temperatures of 25 and 30°C). The parameters evaluated were germination, growth, and oxidative damage indicators at the seedling phase. The germinative decline and initial development of purple corn seedlings, regardless of the temperature (25 and 30°C), reflected oxidative damage resulting from saline stress. Although deleterious effects of salinity were observed, a temperature of 30°C provided greater length and accumulation of dry mass of purple corn seedlings compared to the effects at 25°C. Total chlorophyll, chlorophyll *a*, chlorophyll *b*, total carotenoids, and lipid peroxidation, regardless of the temperature (25 and 30°C), were identified as sensitive biochemical indicators for the detection of physiological quality of purple corn seedlings subjected to NaCl.

Keywords: *Zea mays*; anthocyanins; physiological potential; biochemical indicators.

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Introduction

Corn (*Zea mays* L.) is one of the most important cereals in the world economy. It is characterized by different forms of use, ranging from human and animal food to industrial uses. Pigmented corn and its products have received increasing attention from a nutraceutical point of view because of their potential health benefits (Chavarín-Martínez et al., 2019). Maize varieties with grains of different colors exist, including purple corn, a variety whose color is caused by the high concentration of anthocyanins (Mansilla, Nazar, & Pérez, 2020).

The endosperm of the purple corn caryopsis contains cyanidin-3-glucoside, which is an anthocyanin used as a natural dye and has antioxidant (Harakotr, Suriharn, Tangwongchai, Scott, & Lertrat, 2014), antidiabetic (Thiraphatthanavong et al., 2014), anti-inflammatory (Liu, Li, Zhang, Sun, & Xia, 2014), and anticancer properties (Joshi et al., 2017). Anthocyanins are used as food colors, mainly in the beverage and food industries. Currently, there is a concern among consumers and manufacturers regarding the use of artificial food colors, creating a demand for natural food-based dyes (Lao, Sigurdson, & Giusti, 2017).

One of the obstacles to the cultivation of purple corn may be the salinity in agricultural land or irrigation water. Salinity reduces growth and biomass, directly affecting the operation of stomatal functions and modifying sweat and respiration (El-Esawi & Alayafi, 2019). Additionally, excess salts promote oxidative damage in lipids, proteins, and nucleic acids (Elkelish et al., 2019).

Thus, more research in this area will be useful in the elucidation of salinity damage in industrially important species. Therefore, this study evaluated the effect of salinity concentrations associated with different temperatures on germination, initial development, and oxidative damage indicators of purple corn seedlings.

Material and methods

The experiments were conducted at the Biotechnology Laboratories and the Graduate Program in Plant Production at the Federal Rural University of Pernambuco, Serra Talhada Academic Unit, Pernambuco State, Brazil. Purple corn seeds, produced in the 2018/2019 harvest, were obtained from a rural producer residing in the Sabino Ranch, Tupanaci District of the City of Mirandiba, Pernambuco State, Brazil.

Initially, the water content of seeds was evaluated using the oven method at 105 ± 3°C for 24h, according to the Rules for Seed Analysis (Brasil, 2009). The average water content of the seeds at the time of installation of the experiments was 11.76%.

During the germination process, purple corn seeds were subjected to different levels of salinity (0, 25, 50, 75, and 100 mM NaCl) at temperatures of 25 and 30°C.

Evaluations

Germination

Paper towel sheets for germination were previously moistened with different concentrations of sodium chloride solution equivalent to 2.5 times the dry paper mass; distilled water was used as a control. Before sowing, the seeds were disinfected with 2.5% sodium hypochlorite for 4 min. (Brasil, 2009). The treatments were kept in a biochemical oxygen demand (BOD) germination chamber (Marconi MA 1402/546) for 7 days at constant temperatures of 25 and 30°C and a photoperiod of 12h. As a germination criterion, the number of normal seedlings was evaluated on the seventh day after sowing (Brasil, 2009).

Seedling length

Paper towel sheets for germination were previously moistened with different quantities of sodium chloride solution equivalent to 2.5 times the dry paper mass. After sowing, the treatments were kept in a BOD for 7 days under constant temperatures of 25 and 30°C and a 12h photoperiod. Measurements of normal seedlings occurred on the seventh day after sowing. The shoot length (SL) was evaluated using a millimeter ruler from the base to the end of the largest leaf. The root system length (RSL) was measured from the base to the tip of the root, with average results expressed in cm seedling^{-1}.

Seedling dry matter

After the length evaluation, all normal seedlings were sectioned at the base region to separate the shoots from the root system, in addition to removing traces of caryopsis. The dry mass was determined using a forced air circulation oven for 24h at 80°C. Thereafter, the shoot dry matter (SDM), root system dry matter (RSDM), and total dry matter (TDM) of normal seedlings were determined.

Indicators of oxidative damage

Photosynthetic pigment content

To determine the chlorophyll content, 0.1 g of fresh leaf mass was weighed and placed in identified test tubes. Chlorophyll was extracted by adding 5 mL of acetone (80% v/v). The tubes were hermetically sealed, covered with aluminum foil, and kept refrigerated for 48h. After this period, spectrophotometer readings were taken at wavelengths of 645, 652, and 663 nm for the determination of chlorophyll (Arnon, 1949) and at a wavelength of 470 nm (Lichtenthaler & Buschmann, 2001) for the determination of total carotenoids.

Lipid peroxidation

Lipid peroxidation was estimated by the content of thiobarbituric acid reactive substances (TBARS) according to Heath and Packer (1968). Analyses of shoots and the root system of normal seedlings were conducted using 0.1 g of plant material per replication. The plant material was mashed in a mortar in the presence of liquid nitrogen followed by the addition of 6% trichloroacetic acid (TCA); this was macerated for an additional 3 min., and the extract was centrifuged at 12,000 × *g* at 4°C for 15 min. Then, 0.5 mL of the supernatant was added to 2.0 mL of 20% TCA solution and 0.5% thiobarbituric acid (TBA) (w/v), and the solution was heated in a water bath at 95°C in hermetically sealed tubes for 1h. Next, the reaction was stopped in an ice bath, and readings were taken at 532 nm and 660 nm using a spectrophotometer. The TBARS content was estimated using the molar extinction coefficient of 155 mM^{-1} cm^{-1} after subtracting the absorbance obtained at 660 nm from that at 532 nm.

Experimental design

The experiment was completely randomized in a 5 × 2 factorial design (concentrations 0, 25, 50, 75, and 100 mM NaCl × temperatures of 25 and 30°C), with four replications of 50 seeds (germination), four replications of 20 seeds (seedling length and dry matter tests), and three replicates of 5 normal seedlings from the germination test (oxidative damage indicators). Thereafter, an analysis of variance was performed using the F test ($p < 0.05$). When a significant effect of the interaction was verified in the analysis of variance, the treatments were subjected to regression analysis. In the absence of a significant interaction, the averages of the variables by temperature were compared via Tukey's test ($p < 0.05$), and the means of the concentrations were analyzed by regression analysis. All analyses were performed using the statistical software SISVAR v. 5.6 (Ferreira, 2011). To plot the graphics, the software Sigma Plot 10.0 was used.

Results

According to the statistical analysis, significant differences occurred for all variables studied in relation to saline concentrations. Considering the individual effects of air temperature, only the variable RSL was nonsignificant. Regarding the interaction between salt concentrations and temperatures, the GP, SDM, and TDM did not show significant effects. The germination of purple corn seeds decreased as salt concentrations increased, with lower germination percentages at the concentrations of 75 and 100 mM (Figure 1). The temperatures 25 and 30°C resulted in significant differences in the germination of purple corn seeds, and 30°C resulted in a higher percentage of germination (Figure 2).

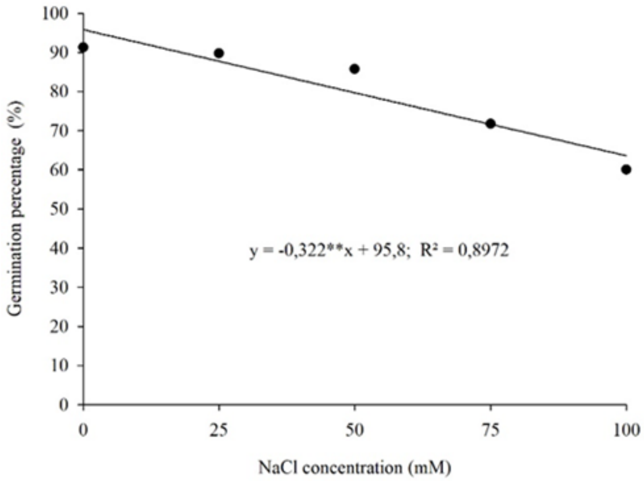

Figure 1. Germination percentage of purple corn seeds submitted to different salt concentrations.

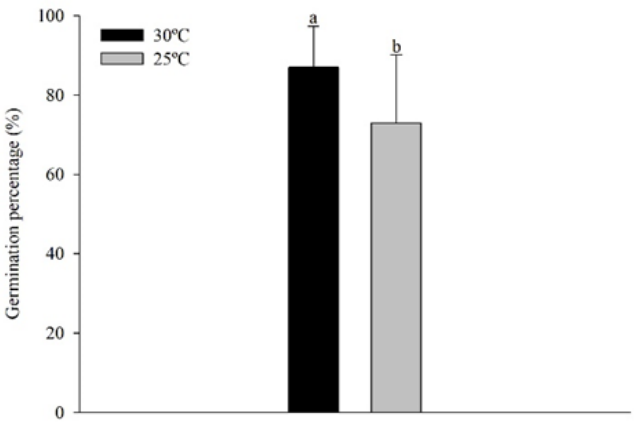

Figure 2. Germination of purple corn seeds submitted to different temperatures. Mean comparison using Tukey's test ($p < 0.05$).

The interaction between salt concentrations and temperatures indicated a significant difference for the variables SL and RSL. At 30°C in both salt concentrations, there was greater SL and RSL (Figures 3A, 3B, and 4). Additionally, the shoots of the seedlings were less affected at concentrations of 25, 50, and 75 mM NaCl at 30°C than at 25°C.

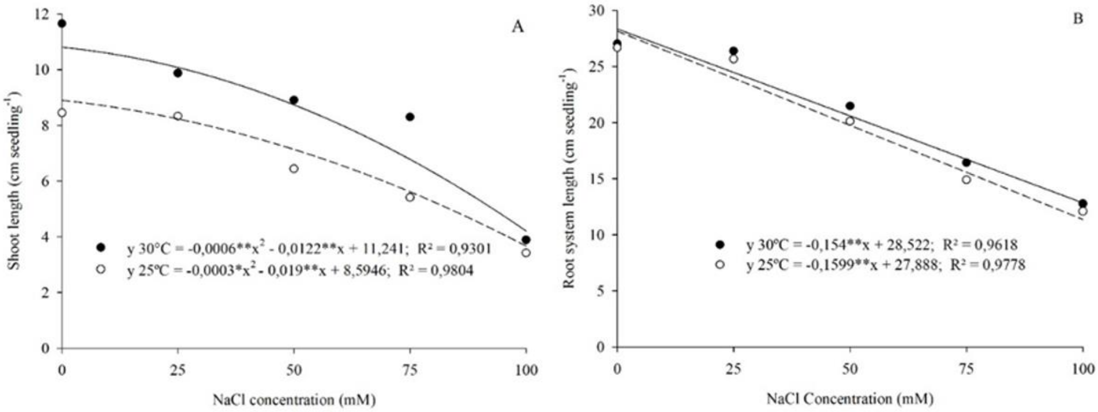

Figure 3. Shoot length (A) and root system (B) of purple corn seedlings submitted to different salt concentrations and temperatures.

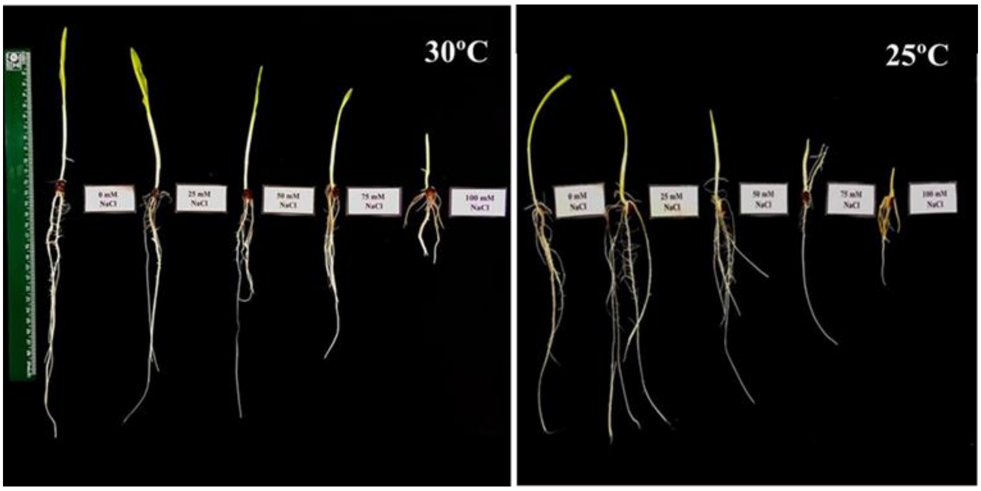

Figure 4. Length of purple corn seedlings submitted to different salt concentrations and temperatures.

The TDM and SDM of seedlings were affected linearly and negatively with the increase in saline concentrations (Figure 5A). The temperature of 30°C promoted a greater accumulation of TDM, SDM, and RSDM of seedlings. By comparing the SDM and the RSDM, a greater accumulation of reserves for roots occurred in the purple corn seedlings (Figure 5B). Regarding the interaction at both temperatures, there was a reduction in the RSDM with the increase in saline concentrations (Figure 6), and 25°C resulted in a lower RSDM compared to that at 30°C.

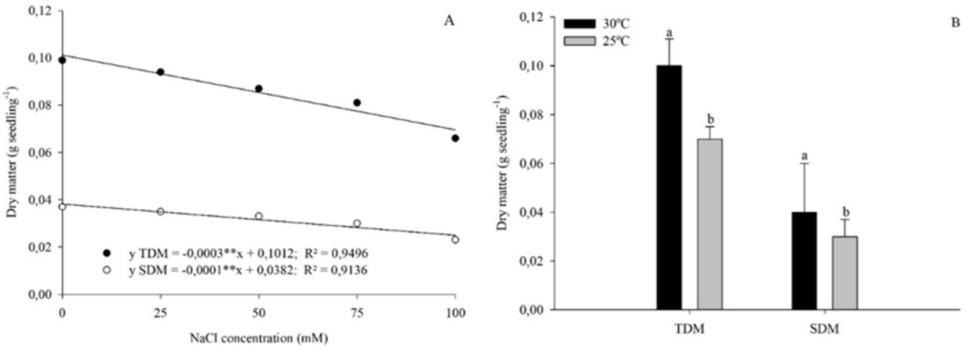

Figure 5. Total dry matter (TDM) and shoot dry matter (SDM) (A) of purple corn seedlings submitted to different salt concentrations and TDM and SDM (B) of purple corn seedlings submitted to different temperatures. Mean comparison using Tukey's test ($p < 0.05$).

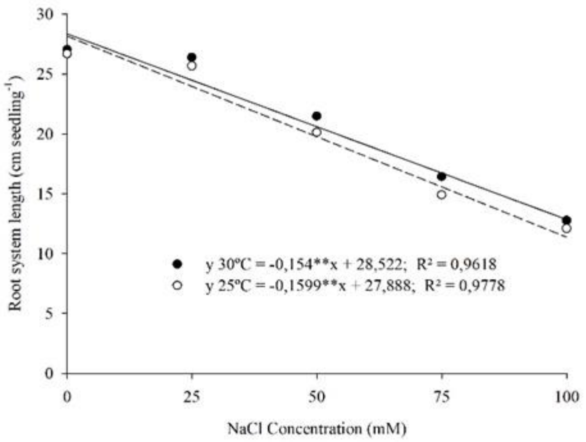

Figure 6. Root system dry matter (RSDM) of purple corn seedlings submitted to different salt concentrations and temperatures.

For total chlorophyll, chlorophyll *a*, chlorophyll *b*, total carotenoids, and lipid peroxidation (TBARS), there were no significant effects of air temperature or its interaction with saline concentrations. The contents of total chlorophyll, chlorophyll *a*, chlorophyll *b*, and total carotenoids were negatively and linearly affected by increases in saline concentrations (Figure 7A and B). In the evaluation of lipid peroxidation (TBARS), the shoots and the root system of purple corn seedlings showed higher values at higher salt concentrations (Figure 8).

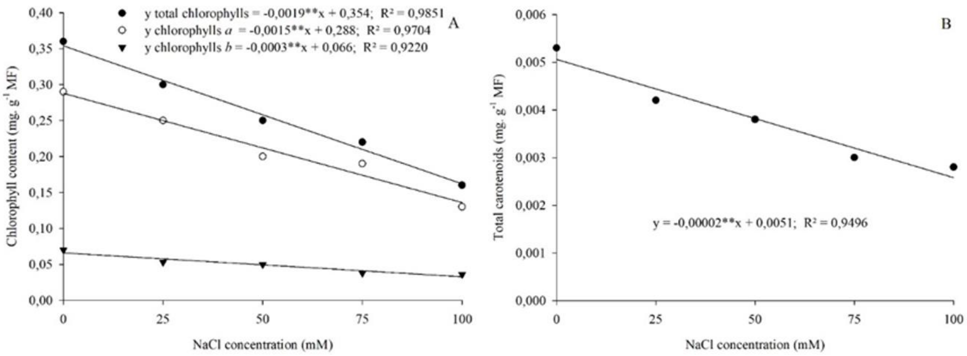

Figure 7. Contents of total chlorophyll, chlorophyll *a*, and chlorophyll *b* (A) and total carotenoids (B) in leaves of purple corn seedlings submitted to different salt concentrations, independent of temperature.

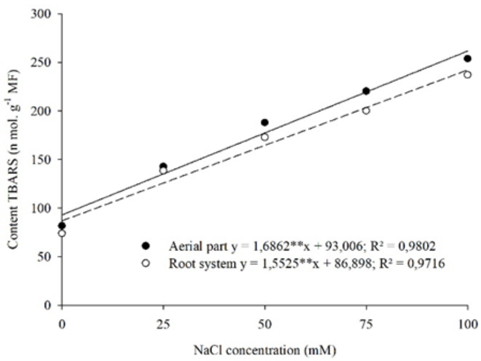

Figure 8. Peroxidation of lipids from the aerial part and root system of purple corn seedlings submitted to different salt concentrations, independent of temperature.

Discussion

The reduction in the germination of purple corn seeds with the increase in the concentration of NaCl (Figure 1) was associated with the toxic effects caused by the salt ions. The decline in vigor promoted by salt stress resulted from increased immobilization of reserves and dysfunction in cell membranes (Taiz, Zeige, Moller, & Murphy, 2017). The use of saline water for irrigation and soil salinity are among the most serious environmental stresses in arid and semiarid areas (Abido & Zsombik, 2019), directly affecting germination and seedling emergence, the critical stages of plant life cycle. The lower osmotic potential of the soil solution slows water absorption by seeds. Additionally, salt stress induces oxidative damage of cells (Shahid et al., 2020).

The decrease in temperature promoted a lower rate of biochemical and physiological activities involved in the metabolism of cells that act in the germinative process of seeds. Thus, 30°C resulted in greater germination and growth of shoots and the root system of purple corn seedlings compared to 25°C (Figures 2, 3A, 3B, and 4). High temperatures up to acceptable limits lead to rapid imbibition, and this phenomenon can be explained by reduced viscosity and increased kinetic energy of water, which accelerate the speed of metabolic reactions of seeds (Marcos-Filho, 2015).

The optimum temperature for seed germination of purple, red, yellow, and white corn was 30°C (Deng, Yang, Zhang, & Li, 2015); the purple corn had a higher germination rate than others when subjected to 40°C. Moreover, Paucar-Menacho et al. (2016) found that the germination of purple corn at 26°C for 63 h allowed seedlings with high levels of phytochemicals, indicating the potential of consumption of seedlings as beneficial food for health promotion. At the initial stage of development, corn seedlings have a survival strategy under saline stress: they invest more energy in the growth of shoots because these structures are responsible for photosynthesis (Silva et al., 2016). This survival mechanism was also observed in this study, in which the SL of purple corn seedlings was less affected by the saline concentrations (25, 50, and 75 mM NaCl) at 30°C (Figure 3A). Bose and Snehasish (2018) evaluated four corn varieties under salt stress and found that seedling growth and initial development decreased at a salinity level of 8 dS m^{-1} (approximately 100 mM), corroborating the results obtained in the present work.

The reduction in TDM and SDM of purple corn seedlings (Figure 5A) occurred because of the increase in the salt content in the substrate that hindered the absorption of water by the roots and provided greater absorption of toxic ions. Similar results were found by AbdElgawad et al. (2016), who reported a decrease in the biomass of roots and leaves of corn with an increase in salinity. Additionally, the authors reported that the accumulation of Na^+ and Cl^- ions in leaf tissues may have caused stomatal closure and damage to the photosynthetic machinery, which, in turn, resulted in lower assimilation of CO_2, growth, and accumulation of biomass.

Under the conditions of saline stress associated with temperature, the RSDM was negatively affected by the increase in sodium chloride concentrations (Figure 6). This reduction in dry matter may have been caused by the competition established by sodium salts for the absorption sites in the plasma membrane of roots.

The higher percentages of germination, SL, RSL, and RSDM of purple corn seedlings at different salt concentrations at 30°C in relation to that at 25°C (Figures 2, 3A, 3B, 4, and 6) can be attributed to more favorable conditions for triggering the biochemical and physiological processes associated with corn germination, thus emphasizing that the germination parameters can be used for the selection of plants tolerant to salt stress (Aflaki, Sedghi, Pazuki, & Pessarakli, 2017).

Corn is grown in regions with temperatures of approximately 18-27°C; however, it can be grown at 33-38°C with excellent yield (Koirala et al., 2017). Therefore, 30°C is within the temperature range for the growth of primary roots. It is known that a drop in temperature slows the rhythm of biochemical and physiological activities involved in the metabolism of cells, thus delaying the development of organs, such as roots, leaves, and stems. Carvalho, Silva, Reisa, Guimarães, and Santos (2017) studied different varieties of corn and found that a temperature of 30°C provided the best results regarding the morphological formation of plants.

Purple corn seedlings when subjected to salt stress caused by increasing concentrations of sodium chloride showed a reduction in total chlorophyll, chlorophyll *a*, chlorophyll *b*, and total carotenoid contents (Figures 7A and 7B). The deleterious effects of salinity caused by osmotic stress, nutritional imbalance, and oxidative stress promote decreases in chlorophyll content, leading to a decrease in the photosynthetic efficiency of plants (Çiçek, Oukarroum, Strasser, & Schansker, 2017). Similar results were observed by Torğut and Akbulut (2018), who found a reduction in total chlorophyll, chlorophyll *a*, chlorophyll *b*, and total carotenoid contents in corn plants under high salt stress.

It appeared that the increase in concentrations of sodium chloride induced higher peroxidation of lipids in shoots and the root system of purple corn seedlings (Figure 8). This likely occurred because of the addition of several reactive oxygen species (ROS) in plants under stressed conditions that possibly caused the rupture of the membrane system. According to AbdElgawad et al. (2016), corn plants under stress conditions generated many ROS, which subsequently induced membrane lipid peroxidation, increasing the content of malondialdehyde (MDA), one of the main products of lipid peroxidation (Ayala, Muñoz, & Argüelles, 2014). Torğut and Akbulut (2018) found that salt stress caused an increase in MDA content in corn plants.

The proper establishment of corn culture in the field, including the occurrence of favorable conditions for germination and seedling emergence, is essential to ensure an ideal plant stand and obtain high yields. However, the results of this research revealed that the environmental factors such as soil salinity and temperature can affect the germination and initial development of purple corn seedlings. The results obtained under laboratory conditions revealed that the increase in concentrations of NaCl reduced germination, initial seedling development, and oxidative damage indicators of purple corn seedlings. However, salt stress conditions favored germination at 30°C compared with that at 25°C, providing a greater length and accumulation of SDM. Furthermore, the results indicated that total chlorophyll, chlorophyll *a*, chlorophyll *b*, total carotenoids, and lipid peroxidation are sensitive biochemical indicators for detect the physiological quality of seeds and vigor of purple corn seedlings.

Conclusion

The germinative decline and the initial development of purple corn seedlings, regardless of the temperature (25 and 30°C), reflected oxidative damage resulting from saline stress. Although there were deleterious effects of salinity, 30°C provided greater length and accumulation of dry mass of purple corn seedlings compared to 25°C. Total chlorophyll, chlorophyll *a*, chlorophyll *b*, total carotenoids, and lipid peroxidation, regardless of the temperature (25 and 30°C), were found to be sensitive biochemical indicators for detection of the physiological quality of purple corn seedlings subjected to NaCl.

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