

Salt stress in popcorn genotypes trigger changes of antioxidant enzymes

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Abstract

Salinity is a major problem in agriculture because it can alter the metabolism of plants and affect crop yield. This study aimed to evaluate the effect of NaCl on growth, key antioxidants and changes in the expression of genes encoding antioxidant enzymes. Two popcorn genotypes, IAC125 and UFMV2, experienced reduction in growth as the salt concentration increased. Increase in chlorophyll content and damage to the plasma membrane was observed. Consequently, changes in osmotic activity led to reduced water content in the leaves. Increased concentration of salt increased the activity of antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) in two popcorn genotypes but maximum activity was observed in the IAC125 genotype. Such enzymatic activities occur in order to maintain the levels of lipid peroxidation under salt stress, indicating that this genotype is tolerant to salinity conditions. The *ZmAPX*, *ZmCAT*, *ZmSOD (Cu/Zn)* and *ZmSOD (Mn)* genes increased their expression as salinity increased. The *ZmSOD (Fe)* gene was highly regulated in the IAC125 genotype under salt stress, but low regulation was observed in the UFM2 genotype, regardless of the salt concentration. The enhancement in tolerance against salt stress indicates that the genes involved in the antioxidative process are triggered by oxidative stress induced by abiotic stresses. These results showed that the popcorn varieties have different levels of salt tolerance due to the differential expression pattern of the antioxidant genes. The up-regulation of antioxidant enzymatic activity could lead to increased scavenging of excessive free radicals and reduce oxidative stress.

Keywords: *Zea mays everta*; abiotic stress; salinity; reactive oxygen species (ROS); RT-PCR.

Abbreviations: ROS_reactive oxygen species, CAT_catalase, APX_ascorbate peroxidase, SOD_superoxide dismutase, MDHAR_monodehydroascorbate reductase, DHAR_dehydroascorbate reductase, GR_glutathione reductase, GSH_glutathione, ASA_ascorbate, O₂[•]_superoxide, H₂O₂_hydrogen peroxide, [•]OH_hydroxyl radical, SH_shoot height, SFM_shoot fresh mass, RWC_relative water content, A_w_water activity, DM_membrane plasma damage, FM_fresh mass weight, DM_dry mass weight, TM_turgid mass weight, PVP_polyvinylpyrrolidone, NBT_nitro-blue tetrazolium, ANOVA_analysis of variance, PCA_principal component analysis.

Introduction

Salt stress is considered one of the most-limiting abiotic factors in agriculture. It significantly impacts plant development, reduces seed germination, root length, plant height and fruit production (Liang et al., 2014; Qadir et al., 2014; Munns and Gilliam, 2015). While there are many naturally saline areas, salinity can also be a consequence of inappropriate irrigation, inadequate fertilization, and deforestation practices (Carvalho et al., 2012; Silva et al., 2014). Under environmental stress, plant behavior varies depending on ionic and osmotic impact on nutrient uptake and metabolism. Such effects can be negative to the point of modifying cell metabolism and cell elongation, leading to plant death (Freitas et al., 2014). Each species has a level of

salt tolerance, which is defined based on the tolerance to salt level, genotype, and stress intensity (Sá et al., 2013; Brito et al., 2014; Oliveira et al., 2015).

Stress conditions lead to the increased production of reactive oxygen species (ROS) such as superoxide (O₂[•]), hydrogen peroxide (H₂O₂) and hydroxyl radical ([•]OH), which are spin-off of the redox metabolism of plant cells that react with biological molecules, causing irreversible damage (Gill and Tuteja, 2010; Barbosa et al., 2014; Choudhury et al., 2017). However, plants activate an antioxidant system to prevent damages from such deleterious effects by removing or degrading free radicals and avoiding cell damages (Serkedjieva, 2011). Among the antioxidant enzymes, the

superoxide dismutase (SOD; EC 1.15.1.1) is responsible for the conversion of superoxide to peroxide radical. The SODs are classified based on the metal present in the catalytic site, i.e., SOD (Cu/Zn) is in the cytosol and chloroplasts, SOD (Mn) in the mitochondrial matrix and peroxisomes and SOD (Fe) in the chloroplasts (Fernandez-Ocana et al., 2011). The catalase (CAT; EC 1.11.1.6) converts hydrogen peroxide to water, while the ascorbate peroxidase (APX; EC 1.11.1.11) also catalyzes the conversion of hydrogen peroxide to water (Demidchik, 2015).

Antioxidant enzymes participate in important signaling pathways in response to stress conditions, as well as in gene regulation. They are involved in metabolism and signal transduction pathways, and act as 'signaling molecules' or 'secondary messengers' (Barbosa et al., 2014). The major ROS-producing sites, for example, during abiotic stresses are the chloroplast, mitochondria, peroxisome and apoplast (Dietz et al., 2016; Huang et al., 2016; Kerchev et al., 2016; Rodriguez-Serrano et al., 2016; Takagi et al., 2016). Antioxidant enzymes are encoded by multiple genes (Vighi et al., 2017). In *Oryza sativa*, eight genes are described for the SOD enzyme, which were classified based on the associated cofactor (copper/zinc, manganese, and iron) and the location in the cell compartment (Gill and Tuteja, 2010). CAT has three different isoforms (Menezes-Benavente et al., 2004) while APX has eight genes with two cytosolic, one mitochondrial, two peroxisomal, and four in the chloroplasts (Teixeira et al., 2004, 2006). In *Hordeum vulgare* two CAT isoforms exist (Skadsen et al., 1995) and in *Zea mays* three isoforms (Cat1, Cat2, and Cat3) exist (Guan and Scandalios, 1995).

In corn, specifically popcorn (*Zea mays everta*) has a differentiated market price (Oliveira et al., 2016). Although this crop is profitable and valued in Brazil (Moterle et al., 2012; Ribeiro et al., 2012; Vittorazzi et al., 2013), further studies are necessary to understand the effects of abiotic stress on popcorn growth and production. Some authors (Oliveira et al., 2016; Zhu et al., 2015) have reported a reduction in growth parameters and changes in physiology of popcorn seedlings in response to salt stress and flooding. Thus, studying the gene regulatory mechanisms of antioxidant enzymes opens a new line of research, considering that no such prior work on popcorn exists. This study aimed at evaluating the regulation of genes encoding SOD, CAT and APX antioxidant enzymes and the ROS production in two popcorn genotypes subjected to different salinity levels.

Results

To understand better the plant responses to salt stress, we subjected two distinct genotypes of popcorn to different concentrations of salt. We observed that shoot height (SH), shoot fresh mass (SFM) and catalase activity (CAT) were the variables most significantly responsive ($p < 0.05$) to salt stress in both concentrations and genotypes. Only the relative water content (RWC) was similar in all cases. Water activity (a_w) was statistically different for salt concentration and membrane plasma damage (DM) in both genotypes. Analyses of SFM, APX and SOD activities revealed significant

differences in the concentration \times genotype interaction (Table 1).

Biomass

Plant growth decreased in both genotypes as the salt concentration increased. The SH of the UFVM2 genotype had a 41.6% reduction at the highest concentration of NaCl when compared with that of the control. The SFM of the IAC125 genotype decreased as salt concentration increased. The chlorophyll index (CI) of the UFVM2 genotype was the highest (Table 2). The water activity (a_w) of the UFVM2 genotype reduced only when treated with 200 mM NaCl (Table 3). Maximum electrolyte leakage was observed in both genotypes when treated with 200 mM NaCl, indicating maximum plasma membrane damage when compared with that of the control (Table 3).

Enzymatic antioxidants

The SOD, CAT and APX enzyme activities in UFVM2 genotype increased when the plants were submitted to salt stress. The highest activities were found at 100 and 200 mM NaCl concentrations. The genotype IAC125 recorded the highest enzyme activity when treated with 100 and 200 mM NaCl, except for CAT with high activity at 200 mM NaCl. In the control conditions (0 mM) SOD, CAT and APX enzyme activity in UFVM2 genotype was at the highest than IAC125 genotype. These data indicated that the IAC125 genotype was more responsive to salt stress, and mainly for SOD, CAT and APX activities that are associated with maintaining the levels of lipid peroxidation under salt stress, offering a greater tolerance to the genotypes in saline soils (Figure 1).

Transcript levels of the antioxidant genes in leaves under salt stress

The transcript levels of the corresponding genes were measured to understand gene regulation of the antioxidant enzymes (SOD, CAT and APX) under salt stress (Figure 2). The *ZmAPX* gene was found to have low expression in the IAC125 genotype despite subjecting to different NaCl concentrations. However, in the UFVM2 genotype, the same gene had higher expression levels, mainly when treated with 200 mM NaCl. The *ZmCAT* gene was expressed at high levels with compared with that of the control. The greatest accumulation of transcripts occurred when treated with 100 and 200 mM NaCl in the IAC125 and UFVM2 genotypes, respectively (Figure 2).

Three different gene isoforms were monitored for SOD. The *ZmSOD (Fe)* gene had higher expression levels in the leaves of IAC125 under control conditions, in comparison to the treatment with 200 mM NaCl. Unlike UFVM2, the *ZmSOD (Fe)* gene had low levels of expression upon exposure to stress. The transcripts of *ZmSOD (Mn)* and *ZmSOD (Cu/Zn)* were abundant and accumulated as stress levels increased (Figs. 2A and 2B).

Principal component analysis (PCA) allowed the evaluation of all variables in the genotypes (Figure 3). The PCA for A and B had a total variance of 100%, while those for C and D had variances of 99.91 and 99.25%, in which the

components PC1 and PC2 could account for 99.46 and 0.45% (Fig. 3C) and 93.16 and 6.09% (Fig. 3D) of the total variance, respectively. The analysis identified three distinct groups, and the SOD enzyme activity was similar in both the genotypes (Figs. 3A and B). SOD activity levels were more evident at 100 mM NaCl, but the *ZmSOD (Fe)* expression decreased at 200 mM NaCl. The *ZmSOD (Cu/Zn)* and *ZmSOD (Fe)* expression did not change due to salt stress (Figs. 3A and 3C). The decrease in APX activity was less pronounced than its gene expression and the inverse was true for CAT.

Discussion

The salt stress induced seemed to affect plant development by decreasing the biomass and plant growth. These responses can be explained by two major findings: first, under salinity conditions, plants reduce water uptake. Second, the Na^+ and Cl^- accumulated in the foliar tissue could cause closure of the stomata. This could impair photosynthesis, result in less CO_2 assimilation and nutrient uptake, and therefore, reduce biomass accumulation (Jouyban, 2012; Munns and Gilliam, 2015; AbdElgawad et al., 2016). In addition, when high salt levels are absorbed by plants, the amounts exceed the capacity of the cell vacuole compartment, thereby filling the cytoplasm and inhibiting the enzymatic activities in diverse metabolic pathways (Willadino et al., 1996; Prisco and Gomes Filho, 2010). Oliveira et al. (2016) also observed reduced growth of *Zea mays* L. var. *everta* when cultivated under salinity conditions, and thus corroborating our results.

The concentration of 200 mM NaCl has been commonly used for plant salt tolerance evaluations (Wu et al., 2013; Kumar et al., 2017). Water activity and relative water content, which represents the available water in the leaf were altered by salinity. This was clear when we compared the control to the 200 mM NaCl treatment. Water uptake is also impaired by excessive ion flow. According to Willadino and Camara (2010), leaves are more vulnerable to Na^+ and Cl^- flows than the root because the accumulated ions are transported via xylem for storage in the leaves as water gets evaporated. This may explain the increase in electrolyte content as stress intensifies and damages the plasma membrane. It is known that the greater the electrolytic leakage, the greater the damage to the plasma membrane. Alterations in the relative water content and plasma membrane integrity are the first signals of response to salt stress. When plants are exposed to high salinity conditions, water stress occurs (Akrami and Arzani, 2018).

In our studies, we observed contrasting results on chlorophyll index in relation to literature. A reduced chlorophyll index in plants under salinity conditions has been reported (Karlidag et al., 2009; Willadino et al., 2011; Amirjani, 2011). The amount of pigment varies with salt stress depending on the species and genotype. In this study, the chlorophyll index increased with salt stress (100 mM and 200 mM NaCl) when compared with that of the control (Table 3). Pandolfi et al. (2012) suggested that stress could trigger a sequence of physiological alterations that enable plants to tolerate salt stress. As observed in this study, the chlorophyll content increased in plants subjected to salt stress, probably to preserve the proper functioning of the

photosynthetic system. Biosynthesis of chlorophyll and carotenoids will increase under conditions of moderate salt stress (Shah et al, 2017). In addition, our results probably indicate some salinity tolerance in the genotypes studied since the increased chlorophyll content is probably a biochemical indicator of plant tolerance to salinity (Ashraf and Harris, 2013; Akrami and Arzani, 2018).

Plants possess protective mechanisms to eliminate the excess ROS (Choudhury et al., 2017), including the enzymatic action of superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT; EC 1.11.1.6), ascorbate peroxidase (APX; EC 1.11.1.11), monodehydroascorbate reductase (MDHAR; EC 1.6.5.4), dehydroascorbate reductase (DHAR; EC 1.15.1.1), glutathione reductase (GR; EC 1.6.4.2); and non-enzymatic action such as ascorbate-ASA, glutathione-GSH, α -tocopherol and flavanols. Plants have developed enzymatic mechanisms to neutralize toxic effects of the formation of ROS (Shapiguzov et al., 2012; Barbosa et al., 2014). This defense system occurs in the form of enzyme synthesis to remove or degrade free-radicals to minimize cell damage (Serkedjieva, 2011). Relief from oxidative stress is usually attributed to increased enzymatic activities and the formation of ROS, which are eliminated under stress conditions (Foyer and Noctor, 2005). Thus, the production of ROS is typically analyzed and compared at toxic and non-toxic levels, being used as a signaling molecule of stress (Barbosa et al., 2014).

It is important to note that O_2^- , H_2O_2 and $\cdot\text{OH}$ are produced in many sub-cellular compartments (Waszczak et al., 2018). The production of ROS is closely related to the stress condition causing membrane damage and electrolytes leakage (Kumar et al., 2015). In this study, salinity led to increased production of O_2^- , H_2O_2 and $\cdot\text{OH}$ in the foliar tissues of popcorn plants and corroborating the results of Azevedo Neto et al. (2006). The authors verified that SOD activity increased with salinity in corn genotypes and that CAT and APX activities were higher in the genotype tolerant to NaCl. Willadino et al. (2011) also reported an increased activity of APX and CAT in sugarcane (*Saccharum officinarum* L.) subjected to up to 100 mM NaCl, but this enzymatic activity decreased at higher concentrations.

Plant response to salt stress results from a sequence of biochemical events and gene expression, provoking modifications in transcription factors (Farooq et al., 2016). The presence of external Na^+ increases the Ca^{2+} free-content in the cytosol, which modulates the activity of proteins that depend on this ion. Therefore, the signal transduction of the ionic saline stress promotes modulation of the expression of genes related to activities of carrier proteins involved in the removal of Na^+ from the cytosol (Zhu, 2003).

It should be emphasized that salinity tolerance can trigger and/or alter the mechanism and expression of several genes, which are divided into different functional groups responsible for minimizing the effects of salinity (Munns and Gilliam, 2015). The regulation of the gene expression of antioxidant enzymes increased the transcription under salt stress. The expression of *ZmSOD (Fe)* and *ZmSOD (Mn)* genes were altered at the highest salt concentration in the IAC125 genotype. However, in the UFVM2 genotype only the *ZmSOD (Fe)* gene remained unchanged and did not alter the expression even under stress. Roy et al. (2014) reported that

Table 1. The P-values (ANOVA) for shoot height (SH) (cm), shoot fresh mass (SFM) (g), chlorophyll index (CI), water activity (WA), relative water content (WRC) (%), Membrane damage (DM) (%), ascorbate peroxidase activity (APX) ($\mu\text{mol ascorbic acid g}^{-1} \text{FM min}^{-1}$), catalase (CAT) ($\mu\text{mol H}_2\text{O}_2 \text{g}^{-1} \text{FM min}^{-1}$) and superoxide dismutase (SOD) ($\text{U SOD g}^{-1} \text{FM min}^{-1}$) in the IAC125 and UFVM2 popcorn genotypes subjected to different concentrations of NaCl and the interaction effects.

Variables	Concentration	Genotype	Concentration \times Genotype
SH	< 0.001	< 0.001	0.492
SFM	< 0.001	< 0.001	0.042
CI	0.015	0.028	0.172
WA	0.002	0.091	0.218
WRC	0.380	0.428	0.949
DM	0.560	0.015	0.111
APX	<0.001	0.024	<0.001
CAT	<0.001	<0.001	0.212
SOD	<0.001	0.342	0.045

*Bold values indicate significance.

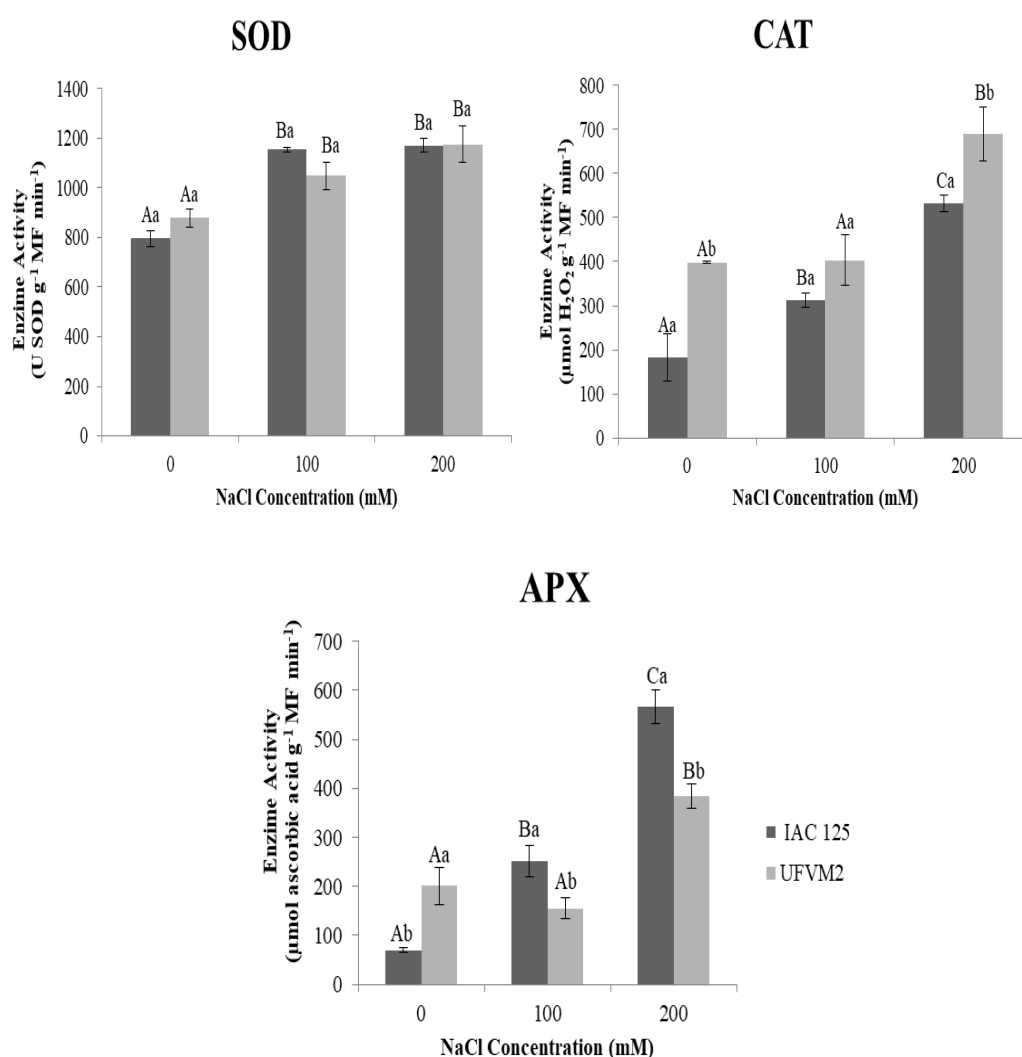


Fig 1. Antioxidant responses of superoxide dismutase (SOD) ($\text{U SOD g}^{-1} \text{MF min}^{-1}$), catalase (CAT) ($\mu\text{mol H}_2\text{O}_2 \text{g}^{-1} \text{MF min}^{-1}$) and ascorbate peroxidase (APX) ($\mu\text{mol ascorbic acid g}^{-1} \text{MF min}^{-1}$) to saline stress caused by different concentrations of NaCl. ¹Mean values followed by uppercase show significant differences among the salt concentrations in the same column. Mean values followed by lowercase show significant differences among the genotypes within the same line as depicted by Tukey test ($p \leq 0.05$).

Table 2. Shoot height (SH) (cm), shoot fresh mass (SFM) (g) and chlorophyll index (CI) of the IAC125 and UFVM2 popcorn genotypes subjected to different NaCl concentrations (mM).

NaCl	SH		SFM		CI	
	IAC125	UFVM2	IAC125	UFVM2	IAC125	UFVM2
0	30.65 Aa	26.50 Ab	1.136Aa	0.702Ab	29.99Aa	22.08Bb
100	27.35 Ba	20.78 Bb	0.699Ba	0.429Bb	31.02Aa	27.52ABa
200	19.70 Ba	15.47 Cb	0.403Ca	0.275Ba	32.75Aa	32.52Aa

Mean values followed by uppercase differ significantly among the salt concentrations in the same column. Mean values followed by lowercase indicate significant difference among the genotypes within the same line as depicted by Tukey test ($p \leq 0.05$).

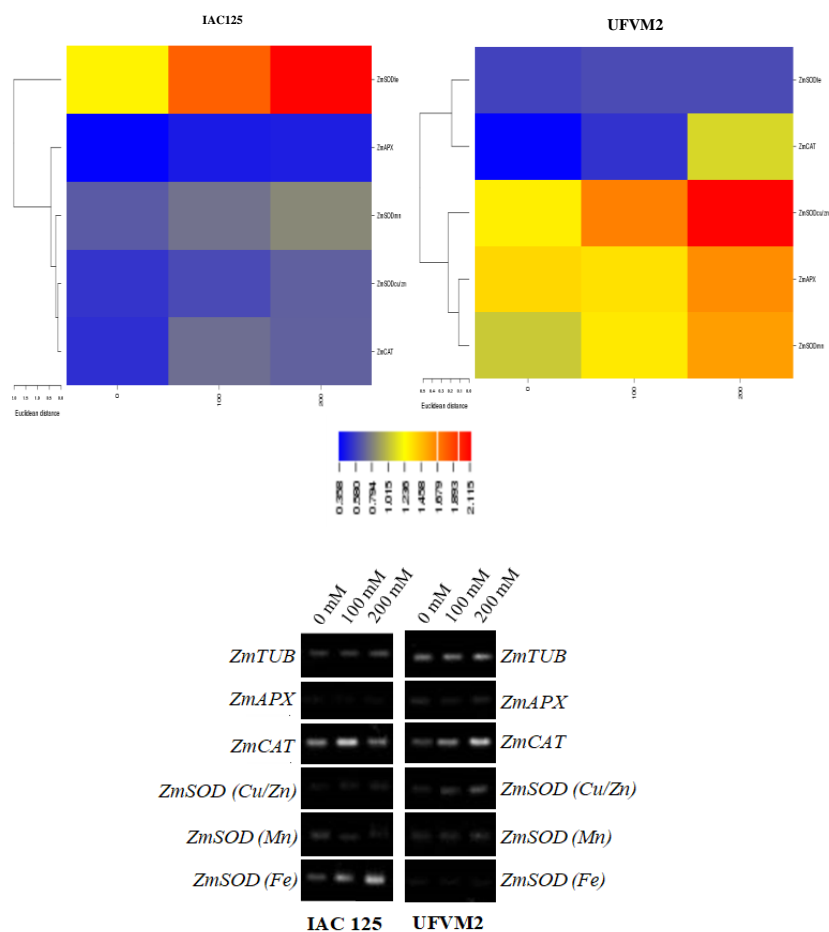


Fig 2. The transcript level of the of antioxidant genes in response to salt stress in popcorn leaves. **A.** Heatmap and Euclidean distance of gene expression of different antioxidant enzymes *ZmSOD (Fe)*, *ZmSOD (Mn)*, *ZmSOD (Cu/Zn)*, *ZmAPX* and *ZmCAT* in the IAC125 and UFVM2 popcorn genotypes subjected to 0, 100 and 200 mM NaCl concentrations. **B.** Expression of the tubulin normalizer gene (*ZmTUB*) and *ZmAPX*, *ZmCAT*, *ZmSOD (Fe)*, *ZmSOD (Mn)*, *ZmSOD (Cu/Zn)* detected by RT-PCR in the IAC125 and UFVM2 popcorn genotypes subjected to 0, 100 and 200 mM NaCl concentrations.

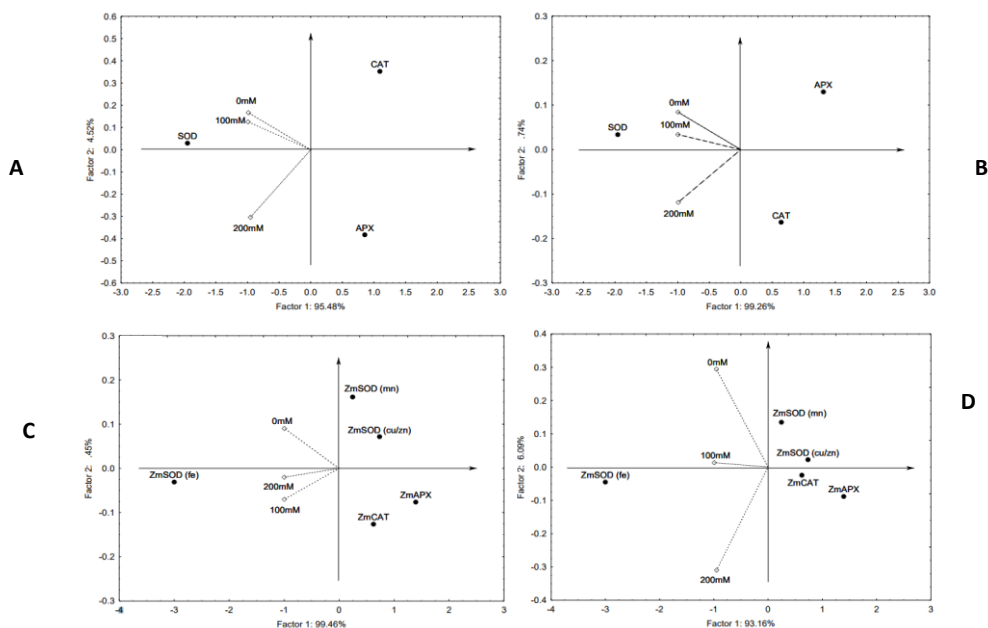
Table 3. Water activity (a_w), relative water content (WRC) (%) and membrane damage (DM) (%) in the IAC125 and UFVM2 popcorn genotypes subjected to different NaCl concentrations (mM).

NaCl	a_w		WRC		DM	
	IAC125	UFVM2	IAC125	UFVM2	IAC125	UFVM2
0	0.947Aa	0.938Aa	85.79Aa	88.15Aa	30.73Aa	9.15Ab
100	0.936Aa	0.938Aa	86.73Aa	92.30Aa	18.50Aa	17.70Aa
200	0.926Aa	0.904Bb	75.60Aa	82.68Aa	27.50Aa	18.62Aa

Mean values followed by an uppercase indicate significant difference among salt concentrations in the same column. Mean values followed by a lowercase indicate significant difference among the genotypes within the same line as depicted by Tukey test ($p \leq 0.05$).

Table 4. Sequence of primers used to quantify the transcripts in *Zea mays* by RT-PCR.

Genes	Forward 5'-3'	Reverse 5'-3'	Tm (°C)	ID
ZmAPX	GGAAGTCTGAGTGGTATAAGG	TTCTCATCCGACGATATTTCTC	58	GRMZM2G137839
ZmCAT	ACGAGAATCTTCTCTATGCTG	ATCATGGTGGTTATTGTGGTGG	58	NM_001111946.1
ZmSOD (Fe)	TTCAAATGTGTCCTATTACGGTCTC	TAGCCAGCTGATTATCAAGCC	58	GRMZM2G081585
ZmSOD (Mn)	GTCATGTGAACCATCAATCTTCTG	CATTCATCTTTTACAAGTGCCTC	58	GRMZM2G059991
ZmSOD (Cu/Zn)	GGTATGCTGACTAGACATGCTG	CTTCACCATTGTCTCTGTGATCTG	58	GRMZM2G025992
ZmTUB	GCGACCATCCAGTTCGT	CTGGTAGTTGATCCGCACTTG	58	GRMZM2G152466

**Fig 3.** Principal components analysis (PCA) of antioxidant enzymes and transcript level of antioxidant genes produced by *Zea mays everta* during salt stress. PCA of SOD, CAT and APX activities (A and B). *ZmAPX*, *ZmCAT*, *ZmSOD (Fe)*, *ZmSOD (Cu/Zn)* and *ZmSOD (Mn)* gene expressions (C and D) of the IAC125 (A and C) and UFMV2 (B and D) popcorn genotypes subjected to 0, 100 and 200 mM NaCl concentrations.

the super expression of genes involved in the ROS removal diminished cell damages, helped the maintenance of photosynthetic energy and improved root growth in saline conditions. According to Silveira et al. (2010), plants subjected to excessive salt concentrations activate biochemical reactions of perception and expression of genes that are linked to stress-moderation factors. It is important to note that gene expression is a cumulative result of all genes in different compartments and isoforms that encode the same enzyme (Ara et al., 2013). This is the reason why some genes did not correspond to the enzyme activity, as observed for *ZmAPX* in the IAC125 genotype and *ZmSOD (Fe)* in the UFMV2 genotype.

The mechanisms that regulate the expression and activity of different antioxidant genes are complex and respond differently to environmental signals. Fortes and Gallusci (2017) suggested that external stimulus such as salt stress can induce dynamic changes that eventually increase the phenotypic plasticity of plants. Some studies demonstrate the association between DNA methylation and stress

tolerance by observing the difference in DNA methylation between contrasting genotypes (Ferreira et al., 2015; Garg et al., 2015). Other studies reported that epigenetic variations between two genotypes are common (Karan et al., 2012; Garg et al., 2015; Wang et al., 2016). However, environmental stresses can significantly reduce crop yields, depending on the severity of the stress, duration as well as on the physiological phase and genetic and epigenetic characteristics of the plant experiencing the stress (Formentin et al., 2018; Ferrer et al., 2018). Genetic and epigenetic analysis conducted by Kumar et al. (2017) in wheat indicate that these mechanisms are associated with different responses of the genotypes cultivated under saline conditions. Our data presents emerging evidence that suggest the role of redox mechanisms in the epigenetic control of gene expression in the IAC125 genotype, which seems to imply hereditary characteristics of plant stress tolerance.

Therefore, this study forms the basis for future work on the expression of genes related to antioxidant enzymes in

popcorn, and it is important to investigate which transcription factors influence the activation or repression of gene expression in response to saline stress.

Materials and methods

Plant material

Three seeds of the IAC125 and Barão Viçosa (UFVM2) genotypes were sown in 500 mL pots containing commercial substrate (Carolina Padrão®). Thinning was done on the 7-day old seedlings, to leave only one plant per pot. The experiment (n = 10) was conducted in a growth chamber maintained at 25 ± 2°C, with lighting at 200-250 μmol m⁻²s⁻¹, 60 ± 5% relative humidity and 16 h-light/8 h-dark photoperiod. Seedlings were irrigated daily with sodium chloride solution (0, 100 and 200 mM NaCl) and every three days with Hoagland solution (Hoagland and Arnon, 1950). The plant material was sampled after subjecting the plants to 15 days of stress and stored at -80°C in ultra-freezer until analysis.

Biomass

Shoot fresh mass and height: Plant shoot height (SH) (cm) was measured using a tape, and the height was calculated from the base of the plant to the apex. The shoot fresh mass (SFM) (g) was determined by weighing the samples in an analytical balance.

Physiological analysis

Total chlorophyll content: The middle third leaves were sampled, and total chlorophyll content was measured in ten biological replications for each treatment using the ClorofiLOG® chlorophyll meter (Model CFL 1030), according to the manufacturer's instructions (Falker®).

Relative water content: The relative water content (RWC, %) was obtained from five leaves taken from the middle third portion and from three biological replications for each treatment and following Rouached et al. (2013). The RWC was calculated using the following equation (Schonfeld et al., 1988):

$$RWC\% = (FM - DM / TM - DM) \times 100$$

Where;

RWC % = relative water content; FM = fresh mass weight; DM = dry mass weight; and TM = turgid mass weight.

Water activity: The water activity (a_w) was determined in leaves using the Lab Master-a_w apparatus, Novasina-Tecnal®. From three leaves, 2 cm-pieces was collected from the middle third area. Three biological replications for each treatment was used, and these leaf samples were placed in the apparatus for wa analysis.

Plasma membrane damage: The plasma membrane damage (DM %) was determined through electrolyte leakage using a ITMCA 150P® micro-processed portable electric conductivity meter, and according to the method described by Blum and Ebercon (1981) and Silveira et al. (2001). Ten discs measuring 1 cm in diameter were collected from the middle third portion with three biological replications for each

treatment for the analysis. The plasma membrane damage (DM %) was obtained using the following equation:

$$DM\% = (L1/L2) \times 100$$

where: DM% = plasma membrane damage; L1 = initial electric conductivity of the extract; and L2 = final electric conductivity of the extract.

Biochemical activity

Fresh leaves (0.2 g) were ground in a chilled mortar with liquid nitrogen and homogenized in 400 mM potassium phosphate buffer (pH 7.8), 10 mM EDTA, 200 mM ascorbic acid and 10% of polyvinylpyrrolidone (PVPP). The homogenate was centrifuged at 12,000 × g for 15 min at 4°C. The supernatant was collected and stored at -80°C until further use. The extracts were used for assaying the antioxidant enzymes: superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX). All assays were performed with three biological replications, with each treatment in triplicate.

SOD enzyme (EC 1.15.1.1): The SOD activity was measured based on its ability to inhibit the photoreduction of nitro-blue tetrazolium (NBT), as described by Giannopolitis and Reis (1977). The reaction medium (200 μL) consisted of 100 mM KPO₄ buffer (pH 7.8), 120 mM L-methionine, 0.1 mM EDTA, 750 μM nitro-blue tetrazolium (NBT), 20 μM riboflavin and 20 μL of the crude sample extract. Spectrophotometry was performed at 560 nm, in which a unit of (U) SOD activity was defined as the amount of enzyme required to inhibit 50% of the NBT reduction. The SOD activity was expressed in U SOD g⁻¹ FW min⁻¹. Analyses were performed in triplicate.

CAT enzyme (EC 1.11.1.6): The CAT activity was determined according to the methodology described by Havir and McHale (1987). The reaction medium (200 μL) consisted of 100 mM KPO₄ buffer (pH 7.0), 125 mM H₂O₂, autoclaved H₂O and 20 μL of the crude sample extract. Catalase activity was determined by the H₂O₂ consumption as monitored by spectrophotometry at 260 nm for 3 minutes and quantified using the 36 M⁻¹ cm⁻¹ molar extinction coefficient (Anderson et al., 1995). CAT activity was expressed in μmol H₂O₂ g⁻¹ MF min⁻¹.

APX enzyme (EC 1.11.1.11): The APX activity was determined according to the methodology described by de Nakano and Asada (1981). The reaction medium (200 μL) consisted of 200 mM KPO₄ buffer (pH 7.0), 5 mM ascorbic acid, 1 mM H₂O₂, autoclaved H₂O and 20 μL of the crude sample extract. The APX activity was determined by the H₂O₂ degradation as monitored by spectrophotometry at 290 nm for 3 minutes and quantified using the 2,8 mM⁻¹ cm⁻¹ molar extinction coefficient (Nakano and Asada, 1981). The APX activity was expressed in μmol ascorbic acid g⁻¹ MF min⁻¹.

Gene expression

Primers

Primers were designed based on sequences obtained from *Arabidopsis thaliana* and *Oryza sativa* from the Phytozome database. Primers were designed using the PerlPrimer 5.8 software (Marshall, 2004) (Table 4).

Extracting RNA and obtaining cDNA

Total RNA was extracted from popcorn leaves using the SV Total RNA Isolation System Kit[®] (Promega[™]) and subjected to DNase digestion (Promega[™]) according to the manufacturer's instructions. The integrity and quality of the total RNA was analyzed by electrophoresing the RNA in 1% agarose gel. Quantification was performed by QuantiFluor assay (Promega[™]). The first cDNA strand was synthesized using 2 µg total RNA using the GoScript[®] Reverse Transcription System (Promega[™]) along with the oligo (dT)₁₅ primer and following the manufacturer's instructions. Total RNA extraction was performed with three biological replicates per treatment.

RT-PCR analysis: The RT-PCR was performed in 15 µL-prepared reactions containing 2 µL cDNA, 10X PCR buffer, 0.8 µL MgCl₂ (50 mM), 0.5 µL dNTP (10 mM), 0.5 µL of each primer (10 mM), and 0.4 µL Taq DNA polymerase (5 U). Amplifications were performed in AG 22331 Thermocycler (Eppendorf, Hamburg, Germany) using the following cycling parameters: 94°C for 5 min, 30 cycles at 94°C for 30 s, 58°C for 40 s and 72°C for 30 s, and a final cycle at 72°C for 10 min. The quantitation of transcripts was standardized using the *ZmTUB* constitutive gene as the normalizer. PCR products were analyzed on a 1.2% agarose gel stained with ethidium bromide. Images were captured by a photographic documentation system, L-PIX Molecular Imaging (Loccus Biotecnologia, Cotia, Brazil), and analyzed by densitometry (Freschi et al., 2009). Band intensities were quantified using the ImageJ[®] 1.46 software (<http://rsbweb.nih.gov/ij/download.html>). The RT-PCR reactions were performed using three biological replicates.

Statistical analysis

The completely randomized statistical design (n=10) was applied for the three concentrations of NaCl (0, 100 and 200 mM) and two popcorn genotypes (IAC125 and UFVM2). Data on agronomic, physiological, and biochemical characteristics were subjected to analysis of variance (ANOVA) and the mean values were compared using Tukey mean test (p<0.05) in Sisvar v 5.6 software (Ferreira, 2011). Gene expression in heatmap was analyzed using CIMminer (<https://discover.nci.nih.gov/cimminer/>). Enzymatic activity and gene expression were discriminated by principal component analysis (PCA) using the Statistica v 13.3 software (Statsoft, 2017).

Conclusion

Our results suggest that salt stress negatively impacts the growth of popcorn plants and induces the production of ROS. This causes oxidative stress. The genes *ZmAPX*, *ZmCAT*, *ZmSOD (Fe)*, *ZmSOD (Cu/Zn)* and *ZmSOD (Mn)* encoding antioxidant enzymes are activated to play important roles in eliminating ROS. The studied genotypes had varying levels of tolerance to salinity. The IAC125 genotype had higher levels of enzymatic activity under stress, indicating that this genotype has stress mechanisms and has tolerance to salt. These variations indicate that epigenetic factors not only control plant development but also influence the phenotypic plasticity under stress

conditions. The UFVM2 genotype had higher levels of genes of antioxidant-enzymes, but such response was likely due to the different isoforms that encode these enzymes.

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References

- AbdElgawad H, Zinta G, Hegab MM, Pandey R, Asard H, Abuelsoud W (2016) High Salinity induces different oxidative stress and antioxidant responses in maize seedlings organs. *Front Plant Sci.* 7:276.
- Akrami M, Arzani, A (2018) Physiological alterations due to field salinity stress in melon (*Cucumis melo* L.). *Acta Physiol Plant.* 40:1-14.
- Amirjani, Mohammad R (2011) Effect of salinity stress on growth, sugar content, pigments and enzyme activity of rice. *Int J Botany.* 7(1):73-81.
- Anderson MD, Prasad TK, Stewart CR (1995) Changes in isozyme profiles of catalase, peroxidase, and glutathione reductase during acclimation to chilling in mesocotyls of maize seedlings. *Plant Physiol.* 109(4):1247-1257.
- Ara N, Nakkhong K, Lv W, Yang J, Hu Z, Zhang M (2013) Antioxidant enzymatic activities and gene expression associated with heat tolerance in the stems and roots of two cucurbit species ("Cucurbita maxima" and "Cucurbita moschata") and their interspecific inbred line "Maxchata". *Int J Mol Sci.* 14(12):24008-24028.
- Ashraf MHPJC, Harris PJC (2013) Photosynthesis under stressful environments: an overview. *Photosynthetica.* 51(2):163-190.
- Azevedo Neto AD, Prisco JT, Enéas-Filho J, De Abreu CEB, Gomes-Filho E (2006) Effect of salt stress on antioxidative enzymes and lipid peroxidation in leaves and roots of salt-tolerant and salt-sensitive maize genotypes. *Environ Exp Bot.* 56(1):87-94.
- Barbosa MR, Medeiros De Araújo Silva M, Willadino L, Ulisses C, Rangel Camara T (2014) Geração e desintoxicação enzimática de espécies reativas de oxigênio em plantas. *Cienc Rural.* 44 (3):453-460.
- Blum A, Ebercon A (1981) Cell membrane stability as a measure of drought and heat tolerance in wheat. *Crop Sci.* 21(1):43-47.
- Brito MEB, Dantas Fernandes PEDRO, Raj Ghey HANS, Soares De Melo A, Dos Santos Soares Filho W, Tabolka Dos Santos R (2014) Sensibilidade à salinidade de híbridos trifoliados e outros porta-enxertos de citros. *Rev Caatinga.* 27(1):17-27.
- Carvalho JF, Tsimpho CJ, Silva EFF, Medeiros PRF, Santos MHV, Santos NA (2012) Produção e biometria do milho verde irrigado com água salina sob frações de lixiviação. *Rev Bras Eng Agríc.* 16(4):368-374.
- Choudhury FK, Rivero RM, Blumwald E, Mittler R (2017) Reactive oxygen species, abiotic stress and stress combination. *Plant J.* 90(5):856-867.
- Demidchik, V (2015) Mechanisms of oxidative stress in plants: from classical chemistry to cell biology. *Environ Exp Bot.* 109:212-228.
- Dietz KJ, Turkan I, Krieger-Liszskay A (2016) Redox- and reactiveoxygen species-dependent signaling in and from the photosynthesizing chloroplast. *Plant Physiol.* 171(3):1541-1550.

- Farooq MA, Gill RA, Islam F, Ali B, Liu H, Xu J, He S, Zhou W (2016) Methyl jasmonate regulates antioxidant defense and suppresses arsenic uptake in *Brassica napus* L. *Front Plant Sci.* 7:468.
- Fernández-Ocaña A, Chaki M, Luque F, Gómez-Rodríguez MV, Carreras A, Valderrama R, Begaramorales JC, Hernández RE, Barroso JB (2011) Functional analysis of superoxide dismutases (SODs) in sunflower under biotic and abiotic stress conditions. Identification of two new genes of mitochondrial Mn-SOD. *J Plant Physiol.* 11:1303-1308.
- Ferreira LJ, Azevedo V, Maroco J, Oliveira MM, Santos AP (2015) Salt tolerant and sensitive rice varieties display differential methylome flexibility under salt stress. *PLoS One.* 10(5):1-19.
- Ferreira DF (2011) Sisvar: a computer statistical analysis system. *Ciênc Agrotec.* 35(6):1039-1042.
- Ferrer MA, Cimini S, Lopez-Orenes A, Calderón AA, Gara L (2018) Differential Pb tolerance in metallicolous and non-metallicolous *Zygophyllum fabago* populations involves the strengthening of the antioxidative pathways. *Environ Exp Bot.* 150:141-151.
- Formentin E, SUDiro C, Perin G, Riccadonna S, Barizza E, Baldoni E, Lavezzo E, Stevanato P, Sacchi Gian A, Fontana P Toppo S, Morosinotto T, Zottini M, Lo Schiavo F (2018) Transcriptome and cell physiological analyses in different rice cultivars provide new insights into adaptive and salinity stress responses. *Front Plant Sci.* 9:204.
- Fortes AM, Gallusci P (2017). Plant stress responses and phenotypic plasticity in the epigenomics era: perspectives on the grapevine scenario, a model for perennial crop plants. *Front Plant Sci.* 8:82.
- Foyer CH, Noctor G (2005) Oxidant and antioxidant signalling in plants: a re-evaluation of the concept of oxidative stress in a physiological context. *Plant Cell Environ.* 28(8):1056-1071.
- Freitas MAC, Amorim AV, Bezerra AME, Pereira MS, Bessa MC, Nogueira Filho FP, Lacerda CF (2014) Growth and salinity tolerance in three medicinal species of the genus *Plectranthus* exposed to different levels of solar radiation. *Rev Bras PI Med.* 16(4):839-849.
- Freschi L, Nievola CC, Rodrigues MA, Domingues DS, Van Sluys MA, Mercier H (2009) Thermoperiod affects the diurnal cycle of nitrate reductase expression and activity in pineapple plants by modulating the endogenous levels of cytokinins. *Physiol Plant.* 137(3):201-212.
- Garg R, Chevala VVSN, Shankar R, Jain M (2015) Divergent DNA methylation patterns associated with gene expression in rice cultivars with contrasting drought and salinity stress response. *Sci Rep.* 5:14922.
- Giannopolitis I, Reis SK (1977) Superoxide dismutases: I. Occurrence in higher plants. *Plant Physiol.* 59(2):309-314.
- Gill SS, Tuteja N (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol Biochem.* 48(12):909-930.
- Guan L, Scandalios JG (1995) Developmentally related responses of maize catalase genes to salicylic acid. *Proc Natl Acad Sci U S A.* 92(13):5930-5934.
- Havir EA, Mchale NA (1987) Biochemical and developmental characterization of multiple forms of catalase in tobacco leaves. *Plant Physiol.* 84(2):450-455.
- Hoagland DR, Arnon DI (1950) The water culture method for growing plants without soils. *Calif Agric Exp Stn Circ.* 347:1-32.
- Huang S, Van Aken O, Schwarzländer M, Belt K, Millar A (2016) The roles of mitochondrial reactive oxygen species in cellular signaling and stress responses in plants. *Plant Physiol.* 171:1551-1559.
- Jouyban Z (2012) The Effects of Salt stress on plant growth. *Eng Appl Sci Res.* 2(1):7-10.
- Karan R, DeLeon T, Biradar H, Subudhi PK (2012) Salt stress induced variation in DNA methylation pattern and its influence on gene expression in contrasting rice genotypes. *PLoS one,* 7(6):40203.
- Karlidag H, Yildirim E, Turan M (2009) Salicylic acid ameliorates the adverse effect of salt stress on strawberry. *Sci Agric.* 66(2):180-187.
- Kerchev PI, Waszczak C, Lewandowska A, Willems P, Shapiguzov A, Zhen L, Alseekh S, Mühlenbock P, Hoerberichts FA, Huang J, Kelen KVD, Kangasjärvi J, Fernie AR, Smet RD, Peer, Messens J, Breusegem FV (2016) Lack of glycolate oxidase 1, but not glycolate oxidase 2, attenuates the photorespiratory phenotype of CATALASE2-deficient Arabidopsis. *Plant Physiol.* 171:1704-1719.
- Kumar M, Hasan M, Arora A, Gaikwad K, Kumar S, Rai RD, Singh A. (2015) Sodium chloride-induced spatial and temporal manifestation in membrane stability index and protein profiles of contrasting wheat (*Triticum aestivum* L.) genotypes under salt stress. *Ind J Plant Physiol.* 20:271-275.
- Kumar S, Beena AS, Awana M, Singh A (2017) Physiological, biochemical, epigenetic and molecular analyses of wheat (*Triticum aestivum*) genotypes with contrasting salt tolerance. *Front Plant Sci.* 8:1151.
- Liang W, Cui W, Ma X, Wang G, Huang Z (2014) Function of wheat Ta-UnP gene in enhancing salt tolerance in transgenic Arabidopsis and rice. *Biochem Biophys Res Commun.* 450(1):794-801.
- Marshall OJ (2004) PerlPrimer: cross-platform, graphical primer design for standard, bisulphite and real-time PCR. *Bioinformatics.* 20(15):2471-2472.
- Menezes-Benavente L, Teixeira FK, Kamei CLA, Margis-Pinheiro M (2004) Salt stress induces altered expression of genes encoding antioxidant enzymes in seedlings of a Brazilian indica rice (*Oryza sativa* L.). *Plant Sci.* 166(2):323-331.
- Moterle LM, De Lucca E, Braccini A, Scapim CA, Barth Pinto RJ, Gonçalves LSA, Rodrigues R, Amaral Júnior AT (2012) Combining ability of popcorn lines for seed quality and agronomic traits. *Euphytica.* 185(3):337-347.
- Munns R, Gilliam M (2015) Salinity tolerance of crops—what is the cost? *New Phytol.* 208(3):668-673.
- Nakano Y, Asada K (1981) Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. *Plant Cell Physiol.* 22(5):867-880.
- Oliveira FDA, De Medeiros JF, Alves RDC, Lima LA, Dos Santos ST, De Régis LR (2015) Produção de feijão caupi em função da salinidade e regulador de crescimento. *Rev Bras Eng Agríc.* 19(11):1049-1056.
- Oliveira FDAD, Medeiros JFD, Cunha RCD, Souza MWDL, Lima LA (2016) Use of biostimulants in relieving salt stress in popcorn. *Rev Ciênc Agron.* 47(2):307-315.
- Pandolfi C, Mancuso S, Shabala S (2012) Physiology of acclimation to salinity stress in pea (*Pisum sativum*). *Environ Exp Bot.* 84:44-51.
- Prisco JT, Gomes Filho E (2010) Fisiologia e bioquímica do estresse salino em plantas. In: Gheyi HR, Dias NS, Lacerda CF (ed) Manejo da salinidade na agricultura: Estudos básicos e aplicados. 1st edn. Fortaleza: INCTSal, 143-150.
- Qadir M, Quillérou E, Nangia V, Murtaza G, Singh M, Thomas RJ, Drechsel P, Noble AD (2014) Economics of salt-induced land degradation and restoration. *Nat Resour Forum.* 38(4):282-295.

- Ribeiro RM do Amaral Júnior AT, Gonçalves LSA, Candido LS, Silva TRC, Pena GF (2012) Genetic progress in the UNB-2U population of popcorn under recurrent selection in Rio de Janeiro. *Genet Mol Res*. 11:1417-1423.
- Rodriguez-Serrano M, Romero-Puertas MC, Sanz-Fernandez M, Hu J, Sandalio LM (2016) Peroxisomes extend peroxules in a fast response to stress via a reactive oxygen species-mediated induction of the peroxin PEX11a. *Plant Physiol*. 171:1665-1674.
- Roy SJ, Negrão S, Tester M (2014) Salt resistant crop plants. *Curr Opin Biotechnol*. 26:115-124.
- Rouached A, Slama I, Zorrig W, Jdey A, Cukier C, Rabhi M, Talbi O, Limami AM, Abdely C. Differential performance of two forage species: *Medicago truncatula* and *Sulla carnosa* under water deficit stress and recovery. *Crop Pasture Sci*. 64(3):254-264.
- Sá FDS, Brito ME, Melo AD, Antônio Neto P, Fernandes PD, Ferreira I B (2013) Produção de mudas de mamoeiro irrigadas com água salina. *Rev Bras Eng Agríc*. 17(10):1047-1054.
- Schonfeld MA, Johnson RC, Carver BF (1988) Water relations in winter wheat as drought resistance indicators. *Crop Sci*. 28:521-31.
- Serkedjjeva J (2011) Antioxidant effects of plant polyphenols: a case study of a polyphenol-rich extract from *Geranium sanguineum* L. In: Gupta SD (ed) *Reactive oxygen species and antioxidants in higher plants*. 1st edn. Enfield: Science Publishers, New Hampshire. 13.
- Shah SH, Houborg R, McCabe MF (2017) Response of chlorophyll, carotenoid and SPAD-502 measurement to salinity and nutrient stress in wheat (*Triticum aestivum* L.). *Agronomy*. 7(3):61.
- Shapiguzov A, Vainonen JP, Wrzaczek M, Kangasjarvi J (2012) Ros-talk-how the apoplast, the chloroplast, and the nucleus get the message through. *Front Plant Sci*. 3: 292.
- Silva JLA, Medeiros JF, Alves SSV, Oliveira FA, Silva Júnior MJ, Nascimento IB (2014) Uso de águas salinas como alternativa na irrigação e produção de forragem no semiárido nordestino. *Rev Bras Eng Agríc*. 18:66-72.
- Silveira JAG, Silva SLF, Silva EN, Viégas RA (2010) Mecanismos biomoleculares envolvidos com a resistência ao estresse salino em plantas. In: Gheyi HR, Dias NS, Lacerda CF (ed) *Manejo da salinidade na agricultura: estudos básicos e aplicados*, 1 st ed. Fortaleza: INCTSal. 11.
- Silveira JAG, Melo ARB, Viégas RA, Oliveira JTA (2001) Salt-induced effects on the nitrogen assimilation related to growth in cowpea plants. *Environ Exp Bot*. 46(2):171-179.
- Skadsen RW, Schulze-Lefert P, Herbst JM (1995) Molecular cloning, characterization and expression analysis of two catalase isozyme genes in barley. *Plant Mol Biol*. 29(5):1005-1014.
- STATSOFT (2017) *Statistica for Windows [Computer program manual]*. v. 13.3. Tulsa: StatSoft. Available at: <<http://www.statsoft.com>>. Accessed 15 Aug 17.
- Takagi D, Takumi S, Hashiguchi M, Sejima T, Miyake C (2016) Superoxide and singlet oxygen produced within the thylakoid membranes both cause photosystem I photoinhibition. *Plant Physiol*. 171:1626-1634.
- Teixeira FK, Menezes-Benavente L, Galvão VC, Margis R, Margis-Pinheiro M (2006) Rice ascorbate peroxidase gene family encodes functionally diverse isoforms localized in different subcellular compartments. *Planta*. 224(2):300-314.
- Teixeira FK, Menezes-Benavente L, Margis R, Margis-Pinheiro M (2004) Analysis of the molecular evolutionary history of the ascorbate peroxidase gene family: inferences from the rice genome. *J Mol Evol*. 59:761-770.
- Vighi IL, Benitez LC, Amaral MN, Moraes GP, Auler PA, Rodrigues GS, Deuner S, Maia LC, Braga EJB (2017) Functional characterization of the antioxidant enzymes in rice plants exposed to salinity stress. *Biol Plantarum*. 61(3):540-550.
- Vitorazzi C, Amaral Júnior AT, Gonçalves LSA, Candido LS, Silva TRC (2013) Selecting pre-cultivars of popcorn maize based on nonparametric indices. *Rev Cienc Agron*. 44:356-362.
- Wang W, Huang F, Qin Q, Zhao X, Li Z, Fu B (2015) Comparative analysis of DNA methylation changes in two rice genotypes under salt stress and subsequent recovery. *Biochem Biophys Res Commun*. 465(4):790-796.
- Waszczak C, Carmody M, Kangasjärvi J (2018) Reactive oxygen species in plant signaling. *Annu Rev Plant Biol*. 69: 209-236.
- Willadino L, Camara TR (2010) Tolerância das plantas à salinidade: aspectos fisiológicos e bioquímicos. *Enciclopédia Biosfera*. 6(11):1-23.
- Willadino L, Camara T, Boget N, Claparols I, Santos M, Torne JM (1996) Polyamine and free amino acid variations in NaCl-treated embryogenic maize callus from sensitive and resistant cultivars. *J Plant Physiol*. 149(2):179-185.
- Willadino L, De Oliveira Filho RA, Da Silva Junior EA, Neto AG, Camara TR (2011) Estresse salino em duas variedades de cana-de-açúcar: enzimas do sistema antioxidativo e fluorescência da clorofila. *Rev Ciênc Agron*. 42(2):417-422.
- Wu D, Cai S, Chen M, Ye L, Chen Z, Zhang H, Dai F, Wu F, Zhang G (2013) Tissue metabolic responses to salt stress in wild and cultivated barley. *PLoS one*, 8(1): e55431.
- Zhu JK (2003) Regulation of ion homeostasis under salt stress. *Curr Opin Plant Biol*. 6: 441-445.
- Zhu M, Wang J, Li F, Shi Z (2015) Physiological and photosynthesis response of popcorn inbred seedlings to waterlogging stress. *Pak J Bot*. 47(6):2069-2075.