Anatolian Journal of Botany
8(1): 21-29 (2024)
Research article *doi: 10.30616/ajb.1387695*

Improving salt stress tolerance in *Zea mays* L. by modulating osmolytes accumulation and antioxidant capacity with Rutin

Asiye SEZGİN MUSLU
Karadeniz Technical University, Science Faculty, Department of Biology, 61080, Trabzon, Türkiye
asiyeszgn@outlook.com

Received : 08.11.2023
Accepted : 03.01.2024
Online : 23.02.2024

Rutin ile osmolit birikimi ve antioksidan kapasite modüle edilerek *Zea mays* L.'de tuz stresi toleransının geliştirilmesi

Abstract: The growth and productivity of maize are severely affected by stress factors**.** Maize seedlings under salt stress were grown hydroponically to study the effect of rutin (Rut), a flavonoid, on changes in the stress parameters (thiobarbituric acid reactive substances (TBARS), hydrogen peroxide (H_2O_2), total chlorophyll), water status (leaf relative water content (RWC), osmolytes; proline, total soluble sugar), and activities of the main antioxidant enzymes (superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and peroxidase (POD)). After 21 days of growth, plants were applied with Rut as foliar spray. After 24 hours, seedlings were exposed to osmotic stress by 100 and 200 mM NaCI in the Hoagland's Solution for 72 hours. Six groups were designed including a control (without NaCl or Rut), 150 mM NaCl, 200 mM NaCl, Rut, Rut+150 mM NaCl, and Rut+200 mM NaCl. Plant leaves were harvested 25 days after treatments. Exogenous significantly decreased TBARS and H_2O_2 contents in leaves of salt-stressed seedlings compared to salt stresses, enhanced the level of osmolytes, leaf RWC, activities of SOD, CAT, APX, and POD, and relative expression levels of *SOD*, *CAT1*, and *APX1*. As a result, findings from the study present reveal the effect of Rut on salt stress tolerance in maize seedlings under different osmotic stress. Here, it was clear that Rut played an active role in stress-alleviating. This application under salt stress can be useful in developing salt stress tolerance in crops for the agriculture sector.

Key words: Antioxidants, gene expression, osmolytes, rutin, salt stress

Özet: Mısırın büyümesi ve verimliliği stres faktörlerinden ciddi şekilde etkilenir. Tuz stresi altındaki mısır fideleri, bir flavonoid olan rutinin (Rut) stres parametrelerindeki (tiyobarbitürik asit reaktif maddeleri (TBARS), hidrojen peroksit (H_2O_2), toplam klorofil), su durumu (yaprak nisbi su içeriği(NSİ), osmolitler; prolin, toplam çözünebilir şeker) ve ana antioksidan enzimlerin (süperoksit dismutaz (SOD), katalaz (CAT), askorbat peroksidaz (APX) ve peroksidaz (POD)) aktiviteleri üzerine etkisini incelemek için hidroponik olarak büyütüldü. 21 günlük büyümenin ardından yapraklara rutin sprey olarak gerçekleştirildi 24 saatin ardından fideler, 72 saat boyunca Hoagland Solüsyonunda 100 ve 200 mM NaCl'nin indüklediği ozmotik strese maruz bırakıldı. Deney grupları, Bir kontrol (NaCl veya Rut içermeyen), 150 mM NaCl, 200 mM NaCI, Rut, Rut+150 mM NaCl ve Rut+200 mM NaCl dahil olmak üzere üç tekrarlı altı grup şeklinde dizayn edildi. Bitki yaprakları uygulamalardan 25 gün sonra hasat edildi. Eksojen Rut, tuz stresine kıyasla tuz stresi altındaki fidelerin yapraklarındaki TBARS ve H_2O_2 içeriğini önemli ölçüde azalttı, osmolit seviyesini, yaprak NSİ'ni, SOD, CAT, APX ve POD aktivitelerini ve *SOD*, *CAT1* ve *APX1*'in nisbi ekspresyon seviyelerini artırdı. Sonuç olarak, mevcut çalışmadan elde edilen bulgular, farklı ozmotik stres altındaki mısır fidelerinde Rut'un tuz stresi toleransı üzerindeki etkisini ortaya koymaktadır. Burada Rut'un stresi azaltmada aktif rol oynadığı açıktı. Tuz stresi altında yapılan bu uygulama, tarım sektörü için bitkilerde tuz stresine toleransın geliştirilmesinde faydalı olabilir.

Anahtar Kelimeler: Antioksidanlar, gen ifadesi, osmolitler, rutin, tuz stresi

Citation: Sezgin Muslu A (2024). Improving salt stress tolerance in *Zea mays* L. by modulating osmolytes accumulation and antioxidant capacity with Rutin . Anatolian Journal of Botany 8(1): 21-29.

1. Introduction

A significant environmental stressor that limits the production and sustainability of agricultural enterprises in arid and semiarid environments is excessive salinity (Yu et al., 2020; Wei et al., 2020; Mukhopadhyay et al., 2021). The extensive use of improper irrigation techniques that we have recently seen, particularly the excessive use of fertilizers and pesticides, has contributed to a notable deterioration of soil salinization throughout the world in addition to amplifying the consequences of global warming (Farooq et al., 2015; Meng et al., 2016).

One of the most significant food crop species is maize, and maize production has been rising. However, because maize is a somewhat salt-sensitive plant species, its growth is severely constrained by salinity, particularly when it is in the seedling stage (Kang et al., 2004; Zelm et al., 2020). Moreover, salinity negatively affects senescence, transpiration, ion transport, osmotic status, and photosynthetic activity in maize seedlings (Negrão et al., 2017; Zhong et al., 2020; Feng et al., 2021; Ju et al., 2021). In addition, excessive salinity can produce reactive oxygen species (ROS), including hydrogen peroxide, superoxide anions, etc. (Mittova et al., 2004; Huo et al., 2020). Therefore, in order to develop tolerance to salt, maize seedlings have developed various strategies, such as enhanced osmolyte accumulation and activated ROS scavenging systems (Acosta-Motos et al., 2015; Zhu et al., 2016).

Salt-induced osmotic effects change the overall metabolic processes and enzyme activity levels in maize plant cells, which results in excessive ROS accumulation and oxidative stress (AbdElgawad et al., 2016; Ali et al., 2021). Plant cells typically establish a complex antioxidant system that controls redox homeostasis in response to oxidative stress. This system frequently includes the enzymes SOD, CAT, APX, and POD, as well as other free radical scavengers (Liang et al., 2017; Sánchez-McSweeney et al., 2021).

Some compounds, including osmolytes, antioxidants, and signal molecules, have been applied to plants subjected to various stress factors to enhance their stress tolerance. One example is rutin, which is one of the flavonoids and a member of the large group of herbal phenolics. Flavonoids are secondary metabolites and have been reported to provide herbal pigmentation and protection from UV rays as a defense mechanism against herbivores and pathogens (Taiz and Zeiger, 2008). Flavonoids also have antioxidant properties (Yang et al., 2008) and have been reported to inhibit lipid peroxidation under drought stress and act as membrane stabilizers (Singh et al., 2017). Most varieties of flavonoids exist in the form of o-glycosides, a sugar linked by one or more hydroxyl groups, and can be stored in the cell vacuole in this form (Jiang et al., 2007). Rutin also calls quercetin -3-0- rutinoside, consists of the aglycone quercetin and the disaccharide rutinose. It is a yellow, crystalline flavonol glycoside ($C_{27}H_{30}O_{16}$) and is converted to quercetin by being catalyzed by rutinosidase, and quercetin is catalyzed by rutinosidase and synthesized to isoquercitrine, and isoquercitrine is synthesized to rutin by rhamnosyl transferase (Suzuki et al., 2015). It has been reported that because young leaves are more sensitive to UV light, they protect the plants from UV rays by storing rutin more intensively (Suzuki et al., 2015). In addition, with its rutin application to the leaves of tomato plants, it was noted that there was an increase in the number of leaves, leaf area, fruit number, fruit weight, shoot and root dry weight, and elongation in the roots (Gorni et al., 2022). Suzuki et al. (2005) found that the concentration of rutin glucosidase and quercetin increased in buckwheat leaves that were subjected to UV-B, osmotic, and cold stresses. It was observed that the amount of rutin and other phenols increased in *Hypericum brasiliense* Choisy. and *Artemisia* plants exposed to drought stress for a long time (Kumar et al., 2021). Overexpression of the drought stress tolerance gene (*NtCHS*) in the drought-stress-tolerant *Nicotiana tabacum* L. resulted in the accumulation of rutin and other flavonoids. The results of this study showed that rutin increases drought stress tolerance (Chen et al., 2019). As with most flavonoids, rutin also has a reducing effect on lipid peroxidation (Jiang et al., 2007). It has been suggested that this property of rutin may be related to the scavenging of hydroxyl groups. Also by the same researchers, it was found that rutin was synthesized at the highest rate among total phenolics in the leaves of halophyte quinoa species. In this study, the activity of antioxidant enzymes did not increase with rutin application (Ismail et al., 2015).

To the best of our knowledge, it is still unclear whether rutin is useful for protecting plants against stressors. Additionally, there are no studies on the protective role of rutin in salt-stressed maize plants. Therefore, the current study has two hypotheses: (1) Rutin could highly stimulate the accumulation of osmolytes to alleviate the salt stress effect in maize seedlings; and (2) Rutin could modulate the antioxidant defense system to develop salt stress tolerance in maize seedlings. Our study will provide new insights into lighting the physiological and molecular mechanisms of rutin in maize seedlings' responses to salt stress.

2. Materials and Method

2.1. Plant material and applications

Maize seedlings were grown hydroponically in a growth chamber (16 h day/8 h night temperature of 25/22, relative humidity (63±2%) and photon flux density (400 µmol $m^{-2}s^{-1}$) with Hoagland's solution (Hoagland and Arnon 1950). After 21 days of growth, rutin (60 ppm) was applied to the leaves as a spray. After 24 hours, seedlings were exposed to osmotic stress induced by 150 and 200 mM NaCl in the Hoagland's Solution for 72 hours.

The experimental pots were designed as six different treatments: the Nutrient Solution for Control (1), 150 mM NaCl (2), 200 mM NaCl (3), Rut (4), Rut+150 mM NaCl (5), and Rut+200 mM NaCl (6). After treatments, 25 day old seedlings were harvested and stored at -80 °C until analysis.

2.2. Determination of Stress Parameters

2.2.1. Thiobarbituric acid reactive substances (TBARS) Assay

The level of lipid peroxidation was measured in terms of TBARS content. A homogenizer was used to homogenize the leaf samples (0.1 g) in 1.8 mL of 0.1 % trichloroacetic acid (TCA). For 5 min, the homogenate was centrifuged at 16,000 xg. 4 mL of thiobarbituric acid prepared in 20% TCA was added to 1 mL of supernatant. The mixture was heated for 30 min at 95 °C before being quickly cooled. The absorbance of the supernatant was measured at 532 and 600 nm (Heath and Packer, 1968).

2.2.2. Hydrogen peroxide (H_2O_2) assay

The extract produced from crushed with nitrogen leaf samples was centrifuged, 1 mL of supernatant was taken and 10 mM potassium phosphate buffer and 1 M potassium iodide were added. The absorbance was then measured at 390 nm (Velikova et al., 2000).

2.2.3. Total chlorophyll assay

The total amount of chlorophyll was determined according to the method of Arnon (1949). 0.1 g of fresh plant leaf was crushed with liquid nitrogen and homogenized with 1.8 mL of 80% acetone. The obtained samples were centrifuged at 4°C for 10 min. It was diluted by adding 4.5 mL of 80% acetone solution to 0.5 mL supernatants. The absorbances of the obtained supernatants were measured at 645 nm and 663 nm.

2.3. Determination of water status

2.3.1. Leaf relative water content (RWC)

To estimate the water saturated weight (SW), maize leaves plucked from seedlings were immediately weighed on balance (about 0.1g) (fresh weight, FW), and then placed in water and rehydrated for 12 hours. The leaves' dry weight (DW) was obtained by drying them in an oven at 65 °C for 48 hours. To compute the leaf RWC, the formula (RWC(%): (FW-DW)/(SW-DW)*100) was employed.

2.3.2. Osmolyte contents

The proline content of dried samples (0.1 g) was evaluated by filtering them following homogenization with 1.8 mL of 3% sulfosalicylic acid. The filtrate was centrifuged for 5 min at 5,000 xg. 1 mL of supernatant was treated with acetic acid (1 mL) and ninhydrin (1 mL). The mixture was then placed in tubes in a water bath at 100 °C for 1 h. 3 mL of toluene was added to the cooled liquid and vortexed. The mixture was placed in sealed tubes and centrifuged at 5,000 xg for 5 min. After centrifugation, the upper phase was pipetted into the cuvette and measured at 520 nm with a spectrophotometer (Bates et al., 1973).

To calculate the total soluble sugar concentration, after homogenizing the dry leaf sample (0.1 g) with 70% ethanol (5 mL), the homogenate was boiled at 80 °C for 3 min. Homogenate was centrifuged at 10,000 xg for 5 min. 900 µL of filtered water was mixed with 100 µL of supernatant and diluted. After adding 1 mL of 5% phenol, this mixture was stirred using a stirrer. 5 mL of sulfuric acid was completely stirred into the same mixture. After cooling to room temperature, the absorbance values of mixture were measured at 490 nm (Dubois, 1956).

2.4. Determination of changes in antioxidant capacity

The ascorbate (AsA) content was calculated using the method provided by Liso et al. (1984). The leaf samples (0.1 g) were homogenized with 5 mL of 5% metaphosphoric acid and centrifuged at 10,000 xg for 10 min. The supernatant (70 µL) was combined with 3 mL of reaction medium (0.1 M citrate-0.2 M phosphate buffer, pH 6.2). AsA content was evaluated after reading the reduction occuring 5 min after the addition of 2 U ascorbate oxidase to the reaction solution. Ascorbate oxidase was blocked once AsA oxidation was complete by adding 10 mM sodium azide. The combination was then treated with 2.5 mM dithiothreitol. The absorbance was measured at 265 nm again after 3 min of reduction with dithiothreitol.

The samples were produced using the modified method of Sezgin et al. (2018) for enzyme and protein extractions. SOD activity was measured at 25°C using the Beauchamp and Fridovich (1973) technique. CAT activity was measured at 240 nm using the Aebi (1983) technique. The activity of APX was assessed using the Nakano and Asada (1987) technique, which is based on ascorbate oxidation at 290 nm. POD activity was measured according to Urbanek et al. (1991) by following the increase in absorbance at 470 nm.

2.5. The analyis of gene expression

To analyze the relative expression levels of *SOD*, *CAT1*, and *APX1* genes, total RNA was obtained using a Plant Total RNA Purification Kit (FavorPrep). The High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) was used for cDNA synthesis. The cDNAs obtained were used in Real Time PCR tests to determine transcript levels. For RT-PCR analysis, SsoFast EvaGreen Supermix and the RT-PCR System (BioRad) were used. The method stages were modified using Solis BioDyne instructions. The actin (*ACT)* gene was used as a control gene The data were normalized to the level of reference gene expression before being displayed as relative gene expression. Table 1 contains a list of primers.

2.6. Statistical analysis

Every experiment was repeated three times with three biological replicates. Duncan's multiple comparison test (one-way ANOVA) was used for statistical analysis in SPSS (Ver. 23.0). Bio-Rad CFX Manager 3.1 was used to examine relative gene expression during qRT-PCR analysis. The vertical bars reflect the standard deviations of three replicated means. At $P < 0.05$, different letters denote significant differences among all treatments.

3. Results

3.1. Effects of rutin on stress parameters under salinity stress condtions

Salt stress applications (150 and 200 mM) drastically increased the membrane damage (TBARS content) of maize seedlings by 34.3 and 69.8%, respectively, as compared with the control: however, Rut application under 150 and 200 Mm NaCl stress decreased TBARS level by 12.5 and 6.6%, respectively, compared to the same stress level alone. Moreover, Rut statistically significant alleviated the salt-induced membrane damage of maize seedlings under both stress conditions compared to salt stress conditions (Fig. 1A). H_2O_2 content of maize seedlings increased when subjected to salt stresss treatments. However, the application of exogenous Rut under salt stress conditions (150 and 200 mM NaCl) decreased statistically significant H_2O_2 level (31.1 and 16.0%, respectively) compared with salt stress conditions (Fig. 1B). Additionally, decrease in TBARS and H_2O_2 levels in Rut combined 150 mM NaCl were higher than Rut combined 200 mM NaCl treatment (Fig. 1A,B). Salinity

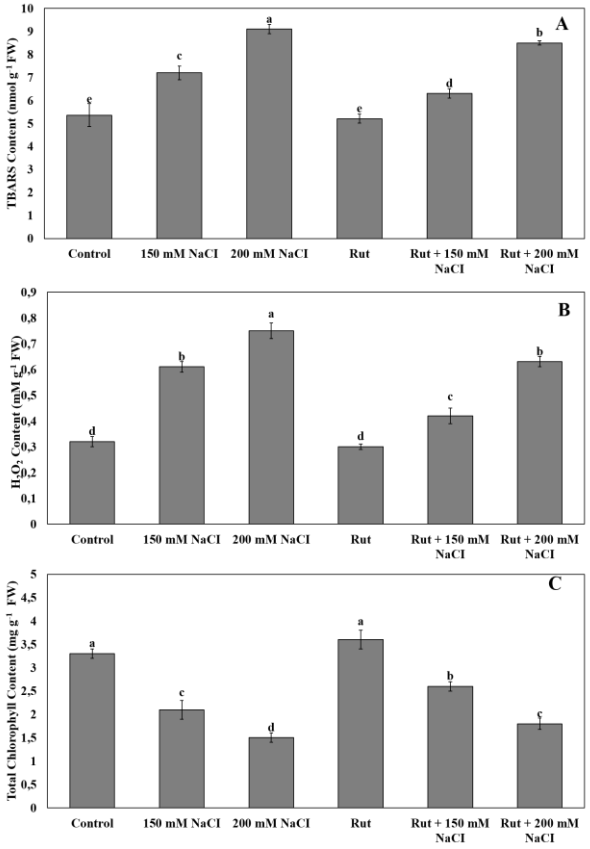

Figure 1. Effects of rutin on stress parameters under salinity stress conditions. TBARS content (**a**), H_2O_2 content (**b**), Total chlorophyll content (**c**).

Table 1. The sequence of gene-specifc primers used for qRT-PCR analysis

Target gene	Oligo name	Sequences 5'-3'
Actin (*ACT*)	ZmACT1_F	ACCAGTTGTTCGCCCACTAG
	ZmACT1_R	GAAGATCACCCTGTGCTGCT
Superoxide dismutase (*SOD)*	ZmSOD_F	TTGTTGCAAATGCTGAGGGC
	ZmSOD_R	AGGCAAGGATGTAACAGCGT
Catalase (*CAT1*)	ZmCAT1_F	TGCTTTCTGCCCAGCGATTA
	ZmCAT1_R	CACTTCTCACGACAGCCTGT
Ascorbate peroxidase (*APX1*)	ZmAPX1_F	GCCTTCTTCAGCTCCCAAGT
	ZmAPX1_R	TGCAAAAGACCACATGCAGC

stress conditions significantly decreased the total chlorophyll content compared with the control but significantly increased in the Rut application under salt stress conditions relative to the salt stress treatments (Fig. 1C).

3.2. Effects of rutin on water status under salinity stress conditions

Both levels of salinity stress decreased Leaf RWC (%), recording as 19.5 and 20.7% higher levels than the control, respectively. When exogenous Rut was applied, there was an increase in the leaf RWC (%) by 14.7 and 8.3% in seedlings subjected to 150 and 200 mM NaCl, respectively, compared to their stress groups. However, leaf RWC (%) was high in Rut combined 150 mM NaCl compared to 150 mM NaCl treatment (Fig. 2A). Total soluble sugar and proline levels markedly increased under both stress conditions compared with the control. Under stress conditions (150 and 200 mM NaCl), Rut application also significantly enhanced total soluble sugar by 14.2 and 30.0%, respectively, compared to the same stress level without Rut. Seedlings treated with rut exhibited an increase proline content by 13.3 and 5.5% under stress conditions (150 and 200 mM NaCl), respectively, compared to the same stress level alone. Additionally, proline content was high in Rut combined 150 mM NaCl in comparison with 150 mM NaCl application, total soluble sugar was high in Rut combined 200 mM NaCl in comparison with 200 mM NaCl application (Fig. 2B,C).

3.3. Effects of rutin on antioxidant capacity under salinity stress conditions

AsA content rose by 25.0 and 55.0 % in the salt applications (150 and 200 mM NaCl compared to the control seedlings. Rut treatment enhanced AsA content by 12.0 and 9.7% in maize seedlings exposed to 150 and 200 mM NaCl in comparison with the salt treatments, respectively. AsA content was high in the Rut combined 200 mM NaCl as compared to 200 mM NaCl treatment (Fig. 3A).

Enzymes activities increased in the both salt treatments as compared to the control seedlings. SOD activities in seedlings treated with Rut under 150 and 200 mM NaCl stress were 75.0 and 14.3% higher, respectively, than the same level of stress without Rut. Exogenous treatment of Rut significantly increased CAT activity by 117.5 and 33.3% under 150 and 200 mM NaCl stress, respectively, compared to the same stress level alone. Additionally, Rut under salt stress conditions (150 and 200 mM NaCl) increased APX activity by 20.0 and 25.0%, respectively, compared to the same stress level. The POD activity in seedlings applied with Rut exposed to 150 and 200 mM NaCl was 50.0 and 4.3%, respectively, higher than the same stress level (Figure 3B,C,D, and E). SOD, CAT, and POD activities also were high in the Rut combined 150 mM NaCl as compared to 150 mM NaCl treatment (Fig. 3B,C, and E)

Relative expression levels of *SOD*, *CAT1*, and *APX1* were significantly up-regulated in the both salt treatments and the Rut treatment in comparison with the control seedlings. Rut+150 mM NaCl and Rut+200 mM NaCl treatments up-regulated the gene expression levels of *SOD* by 30.4 and 30.3%, *CAT1* by 21.2 and 15.2%, and *APX1* by 27.8 and 27.0%, respectively, compared to the same stress levels alone. (Fig. 4A,B, and C).

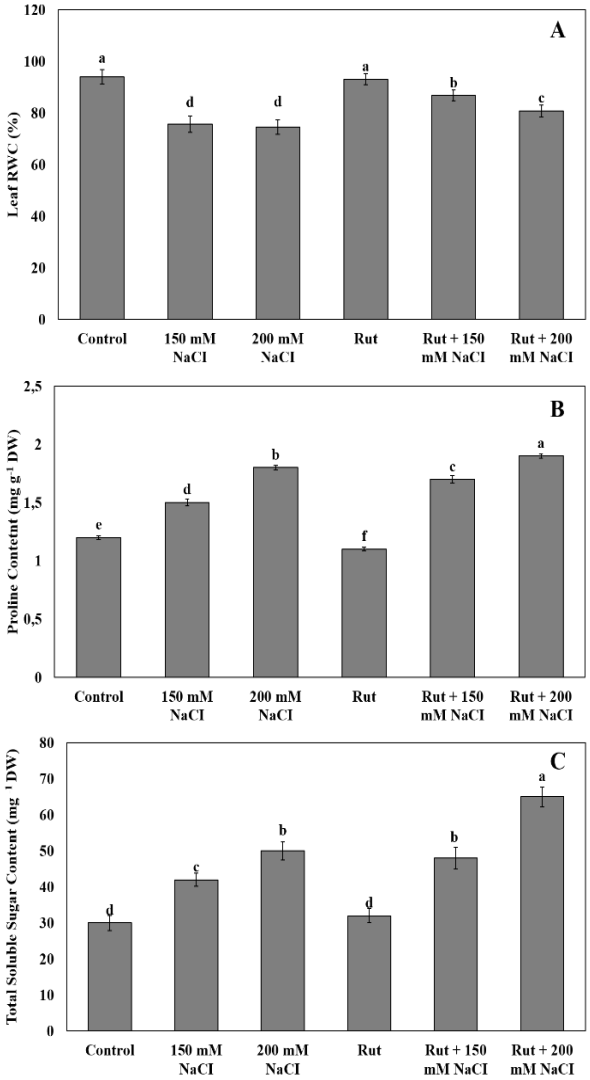

Figure 2. Effects of rutin on water status under salinity stress conditions. Leaf RWC (%) (**a**), Proline content (**b**), Total soluble sugar content (**c**).

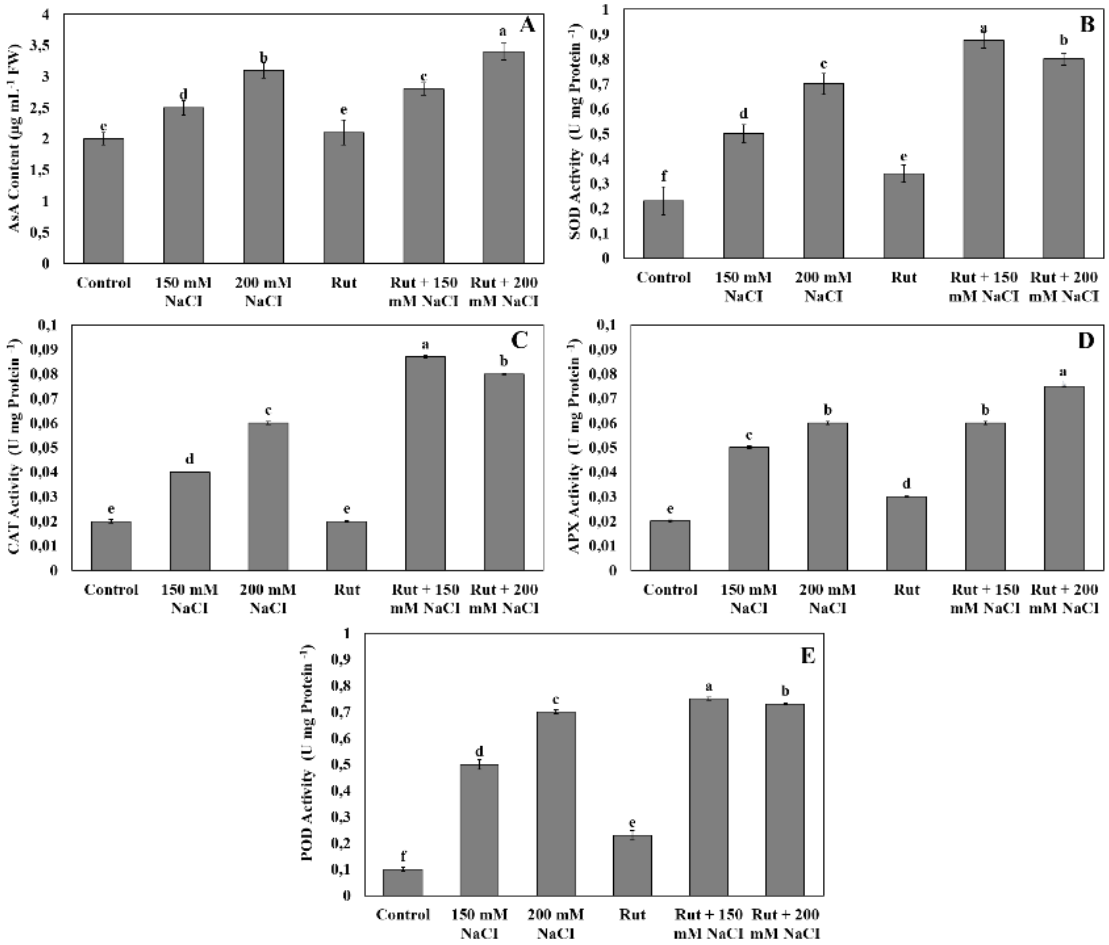

Figure 3. Effects of rutin on antioxidant capacity under salinity stress conditions. AsA content (**a**), SOD activity (**b**), CAT activity (**c**), APX activity (**d**), POD activity (**e**) .

4. Discussions

Our study revealed the effects of foliar Rut application on maize seedlings exposed to various levels of salt stress. We discuss the physiological and molecular mechanisms that seedlings use to tolerate salt stress conditions, as well as how Rut improves salt stress tolerance.

Lipid peroxidation is a process that NaCl, drought, high temperature, etc. cause in cell membranes. In our study, TBARS content increased under salt stress conditions. The use of Rutin reduced the amount of TBARS present as a result of lipid peroxidation brought on by salt stress, demonstrating the role of Rut as a protective and antioxidant molecule against oxidative damage, perhaps as a result of the enhanced activity of antioxidant enzymes. It has been reported that the inhibition of membrane damage increases as the concentration of Rutin increases (Yang et al., 2008). The fact that quercetin, a chemical derivative of Rutin, significantly reduces the amount of TBARS resulting from oxidative stress in salt stress tolerance in tomatoes supports our findings (Parvin et al., 2019). H_2O_2, a ROS produced by cellular metabolism, is a sign of a plant's ability to scavenge ROS in stressful environments. Exogenous Rut application under non-stressed conditions exhibited no obvious effect on H_2O_2 level. According to our findings, salt stress significantly raised the H_2O_2 level, but Rut application (Rut+150 mM NaCl and Rut+200 mM) reduced dramatically the H_2O_2 content under salt stress conditions. Our research further supported the prior papers' results that Rut application reduced H_2O_2 level of tobacco leaves under salt stress (200 mM) (Chen et al., 2019). Similar findings were reported by Parvin et al. (2019) who showed a significant decreased in H_2O_2 content in quercetin applied tomatoes under salt stress. In our current study, we concluded that Rut-treated maize seedlings experienced less cell death when exposed to salt stress, judging from the reduced ROS accumulation (TBARS and H_2O_2). However, the results of the current investigation suggested that Rut application may prevent cell death by lowering the levels of ROS accumulation, TBARS and H_2O_2 that protect against membrane damage under stressful circumstances. These findings suggested that rutin had a potent capacity to scavenge ROS.

Photosynthetic pigments are crucial physiological processes for maintaining plant activity and in solar energy transfer and absorption (Arnao and Hernandez 2010). In our study, salt stress decreased photosynthetic pigment in maize seedlings. However, we observed that pretreatment with rut reduced salt-induced reduction in photosynthesis. Quercetin, a precursor compound of Rutin, has a positive effect by increasing the total amount of chlorophyll in plants under osmotic stress and its antioxidant properties have been supported by studies conducted on wheat (Jańczak-Pieniążek et al., 2021), Arabidopsis (Kurepa et al., 2016) and tomato (Parvin et al., 2019) in which potassium quercetin was applied. Our findings show that exogenous Rut treatment under salinity stress conditions increases chlorophyll content compared to salt stress conditions alone.

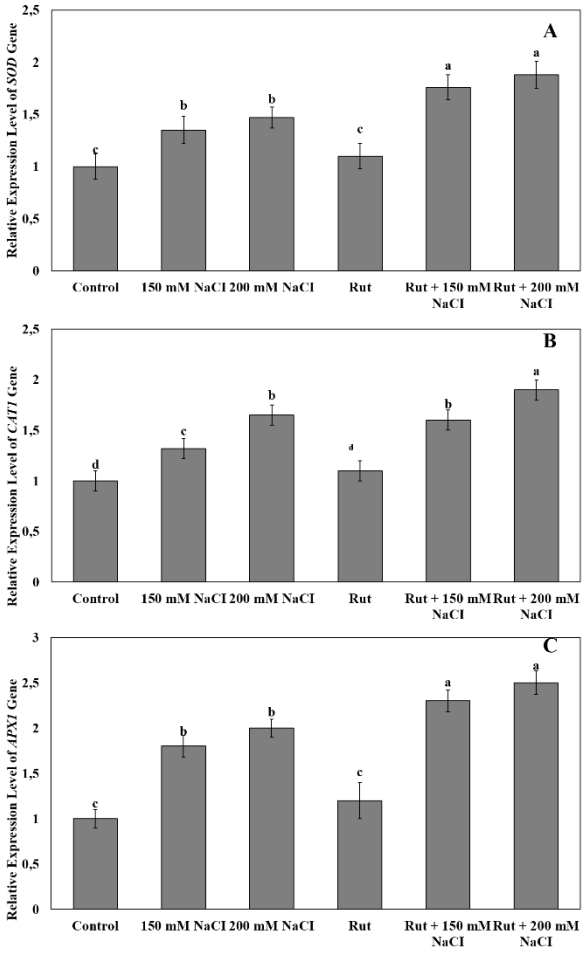

Figure 4. Gene expression analysis. Relative expression levels of *SOD* (**a**), *CAT1* (**b**), *APX1* (**c**).

To adapt to salt stress, plants use a wide range of mechanisms. The activation of the antioxidant defense system, effective ion exclusion, and osmolytes and secondary metabolite accumulation are a few of these tolerance mechanisms (Isayenkov and Maathuis, 2019). Plants have distinct adaptive physio-molecular responses to stress stimuli, such as osmotic adjustment and improved antioxidant capability (Linh et al., 2021). Osmotic adjustment is produced through the assimilation of numerous osmolytes, including proline and glycine betaine, soluble sugar as well as inorganic ions (Safwat and Salam, 2022). Abiotic stress often results in a decrease in leaf water status and an increase in osmotic regulators (Jiang et al., 2016). For evaluating plant tolerance to salt stress, relative water content has been used as an efficient method of water status (Suriya-arunroj et al., 2004). Our findings showed that rut enhanced leaf RWC, proline and total soluble sugar content in the salt stress conditions (150 and 200 mM NaCl). A previous study reported that the total soluble sugar content increased with Rut application to tomato seedlings under drought stress (Gorni, 2022). It was determined that rut application enhanced proline content in quinoa plants under salt stress (400 mM NaCl) (Ismail et al., 2015). In addition, when quercetin, a chemical derivative of Rutin, was applied to tomato seedlings exposed to salt stress, it was found that the amount of proline in the leaf increased as the amount of quercetin increased (Parvin et al., 2019). Our research supported the previous report's results that proline and soluble sugars are soluble compounds that shield plants from environmental challenges through osmoregulation, which scavenges ROS and preserves the integrity of the plasma membrane (Ashraf and Foolad, 2007).

Another important plant tolerance strategy under salt stress is the activation of the antioxidant defense system. The antioxidant system includes SOD, CAT, peroxidases, reductases, AsA, glutathione, polyphenols, etc. (Ren et al., 2020; Joshi et al., 2022). Our results showed that exogenous Rut enhanced the AsA content and the antioxidant enzyme activities such as SOD, CAT, APX, and POD. AsA, a non-enzymatic antioxidant, is used as a reductant in the conversion of H_2O_2 to water by APX enzyme activity (Das and Roychoudhury, 2014). It has been reported that quercetin application increased AsA content in tomato seedlings exposed to salt stress (Parveen et al., 2019). Ismail et al. (2015) in their study, investigated salt stress tolerance of enzymatic and non-enzymatic antioxidants of halophyte and normal genotypes of *Chenopodium quinoa* Willd. species with high flavonoid content. It was found that the flavonoid content, and mostly Rutin, increased when Titicaca plants were exposed to salt stress. The reason for this has been determined that salt stress tolerance is achieved by Rut by regulating K^+ accumulation and Na^+ exclusion in the leaf mesophyll. Superoxide radicals, which increase during osmotic stress, stimulate SOD enzyme activity and try to reduce reactive compounds by converting superoxide into H_2O_2. By converting the H_2O_2 produced into water by APX, CAT, POD enzymes, tolerance is achieved by keeping the amount of ROS below a certain limit (Asada, 1999). APX is an important enzyme of the ascorbate-glutathione cycle. While CAT mostly clears H_2O_2 in peroxisomes, APX serves the same purpose in the cytosol and chloroplasts. APX reduces H_2O_2 to H_2O and dehydroascorbic acid using AsA as the reducing agent. In stressful situations, APX was found to be more effective in scavenging H_2O_2 from CAT (Sharma et al., 2012). Contrary to our findings, a decrease in APX activity was found with quercetin application in tomato seedlings exposed to salt stress (Parvin et al., 2019). When POD is active inside the cell (cytosol, vacuole), cell wall and extracellularly, it is known to play a role as a key enzyme in the scavenging of H_2O_2 (Das and Roychoudhury, 2014). It has been found that excessive increase in *GPX* gene expression increases plant tolerance (Ozyigit et al., 2016). Therefore, Rut treatment might lessen the negative effects of the active oxygen scavenging system of maize seedlings caused by salt and can boost a plant's tolerance to stress. Additionally, in the current study, when we evaluated the gene expressions of *SOD*, *CAT 1*, and *APX* 1 enzymes, it was found to be compatible with their enzyme activities.

According to the findings, exogenous rutin causes changes in physico-biochemical and molecular properties in seedlings and may be a beneficial practice under salt stress conditions. Our findings demonstrated that exogenous rutin treatment in maize seedlings strengthened the antioxidant defense system by reducing ROS accumulation, as indicated by lower TBARS and H_2O_2 levels under salt stress conditions. Rutin supplementation improved seedling chlorophyll content and water status by increasing osmolytes content and leaf RWC.

Conflict of Interest

Author has declared no conflict of interest.

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 Science 7: 276.

Acosta-Motos JR, Diaz-Vivancos P, Álvarez S, Fernández-García N, Sanchez-Blanco MJ, Hernández JA (2015). Physiological and biochemical mechanisms of the ornamental Eugenia myrtifolia L. Plants for coping with NaCl stress and recovery. Planta 42: 829-46.

Aebi HE (1983). Catalase. Methods of enzymatic analysis. New York: Akademic press.

Ali M, Afzal S, Parveen A, Kamran M, Javed MR, Abbasi GH (2021). Silicon mediated improvement in the growth and ion homeostasis by decreasing Na+ uptake in maize (*Zea mays* L.) cultivars exposed to salinity stress. Plant Physiology Biochemistry 158: 208-18.

Arnao MB, Hernandez RJ (2010). Protective effect of melatonin against chlorophyll degradation during the senescence of barleyleaves. Journal of Pineal Research 46: 58-63.

Arnon DI (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant physiology 24(1): 1.

Asada K (1999). The water-water cycle in chloroplasts: Scavenging of active oxygens and dissipation of excess photons. Annual Review of Plant Physiology and Plant Molecular Biology 50: 601-639.

Ashraf M, Foolad MR (2007). Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environmental and Experimental Botany 59: 206-216.

Bates LS, Waldren RP, Teare ID (1973). Rapid determination of free proline for water-stress studies. Plant and soil 39: 205-207.

Beauchamp C, Fridovich I (1971). Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Analytical Biochemistry 44: 276-287.

Chen S, Wu F, Li Y, Qian Y, Pan X, Li F and Yang A (2019). NtMYB4 and NtCHS1 are critical factors in the regulation of flavonoid biosynthesis and are involved in salinity responsiveness. Frontiers in plant science 10: 178-190.

Das K and Roychoudhury A (2014). Reactive oxygen Species, ROS and response of antioxidants as ROS-scavengers during environmental stress in plants. Frontiers in Environmental Science 2: 53.

Dubois M, Gilles KA, Hamilton JK, Rebers PT, Smith F (1956). Colorimetric method for determination of sugars and related substances. Analytical Chemistry 28: 350-356.

Farooq M, Hussain M, Wakeel A and Siddique KHM (2015). Salt stress in maize: effects, resistance mechanisms, and management. A review. Agronomy for Sustainable Development 35: 461-481.

Feng XH, Hussain T, Guo K, An P, Liu XJ (2021). Physiological, morphological and anatomical responses of Hibiscus moscheutos to non-uniform salinity stress. Environmental and Experimental Botany 182: 104301.

Gorni PH, Lima GR, Oliveira Pereira LM, Spera KD, Macros LA and Pacheco AC (2022). Increasing plant performance, fruit production and nutritional value of tomato through foliar applied rutin. Scientia Horticulturae 294: 110755.

Heath RL, Packer L (1968). Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. Archives of Biochemistry and Biophysics 125: 189-198.

Hoagland DR, Arnon DI (1950). The water-culture method for growing plants without soil. Circular. California Agricultural Experiment Station Circular 347: 1-32.

Huo LQ, Guo ZJ, Wang P, Zhang ZJ, Jia X, Sun YM (2020). *MdATG8i* functions positively in apple salt tolerance by maintaining photosynthetic ability and increasing the accumulation of arginine and polyamines. Environmental and Experimental Botany 172: 103989.

Isayenkov SV, Maathuis FJM (2019) Plant salinity stress: Many unanswered questions remain. Frontiers of Plant Science 10: 80

Ismail H, Maksimović JD, Maksimović V, Shabala L, Živanović BD, Tian Y and Shabala S (2015). Rutin, a flavonoid with antioxidant activity, improves plant salinity tolerance by regulating K+ retention and Na+ exclusion from leaf mesophyll in quinoa and broad beans. Functional Plant Biology 43(1): 75-86.

Jańczak-Pieniążek M, Migut D, Piechowiak T, Buczek J and Balawejder M (2021). The Effect of exogenous application of quercetin derivative solutions on the course of physiological and biochemical processes in wheat seedlings, International Journal of Molecular Sciences 22 (13): 6882.

Jiang P, Burczynski F, Campbell C, Pierce, G, Austria JA, Briggs CJ (2007). Rutin and flavonoid contents in three buckwheat species *Fagopyrum esculentum, F. tataricum,* and *F. homotropicum* and their protective effects against lipid peroxidation. Food research international 40(3): 356-364.

Jiang C, Cui Q, Feng K, Xu D, Li C, Zheng Q (2016). Melatonin improves antioxidant capacity and ion homeostasis and enhances salt tolerance in maize seedlings. Acta Acta Physiologiae Plantarum 38: 82.

Joshi S, Nath J, Singh AK, Pareek A, Joshi R (2022). Ion transporters and their regulatory signal transduction mechanisms for salinity tolerance in plants. Physiologia Plantarum 174: 13702.

Ju FY, Pang JL, Huo YY, Zhu JJ, Yu K, Sun LY (2021). Potassium application alleviates the negative effects of salt stress on cotton (*Gossypium hirsutum* L.) yield by improving the ionic homeostasis, photosynthetic capacity and carbohydrate metabolism of the leaf subtending the cotton boll. Field Crop Research 272: 108288.

Kang SZ, Su XL, Tong L, Shi PZ, Yang XY, Abe YK (2004). The impacts of human activities on the water-land environment of the Shiyang River basin, an arid region in Northwest China. Hydrological Sciences Journal 49(3): 427.

Kumar M, Kumar PM, Kumar N, Bajpai AB and Siddique KHM (2021). Metabolomics and Molecular Approaches Reveal Drought Stress Tolerance in Plants. International Journal of Molecular Sciences 22(17): 9108.

Kurepa, J, Shull T, E and Smalle JA (2016) Quercetin feeding protects plants against oxidative stress. Research 5: 2430.

Liang W, Ma X, Wan P, Liu L (2017). Plant salt-tolerance mechanism: a review. Biochemical Biophysical Research Communications 495: 286-291.

Linh NT, Cham LTT, Thang VN (2021) Effects of salinity stress on the growth, physiology, and yield of soybean (*Glycine max* (L.) Merrill). Vietnam Journal of Agricultural Science 4: 1043-1055.

Liso R, Calabrese G, Bitonti MB, Arrigoni O (1984). Relationship between ascorbic acid and cell division. Experimental Cell Research 150: 314-320.

Meng Q, Chen X, Lobell DL, Cui Z, Zhang Y, Yang H, Zhang F (2016). Growing sensitivity of maize to water scarcity under climate change. Scientific Reports 6: 19605.

Mittova V, Guy M, Tal M, Volokita M (2004). Salinity up-regulates the antioxidative system in root mitochondria and peroxisomes of the wild salt-tolerant tomato species *Lycopersicon pennellii*. Journal Experimental Botany 55: 1105-13.

Mukhopadhyay R, Sarkar B, Jat HS, Sharma PC, Bolan NS (2021). Soil salinity under climate change: challenges for sustainable agriculture and food security. Journal of Environmental Management 280: 111736.

Nakano Y, Asada K (1981). Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant and Cell Physiology. 22: 867-880.

Negrão S, Schmöckel SM, Tester M (2017). Evaluating physiological responses of plants to salinity stress. Annals of Botany 119: 1-11.

Ozyigit II, Filiz E, Vatansever R, Kurtoglu KY, Koc I, Öztürk MX and Anjum NA (2016). Identification and comparative analysis of H_2O_2-scavenging enzymes, ascorbate peroxidase and glutathione peroxidase in selected plants employing bioinformatics approaches. Frontiers in Plant Science 7.

Parveen A, Liu W, Hussain S, Asghar J, Perveen S, Xiong Y (2019). Silicon priming regulates morpho-physiological growth and oxidative metabolism in maize under drought stress. Plants *8*(10): 431.

Parvin K, Hasanuzzaman M, Bhuyan MB, Mohsin SM and Fujita M (2019). Quercetin mediated salt tolerance in tomato through the enhancement of plant antioxidant defense and glyoxalase systems. Plants 8 (8): 247.

Ren J, Ye J, Yin L, Li G, Deng X, Wang S (2020). Exogenous melatonin ımproves salt tolerance by mitigating osmotic, ion, and oxidative stresses in maize seedlings. Agronomy 10: 663.

Safwat G, Salam HSA (2022). The effect of exogenous proline and glycine betaine on phyto-biochemical responses of salt-stressed basil plants. Egyptian Journal of Botany 62: 537-547.

Sánchez-McSweeney A, González-Gordo S, Aranda-Sicilia MN, Rodríguez- Rosales MP, Venema K, Palma JM (2021). Loss of function of the chloroplast membrane K(+)/H(+) antiporters AtKEA1 and AtKEA2 alters the ROS and NO metabolism but promotes drought stress resilience. Plant Physiology and Biochemistry 160: 106-19.

Sezgin A, Altuntaş C, Sağlam A, Terzi R, Demiralay M, Kadıoğlu A (2018). Abscisic acid cross-talking with hydrogen peroxide and osmolyte compounds may regulate the leaf rolling mechanism under drought. Acta Physiologiae Plantarum. 40: 1-12.

Sharma P, Jha AB, Dubey RS and Pessarakli M (2012). Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. Journal of Botany 2012: 1-26.

Singh A, Gupta R, Pandey R (2017). Exogenous application of rutin and gallic acid regulate antioxidants and alleviate reactive oxygen generation in *Oryza sativa* L. Physiology and Molecular Biology of Plants. 23: 301-309.

Suriya-arunroj D, Supapoj N, Toojinda T, Vanavichit A (2004). Relative leaf water content as an efficient method for evaluating rice cultivars for tolerance to salt stress. Science Asia 30: 411-415.

Suzuki T, Honda Y and Mukasa Y (2005). Effects of UV-B radiation, cold and desiccation stress on rutin concentration and rutin glucosidase activity in tartary buckwheat (*Fagopyrum tataricum*) leaves. Plant Science 168(5): 1303-1307.

Suzuki T, Morishita T, KIM SJ, Park SU, Woo SH, Noda T, Takigawa S (2015). Physiological roles of rutin in the buckwheat plant. Japan Agricultural Research Quarterly: JARQ 49(1): 37-43.

Taiz L, Zeiger E (2008). Plant Physiology. Ankara: Palme Publishing.

Urbanek H, Kuzniak-Gebarowska E, Herka K (1991). Elicitation of defence responses in bean leaves by *Botrytis cinerea* polygalacturonase. Acta Physiologiae Plantarum 13.

Velikova V, Yordanov I, Edreva A (2000). Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines. Plant science 151: 59-66.

Wei TL, Wang Y, Liu JH (2020). Comparative transcriptome analysis reveals synergistic and disparate defense pathways in the leaves and roots of trifoliate orange (Poncirus trifoliata) autotetraploids with enhanced salt tolerance. Horticulture Research 7: 88.

Yang J, Guo J, Yuan J (2008). In vitro antioxidant properties of rutin. Lebensm. Wiss Technology 41: 1060-1066.

Yu ZP, Duan XB, Luo L, Dai SJ, Ding ZJ, Xia GM (2020). How plant hormones mediate salt stress responses. Trends in Plant Science 25: 1117-1130.

Zelm EV, Zhang YX, Testerink C (2020). Salt tolerance mechanisms of plants. Annual Review Plant Biology 71:403-433.

Zhong M, Song R, Wang Y, Shu S, Sun J, Guo SR (2020). TGase regulates salt stress tolerance through enhancing bound polyaminesmediated antioxidant enzymes activity in tomato. Environmental and Experimental Botany 179: 104191.

Zhu JK (2016). Abiotic stress signaling and responses in plants. Cell 167: 313-324.