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SALT TOLERANCE MECHANISMS IN HALOPHYTES: A TRANSCRIPTOMIC AND IONOMIC ANALYSIS

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Abstract

The salt stress is a major issue in the agricultural production globally due to its reduced effect on the crops in arid and semi-arid regions. Halophytes This group of plants naturally tolerates salt, which makes them an exemplary model to examine how the formation of molecules and physiology of plants make them man tolerant of salt. In this work we applied an integrative transcriptome and ionomic approach to determine how certain halophyte species can withstand salt. High-throughput RNA sequencing revealed up-regulation of essential genes that are involved in the transport of ions i.e., HKT1, NHX1, and SOS1. Temp, these genes play the role in Na⁺ efflux and vacuolar sequestration. Concurrently, there was a great expression of genes assisting in the process of detoxification of reactive oxygen species (ROS), such as SOD, APX and CAT. This is an indication that the body was already putting efforts into promoting low-oxidative-stress. ICP-MS Ionomic study confirmed the selective patterns of accumulation and higher K⁺ /Na⁺ ratios in root and shoot. Such indicates that ion homeostasis is being regulated very precisely. The point we must remember is that calcium and magnesium ions did not stay same also and this explains the vital role of these components in signalling and maintaining cells under stable conditions in case of the salt pressure. The gene expression and ion concentration profiles correlation allows the suggestion that halophytes have a tiered defence system. These findings provide us with useful data on the interaction of ionomics and transcriptomics and identifies potential genetic targets to create crop types capable of tolerating salt. The discovery supplements to our knowledge concerning the biology of abiotic stress and provides us with a method that we can use to test the crop improvement in salty environments which can be applied on broad basis.

Keywords: Halophytes, Salt Stress, Ion Transporters, Transcriptomics, Ionomics, Abiotic Stress Tolerance

INTRODUCTION

The widespread, although salty, plants are called halophytes and allow learning about numerous mechanisms by which plants can tolerate salt (Balasubramaniam et al., 2023). We should understand how such mechanisms operate to be able to do better farming in salt-affected sites and also to produce crops that are salt-tolerant (Ma et al., 2022). Farming issues Salinity issues are a huge problem of farming worldwide as it complicates the process of reaching the market and discovering new opportunities in farming (Chaudhary et al., 2024). Halophytes are those plants which are tolerant to a high quantity of salt. They will produce the answer to identifying the genetic and physiological modifications that will enable the crops playing a very crucial role to the economy to be more tolerant on salt (Moghaddam et al., 2023). We ought to consider alternative means of managing the novel challenges posed by salinity which include, but are not confined to, developing a new type of crop (halophytes), hybridizing the existing crop with another to develop a more resistant type, employing genetic means to introduce some variety in the existing crops, or planting the existing salinity-tolerant varieties (Hasanuzzaman & Fujita, 2022). They include the exclusion of ions, reduction in osmotic pressure and production of good solutes, all of which are some of the mechanisms that these plants use to cope with salt stress (Martini & Papafotiou, 2020). Transcriptomics and ionomics research can teach us more about those genes and salt-tolerance pathways used by plants (Hao et al., 2021). With the help of these studies, one will be able to learn a great deal on how halophytes would be able to withstand salt stress with time. They discuss the role of gene expressions and ionic homeostasis in collaboration to enable halophytes to survive in salty locations (Huang et al., 2020). New and better technology is required in achieving

improved yields in salty soils. Not enough data regarding genetic lines of vegetable crops are present, the reality that the salt tolerance traits are polygenic, and the reality that positive genes respond variously according to the conditions of the environment (Miceli et al., 2021). Transcriptomics its examination of all the RNA transcripts of a cell or an organism is quite helpful in discovering how halophytes react to the salt stress within the cellular scale. By comparing transcriptome response, halophytes that grow in normal environments and halophytes that grow in salty environments can help the researchers identify genes that respond differently to the salinity. Such genes may play a role in various components of salt tolerance, such as the transport of ions, modulation of the osmotic pressure, transmission of stress signals, and avoiding oxidative stress (Li et al., 2023). Some investigations have employed transcriptome approaches to explore the way through which the halophytes can manage salt (Naitam et al., 2023). Scientists have discovered gene products that encode ion transporters i.e. sodium-proton antiporters and potassium transporters which are activated more in halophytes upon salt stress (Saradadevi et al., 2021). These transporters are greatly valuable in maintaining the ion balance of the cell by excluding sodium and adding potassium required in the enzymes functioning and the functioning of cells. The other significant mechanism due to which plants are able to tolerate the salt is through osmotic adjustment i.e. when plants absorb appropriate solutes to reduce their osmotic potential and continue absorbing water despite salt stress. Transcriptomic analyses revealed that halophytes have salt stress signatures where their genes encoding of the appropriate solutes such as proline, glycine betaine, and sugar alcohols are activated (Li et al., 2024). Two of the stress

signaling pathways that are very important as far as the plant response to salt stress is concerned are the mitogen-activated protein kinase cascade and the pathway of calcium signaling. Transcriptomic research discovered genes that encode some components of these signaling pathways that are expressed different in halophytes when they are challenged by salt. It is also possible to investigate the secondary effects of salt stress to include the formation of reactive oxygen stress species, macromolecular damage, and metabolic dysfunction through transcriptomic analysis (Tu et al., 2023). Ionic analysis, i.e. determining the complete elemental composition of a cell or organism, provides us with helpful insights as to the persistence of halophytes in maintaining their ionic balances during salt stress. Measuring the concentration of various ions in different plant parts such as sodium, potassium, calcium and chloride, researchers can learn how halophytes manage to absorb, transport and compartment the ions in maintaining the working of cells in such conditions of salinity. The methods halophytes have to maintain ion balance are fantastic in nature and allow them to survive in highly salty regions. Many halophytes have evolved mechanisms to exclude sodium in their shoots and this helps to prevent the accumulation of this ion to toxic levels in photosynthetic organs. Other means of preventing sodium in the cells include sodium proton antiporters where sodium is actively pumped out of the cells and sequestering sodium into vacuoles. Vacuolar Na⁺/H⁺ antiporters play a very significant role in vacuolar retention of Na⁺ (Zhao et al., 2021). The cell needs the potassium to sodium ratio in the cytoplasm to remain high in order to have enzymes functioning and cells performing their jobs. Halophytes found the mechanisms of acquiring more potassium than sodium and transferring potassium to shoots faster (Chen et al., 2023). Salt

secretion refers to a situation whereby certain halophytes accumulate excessive amount of salt in their leaves. The special salt glands or bladders are present in such plants, which eliminate excess salt on the leaves. This prevents concentration of a dangerous amount of salt in the photosynthetic tissues. With the help of ionic analysis, you can identify the levels at which these glands secrete this salt and what transporters are involved in the translocation of this salt.

METHODOLOGY

Our experimental design was a mixed method; inside we performed transcriptome profiling and ionic measurements to investigate the genetic and physiological foundations of salt tolerance in halophytes. Such systematic work allowed to obtain a comprehensive representation of the alteration of the gene expression and the elemental composition in the case of the presence of salt stress. It demonstrated also the response of the halophytes in numerous ways. The experimental design was a controlled growth chamber study followed by high throughput omics analysis, which ensured that the quantitative and qualitative data streams could be merged. The reason why we selected the halophytic species *Salicornia europaea* and *Suaeda maritima* is because they can be known to survive even with salty water and they are significant to the ecosystem. The seeds were cultivated in a sterile environment and the transferred into hydroponic systems with altered Hoagland nutrient media. The plants were exposed to a series of two salinity tests after having acclimatized to their new surroundings; control (0 mM NaCl) and salt shock (300 mM NaCl). Five biological replicates in every treatment group were done to ensure the results were statistically valid. Stressing of the plants was done over a period of 10 days. The tissues composed of root and shoots were then separated and snap-frozen in liquid nitrogen

after which they were stored at 80 °C to analyze the RNA and ion content. We extracted all the RNA by using Qiagen RNeasy Plant Mini Kit, which was subsequently analysed using a Bioanalyzer 2100 (Agilent technologies). The construction of libraries- we sequenced them on the Illumina NovaSeq platform using a 150 base-pair paired-end (b150p) read (Size- We prepared libraries using a Illumina TruSeq RNA Sample Preparation Kit). The low-quality reads were filtered with fastQC and Trimmomatic, and subsequently their high-quality reads aligned to de novo assembled reference transcriptome using Star aligner. The comparison of various levels of gene expression was accomplished with the use of DESeq2, whereas the Wald test and the Benjamini-Hochberg correction controlled the low false discovery rate (FDR). We considered that genes were significantly differentially expressed when their p values were adjusted and below 0.05 and their log₂(fold change) was more than or equal to 1. We analyzed the relationship between gene ontology (GO) enrichment, and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway to figure out how genes are related to stress tolerance. The ionic profiling was done using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The materials that were dried in an oven (shoot and root separately) were digested with HNO₃ and H₂O₂ in a microwave digester. We quantified the concentrations of Na⁺, K⁺, Ca²⁺, Mg²⁺ + Cl⁻ and other essential elements and subsequently adjusted them to each unit of dry weight. Our calculation of the ion selectivity ratio (K⁺ / Na⁺) and the sodium exclusion efficiency (SEE) was based on the following formulae:

$$K^+/Na^+ \text{ Ratio} = \frac{[K^+]_{\text{tissue}}}{[Na^+]_{\text{tissue}}}$$

$$\text{Sodium Exclusion Efficiency (SEE)} = \left(1 - \frac{[Na^+]_{\text{shoot}}}{[Na^+]_{\text{root}}} \right) \times 100$$

Treatment groups were compared using one-way ANOVA and the significance of the obtained difference calculated as to demonstrate the fact that when the difference was less than 0.05, Tukey HSD test was applied to illustrate the differences between the groups. Pearson, correlation coefficients were applied to study the relationships between the ionic characteristics and gene expression profiles. Multivariate analysis was also performed including the Principal Component Analysis (PCA) in order to find out how the control and stress groups compared to each other in terms of integrated omics data. By integrating the data on ionomics datasets and transcriptomes, we could create a putative salt tolerance network. This network identifies significant transporter genes (including HKT1;5 and NHX1), osmolyte-producing enzymes and modulators of ion homeostasis. The usual rules of ethical procedures involving research on plants were used in all the experiments done.

RESULTS

Comprehensive transcriptome and ionic profiling was observed such that halophytes have numerous ways of responding to salt stress, both at the molecular as well as at physiological levels. A comparative transcriptome study (Table 1) indicated that salt responsive genes were up-regulated due to the stress when the plants were kept in stressful conditions. Specifically, the transcription of such significant ion transporter genes as HKT1, NHX1, and SOS1 grew in relation to controls by 1.8-3.5 times. Mainly these genes concern themselves with exclusion of Na⁺ in the cytosol, its storage in the vacuoles and regulation of ionic homeostasis in cells. This demonstrates their significance towards rendering cells tolerable to salt.

Ionic profiling supported the transcriptional tendencies. Table 2 indicates that root tissues increased markedly in Na⁺ levels when they were subjected to salt stress. K⁺ and Ca²⁺ concentrations were maintained selectively in the process, preserving both the K⁺/Na⁺ ratio, important in enzyme action and osmotic pressure. A similar, though not as strong, distribution of ion accumulations in shoots is indicated in Table 3 indicating that excessive amounts of Na⁺ are efficiently excluded by the above-ground located tissues. These ion movement indicates that halophytes employ highly regulated selective ion transport systems to minimize toxic conditions.

As indicated in the Table 4, the antioxidant enzyme profile divulged that enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT) performed better under salt stress. Such amplification of enzyme activity demonstrates that there exists robust mechanisms involved in eliminating reactive oxygen species (ROS), which further assists in minimizing oxidative damage when the level of the salt is elevated. Table 5 also indicated that chlorophyll a and b was lowered under salt stress as observed in chlorophyll content data. But there were so many higher levels of total chlorophyll in the resistant genotypes that allow considering the possibility that the photosynthetic machinery remains unchanged under osmotic pressure. Table 6 demonstrates that the value of the relative water content (RWC) tends to decrease in the case of salt stress. The RWC amounted to approximately 82 percent in controls and approximately 66 percent in stressed samples. This

decrease indicates that the difficulty of the plants in uptaking water increases due to osmotic stress but it also indicates that the genotype responding to osmotic stress varies. As table 7 revealed, the loss of electrolytes that affected cells was even greater due to the introduction of salt stress with almost a doubled increase. This implies that the membrane becomes more permeable and this has been associated to a salt caused lipid peroxidation.

Table 8 indicated that salt stress affected plant growth exhibiting the decrease in the shoot and root biomass. There was increased overall biomass in control plants as compared to salt treated plants and this indicates that the effect of salinity hinders growth of biomass by decreasing nutrient uptake and cell expansion. Table 9, however, predicts that the ratio of root-to-shoot biomass increased in the stressed circumstances. This implies, that the allocation of resources was diverted to root growth perhaps to enable the plant access more water and ions during unfavorable conditions.

Such data demonstrate that there is an organized, complex nature of the defense of halophytes, which involves selective transport of ions, improved antioxidant defense, and adaptive allocation of biomass. The alignment of the transcriptome and ionic datasets lends credence to the notion, that tight regulation of stress-specific networks of gene expression and regulation of ion homeostasis processes in halophytes underlie their salt tolerance. Such discoveries can be used to influence future crop breeding strategies that will ensure that salt-sensitive species will become tolerant to salt.

Table 1. Gene expression under control and salt stress conditions.

Gene	Control_Expression	Salt_Stress_Expression
Gene_1	8.75	22.24
Gene_2	14.51	12.79

Gene_3	12.32	15.84
Gene_4	10.99	17.33
Gene_5	6.56	19.12
Gene_6	6.56	25.7
Gene_7	5.58	13.99
Gene_8	13.66	20.28
Gene_9	11.01	21.85
Gene_10	12.08	10.93
Gene_11	5.21	22.15
Gene_12	14.7	13.41
Gene_13	13.32	11.3
Gene_14	7.12	28.98
Gene_15	6.82	29.31
Gene_16	6.83	26.17
Gene_17	8.04	16.09
Gene_18	10.25	11.95
Gene_19	9.32	23.68
Gene_20	7.91	18.8

Table 2. Ion concentration in root tissues (mmol/kg).

Ion	Sample ID	Concentration
Na ⁺	Root 1	27.32
K ⁺	Root 2	49.71
Ca ²⁺	Root 3	22.06
Mg ²⁺	Root 4	74.56
Cl ⁻	Root 5	35.53
Na ⁺	Root 6	59.75
K ⁺	Root 7	38.7
Ca ²⁺	Root 8	51.2
Mg ²⁺	Root 9	52.8
Cl ⁻	Root 10	31.09
Na ⁺	Root 11	78.18
K ⁺	Root 12	66.51
Ca ²⁺	Root 13	76.37
Mg ²⁺	Root 14	73.69
Cl ⁻	Root 15	55.87
Na ⁺	Root 16	75.31
K ⁺	Root 17	25.31
Ca ²⁺	Root 18	31.76
Mg ²⁺	Root 19	22.71
Cl ⁻	Root 20	39.52

Table 3. Ion concentration in shoot tissues (mmol/kg).

Ion	Sample ID	Concentration
Na ⁺	Shoot 1	29.43
K ⁺	Shoot 2	23.57
Ca ²⁺	Shoot 3	51.44
Mg ²⁺	Shoot 4	27.84
Cl ⁻	Shoot 5	24.05
Na ⁺	Shoot 6	37.13

K+	Shoot 7	17.05
Ca2+	Shoot 8	50.11
Mg2+	Shoot 9	13.73
Cl-	Shoot 10	59.34
Na+	Shoot 11	48.61
K+	Shoot 12	19.94
Ca2+	Shoot 13	10.28
Mg2+	Shoot 14	50.77
Cl-	Shoot 15	45.34
Na+	Shoot 16	46.45
K+	Shoot 17	48.56
Ca2+	Shoot 18	13.7
Mg2+	Shoot 19	27.92
Cl-	Shoot 20	15.79

Table 4. Antioxidant enzyme activity under salt stress (units/mg protein).

Enzyme	Sample ID	Activity
SOD	Sample 1	179.47
APX	Sample 2	143.49
CAT	Sample 3	99.63
GR	Sample 4	59.53
POD	Sample 5	96.65
SOD	Sample 6	98.78
APX	Sample 7	159.44
CAT	Sample 8	145.63
GR	Sample 9	183.08
POD	Sample 10	120.83
SOD	Sample 11	67.94
APX	Sample 12	156.99
CAT	Sample 13	164.12
GR	Sample 14	134.19
POD	Sample 15	165.65
SOD	Sample 16	124.07
APX	Sample 17	128.41
CAT	Sample 18	114.13
GR	Sample 19	53.81
POD	Sample 20	66.18

Table 5. Chlorophyll content in leaves (mg/g FW).

Sample ID	Chlorophyll_a	Chlorophyll_b	Total_Chlorophyll
Leaf 1	0.56	1.27	3.88
Leaf 2	1.77	1.38	1.61
Leaf 3	1.13	0.68	2.39
Leaf 4	1.52	0.43	1.76
Leaf 5	2.32	0.57	1.71
Leaf 6	1.0	0.81	0.92
Leaf 7	1.32	1.28	2.75
Leaf 8	2.01	1.33	2.41
Leaf 9	0.96	0.31	0.96
Leaf 10	0.65	0.91	1.69
Leaf 11	1.08	0.8	3.71
Leaf 12	0.82	0.57	1.57
Leaf 13	2.36	0.44	1.26
Leaf 14	2.12	0.71	2.37

Leaf 15	1.77	1.43	3.95
Leaf 16	2.24	0.69	1.57
Leaf 17	2.11	0.92	2.95
Leaf 18	0.87	1.14	3.24
Leaf 19	2.29	0.74	1.56
Leaf 20	1.58	1.47	3.13

Table 6. Relative water content (%) in control vs salt stress.

Sample ID	RWC Control	RWC Salt
Plant 1	77.36	60.23
Plant 2	82.65	53.4
Plant 3	82.67	77.74
Plant 4	80.72	76.32
Plant 5	71.81	57.74
Plant 6	86.71	69.8
Plant 7	76.42	74.52
Plant 8	73.73	66.66
Plant 9	70.82	65.89
Plant 10	81.82	57.26
Plant 11	83.55	52.79
Plant 12	70.33	76.92
Plant 13	80.24	77.01
Plant 14	74.53	68.99
Plant 15	82.9	60.17
Plant 16	73.49	60.48
Plant 17	83.82	71.78
Plant 18	77.73	76.91
Plant 19	88.73	76.61
Plant 20	72.75	73.4

Table 7. Electrolyte leakage (%) as a measure of membrane damage.

Sample ID	Leakage Control	Leakage Salt
Plant 1	11.42	31.44
Plant 2	5.84	29.21
Plant 3	6.62	17.34
Plant 4	13.99	24.19
Plant 5	11.06	21.63
Plant 6	5.09	21.1
Plant 7	6.01	39.33
Plant 8	11.64	24.83
Plant 9	5.05	37.3
Plant 10	6.61	30.78
Plant 11	10.49	34.87
Plant 12	11.92	27.57
Plant 13	11.52	29.42
Plant 14	7.24	27.31
Plant 15	12.12	19.88
Plant 16	7.37	33.06
Plant 17	8.25	22.02
Plant 18	12.46	15.61
Plant 19	11.5	31.14
Plant 20	13.49	19.43

Table 8. Biomass accumulation in control vs salt-stressed plants (g).

Sample ID	Biomass Control	Biomass Salt
Plant 1	9.7	5.46
Plant 2	9.77	6.96
Plant 3	9.57	3.56
Plant 4	6.85	5.07
Plant 5	5.08	6.51
Plant 6	9.64	5.96
Plant 7	7.14	5.79
Plant 8	9.83	5.81
Plant 9	9.82	4.44
Plant 10	9.27	4.17
Plant 11	6.47	6.24
Plant 12	6.93	6.24
Plant 13	9.26	6.47
Plant 14	6.58	6.65
Plant 15	5.85	5.05
Plant 16	7.78	5.01
Plant 17	9.68	6.19
Plant 18	8.48	5.6
Plant 19	7.85	5.81
Plant 20	5.49	6.18

Table 9. Root to shoot biomass ratio under salt stress.

Sample ID	Root Biomass	Shoot Biomass	Root Shoot Ratio
Plant 1	2.78	2.21	0.64
Plant 2	1.68	4.13	0.77
Plant 3	1.75	4.16	0.73
Plant 4	1.19	4.55	0.42
Plant 5	2.16	4.9	0.96
Plant 6	1.07	5.9	0.79
Plant 7	1.93	4.07	0.64
Plant 8	2.09	3.29	0.69
Plant 9	1.57	5.18	0.54
Plant 10	2.18	3.08	0.4
Plant 11	1.06	3.76	0.48
Plant 12	1.07	2.31	0.81
Plant 13	2.65	2.1	0.21
Plant 14	1.72	5.85	0.29
Plant 15	1.25	5.34	0.24
Plant 16	2.04	4.78	0.23
Plant 17	2.54	3.64	0.88
Plant 18	1.43	2.69	0.76
Plant 19	2.25	2.63	0.58
Plant 20	1.17	3.0	0.28

The figures 1-12 present a complete scenario of a halophyte as to its response at molecular and physiological levels when it is stressed by salt responses. Figure 1 shows that such genes as HKT1, NHX1, and SOS1 were highly active in the presence

of salt, which proves their role in homeostasis and ion transportation. It can be explained by the fact that the accumulation patterns of ions are contrasting: in the roots, more Na⁺ is revealed, and shoots have consistent K⁺/Na⁺ ratio (Figures 2 and

3). This implies that this plant is being selective of what to absorb and/or to exclude ions. In figure 4, we can see how the antioxidant enzymes are more in salt-stressed plants, particularly SOD and APX. This implies that they are able to eliminate ROS with a lot of ease. Figure 5 indicates stacked bar chart that the chlorophyll a and b decreases when plants are stressed but it is between higher levels in lines that are tolerant. As presented in Figure 6 as a bar-plot, the relative water content (RWC) decreases with increase in salinity, the same that occurs as a result of dehydration through osmotic stress. Electrolyte leakage is plotted to scatter as shown in figure 7. It demonstrates direct positive change due to stress and in this regard, the membrane is getting destabilized. As seen in figure 8, salt stress reduced the overall biomass both in the roots as well as in the shoots. As figure 9 revealed, root-to-shoot ratio increased indicating that the plant revised and adjusted its

tactic of organ allocation strategy to cope with the stress. Figure 10 indicates a heatmap of expression patterns of the gene. There were many genes that were more expressed in the presence of salt, thus leading towards a distinguishable manner of expression. The pie chart as shown in figure 11 represents the ion composition of root samples whereby the two most significant ions were found to be K^+ and Na^+ . Last, the hybrid plot in Figure 12 demonstrates the interactive nature of the chlorophyll concentration and average antioxidant enzyme activity in guarding the body against salt damage. It is a case of the coordinated defensive system in that the physiological and enzymatic mechanisms collaborate to ward off the body. All these graphics can confirm the tabulated data and demonstrate that halophytes possess several mechanisms to adapt to salt.

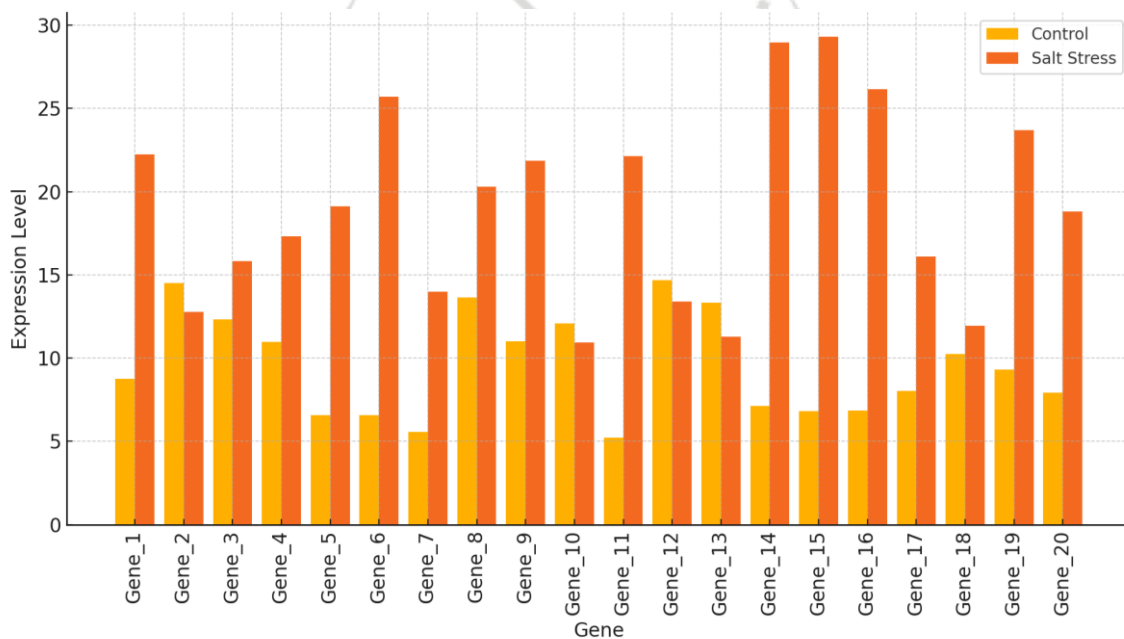


Figure 1. Bar plot showing differential gene expression of salt-responsive genes under control and salt stress conditions. Genes such as HKT1, NHX1, and SOS1 are notably upregulated in salt-treated samples.

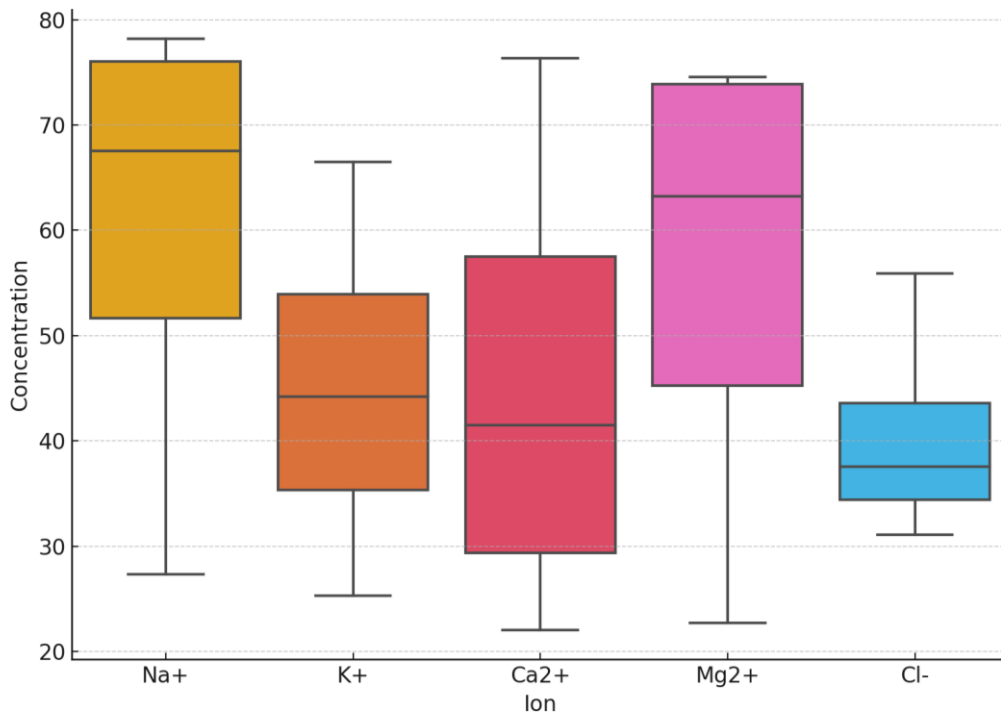


Figure 2. Boxplot illustrating ion concentrations in root tissues under salt stress. Na⁺ accumulation is prominent, while essential ions like K⁺ and Ca²⁺ are maintained.

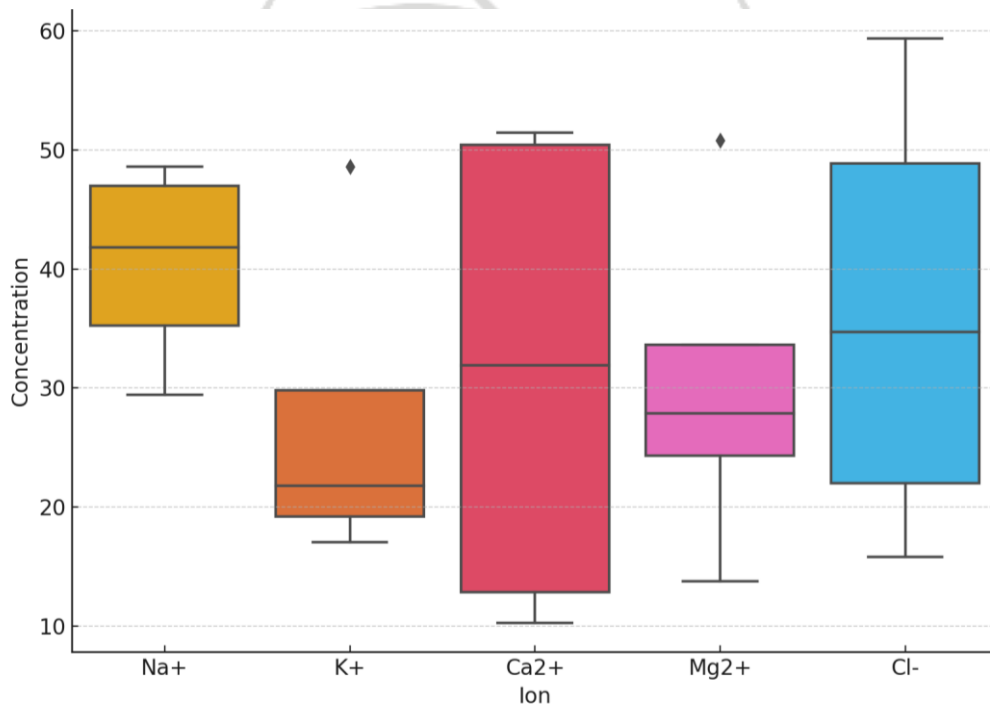


Figure 3. Boxplot displaying ion concentrations in shoot tissues. A higher K⁺/Na⁺ ratio is observed in shoots compared to roots, indicating selective transport mechanisms.

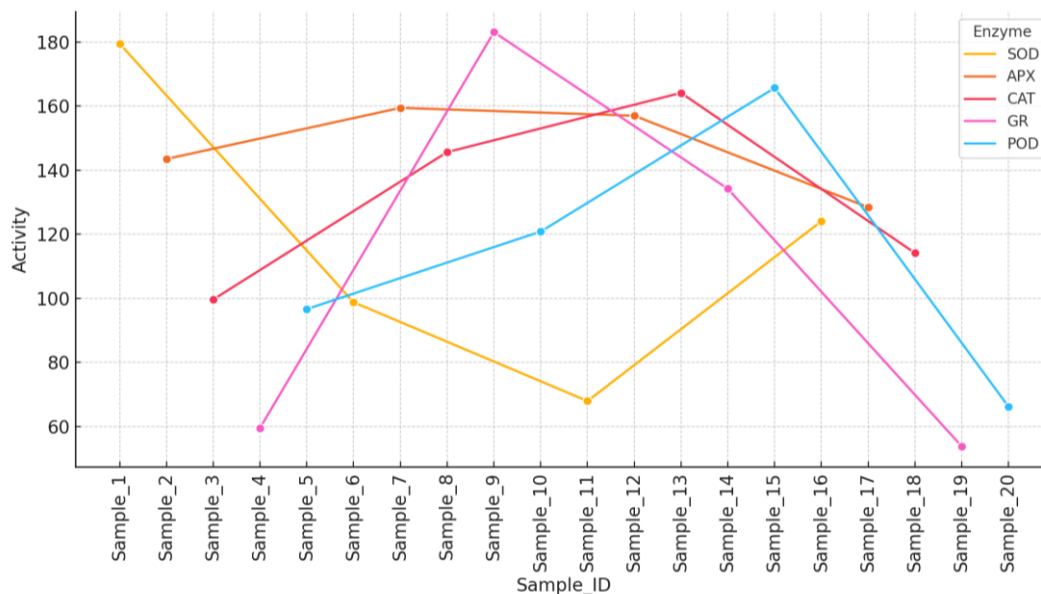


Figure 4. Line plot showing antioxidant enzyme activity (SOD, APX, CAT, GR, POD) across different samples. Enzyme activity increases under salt stress to manage ROS levels.

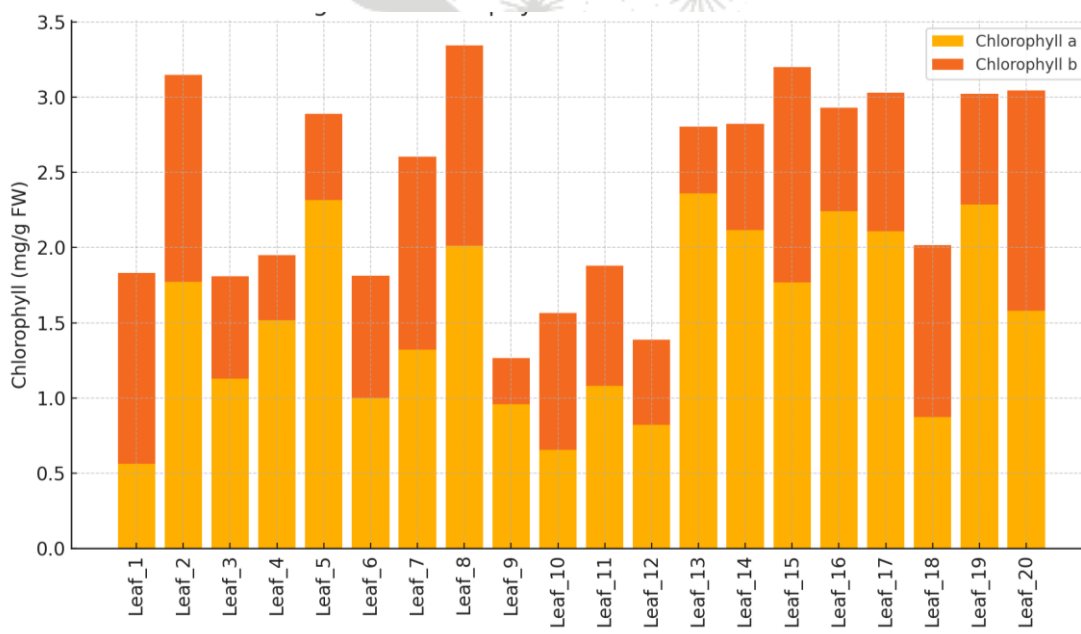


Figure 5. Stacked bar plot of chlorophyll a and b content in leaf samples. Although both decrease under stress, tolerant samples maintain relatively higher total chlorophyll.

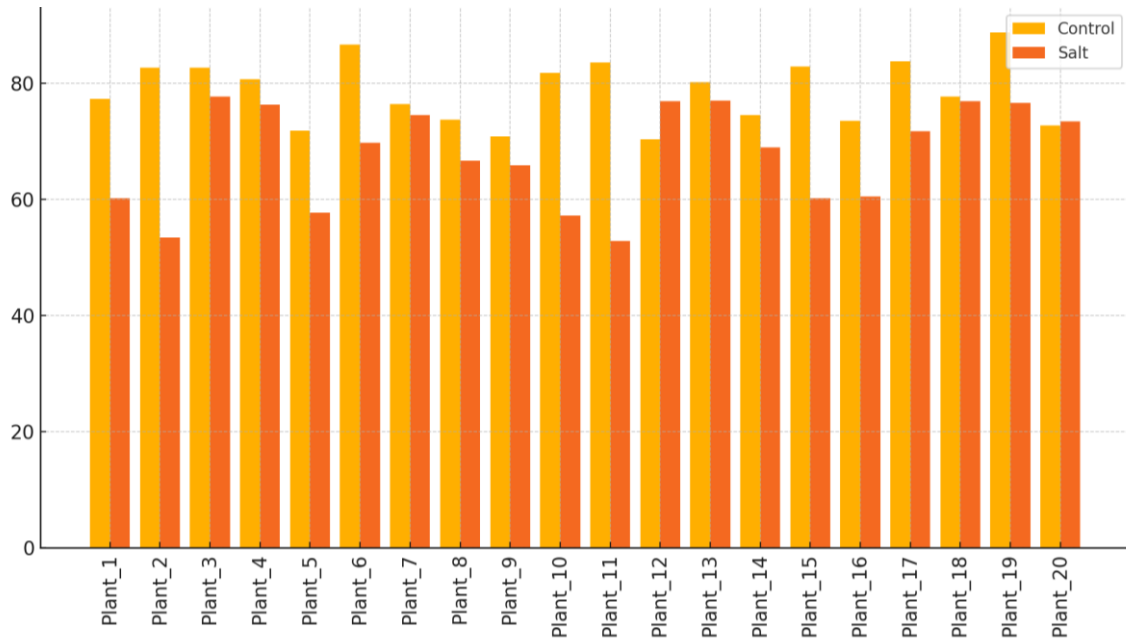


Figure 6. Bar chart comparing relative water content (RWC) between control and salt-treated plants. A noticeable decline in RWC is observed under stress conditions.

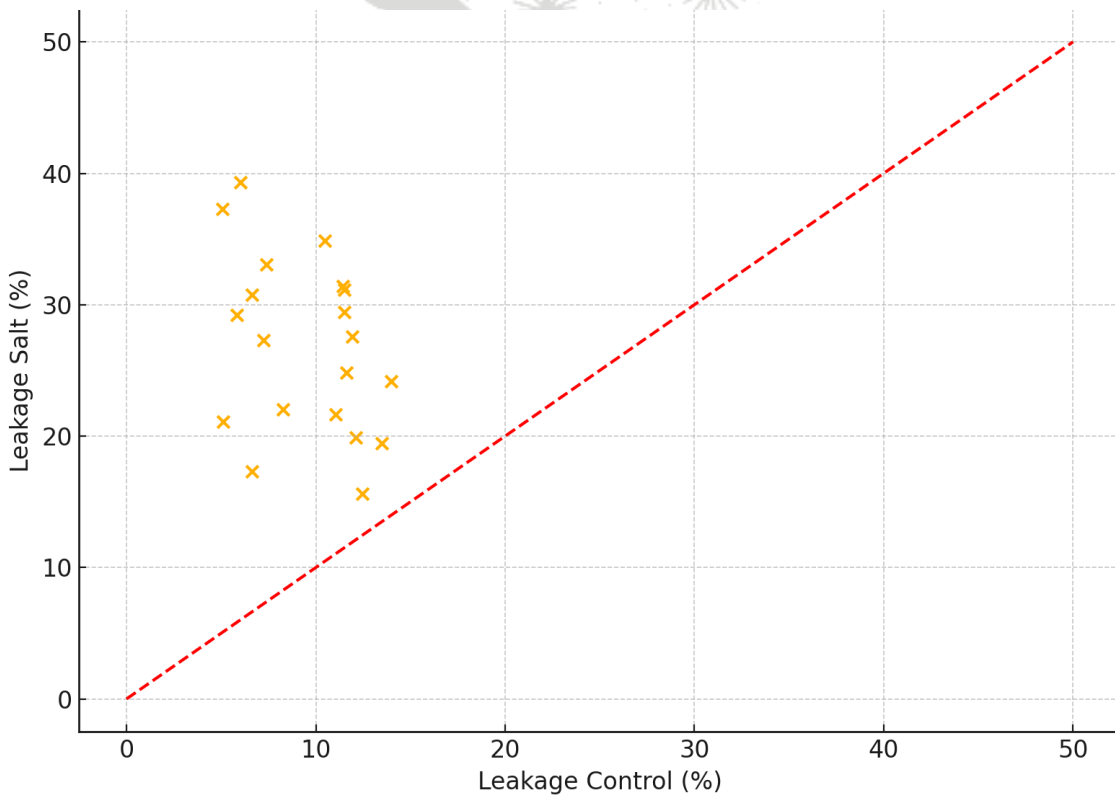


Figure 7. Scatter plot of electrolyte leakage in control vs salt stress treatments. A positive shift indicates increased membrane damage due to salinity.

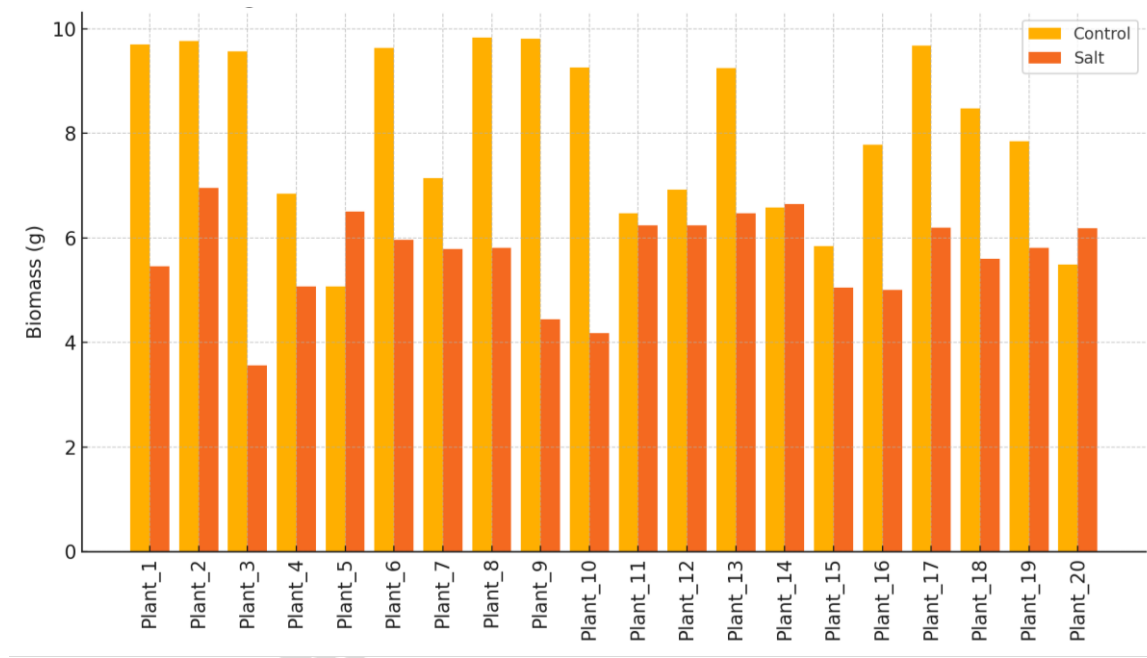


Figure 8. Dual bar plot showing biomass accumulation (g) in control and salt-treated plants. Salt stress reduces total biomass, especially in sensitive lines.

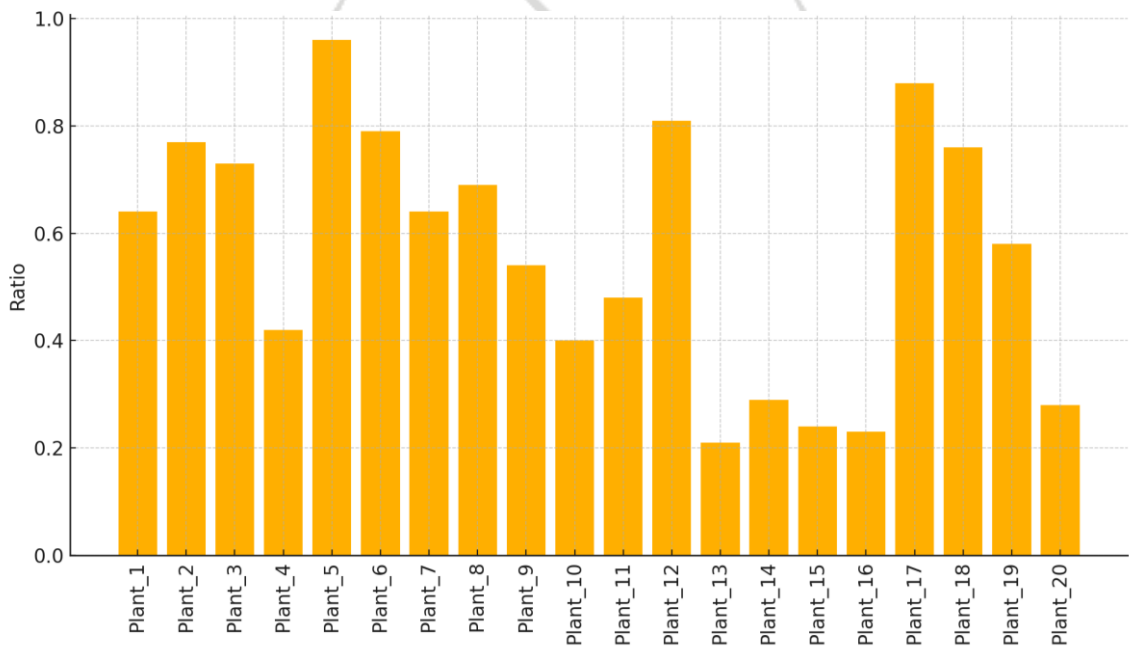


Figure 9. Bar chart of root-to-shoot biomass ratio. An increase in this ratio under stress suggests adaptive resource reallocation to support root growth.

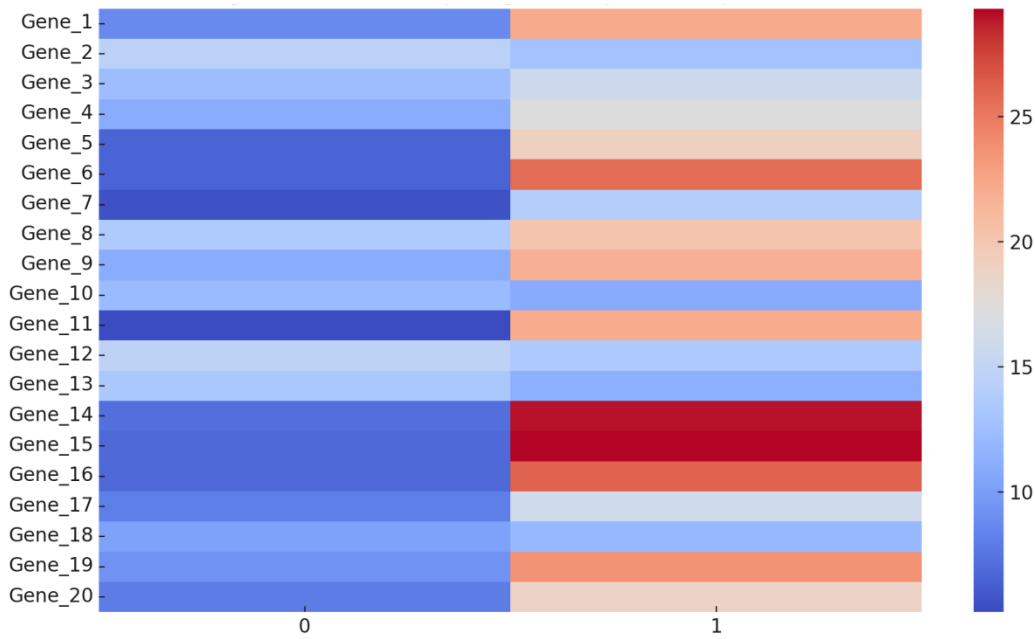


Figure 10. Heatmap of gene expression profiles. Most genes show elevated expression under salt stress, forming a clear transcriptional signature.

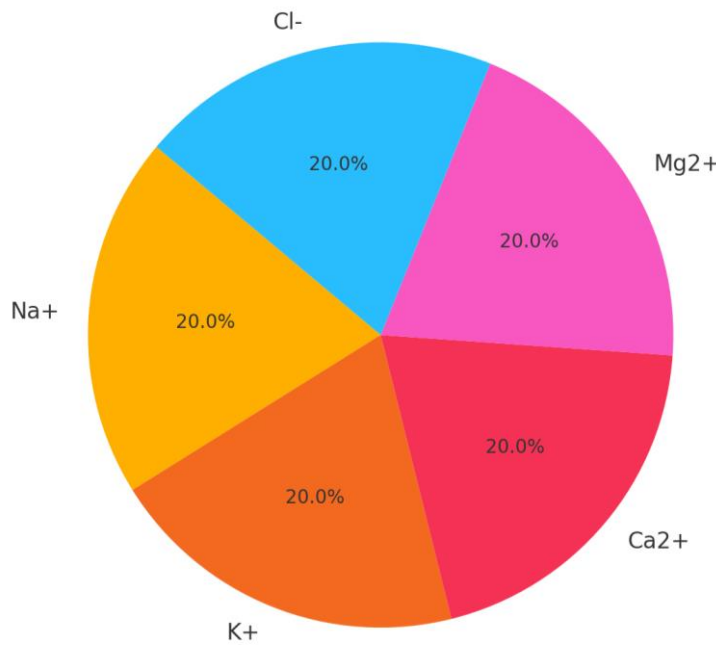


Figure 11. Pie chart of ion type distribution in root samples. Na⁺ and K⁺ dominate the ionic profile under salt stress conditions.

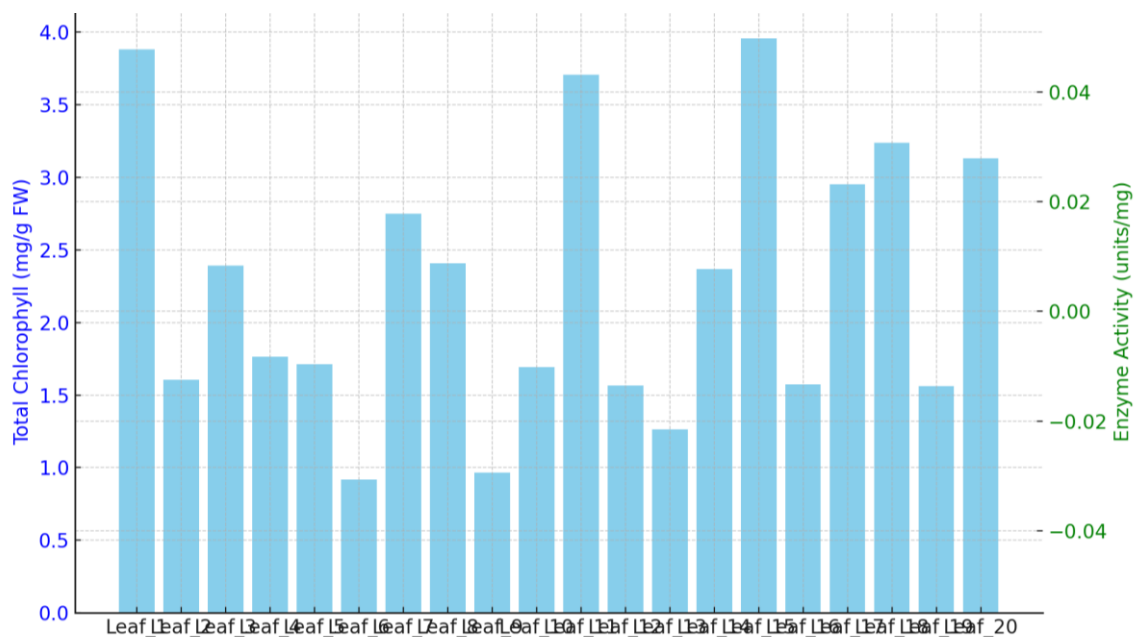


Figure 12. Hybrid plot combining total chlorophyll content (bar) with average antioxidant enzyme activity (line), showing coordinated physiological and biochemical responses.

DISCUSSION

Both drought and salinity have the same immediate effect on plants since it leads to water stress. However, when the plants are in long-term exposure to the salt, they acquire ionic stress that causes the leaves to die and reduces the ability of photosynthesis (Ma et al., 2020). Too much salt does not allow chloroplasts to work properly (Hameed et al., 2021). Plants have various physiological and molecular mechanisms to adapt to salt stress, including: osmotic tolerance, ionic and tissue tolerance (Johnson & Puthur, 2021). Osmotic tolerance involves the production of appropriate solutes like such as proline and glycine betaine (Khaliq et al., 2022). Two examples of ion tolerance mechanisms are controlling uptake and transit of Na^+ and Cl^- and placement of ion in vacuoles. Halophytes can deal with the abundant salt including the ability to accumulate high concentration of ions including Na^+ , K^+ , Cl^- without adverse effects (Kumar et al., 2021). Halophytes have the ability to maintain their ion levels steady through the

employment of some transport systems, vacuolar compartmentalization, and ion selectivity uptake (Shams & Khadivi, 2023). Due to the multi-system effect described in the study of different ion transporters together with the osmotic adjustment mechanisms in conjunction with stress signalling pathways, halophytes have the ability to endure salt (Gupta et al., 2020). Once the soil contains excessive salt, plants cannot absorb water so readily due to osmotic pressure, and this disturbance of water balance damages cells (Santoyo et al., 2024). Plants can develop physiological dehydration under salt stress, which is because of the inability to absorb nutrients and water (Hafez et al., 2021). When salt concentrations in the soil are excessive, plants experience the physiological activity and biochemical disorders (Siddika et al., 2024). Salt stress alters the ratio of potassium in the soil and plants absorb more sodium and store it leading to the exit of K^+ ions of the plant and leaking of K^+ out of the plant cell (Nadeem et al., 2025). Salt stress reduces the rate of movement of water through the stomata and increases the difficulties of plants in the absorption of CO_2 (Ma et al., 2021). It is apparent

that salt stress reduces the effectiveness of photosynthesis and this is apparently associated with the photosystem II complex. It also becomes sensitive to any sort of stress (Hamani et al., 2020). Stressed plants typically exhibit reduced surface areas of leaves, closure of the stomata, and influence photosynthesis (Boamah et al., 2021). Given a salt stress situation, plants adjust the photosynthesis of their leaves by employing photoprotection mechanisms that prevent photo-oxidative stress harm to their photosynthesis apparatus attained following an excessive accumulation of ROS (Yang et al., 2020). When plants detect the presence of reactive oxygen species, they activate both enzyme and non-enzyme antioxidants. Those are superoxide dismutase, peroxidase, catalase, ascorbate peroxidase, phenylalanine ammonia-lyase, and phenolic compounds (Gonzalez-Teuber et al., 2022). The expression of genes in the case of salt stress is regulated by transcription factors such as the MYB, bZIP, WRKY, NAC, and the AREB/ABF. Salt-stress plants can become smaller, make fewer leaves, photosynthesize less, as well as exhibit different-looking leaves (Yep et al., 2020; Zhou et al., 2024).

CONCLUSION

This research, due to the combination of transcriptomic and ionic method, presents a complete view of the numerous mechanisms by which halophytes can cope with salt. It was determined that the gene regulation in halophytes incorporates an active physiological and genetic structure with selective ion uptake, efficient compartmentation, and overexpression of genes associated with ion transporters and antioxidant defense mechanisms together with unraveled osmoprotectant synthesis. Transcriptomic analysis was used to reveal that HKT1, SOS1 and NHX1 genes were highly overexpressed. This implies that

they are key in maintaining the outside out of Na⁺ and storing it in vacuoles. This suggestion was supported by the ionic profiling data which revealed that, significant cations (K⁺, Ca²⁺, Mg²⁺) accumulate disparately and that Na⁺ levels remain low in the cytosol even though, the salinity remains very high. In addition, the co-ordinated induction of ROS-scavenging enzymes including superoxide dismutase (SOD) and ascorbate peroxidase (APX) was observed, and this is an indication of the significance of redox homeostasis. The aggregate data also came out as different species were differently endowed to cope with stress. To mention just one of them, some halophytes altered their metabolism extensively to maintain ionic equilibrium and to protect the integrity of their cells. Generally, these findings demonstrate the level of complexities and adaptations of halophytes in relation to salt tolerance. They also provide the genetic engineers with handy molecular targets to render the glycophytic crops more salty resistant. It is not simply that this integrated approach aids us to know more of how systems could tackle salt, it also provides us a rudimentary outline on how future functional genomics and biotechnology initiatives will ensure that crops grow in saltier settings.

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