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NOSHEEN IFTIKHAR

SHAGUFTA PERVEEN

BABER ALI

MUHAMMAD HAMZAH SALEEM

MOHAMMAD KHALID AI-SADOON

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Research Article

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Physiological and biochemical responses of maize (Zea mays L.) cultivars under salinity stress

Nosheen IFTIKHAR¹, Shagufta PERVEEN¹, Baber ALI^{2,*}, Muhammad Hamzah SALEEM³, Mohammad Khalid Al-SADOON⁴

¹Department of Botany, Government College University, Faisalabad, Pakistan ²Department of Plant Sciences, Quaid-i-Azam University, Islamabad, Pakistan ³College of Plant Science and Technology, Huazhong Agricultural University, Wuhan 430070, China Department of Zoology, College of Science, King Saud University, Rivadh, Saudi Arabia

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Abstract: Salt stress is a serious threatening factor for cereal crops such as maize (Zea mays L.) by affecting their growth and development. In the current era, the requirement for staple crops is increasing, so it is important to screen out salt-tolerant genotypes. For this purpose, a pot experiment was designed within three replications on ten different genotypes of the maize. The plants were planted in plastic pots and salt stress (0, 40, 70, 100 mM) was maintained. The salt stress induced a noticeable reduction in plant growth traits (shoot length, root length, shoot, root fresh and dry weight, and leaf area (LA). The photosynthetic pigments as Chl a, Chl b, total chlorophyll, and carotenoids. The elevated stress levels cause an intensive accumulation rate of MDA (malondialdehyde) and H₂O₂ (hydrogen peroxide) resulting from stress exposure and ultimately damaged the membrane-bounded organelles. The flavonoid and phenolic contents increased as the salt stress level increased, this increase was higher in Pearl and least in Sadaf. The activity of cellular antioxidants (SOD) is significantly enhanced under stress to quench oxidative stress. Our results revealed the genotype Sadaf as sensitive and salt tolerant genotypes were as Pearl > Sahiwal 2002 > Pioneer > MMRI(Y). Based on screening, the tolerant genotypes have the potential to grow under saline conditions. However, further research is needed to explore the genetic basis of salt tolerance in these genotypes.

Key words: Maize, cereal crop, salt stress, chlorophyll, cellular antioxidant, oxidative stress

Abbreviations: LA- Leaf area, CRD-Completely randomized design, Chl- Chlorophyll, Flavo- Flavonoids, Phenol- Phenolics, Caroten-Carotenoids

1. Introduction

The interaction of plants with the environment is obvious but when plants face stressful conditions, these conditions ultimately lead to a reduction in their growth and development (Zhu, 2016; Abeed et al., 2022). Plants have a variety of tolerance mechanisms that include the accumulation of proteins, osmoprotectants, modifications in ion transporters, and transcriptional regulation and ion homeostasis (Abeed et al., 2022). The signalling cascades are also stimulated to counteract biochemical and molecular changes (Saharan et al., 2022). The current climatic scenarios rapidly exaggerated salt stress, especially in cereal crops. The salt stress imposes a negative effect on seedling growth, photosynthesis, and yield (Salama et al., 2022). Salt stress also disrupts intracellular ion homeostasis and results in osmotic stress, which imbalances intracellular K⁺ in roots, shoots, and leaves. Additionally, promotes the

reactive oxygen species (ROS) and these ROS scavenge the cellular antioxidant and shut down the levels of organic osmolytes and reduce membrane permeability (Ye et al., 2022). To counteract these serious threats, salt-tolerant crops, improved the defensive mechanisms by enhancing the antioxidants, flavonoids and phenolics contents, free amino acids and soluble sugars (Zhao et al., 2020). Nowadays, screening of salt-tolerant genotypes is the main focus of most of the researches of the current era, because these crops are mandatory to fulfil the food demand of the world (Talaat et al., 2022). Overcoming soil salinity is one of the major global issues and needs to be solved by phytoremediation strategies and planting tolerant plant ecotypes (Adhikari et al., 2020).

Salt stress is considered to determine the reason for seed dormancy, nutrient deficiency and low profile foods across the world (Shiade et al., 2020). Salinity



^{*} Correspondence: baberali@bs.qau.edu.pk

affected about considerable portion of the agricultural land of world (Srivastava et al., 2019). Soil salinization is expanding rapidly across the world due to poor drainage and agricultural practices (Rasel et al. 2021; Wang et al., 2020). In contrast, human-induced anthropogenic activities include water irrigation by using a water table, inadequate drainage, and runoff nutrients by rains toward the water reserves (Santpoor 2020; Syed et al., 2021). The expanding salt stress has a severe effect on Pakistan's economy (ur Rehman et al., 2021). Many cereal crops have a negative correlation with salt stress in terms of food production (Arif et al., 2020; ur Rehman et al., 2021).

Maize (Zea mays L.) is an important cereal crop belonging to the family Poaceae and included staple food is required to cope with food deficiency worldwide (Jacob et al., 2020). Maize is a valuable cereal crop and provides food for humans as well as fodder for livestock. It contributes 36% (782 Mt) of global grain production (Kaleem et al., 2021). It is a rich source of nutrition; carbohydrates (18.7%), lipids (1.35%), proteins (3.27%), and vitamins (Kaushal et al., 2023). Maize (Zea mays L.) can adapt changings under unfavourable conditions (salinity, drought, chilling, and heat stress) and environmental changes (Kumar et al., 2022; Lee et al., 2021; Sabagh et al., 2021; ur Rehman et al., 2021). Plant growth, physiology, and biomass are highly affected by an elevated level of abiotic stresses (Dekobe et al., 2021). While, plant growth promotors are requisite to attain the increased growth regeneration (Wu et al., 2022; Asghar et al., 2023). Salt stress is key limiting factor for plant leaf expansion and grain numbers and germination (Hadia et al., 2023). This also causes a reduction in chlorophyll pigments (Chl a, b, and total chlorophyll), and starch levels (Hassanein et al., 2002). Flavonoid accumulation is decreased when a crop is subjected to salt stress (Perveen et al., 2021). Increasing environmental changes have a deleterious effect on Z.mays growth and yield rate ultimately decreasing the food availability and leading to corn deficiency. There is a reduced production rate to meet the need of Z. mays (Kaya et al., 2020).

The salt stress influence on cereal crops is currently expanded, so it is a dire need to overcome this problem by screening salt-stress-tolerant crops to enhance the production of the food. Our present study hypothesized that current results should help to differentiate saltsusceptible and tolerant genotypes by ascertaining the growth rate, photosynthetic pigments and enzymatic and nonenzymatic antioxidants. This work will also provide information for future researchers to consider the most suitable cultivars grown under saline conditions.

2. Materials and methods

A pot (10 cm \times 7 cm) experiment following a completely randomized design (CRD) was performed in the stress

physiology lab of the Department of Botany, Government College University Faisalabad during the month of March. Day to night temperature was 25-30 °C and humidity average was 60%-70%. The seeds of 10 maize (Zea mays L.) varieties; Malika 2016, Sadaf, Agaiti 2002, Akbar, MMRI(Y), Pak afghoi, Neelum, Pioneer, Pearl, and Sahiwal were collected from Ayub Agricultural Research Institute Faisalabad and Maize and Millet Research Institute (MMRI) Sahiwal. Before seed sowing, seeds were well washed with double distilled water. Then healthy seeds were selected for sowing purposes. Pots were filled with washed and air-dried sand. To avoid water logging, a hole was made in the bottom of plastic pots before seed sowing. Five seeds per pot were sown and each variety had three replicates. After 7 days of seed sowing, uniform germination was observed and at this stage, salt (NaCl) stress along with Hogland's solution was applied to plants through the sand medium. For each of the ten maize varieties, four salts (NaCl) stress levels (S1 = 0 mM, S2 = 40 mM, S3 = 70 mM, S4 = 100 mM) were applied. While the control plants were only watered along with Hogland's solution. Plants condition and their stress response were monitored (thinning was also done when needed) on daily bases for up to four weeks. After the 27th day of stress application, plants were uprooted to measure growth and physiological parameters and leaves were placed in airtight bags for further biochemical and antioxidative analysis.

2.1. Sampling and data curation

All plants were uprooted in April 2019. Three plants from each pot were harvested for different morphological measurements. Each of the plants was washed and divided into two parts (root and shoot) to avoid dust. Leaves of plants from all pots were packed in air-tight bags and stored in the freezer for chlorophyll and other physiological traits. Root and shoot length was measured by using a measuring scale. Root and shoot fresh weight was measured through a digital weighing balance. Then, the root and shoot were packed in brown paper and placed in an oven at 105 °C for 1 h. Then at 70 °C for 72 h (3 days and nights) to measure their dry weight.

2.2. Plant analysis and measurements

Leaf area was calculated by using a method proposed by Carleton and Foote (1965). Relative water content was calculated by following the techniques of Jones and Turner (1978). The 0.5 g of fresh leaves were cut into pieces and ground with 80% acetone (10 mL). Then centrifuged and absorbance values were recorded at 480, 645, and 663 nm by Arnon's methods (Arnon 1949) described method. Malondialdehyde (MDA) contents were measured by using TBA (Thiobarbituric acid) by following the method of Cakmak and Horst (1991). Hydrogen peroxide (H_2O_2) was calculated at a wavelength of 390 nm through a UVvisible spectrophotomete. Flavonoids were measured at 510 nm by using $AlCl_3$ (10%) and NaOH (1M) (Karadeniz et al., 2005). The 0.5 g of leaf sample was used for grinding in 10 mL of acetone (80%) and the treatment of Folin-Ciocalteau's phenol reagent absorbance for phenolic content estimation was noted at 750 nm by using a described method of Julkunen-Tiitto (1985). For sodium ion estimation acid digestion by Allen et al. (1986) was applied where sulphuric acid and hydrogen peroxide were used and chloride ion was estimated by AgCl precipitation following the method of Johnson and Ulrich (1959) by using bartend's reagent and value was noted at a wavelength of 460nm. The analysis of variance (ANOVA) was used to examine all the data, and the least significant differences (LSD) test was used to identify any significant differences in arsenic stress at the p < 0.05 level.

3. Results

3.1. Plant growth traits

The present experiment was designed to evaluate the salinity stress effect in various maize (Z. mays) cultivars. The present findings depicted that salt stress has a negative effect on growth rate. The Sadaf variety showed significantly $(p \ge 0.001)$ highest reduction in growth parameters (root length, shoot length, root fresh weight, shoot fresh weight, root dry weight, shoot dry weight, and leaf area) while the Malika 2016, Agaiti 2002 and Pak Afghoi varieties also showed reduced root and shoot length and root and shoot fresh and dry weight but their reduction rate was less as compared to the Sadaf cultivar. Pearl cultivar showed significantly ($p \ge 0.001$) least salt stress effect on growth rate of plants than the MMRI(Y) and Sahiwal 2002 varieties, which showed little effect of sodium chloride on growth attributes of maize (Z. mays) (Figure 1, Table 1). All maize cultivars showed that gradual increased salt stress level have higher damaging effect on growth rate respectively. All cultivars showed least decrease in root and shoot length, fresh and dry weight, LA and RWC when plant was exposed to 0 mM of NaCl and their reduction rate was increased as salt stress level was increased as 0,40, 70, and 10 mM sodium chloride concentration.

3.2. Photosynthetic pigments

Chlorophyll pigments (chlorophyll a, b, carotenoids, and total chlorophyll) were highly reduced in the Sadaf cultivar under sodium chloride stress while Pearl showed the least reducing effect of salinity stress on its photosynthetic pigments (Figure 2, Table 1). The statistically significant ($p \ge 0.001$) effect of salt stress was observed on all *Z. mays* cultivars but according to statistics its greater reducing effect was observed in the Sadaf, Malika 2016, and the Agaiti cultivars while less salinity effect was observed in the Pearl, Sahiwal 2002 and Pioneer varieties. All other *Z. mays* cultivars showed a moderate effect of salinity. Plants grown without stress conditions showed increased

chlorophyll pigments but salinity-subjected plants showed more reduction with elevated salinity stress levels (0 mM, 40 mM, 70 mM, 100 mM).

3.3. Oxidative stress markers and osmoprotectants

In this experiment, ten maize (Zea mays L.) varieties were used to check tolerance levels against salinity stress. MDA and H₂0, were accumulated under salinity stress as compared to control conditions (Figure 2, Table, 1). Under stress-subjected conditions, nonenzymatic antioxidants are increased in concentration to mitigate the oxidative stress effect produced by ROS. The Sadaf cultivar statistically showed more significant (p > 0.001) accumulation under stress exposure as compared to the Pearl variety. So, Pearl is considered salinity tolerant and Sadaf a salt-sensitive variety. The trend of tolerance rate to sodium chloride stress among different maize (Zea mays L.) cultivars was Peal > Sahiwal 2002 > Pioneer > MMRI(Y) > Neelum > Akbar > Pak Afghoi > Agaiti 2002 > Malika 2016 > Sadaf. Salinity stress showed a significant (p > 0.001) increase in these oxidative stress biomarkers.

Phenolics and flavonoids are highly accumulated under different levels of salinity stress. Their accumulation was more (p > 0.001) in salinity tolerant (Pearl) and least in sensitive (Sadaf) cultivar. The salt stress showed a greater changed effect (p > 0.001) on all varieties (Figure 2, Table 1). Some varieties (Pear, Sahiwal 2002, Pioneer) showed higher accumulation under stress exposure while others showed the least accumulation (the Sadaf, Malika 2016, Agaiti 2002 varieties). While, the Pak Afghoi, Akbar and Neelum varieties showed moderately tolerant behaviour towards saline conditions.

3.4. Ionic contents

Salinity stress significantly (p > 0.001) increased sodium and chloride ions in the Sadaf cultivar under highly saline conditions while significantly (p > 0.001) least ion accumulation was observed in Pearl (salt tolerant variety) (Figure 3, Table 1). All other *Z. mays* cultivars showed moderate Na⁺ and Cl⁻ ions accumulation rates in the order of Malika 2016 > Agaiti 2002 > Pak Afghoi > Akbar > Neelum > MMRI(Y) > Sahiwal 2002 > Pioneer.

3.5. Pearson's correlation

A Pearson's correlation graph was constructed to analyze the relationship between various growth characteristics of Z. mays (Malika 2016, Sadaf, Agaiti 2002, Akbar, MMRI(Y), Pak afghoi, Neelum, Pioneer, Pearl and Sahiwal) cultivars with MDA and H_2O_2 formation (Figure 4). MDA and H_2O_2 were positively correlated with each other. Similarly, all growth attributes such as SL, RL, SFW, RFW, SDW, RDW, LA, Chl a, b, T. Chl and carotenoids were positively correlated with each other while, were negatively correlated with MDA and H_2O_2 . Phenolic, flavonoid and relative water content were also negatively correlated with

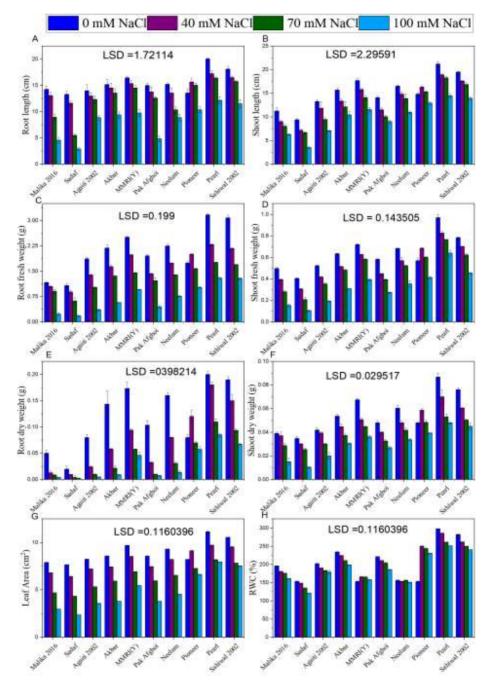


Figure 1. Physio-morphological attributes of maize (*Zea mays* L.) ($p \le 0.05$) between four salt treatments (0, 40, 70, and 100 mM).

MDA and H_2O_2 and were slightly correlated with all other studied attributes. This Pearson's correlation demonstrated a strong connection between plant growth and ROS production.

3.6. Principal component analysis

Principal component analysis (PCA) provides loading plots to assess the effect of NaCl-stress on Z. mays

(Malika 2016, Sadaf, Agaiti 2002, Akbar, MMRI(Y), Pak afghoi, Neelum, Pioneer, Pearl and Sahiwal) cultivars as presented in Figure 5. Among the entire main component PC1 and PC2 provide more than the overall data base and comprises the largest portion of all components (Figure 5). Accordingly, MDA and H₂O₂ were positively correlated with each other. Similarly, all growth attributes such as SL, RL, SFW, RFW, SDW, RDW, LA, Chl a, b, T.

SOV	NaCl stress (S)	Variety (V)	V×S	Error
RL	321.67003***	72.8756***	4.4240***	1.122
SL	170.7645***	165.1286***	0.5629ns	0.665
RFW	12.0579***	2.8955***	0.0220***	0.005
RDW	0.607544***	0.025910***	0.0013154***	0.00012
SFW	1.5542***	0.85096***	0.0158***	0.0026
SDW	0.0041***	0.0019***	3.899***	0.00011
LA	108.9842***	22.9983***	0.5023***	0.0017
RWC	4635.358***	25965.138***	190.2267***	5.240
Chl. a	0.2493***	0.4393***	0.003914***	0.00018
Chl. b	0.56077***	0.32136***	9.5416***	0.00048
Total Chl.	1.5478***	1.4880***	0.0059117***	0.00062
Caroten.	0.01057***	0.00105***	1.255**	0.00061
MDA	207.3744***	235.82***	1.3594***	1.1320
H ₂ O ₂	145.5388***	36.6033***	0.3143***	0.112
Flavo.	6113.13***	4429.14***	146.87***	1.2883
Phenol.	64.3398***	25.9915***	0.2502***	0.6581
Root Na ⁺	1376.5639***	571.39352***	45.144136***	1.3334
Shoot Na ⁺	3138.3778***	624.04074***	53.64321***	1.625
Root Cl-	1087.5556***	470.38519***	51.481481***	1.658
Shoot Cl-	1556.2972***	581.3787***	44.223148***	1.334
Df	3	9	27	80

Table 1. Mean square values of salt induced changes in growth, physiological and ionic traits of maize (Zea mays L.) where *, ** and *** = Significant at 0.05, 0.01, and 0.001 levels respectively; ns = nonsignificant; df = degree of freedom.

Chl and carotenoids were positively correlated with each other while, were negatively correlated with MDA and H_2O_2 . Phenolic, flavonoid and relative water content were also negatively correlated with MDA and H_2O_2 and were slightly correlated with all other studied attributes.

4. Discussion

Salt stress is the most harmful threatening factor for food production because it decreases the growth and production rate of any crop (Li et al., 2020). Sodium chloride stress is a ubiquitous threat to crops of their reduced growth and development. Reclaimation ability of shoot is highly important for effective transformation system (Asghar et al., 2022). Saline areas have consisted of large amounts of soluble salts and exchangeable ions which ultimately reduce the plant growth rate by minimizing the height, weight and biomass of any plant. This stress has a negative effect on commercially produced crops; it harms above 800 million ha of land all over the world (FAO and Rome, 2005). Salt stress may be the cause of reduced morphological, physiological, and biochemical changings and result in altered growth and productivity rate (El-Naim et al., 2012; Moud and Maghsoudi, 2008; Nxele et al., 2017; Rady et al., 2019). In the present experiment noticeable gradual decrease in plant height, fresh and dry weight along with leaf area was noticed with four salt stress levels

(0, 40, 70, 100 mM) (Figure 1, Table 1). The mechanism of this reduced height, fresh and dry matter and LA might be less water uptake by plant leading to reduced growth rate (Deinlein et al., 2014). Plant cell injury and cell death caused by the entrance of an extra amount of salt may be the reason for the reduced growth rate of plants (Muchate et al., 2016). Another reason might be the limited water, nutrient and air supply to plants which ultimately reduces plant biomass (Attia et al., 2008). Reduced root and shoot length might be due to increased osmotic pressure in the root zone under a saline condition which ultimately affects the root water uptaking process and results in short plant height (Aydınşakir et al., 2013). Another reason for the reduced growth and height rate of a plant under salinity stress may be the increased osmotic stress, oxidative stress, nutrient deficiency, and ion imbalance (El-Naim et al., 2022). The same findings were observed in wheat (Gholizadeh et al., 2021) and rice (Sarwar et al., 2022).

Photosynthesis is an important process for the food production of any plant. Photosynthesis is controlled by chlorophyll pigments (chl a, chl b, and carotenoids) and the functioning of these pigments is highly reduced under saline conditions (Riaz et al., 2019). Salt stress minimizes photosynthesis by reducing RUBISCO activity ultimately, the photosystem activity is reduced (Parvin et al., 2019). The present experimental work consisting of four stress

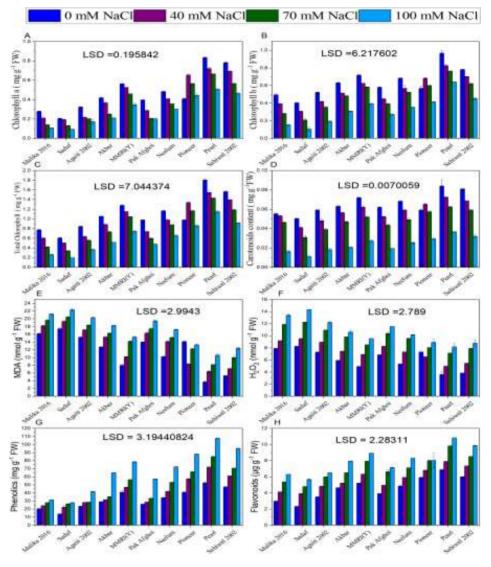


Figure 2. Biochemical traits attributes of maize (Zea mays) ($p \le 0.05$) between four salt treatments (0, 40, 70, and 100 Mm).

levels showed a gradual reduction in chlorophyll pigments in all maize (*Zea mays* L.) cultivars. The Pearl variety showed less effect on chlorophyll pigments while Sadaf cultivars' photosynthetic pigments were highly affected by salinity stress. This effect was gradually increased by increasing stress levels (Figure 1, Table 1).

Reduced chlorophyll contents may be due to the inhibitory effect of accumulated ions (Srinieng et al., 2015). Another reason may be the increased chlorophyllase activity which leads to structural damage in chlorophyll pigments and ultimately reduced the chlorophyll contents in plant (Nazar et al., 2014). This pigment reduction may be due to increased cholorophylase, a chlorophyll degradation enzyme produced as a result of elevated salinity level (Noreen et al., 2009). Another reason might be stomatal closure due to water deficiency by increased nutrient uptake in presence of high salt level (Chatrath et al., 2000). One more reason may be the pigmental variabilities, chlorophyll structural damage and altered carotenoid combinations (Aazami et al., 2021). Another reason for decreased photosynthetic rate might be the reduced PS11 effectiveness and reduced yield of photons under salinity stress (Yang and Lu, 2005). One more reason may be the production of toxic compounds; H_2O_2 which breakdown the thylakoid membrane chlorophyll pigments (Cha-Um and Kirdmanee, 2009). The same findings of reduced photosynthetic pigments under salinity stress were observed in rice (Alam et al., 2022) in pumpkin (Taibi et al., 2016).

Malondialdehyde (MDA) is the pointer of stress introduction which damages the membrane when the plant

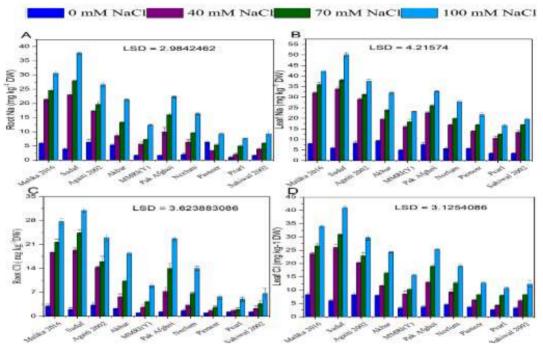


Figure 3. Nutritional status of maize (*Zea mays*) ($p \le 0.05$) between four salt treatments (0, 40, 70, and 100 Mm).

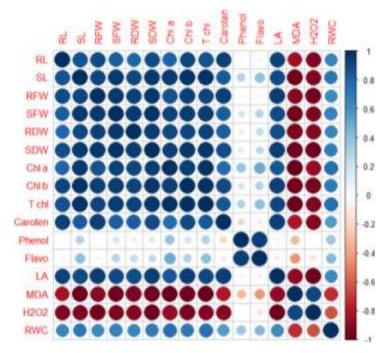


Figure 4. Correlation of several growth and physiological attributes with MDA and H2O2 production in Z. mays plants. RL, root length; SL, shoot length; RFW, root fresh weight; SFW, shoot fresh weight; RDW, root dry weight; SDW, shoot dry weight; Chl a, chlorophyll a; Chl b, chlorophyll b; T. chl, total chlorophyll; Caro, carotenoids; Phenol, phenolic; Flavo, flavonoid; LA, leaf area; MDA, malondialdehyde; H2O2, hydrogen peroxide; RWC, relative water content.

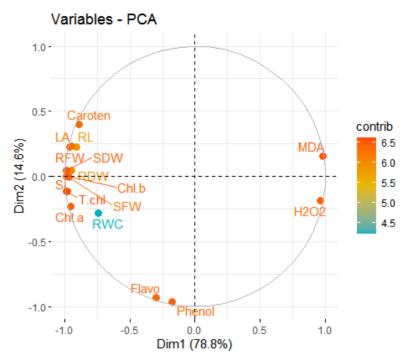


Figure 5. Score and loading plots of principal component analysis (PCA) on different studied attributes of *Z*. mays plants grown under salt stressed environment. The abbreviations are as follows: RL, root length; SL, shoot length; RFW, root fresh weight; SFW, shoot fresh weight; RDW, root dry weight; SDW, shoot dry weight; Chl a, chlorophyll a; Chl b, chlorophyll b; T. chl, total chlorophyll; Caro, carotenoids; Phenol, phenolic; Flavo, flavonoid; LA, leaf area; MDA, malondialdehyde; H2O2, hydrogen peroxide; RWC, relative water content.

is exposed to salt stress (Datir et al., 2020). The present experiment depicted that MDA and H_2O_2 accumulated when the plant was subjected to four different stress levels (0 mM, 40 mM, 70 mM, and 100 mM) and this accumulation gradually increased as the stress level was increased. The Sadaf variety was the most sensitive and the pearl was most tolerant to salinity stress. The accumulation rate of these two nonenzymatic antioxidants was more in Pearl variety than in the Sadaf (Figure 2, Table 1). A possible reason for this MDA and H_2O_2 accumulation might be membrane breakage, ion leakage, lipid peroxidation and nutrient deficiency (Katsuhara et al., 2005). Similar results of accumulated malondialdehyde and hydrogen peroxide accumulation were observed in sorghum by Huang (2018) and in rice (Khan et al., 2002).

Flavonoids are water-soluble pigments that are accumulated on salt stress exposure of plants. These are important for the mitigation effect against oxidative stress (Sacala, 2017). In the present experiment, the most tolerant variety (Pearl) showed more flavonoid accumulation than the salt-sensitive variety (Sadaf) and this accumulation rate gradually increased as the stress level was increased. The reason for this accumulation may be the overproduction of ROS (reactive oxygen species) which is due to oxidative stress exposure resulting in osmotic damage, quenching of reactive oxygen species, and photo-protection (Pervaiz et al., 2017). The same findings were studied in Barley (Ali et al., 2003).

Phenolics are nonenzymatic antioxidants which help plants to survive under unfavourable conditions. In the present experiment, ten maize cultivars were used and they were left to grow under four salt stress levels. Phenolic accumulation was greater in the Pearl cultivar which declared it as a tolerant variety while it was less accumulated in all other varieties, least phenolic accumulation was in the Sadaf cultivar, and its accumulation rate was increased under stress conditions. The mechanism behind phenolic accumulation may be highly produced ROS because these are more accumulated for scavenging deleterious effects of reactive oxygen species (Mechri et al., 2015; Posmyk et al., 2009). Another reason for its accumulation might be the donation of hydrogen ions (Posmyk et al., 2009) restriction to the H₂O₂ conversion into free radicals (Pearse et al., 2005). One more reason may be that this prevents the plasma membrane from being damaged by scavenging the harmful effect of reactive oxygen species (Laus et al., 2021). The same results of phenolic accumulation under salinity stress in cucumber and tomato were studied (Abdel-Farid et al., 2020).

Higher salt ions concentration either in the root or shoot is the result of salinity stress. This ultimately causes water regulation in plant cells due to disturbed ion level in the plant cells and produce ion toxicity (Barros et al., 2021; Katerji et al., 2004). Increase in Cl- ion concentration in the root results in chloride ion elevation in the shoot also (Yousif et al., 1972). The present experiment showed that as sodium chloride stress is applied to different maize (*Zea mays* L.) varieties; the growth rate is highly affected depending on the stress level. The same findings were observed by Turan et al. (2007). The reason may be depolarising of the membrane and ultimate K⁺ ion leakage (Cramer et al., 1985).

5. Conclusion

The current experiment depicted that sodium chloride had a highly negative impact on growth rate and chlorophyll

References

- Aazami MA, Rasouli F, Ebrahimzadeh A (2021). Oxidative damage, antioxidant mechanism and gene expression in tomato responding to salinity stress under in vitro conditions and application of iron and zinc oxide nanoparticles on callus induction and plant regeneration. BMC Plant Biology 21 (1): 597. https://doi.org/10.1186/s12870-021-03379-7
- Abdel-Farid IB, Marghany MR, Rowezek MM, Sheded MG (2020).
 Effect of Salinity Stress on Growth and MetabolomicProfiling of Cucumis sativus and Solanum lycopersicum. Plants 9 (11): 1626. https://doi.org/10.3390/plants9111626
- Abeed AHA, Salama FM (2022a). Attenuating Effect of an Extract of Cd-Hyperaccumulator Solanum nigrum on the Growth and Physio-chemical Changes of Datura innoxia Under Cd Stress. Journal of Soil Science and Plant Nutrition 22(4): 4868-4882. https://doi.org/10.1007/s42729-022-00966-x
- Abeed AH, Mahdy RE, Alshehri D, Hammami I, Eissa MA et al. (2022b). Induction of resilience strategies against biochemical deteriorations prompted by severe cadmium stress in sunflower plant when Trichoderma and bacterial inoculation were used as biofertilizers. Frontiers in Plant Science. https://doi.org/10.3389/fpls.2022.1004173
- Adhikari B, Dhungana SK, Kim I-D, Shin DH (2020). Effect of foliar application of potassium fertilizers on soybean plants under salinity stress. Journal of the Saudi Society of Agricultural Sciences 19 (4): 261-269. https://doi. org/10.1016/j.jssas.2019.02.001
- Afridi MS, Mahmood T, Salam A, Mukhtar T, Mehmood S et al. (2019). Induction of tolerance to salinity in wheat genotypes by plant growth promoting endophytes: Involvement of ACC deaminase and antioxidant enzymes. Plant Physiology and Biochemistry 139: 569-577. https://doi.org/10.1016/j. plaphy.2019.03.041

contents which ultimately results in oxidative stress. It also resulted that Pearl, Sahiwal, and Pioneer varieties are salinity tolerant while the Sadaf, Malika 2016, and Agaiti 2002 cultivars were salt sensitive. All other varieties showed moderate behaviour toward saline conditions.

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- Alam MS, Kong J, Tao R, Ahmed T, Alamin M (2022). CRISPR/ Cas9 mediated knockout of the OsbHLH024 transcription factor improves salt stress resistance in rice (Oryza sativa L.). Plants 11 (9): 1184. https://doi.org/10.3390/ plants11091184
- Ali RM, Abbas HM (2003). Response of salt stressed barley seedlings to phenylurea. Plant Soil and Environment 49(4):158-162. https://pse.agriculturejournals.cz/pdfs/pse/2003/04/04
- Arif Y, Singh P, Siddiqui H, Bajguz A, Hayat S (2020). Salinity induced physiological and biochemical changes in plants: An omic approach towards salt stress tolerance. Plant Physiology and Biochemistry 156: 64-77. https://doi.org/10.1016/j. plaphy.2020.08.042
- Arnon DI (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. Plant physiology 24(1): 1-15. https://doi.org/10.1104/pp.24.1.1
- Asghar S, Ghori N, Hyat F et al. (2023). Use of auxin and cytokinin for somatic embryogensis in plant: a story from competence towards completion. Plant Growth Regulation 99: 413-428. http://doi.org/10.1007/s10725-022-00923-9
- Asghar S, Xiong Y, Che M, Fan X, Li H et al. (2022). Transcriptome analysis reveals the effects of strigolactone on shoot regeneration of apple. Plant Cell Reports 41(7): 1613-1626. https://doi.org/10.1007/s00299-022-02882-x
- Attia H, Arnaud N, Karray N, Lachaâl M (2008). Long-term effects of mild salt stress on growth, ion accumulation and superoxide dismutase expression of Arabidopsis rosette leaves. Physiologia plantarum 132 (3): 293-305. https://doi.org/10.1111/j.1399-3054.2007.01009.x
- Cakmak I, Horst WJ (1991). Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (Glycine max). Physiologia plantarum 83 (3): 463-8. https://doi. org/10.1111/j.1399-3054.1991.tb00121.x

- Aydınşakir K, Erdal Ş, Pamukçu M (2013). The effects of different salt concentrations on germination and seedling parameters of silage corn (Zea mays L.) varieties. Anadolu Tarım Bilimleri Dergisi 28 (2): 94-100.
- Barros NLF, Marques DN, Tadaiesky LBA, de Souza CRB (2021). Halophytes and other molecular strategies for the generation of salt-tolerant crops. Plant Physiology and Biochemistry 162: 581-591. https://doi.org/10.1016/j.plaphy.2021.03.028
- Carleton AE, Foote WH (1965). A comparison of methods for estimating total leaf area of barley plants 1.Crop Science 5(6): 602-3. https://doi.org/10.2135/cropsci1965.0011183X0005000 60041x
- Chatrath A, Mandal PK, Anuradha M (2000). Effect of secondary salinization on photosynthesis in fodder oat (Avena sativa L.) genotypes. Journal of Agronomy and Cro Science 184(1): 13-16. https://doi.org/10.1046/j.1439-037x.2000.00333.x
- Cha-Um S, Kirdmanee C (2009). Effect of salt stress on proline accumulation, photosynthetic ability and growth characters in two maize cultivars. Pakistan Journal of Botany 41(1) : 87-98. https://www.pakbs.org/pjbot/PDFs/41(1)/PJB41(1)087.pdf
- Cramer GR, Läuchli A, Polito VS (1985). Displacement of Ca2+ by Na+ from the plasmalemma of root cells: a primary response to salt stress?. Plant physiology 79(1): 207-211. https://doi. org/10.1104/pp.79.1.207
- Datir S, Singh N, Joshi I (2020). Effect of NaCl-induced salinity stress on growth, osmolytes and enzyme activities in wheat genotypes. Bulletin of environmental contamination and toxicology 104: 351-7. https://doi.org/10.1007/s00128-020-02795-z
- Deinlein U, Stephan AB, Horie T, Luo W, Xu G et al. (2014). Plant salt-tolerance mechanisms. Trends in plant science 19 (6): 371-9. https://doi.org/10.1016/j.tplants.2014.02.001
- Dikobe TB, Mashile B, Sinthumule RR, Ruzvidzo OJAJoPS (2021). Distinct morpho-physiological responses of maize to salinity stress. American Journal of Plant Sciences. 12: 946-959. https:// doi.org/10.4236/ajps.2021.126064
- El-Naim AM, Khaliefa EH, Ibrahim KA, Ismaeil FM, Zaied MM (2012). Growth and yield of Roselle (Hibiscus sabdariffa l.) as influenced by plant population in arid tropic of Sudan under rain-fed. International Journal of Agriculture and Forestry 2(3): 88-91. https://doi.org/10.5923/j.ijaf.20120203.02
- FAO A (2005). Global network on integrated soil management for sustainable use of salt-affected soils. FAO Land and Plant Nutrition Management Service Rome.
- Gholizadeh F, Mirzaghaderi G, Danish S, Farsi M, Marashi SH (2021). Evaluation of morphological traits of wheat varieties at germination stage under salinity stress. Plos one 16(11): e0258703. https://doi.org/10.1371/journal.pone.0258703
- Hadia E, Slama A, Romdhane L, Cheikh M'Hamed H, Fahej MA, Radhouane L (2023). Seed priming of bread wheat varieties with growth regulators and nutrients improves salt stress tolerance particularly for the local genotype. Journal of Plant Growth Regulation 42(1): 304-18. https://doi.org/10.1007/ s00344-021-10548-3

- Hasanuzzaman M, Bhuyan MB, Zulfiqar F, Raza A, Mohsin SM (2020). Reactive oxygen species and antioxidant defense in plants under abiotic stress: Revisiting the crucial role of a universal defense regulator. A ntioxidants 9 (8): 681. https://doi.org/10.3390/antiox9080681
- Huang RD (2018). Research progress on plant tolerance to soil salinity and alkalinity in sorghum. Journal of Integrative Agriculture 17(4): 739-46.
- Jacob PT, Siddiqui SA, Rathore MS (2020). Seed germination, seedling growth and seedling development associated physiochemical changes in Salicornia brachiata (Roxb.) under salinity and osmotic stress. Aquatic Botany 166: 103272. https://doi.org/10.1016/j.aquabot.2020.103272
- Jones MM, Turner NC (1978). Osmotic adjustment in leaves of sorghum in response to water deficits. Plant Physiology 61(1): 122-6. https://doi.org/10.1104/pp.61.1.122
- Julkunen-Tiitto R (1985). Phenolic constituents in the leaves of northern willows: methods for the analysis of certain phenolics. Journal of agricultural and food chemistry 33(2): 213-7. https://doi.org/10.1021/jf00062a013
- Kaleem M, Hussain I, Hameed M, Ahmad MS, Mehmood A et al. (2021). Alleviation of Cadmium Toxicity in Zea Mays L. through Up-Regulation of Antioxidant Defense System and Organic Osmolytes under Supplemental Calcium. PLOS ONE https://doi.org/10.21203/rs.3.rs-611406/v1.
- Kaya C, Ashraf M, Alyemeni MN, Corpas FJ, Ahmad P (2020). Salicylic acid-induced nitric oxide enhances arsenic toxicity tolerance in maize plants by upregulating the ascorbateglutathione cycle and glyoxalase system. Journal of hazardous materials 399: 123020. https://doi.org/10.1016/j. jhazmat.2020.123020
- Karadeniz F, Burdurlu HS, Koca N, Soyer Y (2005). Antioxidant activity of selected fruits and vegetables grown in Turkey. Turkish Journal of Agriculture and Forestry 29(4): 297-303.
- Katerji N, Van Hoorn JW, Hamdy A, Mastrorilli M (2004). Comparison of corn yield response to plant water stress caused by salinity and by drought. Agricultural water management 65(2): 95-101. https://doi.org/10.1016/j. agwat.2003.08.001
- Katsuhara M, Otsuka T, Ezaki B (2005). Salt stress-induced lipid peroxidation is reduced by glutathione S-transferase, but this reduction of lipid peroxides is not enough for a recovery of root growth in Arabidopsis. Plant Science 169: 369-373. https://doi. org/10.1016/j.plantsci.2005.03.030
- Kaushal M, Sharma R, Vaidya D, Gupta A, Saini HK et al. (2023). Maize: an underexploited golden cereal crop. Cereal Research Communications 51(1): 3-14. https://doi.org/10.1007/s42976-022-00280-3
- Khan M, Singha KL, Panda SJAPP (2002): Changes in antioxidant levels in Oryza sativa L. roots subjected to NaCl-salinity stress. Acta Physiologiae Plantarum 24 (2): 145-148. https://doi. org/10.1007/s11738-002-0004-x

- Kumar P, Choudhary M, Halder T, Prakash NR, Singh V et al. (2022). Salinity stress tolerance and omics approaches: Revisiting the progress and achievements in major cereal crops. Heredity 128: 497-518. https://doi.org/10.1038/ s41437-022-00516-2
- Laus MN, De Santis MA, Flagella Z, Soccio M (2021). Changes in antioxidant defence system in durum wheat under hyperosmotic stress: a concise overview. Plants 11(1): 98. https://doi.org/10.3390/plants11010098
- Lee K, Missaoui A, Mahmud K, Presley H, Lonnee M (2021). Interaction between grasses and Epichloë endophytes and its significance to biotic and abiotic stress tolerance and the rhizosphere. Micoorganisms 9 (11): 2186. https://doi. org/10.3390/microorganisms9112186
- Li X, Sun P, Zhang Y, Jin C, Guan C (2020). A novel PGPR strain Kocuria rhizophila Y1 enhances salt stress tolerance in maize by regulating phytohormone levels, nutrient acquisition, redox potential, ion homeostasis, photosynthetic capacity and stress-responsive genes expression. Environmental and Experimental Botany 174: 104023. https://doi.org/10.1016/j.envexpbot.2020.104023
- Mechri B, Tekaya M, Cheheb H, Hammani M (2015). Determination of mannitol sorbitol and myo-inositol in olive tree roots and rhizospheric soil by gas chromatography and effect of severe drought conditions on their profiles. Journal of Chromatographic Science 53 (10): 1631-1638. https://doi.org/10.1093/chromsci/ bmv066
- Moud AM, Maghsoudi K (2008). Salt stress effects on respiration and growth of germinated seeds of different wheat (Triticum aestivum L.) cultivars.World Journal of Agricultural Sciences 4: 351-358. http://www.idosi.org/wjas/wjas4(3)/12.pdf
- Muchate NS, Nikalje GC, Rajurkar NS, Suprasanna P, Nikam TD (2016). Plant salt stress: adaptive responses, tolerance mechanism and bioengineering for salt tolerance. The Botanical Review 82: 371-406. https://doi.org/10.1007/s12229-016-9173-y
- Nadarajah KK (2020). ROS homeostasis in abiotic stress tolerance in plants. International journal of molecular sciences 21: 5208. https://doi.org/10.3390/ijms21155208
- Nazar R, Khan MIR, Iqbal N, Masood A, Khan NA (2014). Involvement of ethylene in reversal of salt inhibited photosynthesis by sulfur in mustard. Physiologia plantarum 152: 331-344. https://doi. org/10.1111/ppl.12173
- Noreen Z, Ashraf M (2009). Changes in antioxidant enzymes and some key metabolites in some genetically diverse cultivars of radish (Raphanus sativus L.). Environmental and Experimental Botany 67(2): 395-402. https://doi.org/10.1016/j. envexpbot.2009.05.011
- Nxele X, Klein A, Ndimba BK (2017). Drought and salinity stress alters ROS accumulation, water retention, and osmolyte content in sorghum plants. South African journal of botany 108: 261-266. https://doi.org/10.1016/j.sajb.2016.11.003
- Parvin K, Hasanuzzaman M, Bhuyan MB, Mohsin SM, Fujita M (2019). Quercetin mediated salt tolerance in tomato through the enhancement of plant antioxidant defense and glyoxalase systems. Plants 8(8): 247. https://doi.org/10.3390/plants8080247

- Pearse IS, Heath KD, Cheeseman JM Cell (2005). Biochemical and ecological characterization of two peroxidase isoenzymes from the mangrove, Rhizophora mangle. Plant, Cell and Environment. 28 (5): 612-622. https://doi.org/10.1111/j.1365-3040.2005.01307.x
- Pervaiz T, Songtao J, Faghihi F, Haider MS, Fang J (2017). Naturally occurring anthocyanin, structure, functions and biosynthetic pathway in fruit plants. Journal of Plant Biochemistry and Physioloy 5 (2): 1-9.
- Perveen S, Hussain SA (2021). Methionine-induced changes in growth, glycinebetaine, ascorbic acid, total soluble proteins and anthocyanin contents of two Zea mays L. varieties under salt stress. Journal of Animal & Plant Sciences 31(1). https:// doi.org/10.36899/JAPS.2021.1.0201
- Posmyk M, Kontek R, Janas KM (2009). Antioxidant enzymes activity and phenolic compounds content in red cabbage seedlings exposed to copper stress. Ecotoxicology and Environmental safety 72 (2): 596-602. https://doi.org/10.1016/j. ecoenv.2008.04.024
- Rady MM, Talaat NB, Abdelhamid MT, Shawky BT, Desoky ES (2019). Maize (Zea mays L.) grains extract mitigates the deleterious effects of salt stress on common bean (Phaseolus vulgaris L.) growth and physiology. The Journal of Horticultural Science and Biotechnology 94 (6): 777-789. https://doi.org/10.1080/14 620316.2019.1626773
- Rasel M, Tahjib-Ul-Arif M, Hossain MA, Hassan L, Farzana S et al. (2021). Screening of salt-tolerant rice landraces by seedling stage phenotyping and dissecting biochemical determinants of tolerance mechanism. Journal of Plant Growth Regulation 40: 1853-1868.
- Riaz M, Arif MS, Ashraf MA, Mahmood R, Yasmeen T et al. (2019). A comprehensive review on rice responses and tolerance to salt stress. Advances in rice research for abiotic stress tolerance 1: 133-158. https://doi.org/10.1016/B978-0-12-814332-2.00007-1
- Sabagh A, Çiğ F, Seydoşoğlu S, Battaglia ML, Javed T et al. (2021). Salinity stress in maize: Effects of stress and recent developments of tolerance for improvement. Cereal Grains 1: 213. https://doi.org/10.5772/intechopen.98745
- Sacała E (2017). The influence of increasing doses of silicon on maize seedlings grown under salt stress. Journal of Plant Nutrition 40 (6): 819-827. https://doi.org/10.1080/01904167.2016.1236948
- Saharan BS, Brar B, Duhan JS, Kumar R, Marwaha S (2022). Molecular and physiological mechanisms to mitigate abiotic stress conditions in plants. Life 12 (10): 1634 https://doi. org/10.3390/life12101634.
- Salama FM, AL-Huqail AA, Ali M, Abeed AH (2022). Cd Phytoextraction Potential in Halophyte Salicornia fruticosa: Salinity Impact. Plants 11 (19): 2556. https://doi.org/10.3390/ plants11192556.
- Santpoort R (2020). The drivers of maize area expansion in Sub-Saharan Africa. How policies to boost maize production overlook the interests of smallholder farmers. Land 9 (3): 68. https://doi.org/10.3390/land9030068.

- Shiade SR, Boelt B (2020). Seed germination and seedling growth parameters in nine tall fescue varieties under salinity stress. Acta Agriculturae Scandinavica, Section B—Soil & Plant Science 70 (6): 485-494. https://doi.org/10.1080/09064710.20 20.1779338
- Syed A, Sarwar G, Shah SH, Muhammad S (2021). Soil salinity research in 21st century in Pakistan: its impact on availability of plant nutrients, growth and yield of crops. Communications in Soil Science and Plant Analysis 52 (3): 183-200. https://doi. org/10.1080/00103624.2020.1854294
- Taïbi K, Taïbi F, Abderrahim LA, Ennajah A, Belkhodja M (2016). Effect of salt stress on growth, chlorophyll content, lipid peroxidation and antioxidant defence systems in Phaseolus vulgaris L. South African Journal of Botany 105: 306-312 https://doi.org/10.1016/j.sajb.2016.03.011
- Talaat NB, Todorova D (2022). Antioxidant machinery and glyoxalase system regulation confers salt stress tolerance to wheat (Triticum aestivum L.) plants treated with melatonin and salicylic Acid. Journal of Soil Science and Plant Nutrition 22 (3): 3527-3540. https://doi.org/10.1007/s42729-022-00907-8
- Turan MA, Turkmen N, Taban N (2007). Effect of NaCl on stomatal resistance and proline, chlorophyll, Na, Cl and K concentrations of lentil plants. Journal of Agronomy. https://doi.org/10.3923/ ja.2007.378.381
- ur Rehman F, Adnan M, Kalsoom M, Naz N, Husnain MG (2021). Seed-borne fungal diseases of Maize (Zea mays L.): A review. Agrinula: Jurnal Agroteknologi dan Perkebunan 4 (1): 43-60. https://doi.org/10.36490/agri.v4i1.123

- Wang H, Liang L, Liu S, An T, Fang Y et al. (2020). Maize genotypes with deep root systems tolerate salt stress better than those with shallow root systems during early growth. Journal of Agronomy and Crop Science 206: 711-721. https://doi. org/10.1111/jac.12437
- Wu L Y, Shang G D, Wang F X, Gao J, Wan M C et al. (2022). Dynamic chromatin state profiling reveals regulatory roles of auxin and cytokinin in shoot regeneration. Developmental Cell 57(4): 526-542. https://doi.org/10.1016/j.devcel.2021.12.019.
- Yang X, Lu C (2005). Photosynthesis is improved by exogenous glycinebetaine in salt-stressed maize plants. Physiologia Plantarum 124: 343-352. https://doi.org/10.1111/j.1399-3054.2005.00518.x
- Ye X, Tie W, Xu J, Ding Z, Hu W (2022). Comparative Transcriptional Analysis of Two Contrasting Rice Genotypes in Response to Salt Stress. Agronomy 12 (5): 1163. https://doi.org/10.3390/ agronomy12051163
- Yousif YH, Bingham FT, Yermanos D (1972). Growth, mineral composition, and seed oil of sesame (Sesamum indicum L.) as affected by NaCl. Soil Science Society of America Journal 36 (3): 450-453. https://doi.org/10.2136/ sssaj1972.03615995003600030025x
- Zhao C, Zhang H, Song C, Zhu J-K, Shabala S (2020). Mechanisms of plant responses and adaptation to soil salinity. The innovation 1: 100017. https://doi.org/10.1016/j.xinn.2020.100017
- Zhu JK (2016). Abiotic stress signaling and responses in plants. Cell 167(2): 313-324. https://doi.org/10.1016/j.cell.2016.08.029