

Physiological and Biochemical Responses of Maize (*Zea mays* L.) Cultivars to Salinity Stress

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Abstract— Salinity is a critical abiotic constraint to maize (*Zea mays* L.) production, affecting growth, photosynthesis and oxidative balance. This study examined the responses of five cultivars (DHM 144, NK 7720, SY 594, Sunny NMH 777 and Dragon NMH 1247) to 150 mM NaCl stress. Measurements at 0, 7 and 14 days after stress initiation included plant height, leaf area, chlorophyll content, stomatal conductance, antioxidant enzyme activities [superoxide dismutase (SOD), catalase (CAT), peroxidase (POD)], proline accumulation and malondialdehyde (MDA) levels. Salinity reduced morphological and physiological traits across genotypes, with Dragon NMH 1247 maintaining higher growth, chlorophyll content and stomatal conductance, coupled with enhanced SOD, CAT and POD activities and lower MDA accumulation. All cultivars had a higher proline content which was not always correlated with stress tolerance. Findings show that antioxidant capacity, chlorophyll retention and low oxidative damage are some of the main factors that determine salinity tolerance in maize. Dragon NMH 1247 emerged as the most tolerant genotype and is an attractive target for saline-prone environments and breeding programs.

Keywords— Maize, salt stress, proline, antioxidant enzymes.

I. INTRODUCTION

Maize (*Zea mays* L.) is a universally significant cereal crop that is a staple food for millions of people, a critical component of livestock feed and a primary industrial raw material. Its capacity for adaptation to different agro-climatic factors has enabled its cultivation in different geographical areas, making it among the most extensively grown crops in the world (FAO, 2020). Nevertheless, its productivity is under threat from various abiotic stresses, especially salinity, which poses a considerable challenge to agricultural sustainability and food security.

Salinity stress negatively impacts plant growth and metabolism by interfering with water uptake, resulting in ionic toxicity and oxidative stress. These effects inhibit photosynthesis, nutrient assimilation and enzyme activity, ultimately leading to reduced yields (Munns and Tester, 2008). Even moderate levels of salt can cause significant losses in physiological performance and biomass gain in glycophytic crops such as maize, which is moderately sensitive to salinity (Cairns et al., 2013).

At the physiological level, salt stress affects plant height, leaf area, stomatal conductance and chlorophyll content—parameters used to measure plant vigor and photosynthetic performance under stress conditions. For example, reduction in stomatal conductance limits CO₂ uptake, thereby decreasing carbon assimilation and plant productivity (Zhang et al., 2006). Simultaneously, ionic and osmotic stress caused by salt induces symptoms including leaf chlorosis and stunted growth.

At the biochemical level, salinity causes excessive production of reactive oxygen species (ROS), leading to oxidative stress on cellular components. Plants respond by activating an antioxidant defense system containing enzymatic activities such as

superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) that mitigate ROS toxicity and maintain cellular integrity (Apel and Hirt, 2004). Additionally, osmolytes like proline accumulate in response to osmotic stress, contributing to osmotic adjustment, membrane stabilization and free radical scavenging (Ashraf and Harris, 2004). Lipid peroxidation byproducts such as malondialdehyde (MDA) serve as effective indicators of oxidative membrane damage and stress severity.

Maize possesses high genetic diversity, resulting in varied physiological and biochemical responses to salt stress among different cultivars. Characterizing this variability is essential for identifying stress-resilient genotypes suitable for salt-affected soils. Previous literature has demonstrated that certain cultivars exhibit enhanced antioxidant capacity, superior osmotic regulation and reduced MDA accumulation, contributing to superior salinity tolerance (Duvick, 2005; CIMMYT, 2019).

Therefore, evaluating a comprehensive range of physiological and biochemical parameters is important for understanding salt tolerance mechanisms in maize. The aim of this study was to examine how salinity influences key plant characteristics including height, leaf area, chlorophyll content, stomatal conductance and biochemical indices such as SOD, CAT and POD activities, proline content, and MDA levels across different maize cultivars. The findings will help identify maize genotypes capable of withstanding saline conditions and guide future breeding initiatives to develop resilient cultivars for saline-prone agroecosystems.

II. MATERIALS AND METHODS

2.1 Plant Material and Experimental Design:

The experiment was conducted with five maize (*Zea mays* L.) cultivars: DHM 144, NK 7720, SY 594, Sunny NMH 777 and Dragon NMH 1247. Seeds of each cultivar were surface sterilized followed by controlled germination to obtain uniform and healthy seedlings. After germination, seedlings were transplanted into pots filled with a homogenized and uniformly mixed soil medium. The experimental design was a completely randomized design (CRD) with two treatments (control and salinity stress), five cultivars and three biological replicates per treatment.

2.2 Salinity Stress Treatment

Salinity stress was imposed by applying 150 mM sodium chloride (NaCl) solution. Before stress initiation, all plants were irrigated with deionized water for seven days to ensure baseline uniformity. On Day 0, the treatment group received NaCl solution while the control group received deionized water. A soil salinity meter was used to monitor soil salinity levels and maintain consistent concentration across all treated pots. NaCl solution was applied every other day to sustain constant salinity stress.

2.3 Sampling Time Points

Physiological and biochemical measurements were performed at three time points: Day 0 (stress onset, baseline), Day 7 (mid-stress) and Day 14 (stress termination). Sample collection at each time point was conducted on both control and salt-treated plants.

2.4 Measurement of Physiological Parameters

- **Plant Height (PH)** was measured from the root-shoot junction to the tip of the youngest leaf using a ruler and recorded in centimeters (cm).
- **Leaf Area (LA)** was estimated using the formula: $LA = L \times W \times 0.75$, where L = leaf length and W = leaf width (both in cm). The coefficient 0.75 accounts for the elliptical shape of the leaf blade.
- **Chlorophyll Content (Chl)** was measured in fresh leaf samples using a SPAD-502 Plus chlorophyll meter (Konica Minolta, Japan).
- **Stomatal Conductance (SC)** was recorded using a portable porometer (SC-1 Leaf Porometer, Decagon Devices, USA) on the abaxial surface of fully expanded leaves.

2.5 Estimation of Biochemical Parameters

- **Superoxide Dismutase (SOD) Activity** was assayed by measuring the photochemical reduction of nitro blue tetrazolium (NBT) following the method of Beauchamp and Fridovich (1971). One unit of SOD activity was defined as the amount of enzyme required to inhibit 50% NBT photoreduction.

- **Catalase (CAT) Activity** was determined by monitoring the decomposition of H₂O₂ at 240 nm according to Aebi (1984).
- **Peroxidase (POD) Activity** was measured using guaiacol as substrate following the method of Chance and Maehly (1955).
- **Proline Content** was quantified using the ninhydrin method (Bates et al., 1973). Absorbance was read at 520 nm and results were expressed as $\mu\text{mol proline g}^{-1}$ fresh weight.
- **Malondialdehyde (MDA) Content**, an index of lipid peroxidation, was determined using the thiobarbituric acid (TBA) reaction as described by Heath and Packer (1968). Absorbance was measured at 532 nm and corrected for non-specific turbidity at 600 nm.

2.6 Statistical Analysis:

Data were analyzed using two-way analysis of variance (ANOVA) with cultivar and treatment as fixed factors using SPSS software (Version XX). Duncan's Multiple Range Test (DMRT) was used to compare means at a significance level of $p \leq 0.05$. Correlation analysis of physiological and biochemical traits was performed using PAST software (Version 4.03).

III. RESULTS

3.1 Effect of Salinity on Morphological Traits:

3.1.1 Plant Height:

Salinity stress differentially affected plant height among maize cultivars over the experimental period (Figure 1). At Day 0, plant heights ranged from 188 cm to 229 cm across cultivars (SY 594 and Dragon NMH 1247, respectively). By Day 14, most cultivars showed either insignificant changes or slight decreases in height. Dragon NMH 1247 exhibited a gradual increase from 229 cm to 243 cm. DHM 144 and NK 7720 recorded slight incremental changes of 4-5 cm. SY 594 showed the highest reduction of 8 cm from its Day 0 height. The minimal reduction, or slight increase, in Dragon NMH 1247 height indicates greater tolerance to salt-induced growth retardation, possibly associated with efficient osmotic homeostasis and cell growth maintenance. Conversely, the height decrease in Sunny NMH 777 and SY 594 suggests higher sensitivity to salinity stress. These observations align with previous reports that stress-tolerant maize genotypes sustain shoot extension through hormonal and osmotic adaptation (Munns and Tester, 2008; Farooq et al., 2009; Hussain et al., 2018; Tang et al., 2023).

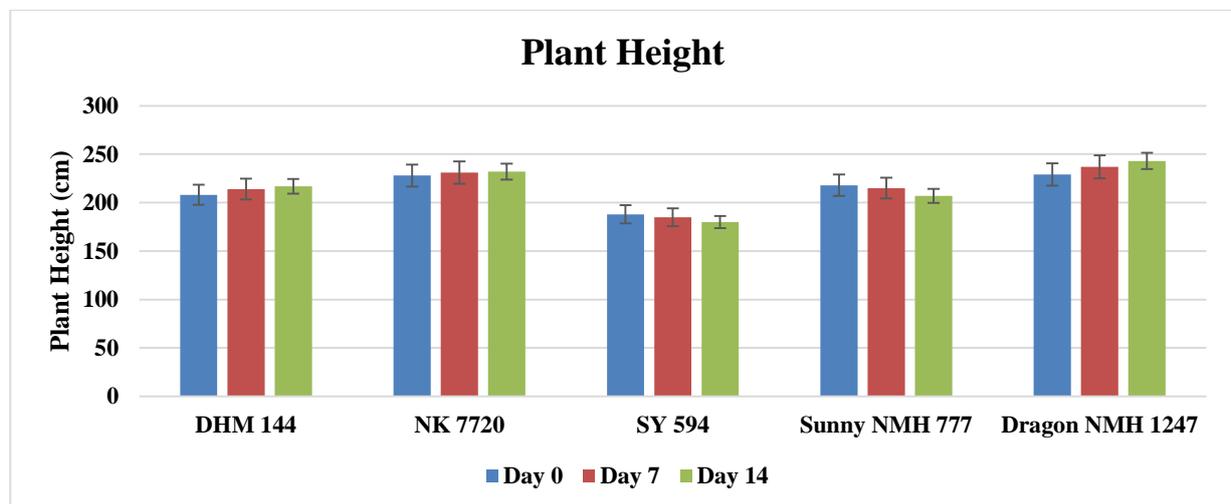


FIGURE 1: Effect of salt stress on the plant height of maize cultivars

3.1.2 Leaf Area:

Salinity caused progressive reduction in leaf area with considerable variation among cultivars (Figure 2). Dragon NMH 1247 maintained relatively greater leaf area under stress with minimal decreases, indicating robust cellular structure and sustained development (Romdhane et al., 2020). Yan et al. (2023) associated similar characteristics with enhanced biomass production in stress-tolerant lines. NK 7720 and DHM 144 exhibited moderate reductions, suggesting intermediate tolerance levels.

Conversely, SY 594 and Sunny NMH 777 showed sharp decreases, reflecting inhibited leaf expansion due to ionic toxicity and premature senescence as observed by Munns and Tester (2008).

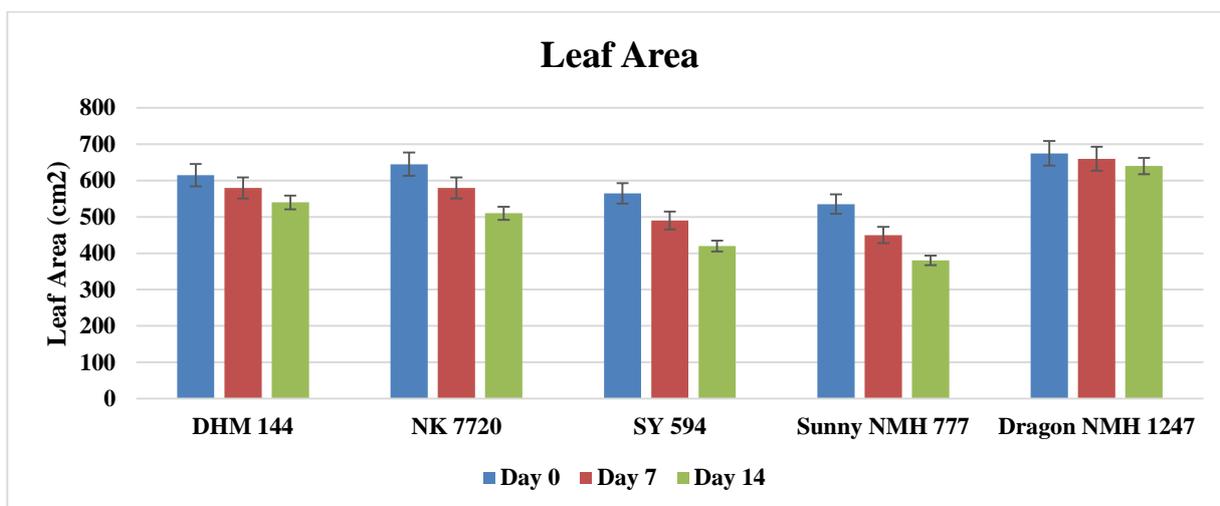


FIGURE 2: Effect of salt stress on the LA of maize cultivars

3.2 Physiological Responses:

3.2.1 Chlorophyll Content:

Salinity significantly reduced chlorophyll content across all genotypes, particularly after Day 14 (Figure 3). Sunny NMH 777 displayed the lowest chlorophyll levels while Dragon NMH 1247 and DHM 144 maintained higher SPAD values. These results are consistent with Ali et al. (2024) and Moharramnejad et al. (2019) who reported enhanced chlorophyll retention as a characteristic of tolerant genotypes. Salt-induced chlorophyll degradation is associated with oxidative damage and impaired pigment biosynthesis (Ashraf and Harris, 2004). Higher chlorophyll levels in resistant genotypes indicate effective antioxidant protection and reduced membrane damage, in agreement with findings by Akhtar et al. (2024).

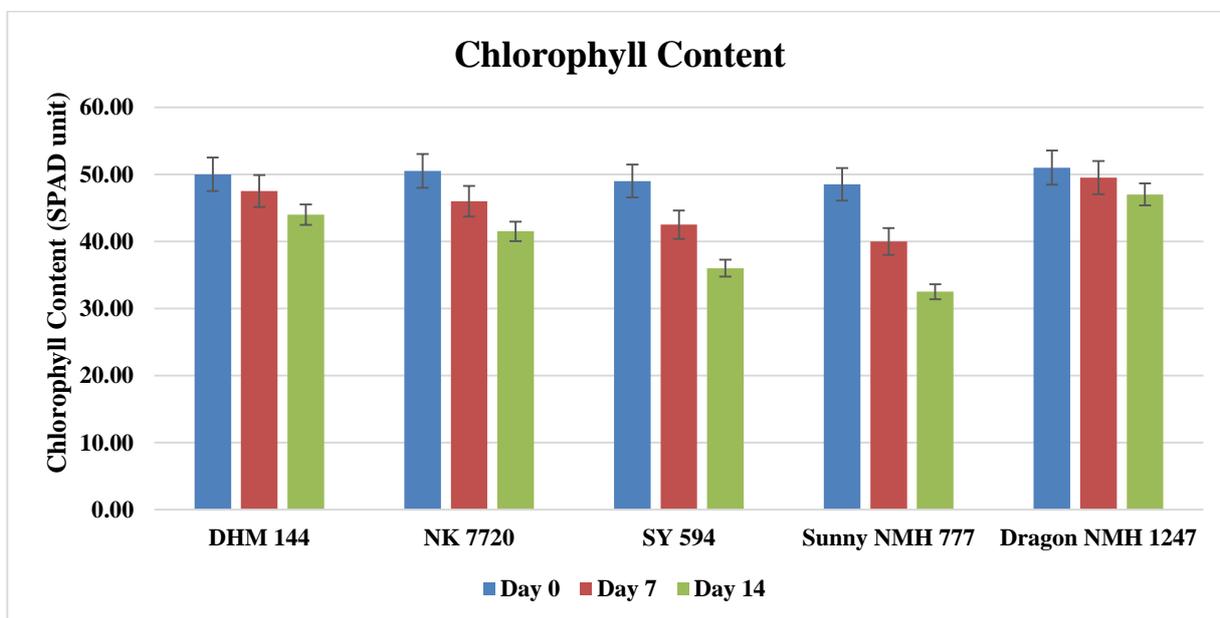


FIGURE 3: Effect of salt stress on the Chlorophyll Content of maize cultivars

3.2.2 Stomatal Conductance:

All cultivars exhibited reduced stomatal conductance under salt stress, indicating decreased gas exchange and photosynthetic activity (Figure 4). However, the reduction was smaller in DHM 144 and Dragon NMH 1247, suggesting better stomatal regulation under stress (Liao et al., 2022). Reduced stomatal conductance is a well-documented protective response to minimize water loss, though it limits carbon assimilation and consequently yield (Beauclaire et al., 2024).

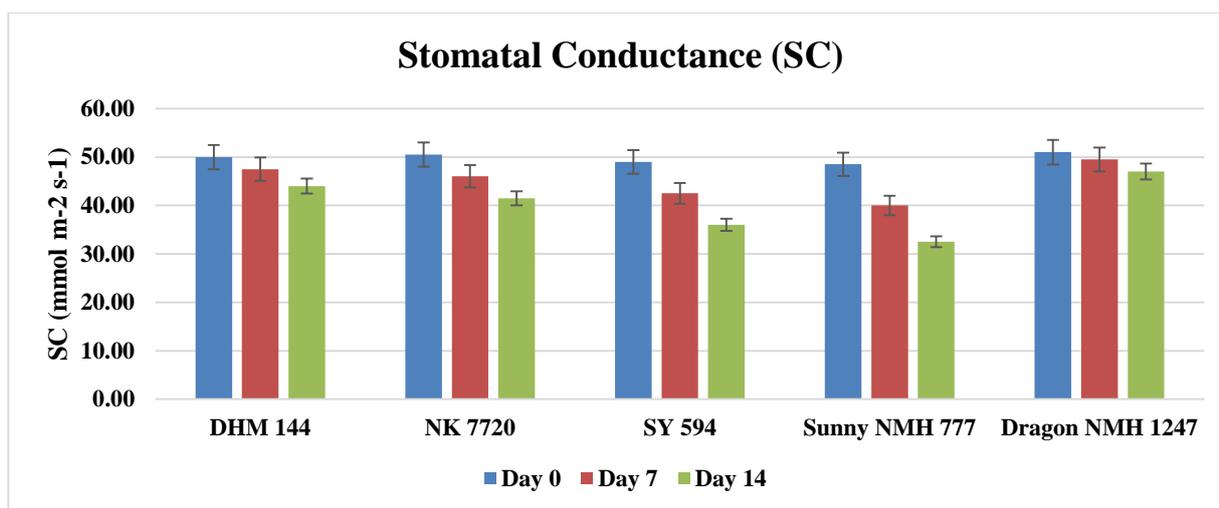


FIGURE 4: Effect of salt stress on the Stomatal Conductance (SC) of maize cultivars

3.3 Biochemical Responses:

3.3.1 Antioxidant Enzyme Activities:

Salt stress induced pronounced increases in antioxidant enzyme activities (SOD, CAT, POD) across all maize cultivars, with the highest activities observed in Dragon NMH 1247 followed by Sunny NMH 777 (Table 1). This elevation reflects effective ROS-scavenging capacity, essential for stress tolerance. SOD provides the initial defense by converting superoxide radicals to hydrogen peroxide, which is subsequently neutralized by CAT and POD (Mittler, 2002). These results align with Shehu et al. (2023) and Ali et al. (2024), who reported that enhanced SOD activity correlates with improved salt and drought tolerance in maize and other cereals.

CAT decomposes toxic H₂O₂ produced by SOD activity into water and oxygen. All maize varieties showed increased CAT activity in response to salt stress, indicating a universal stress response. Similar patterns were reported by Hussain et al. (2018) and Ali et al. (2024), where CAT activity positively correlated with yield stability under abiotic stress.

POD contributes to oxidative stress mitigation by catalyzing H₂O₂ reduction using phenolic substrates, often complementing CAT activity. Up-regulation of POD was observed across all varieties, with NK 7720 showing the highest induction, suggesting enhanced oxidative detoxification potential and synergy between CAT and POD pathways. These findings are supported by Ali et al. (2024) and Munavvar et al. (2025), emphasizing the importance of POD in stress adaptation.

TABLE 1
 EFFECT OF SALT STRESS ON ANTIOXIDANT ENZYME ACTIVITIES IN MAIZE CULTIVARS

| Cultivar | SOD Activity (U mg ⁻¹ protein) | | | CAT Activity (μmol min ⁻¹ mg ⁻¹ protein) | | | POD Activity (μmol min ⁻¹ mg ⁻¹ protein) | | |
|-----------------|---|--------------|--------------|--|-------------|-------------|--|-------------|-------------|
| | Day 0 | Day 7 | Day 14 | Day 0 | Day 7 | Day 14 | Day 0 | Day 7 | Day 14 |
| DHM 144 | 21.13 ± 0.22 | 18.84 ± 0.14 | 16.80 ± 0.24 | 1.41 ± 0.01 | 2.32 ± 0.02 | 3.04 ± 0.02 | 0.70 ± 0.01 | 1.12 ± 0.01 | 1.44 ± 0.02 |
| NK 7720 | 21.38 ± 0.34 | 18.18 ± 0.23 | 16.36 ± 0.36 | 1.45 ± 0.01 | 2.27 ± 0.01 | 3.18 ± 0.03 | 0.70 ± 0.01 | 1.06 ± 0.01 | 1.50 ± 0.08 |
| SY 594 | 21.01 ± 0.46 | 17.39 ± 0.34 | 13.33 ± 0.22 | 1.38 ± 0.01 | 2.26 ± 0.02 | 3.11 ± 0.02 | 0.69 ± 0.01 | 1.04 ± 0.01 | 1.44 ± 0.06 |
| Sunny NMH 777 | 20.74 ± 0.22 | 17.00 ± 0.12 | 11.54 ± 0.32 | 1.33 ± 0.02 | 2.20 ± 0.02 | 2.56 ± 0.02 | 0.67 ± 0.02 | 1.00 ± 0.02 | 1.15 ± 0.07 |
| Dragon NMH 1247 | 21.28 ± 0.43 | 20.00 ± 0.23 | 18.71 ± 0.28 | 1.49 ± 0.02 | 2.48 ± 0.01 | 2.95 ± 0.01 | 0.74 ± 0.01 | 1.21 ± 0.02 | 1.44 ± 0.01 |
| S.Em± | 1.62 | | | 0.032 | | | 0.01 | | |
| CD (5%) | 4.77 | | | 0.092 | | | 0.03 | | |
| CD (1%) | 6.51 | | | 0.125 | | | 0.05 | | |

Values represent mean ± standard error (n=3)

3.3.2 Proline Accumulation:

Salt stress significantly increased proline levels across all cultivars, with the highest concentrations observed in Dragon NMH 1247 and Sunny NMH 777 at Day 14 (Figure 5), indicating activation of osmotic adjustment mechanisms. However, despite high proline content, Sunny NMH 777 showed poor performance in physiological and antioxidant parameters, suggesting that proline accumulation alone is insufficient for effective stress tolerance. Similar findings were reported by Ibrahim et al. (2022) and Shahimoghdam et al. (2024), indicating that proline concentration in sensitive genotypes may reflect stress severity rather than stress resilience. Proline functions as an osmoprotectant, stabilizing proteins and membranes while scavenging free radicals under osmotic stress (Szabados and Saviouré, 2010).

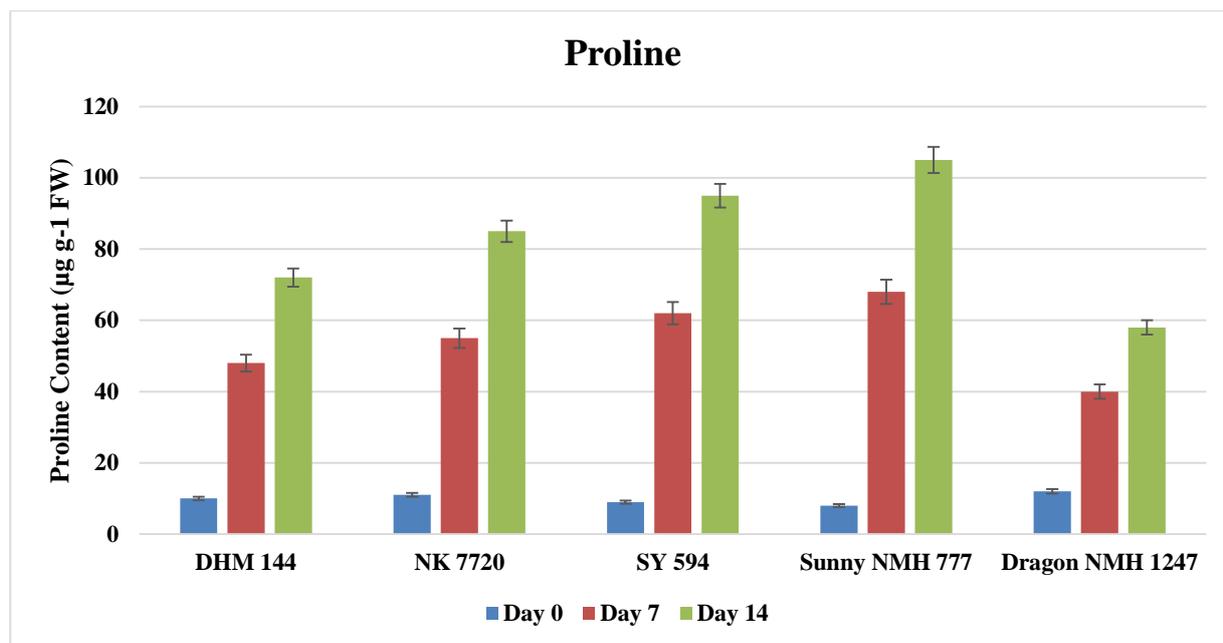


FIGURE 5: Effect of salt stress on the Proline Content of maize cultivars

3.3.3 Malondialdehyde Content:

MDA levels, indicating lipid peroxidation and membrane damage, increased significantly under salt stress (Table 2). The highest MDA content was recorded in Sunny NMH 777 while the lowest was observed in Dragon NMH 1247 at Day 14. This pattern demonstrates superior oxidative damage mitigation in Dragon NMH 1247 compared to other cultivars, consistent with Ali et al. (2024) who emphasized that coordinated enzymatic activity is essential for membrane stability under stress.

TABLE 2
 EFFECT OF SALT STRESS ON MDA CONTENT ($\mu\text{mol g}^{-1}$ FW) IN MAIZE CULTIVARS

| Cultivar | Day 0 | Day 7 | Day 14 |
|-----------------|-------------|-------------|-------------|
| DHM 144 | 2.45 ± 0.12 | 4.89 ± 0.18 | 6.78 ± 0.22 |
| NK 7720 | 2.38 ± 0.10 | 4.76 ± 0.15 | 6.54 ± 0.19 |
| SY 594 | 2.52 ± 0.14 | 5.23 ± 0.21 | 7.45 ± 0.26 |
| Sunny NMH 777 | 2.61 ± 0.13 | 5.67 ± 0.24 | 8.12 ± 0.31 |
| Dragon NMH 1247 | 2.31 ± 0.09 | 4.12 ± 0.14 | 5.23 ± 0.17 |

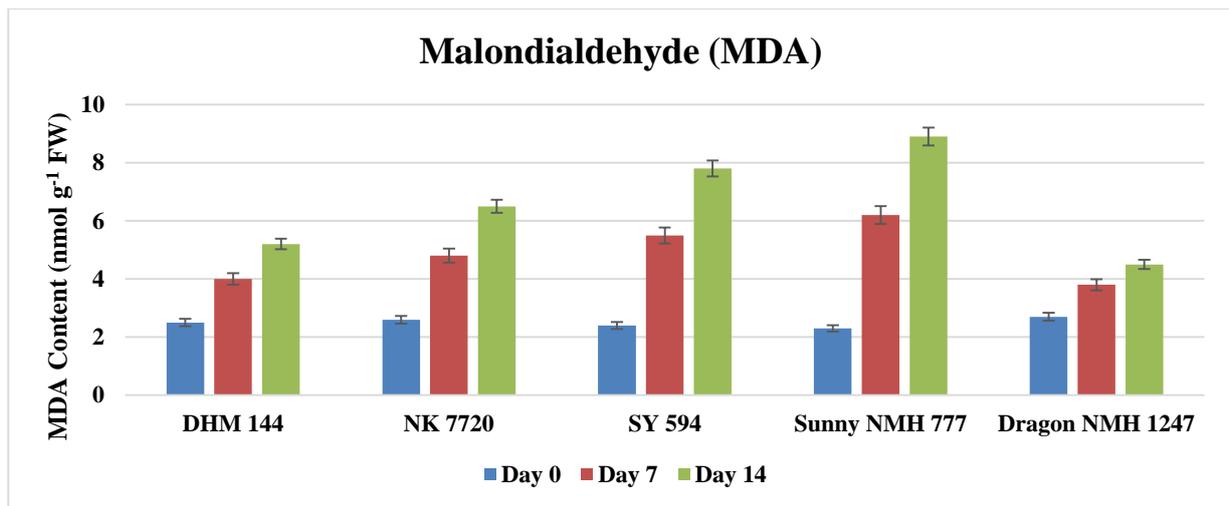


FIGURE 6: Effect of salt stress on the Malondialdehyde of maize cultivars

IV. DISCUSSION

Salinity stress adversely affected morphological, physiological and biochemical parameters in all maize cultivars, though the magnitude of impact varied considerably among genotypes. Dragon NMH 1247 consistently demonstrated superior performance across most measured traits, maintaining greater plant height, leaf area, chlorophyll content and stomatal conductance under stress conditions. This was accompanied by enhanced antioxidant enzyme activities and reduced MDA accumulation, indicating effective ROS scavenging and membrane protection.

The maintenance of plant height and leaf area in Dragon NMH 1247 under salinity suggests efficient osmotic adjustment and sustained cellular division and expansion. Similar observations were reported by Romdhane et al. (2020) in salt-tolerant maize lines, where preserved growth correlated with better ion homeostasis and water relations. The minimal reduction in chlorophyll content in this cultivar indicates protection of photosynthetic pigments from salt-induced degradation, consistent with findings by Moharramnejad et al. (2019) who identified chlorophyll retention as a key tolerance trait.

Stomatal conductance responses revealed that Dragon NMH 1247 and DHM 144 maintained relatively higher stomatal opening under stress, suggesting better control of gas exchange while managing water loss. This partial stomatal opening may enable continued carbon assimilation, contributing to sustained growth. Beauclaire et al. (2024) similarly reported that tolerant genotypes optimize the trade-off between water conservation and CO₂ uptake through modulated stomatal behavior.

The coordinated up-regulation of SOD, CAT and POD activities in Dragon NMH 1247 represents an efficient antioxidant defense system. SOD catalyzes the dismutation of superoxide radicals to H₂O₂, while CAT and POD subsequently detoxify H₂O₂, preventing accumulation of harmful ROS (Mittler, 2002). The simultaneous enhancement of all three enzymes suggests integrated regulation of the antioxidant network, which likely contributed to reduced oxidative damage as evidenced by lower MDA levels. These findings align with Ali et al. (2024) and Shehu et al. (2023), who reported similar antioxidant coordination in stress-tolerant maize genotypes.

Proline accumulation occurred in all cultivars under salinity, confirming its role as an osmotic stress response. However, the lack of correlation between proline content and overall stress tolerance—particularly in Sunny NMH 777 which accumulated high proline but performed poorly—indicates that proline alone is insufficient for stress adaptation. This observation supports the view of Ibrahim et al. (2022) and Shahimoghdam et al. (2024) that proline accumulation in sensitive genotypes may reflect stress severity rather than adaptive capacity. Effective tolerance apparently requires integrated responses including antioxidant defense, osmotic adjustment and membrane stability rather than any single protective mechanism.

MDA content, reflecting lipid peroxidation and membrane damage, was lowest in Dragon NMH 1247 and highest in Sunny NMH 777. This inverse relationship between antioxidant activity and MDA accumulation confirms that efficient ROS scavenging protects membrane integrity under stress. Similar patterns were reported by Ali et al. (2024), where tolerant lines exhibited lower MDA alongside elevated antioxidant enzyme activities.

The observed genotypic variation in salinity responses underscores the importance of genetic diversity in maize for stress adaptation. Dragon NMH 1247 emerged as the most salt-tolerant cultivar, combining sustained growth, chlorophyll retention,

stomatal regulation, coordinated antioxidant defense and membrane stability. Sunny NMH 777 and SY 594 showed greater sensitivity, characterized by growth reduction, chlorophyll degradation, weaker antioxidant responses and higher oxidative damage. DHM 144 and NK 7720 exhibited intermediate responses, suggesting moderate tolerance levels.

V. CONCLUSION

Salinity stress significantly impacted physiological and biochemical characteristics in maize, with considerable genotypic variation among the five cultivars evaluated. Dragon NMH 1247 demonstrated the highest salt tolerance, maintaining superior growth, chlorophyll content, stomatal conductance and antioxidant enzyme activities while accumulating less MDA. Sunny NMH 777 and SY 594 were more sensitive, exhibiting greater growth reduction, chlorophyll degradation, weaker antioxidant responses and higher oxidative damage. DHM 144 and NK 7720 showed intermediate tolerance levels.

The findings indicate that effective salinity tolerance in maize requires integrated mechanisms including sustained photosynthetic pigment retention, modulated stomatal behavior, coordinated antioxidant enzyme activity and membrane stability. Proline accumulation, while universally induced by salinity, does not independently confer tolerance and may reflect stress intensity rather than adaptive capacity in sensitive genotypes.

Dragon NMH 1247 represents a promising genetic resource for cultivation in saline-prone areas and for inclusion in breeding programs aimed at developing salt-tolerant maize varieties. Further research should investigate the molecular mechanisms underlying the superior performance of this cultivar, including expression patterns of stress-responsive genes and ion transport regulation. Field validation under diverse saline conditions would also be valuable to confirm the consistency of these findings across environments.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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