ISSN: 3049-0677 (Online)

PhytoTalks

October 2025; Volume 2: Issue 3: 523-540

https://doi.org/10.21276/pt.2025.v2.i3.9

Research Article

OPEN ACCESS

Physiological and Biochemical Responses of Willow (*Salix* spp.) Clones to Salinity Stress in Semi-Arid Conditions: Identifying Salt-Tolerant Genotypes for Sustainable Land Use

Neha Saini[1]*, T. N. Manohara[1],

[1]Institute of Wood Science and Technology (IWST), 18th Cross, Malleswaram, Bengaluru – 560003, Karnataka, India

*Corresponding author Email: saininehaforestry@gmail.com

Article History
Received: 10.08.2025
Revised: 04.09.2025
Accepted: 12.09.2025
Online
Version: 1

Additional Information
Peer review: The publisher expresses gratitude to the anonymous reviewers and sectional editors for their invaluable contributions during the peer review process.

Reprints and Permissions Information is available at:
https://phytotalks.com/journal/index.php/PT/open_acess_policy

Publisher's note: Regarding jurisdictional claims in published maps and institutional affiliations, PhytoTalks maintains its objectivity.

Cite this article: Saini N, Manohara TN. Physiological and Biochemical Responses of Willow (*Salix* spp.) Clones to Salinity Stress in Semi-Arid Conditions: Identifying Salt-Tolerant Genotypes for Sustainable Land Use. *PhytoTalks*. 2025; 2(3): 523-540.

Abstract

Salinity stress presents a significant challenge to plant productivity, especially in semi-arid regions where soil degradation intensifies due to climate change and unsustainable irrigation practices. This study evaluated ten willow (*Salix* spp.) clones exposed to increasing irrigation salinity levels (4–16 dS/m) to investigate their physiological and biochemical responses over a 105-day period. Key stress indicators—including relative water content (RWC), water potential (Ψ_w), osmotic potential (Ψ_s), relative stress injury (RSI), proline accumulation, lipid peroxidation (MDA), and antioxidant enzyme activities (SOD, POD)—were measured to assess clone performance. Clone 131/25 consistently outperformed the others, showing higher RWC, less negative Ψ_w and Ψ_s, lower RSI and MDA levels, and increased proline, SOD, and POD activities. Clones J799 and SI-64-017 also displayed strong adaptive traits. These genotypes demonstrated superior osmotic adjustment, reduced oxidative stress, and maintained chlorophyll content under salinity, indicating the presence of effective tolerance mechanisms. Correlation analysis confirmed strong positive relationships between survival, growth, proline, and antioxidant activity, as well as negative correlations with RSI and Na^+/K^+ ratio. The results identify 131/25, J799, and SI-64-017 as promising candidates for cultivation on salt-affected lands and for use in agroforestry, bioenergy, and ecological restoration programmes. These findings support their deployment in climate-resilient, sustainable land management in saline-prone semi-arid regions.

Keywords: *Salix* spp., salinity stress, salt tolerance, osmotic adjustment, proline, antioxidant enzymes.

1. Introduction

Willows (*Salix* spp.) comprise a diverse genus with over 400 species worldwide. They are recognised for their rapid growth, ecological adaptability, and various uses in forestry, traditional medicine, and environmental restoration. In India, 33 willow species have been identified, including seven tree species: *Salix tetrasperma*, *S. acmophylla*, *S. alba*, *S. fragilis*, *S. babylonica*, *S. daphnoides*, and *S. excelsa* — mainly found across the temperate Himalayan and sub-Himalayan regions[1]. *S. tetrasperma* is found throughout India, whereas the others are generally limited to specific elevational zones.

Willow trees are highly valued for their diverse purposes, including the extraction of salicin (a medicinal compound) and the production of high-quality wood for cricket bats, veneer, fuelwood, fodder, and bioenergy. Their ability to rapidly produce biomass, combined with strong coppicing ability, makes them suitable for short-rotation forestry (SRF) and agroforestry systems. Notably, *Salix* alba has demonstrated superior biomass yield in high-density SRF plantations, outperforming *S. viminalis* and *S. fragilis*[2].

Soil salinization has become an increasing global concern, particularly in arid and semi-arid regions, where it threatens agricultural productivity and ecological balance. It is estimated that over 20% of the world's irrigated land is affected by salinity, resulting in reduced land-use efficiency and limited plant growth. However, several *Salix* species have demonstrated moderate to high levels of salt tolerance[3], making them promising options for afforestation, ecological restoration, and bioenergy cultivation on salt-affected lands.

Salinity stress reduces plant water uptake by lowering external water potential (Ψw), leading to osmotic stress, ion toxicity, and oxidative damage[4]. In response, plants activate various physiological and biochemical mechanisms to maintain internal balance. Key physiological indicators—such as relative water content (RWC), water potential (Ψw), osmotic potential (Ψs), and relative stress injury (RSI)—are often used to evaluate plant water status and membrane stability under stress conditions. Additionally, salinity-induced oxidative stress results in the overproduction of reactive oxygen species (ROS), which are regulated and detoxified by antioxidant enzymes like superoxide dismutase (SOD) and peroxidase (POD)[5]. Proline accumulation plays a vital role in osmotic regulation and membrane protection, while malondialdehyde (MDA) content serves as a biochemical marker of lipid peroxidation and oxidative membrane damage. The accumulation of proline leads to a decrease in cell potential, facilitating osmotic adjustment and membrane protection, while MDA acts as an indicator of lipid peroxidation and oxidative damage.

Growing global interest in developing salt-tolerant willow genotypes highlights the need for detailed physiological and biochemical assessments under saline conditions, which remain limited in Indian agro-climatic contexts, especially in semi-arid regions. This study evaluates ten genetically diverse willow clones cultivated under controlled saline irrigation in a semi-arid environment. Key physiological parameters (such as relative water content, relative stress injury, water potential, and osmotic potential), biochemical markers (proline and malondialdehyde), and antioxidant enzyme activities (superoxide dismutase and peroxidase) were measured across five salinity levels and multiple time points. The aim is to identify salt-tolerant genotypes with potential for biomass production, agroforestry, and ecological restoration of degraded, saline-affected landscapes. The findings offer valuable insights for climate-resilient land-use planning and the development of sustainable forestry strategies in water-scarce regions worldwide.

2. Materials and Methods

2.1 *Study Site and Climatic Conditions*

The experiment was carried out at Zarifa Farm, situated at 29°41'N, 76°59'E, at an altitude of 252 metres above sea level. This site is within a hot, sub-humid agro-climatic zone characterised by a subtropical to tropical climate. The region receives around 828 mm of average annual rainfall, mainly during the monsoon season from June to September. Extensive meteorological data—including temperature, relative humidity, sunshine duration, evaporation, wind speed, and rainfall—were gathered for 2020–2021. Inside the polyhouse, air temperature was recorded every four hours from March to July to track diurnal fluctuations.

2.2 *Plant Material and Propagation*

Ten genetically distinct willow (*Salix* spp.) clones were chosen for evaluation: J799, SI-64-017, 131/25, PN731, UHFS62, UHFS296, UHFS371, UHFS85, UHFS251, and UHFS221. Eight of these clones were developed at the ICAR–Central Soil Salinity Research Institute (CSSRI), Karnal, in 2016, while UHFS296 and UHFS251 were introduced in 2020.

Propagation was carried out using semi-hardwood cuttings approximately 6 cm long. To encourage root development, cuttings were treated with 1000 ppm indole-3-butyric acid (IBA) before planting in perforated polybags. The potting mixture consisted of equal parts soil, sand, and farmyard manure (FYM). Cuttings were kept under standard nursery and silvicultural practices for 10 weeks. Fertigation was applied periodically to promote optimal growth and establishment.

2.3 *Experimental Design and Salinity Treatments*

The experiment was conducted using a Randomised Block Design (RBD) with five salinity treatments, defined based on the electrical conductivity of the irrigation water (ECiw). A total of ten blocks were established: the first five blocks comprised clones 1–5 and the remaining five blocks comprised clones 6–10. Within each block, salinity treatments (SL0–SL4) were randomly assigned to experimental units to minimize positional and environmental bias.

The salinity levels were selected in accordance with widely used classifications of soil electrical conductivity (ECe): ≥ 4 dS/m (saline), 4–8 dS/m (moderately saline), 8–12 dS/m (strongly saline), and ≥ 16 dS/m (very strongly saline). Accordingly, five irrigation water salinity levels were imposed:

- **SL0** – Control (good-quality water)
- **SL1** – 4 dS/m
- **SL2** – 8 dS/m
- **SL3** – 12 dS/m
- **SL4** – 16 dS/m

All ten willow clones were subjected to each salinity treatment with three replications. For each clone, 15 seedlings were used (5 seedlings × 3 replications), resulting in a total of 750 plants. This factorial arrangement enabled the assessment of genotype × salinity interactions (Table 1).

Table 1. Salinity stress intervals and corresponding stock age of willow clones

S. No.	Interval	Date range	Salinity stress period (days)	Stock age (days)
1	I	20 March – 10 April 2020	21	75–96
2	II	11 April – 01 May 2020	42	96–117
3	III	02 May – 22 May 2020	63	117–138
4	IV	23 May – 12 June 2020	84	138–159
5	V	13 June – 03 July 2020	105	159–180

2.4 *Preparation of Saline Irrigation Water and Irrigation Schedule*

Saline water was prepared by mixing waters of different salinities to reach the targeted EC iw levels. The irrigation schedule followed the IW/CPE (Irrigation Water/Cumulative Pan Evaporation) ratio method to ensure consistent water application across all treatments. To prevent excessive salt build-up in the root zone, each saline irrigation was followed by an application of good-quality water. Salinity treatments commenced in April 2020 and continued until July 2020.

2.5 *Sampling Schedule and Parameters Measured*

Plant samples were collected every 21 days after salinity exposure began, resulting in five sampling intervals: 21, 42, 63, 84, and 105 days (Table 1). At each interval, physiological and biochemical parameters were measured to assess stress responses (Fig. 1). The parameters included:

- Water relations: Relative Water Content (RWC, %), Water Potential (Ψ_w, MPa), Osmotic

Potential (Ψ_s, MPa), and Relative Stress Injury (RSI, %) (Figs. 1–8)

- Biochemical markers: Proline content (mg g^{-1} FW) and Malondialdehyde (MDA) content (µmol g^{-1} FW) (Figs. 9–12)
- Antioxidant enzyme activities: Superoxide Dismutase (SOD) and Peroxidase (POD) (Figs. 13–16)
- Chlorophyll content: Total chlorophyll (mg g^{-1} FW) (Figs. 17–18)

Data from all physiological, biochemical, and antioxidant traits recorded across the five salinity intervals were compiled for multivariate analysis. Standardized Z-scores were calculated for each trait–clone–treatment combination, and Pearson's correlation coefficients were computed to examine interrelationships among traits. These associations and variability patterns are presented as a heat map in the Results section (Fig. 21).

2.6 *Relative Water Content (RWC, %)*

Determined following Barrs and Weatherley (1962):

$$RWC = TW - DW \,/\, FW - DW \times 100$$

Where FW = fresh weight, TW = turgid weight, and DW = dry weight.

2.7 *Relative Stress Injury (RSI, %)*

Calculated from electrolyte leakage as per Dionisio-Sese and Tobita (1998):

$$RSI = EC_a / EC_a + EC_b \times 100$$

Where EC_a = conductivity after 24 h at 25°C, and EC_b = conductivity after boiling for 1 h.

2.8 *Water Potential (Ψw, MPa)*

Measured using a WP4C Dew Point Potential Meter (METER Group Inc., USA).

2.9 *Osmotic Potential (Ψ_s, MPa)*

Measured using a 5100-B Vapour Pressure Osmometer (Wescor, USA). Osmolarity was converted to MPa using:

$$40 \text{ m Osmol} = -1 \text{ bar}$$

$$-10 \text{ bar} = -1 \text{ MPa}$$

2.10 *Proline Content (mg g⁻¹ FW)*

Proline content was estimated using the method of Bates et al.[6]. Fresh leaf tissue (300 mg) was homogenized in 3% sulfosalicylic acid, centrifuged, and reacted with acid ninhydrin reagent. After heating and extraction with toluene, absorbance was measured at 520 nm.

2.11 *Lipid Peroxidation (MDA Content, µmol g⁻¹ FW)*

Lipid peroxidation was measured following Heath and Packer[7]. Fresh leaf tissue was homogenized in 0.1% TCA, centrifuged, and combined with TBA reagent. The reaction mixture was incubated at 100 °C for 30 minutes and then rapidly cooled. Absorbance was read at 532 nm and corrected for nonspecific turbidity at 600 nm. MDA concentration was calculated using an extinction coefficient of 155 mM^{-1} cm^{-1}.

2.12 *Superoxide Dismutase (SOD, Units g⁻¹ FW)*

Assayed using Beauchamp and Fridovich[8] (1971), based on inhibition of nitro blue tetrazolium (NBT) photoreduction at 560 nm. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of NBT reduction.

2.13 *Peroxidase (POD, Units g⁻¹ FW)*

Peroxidase activity was measured following Rao et al.[9] by monitoring the oxidation of guaiacol in the presence of H_2O_2 at 470 nm. Enzyme activity was expressed as µmol guaiacol oxidised min^{-1} g^{-1} FW.

2.14 *Total Chlorophyll (mg g⁻¹ FW)*

Total chlorophyll content was determined according to Arnon [10] using an acetone extraction method. Absorbance values at 645 and 665 nm were recorded and used for chlorophyll calculations.

$$\text{Chl a}=13.19A_{665}-2.57A_{645}$$

$$\text{Chl b}=22.10A_{645}-5.26A_{665}$$

$$\text{Total Chl}=\text{Chl a}+\text{Chl b}$$

3. Results

3.1 *Growth Parameters*

Plant height and collar diameter decreased significantly with increasing salinity stress, with the greatest reduction observed at 105 days (Figures 1 and 2).

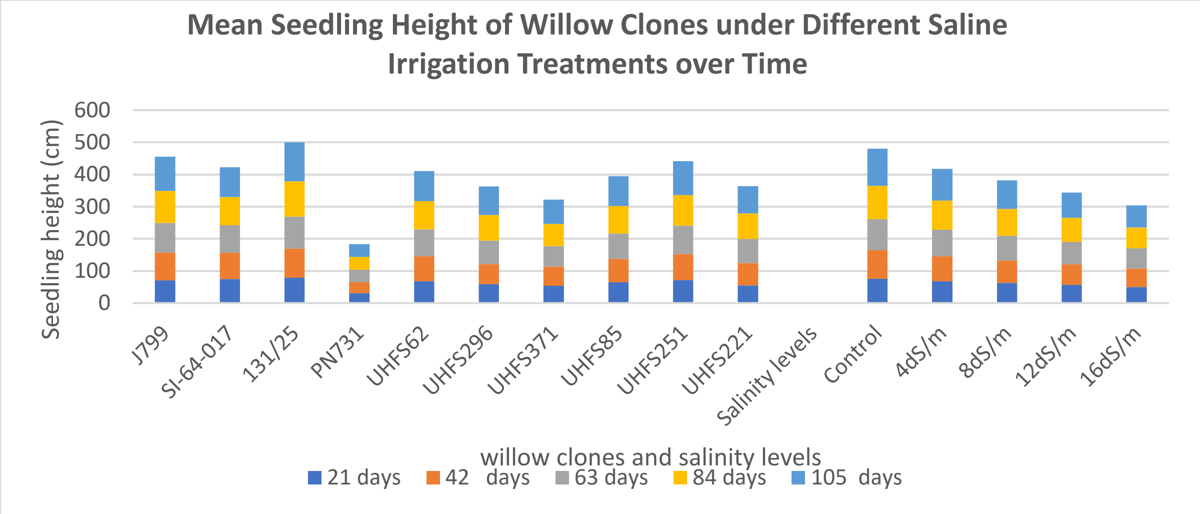

Figure 1: Effect of salinity stress duration (21, 42, 63, 84, and 105 days) on the seedling height of ten willow (*Salix* spp.) clones (J799, SI-64-017, 131/25, PN731, UHFS62, UHFS296, UHFS371, UHFS85, UHFS251, and UHFS221).

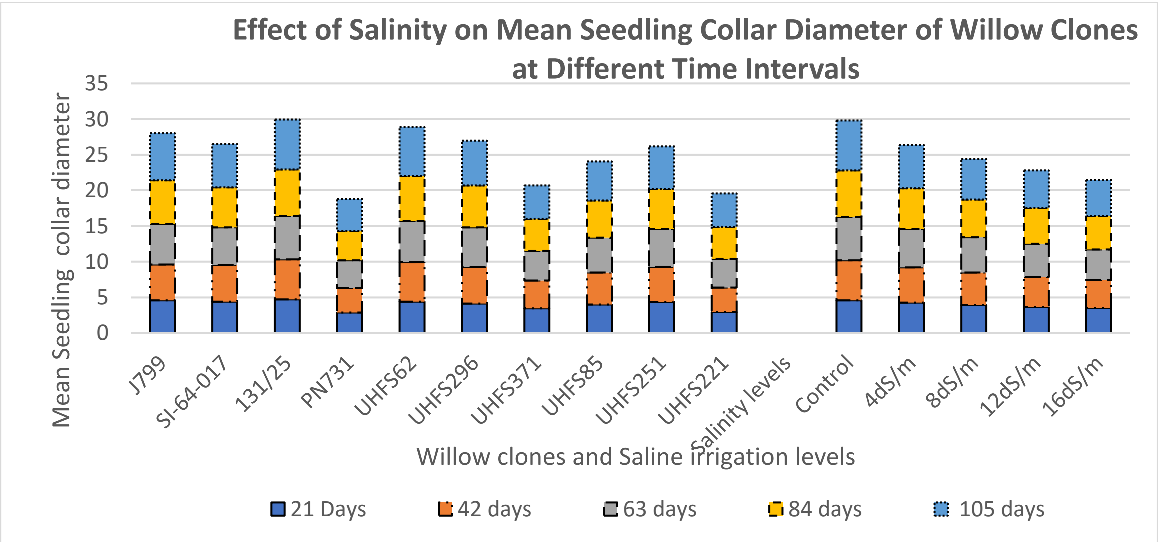

Figure 2: Effect of salinity stress duration (21, 42, 63, 84, and 105 days) on the seedling collar diameter of ten willow (*Salix* spp.) clones (J799, SI-64-017, 131/25, PN731, UHFS62, UHFS296, UHFS371, UHFS85, UHFS251, and UHFS221)

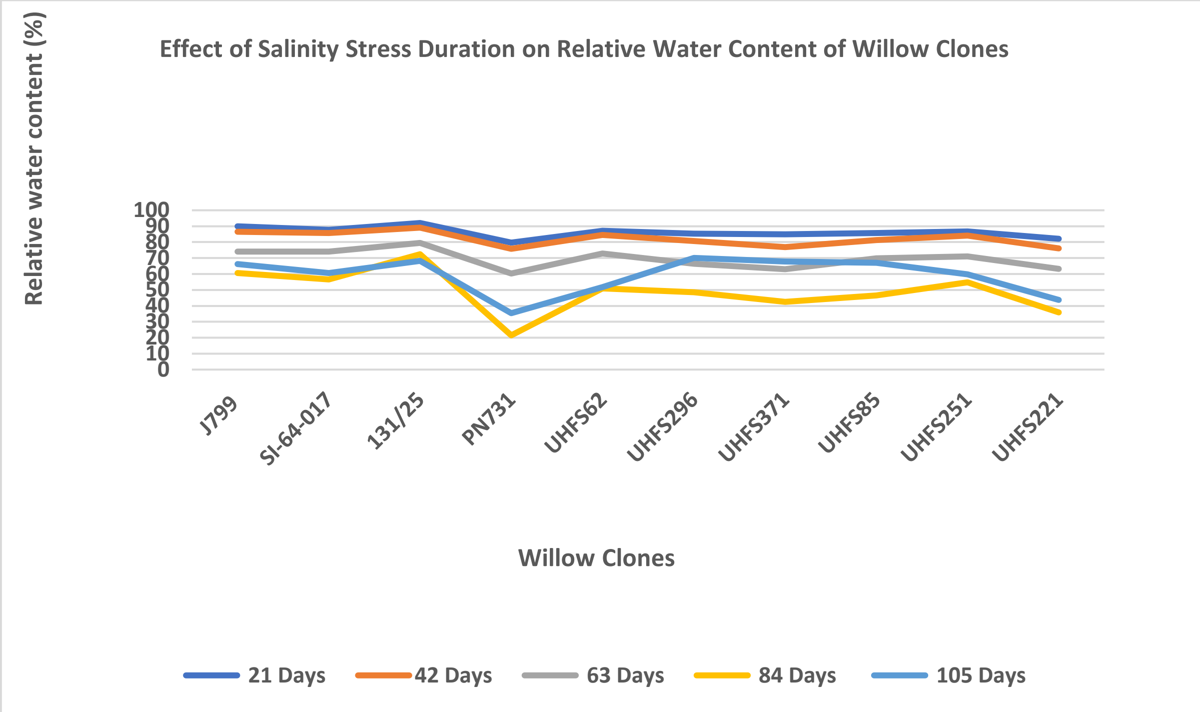

Figure 3: Effect of salinity stress duration (21, 42, 63, 84, and 105 days) on the relative water content (RWC, %) of ten willow (*Salix* spp.) clones (J799, SI-64-017, 131/25, PN731, UHFS62, UHFS296, UHFS371, UHFS85, UHFS251, and UHFS221).

3.2 *Physiological Traits*

3.2.1 Relative Water Content (RWC)

RWC gradually decreased with prolonged salinity exposure (Figure 3) and consistently dropped across different salinity levels and exposure durations (Figure 4). Among the clones, 131/25 retained the highest RWC under stress, while PN731 showed the most substantial decline. For instance, after 21 days at 16 dS/m, RWC decreased by 27.8% in 131/25 but by 52.4% in PN731.

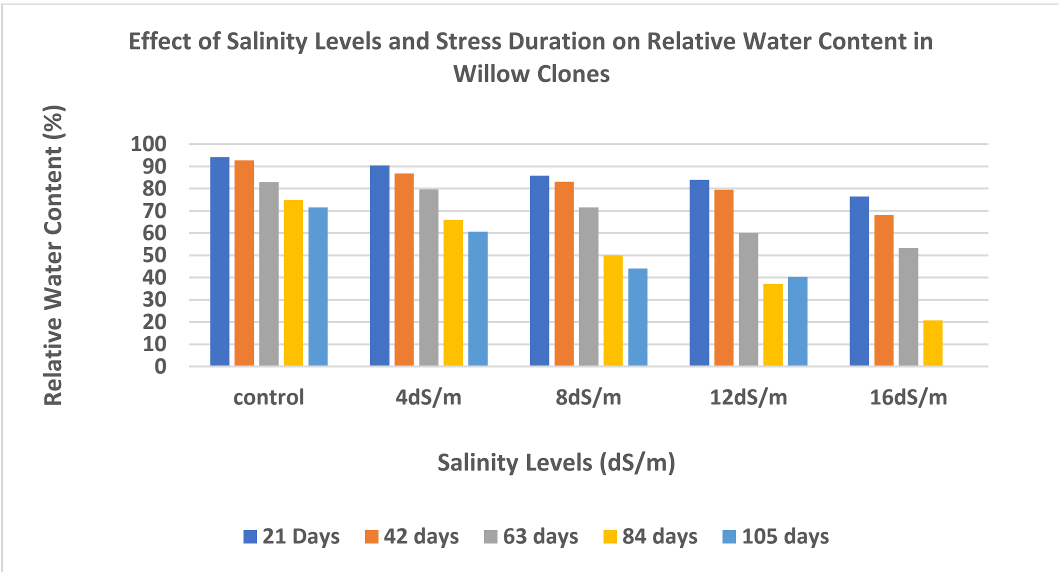

Figure 4: Effect of salinity levels (0, 4, 8, 12, and 16 dS m⁻¹) and stress durations (21, 42, 63, 84, and 105 days) on the relative water content (RWC, %) of willow (*Salix* spp.) clones.

3.2.2 Relative Stress Injury (RSI)

RSI increased significantly with salinity and exposure time (Figure 5), showing similar trends across different salinity levels and durations (Figure 6). PN731 displayed the most membrane damage, while 131/25 had the lowest injury scores, indicating better membrane stability.

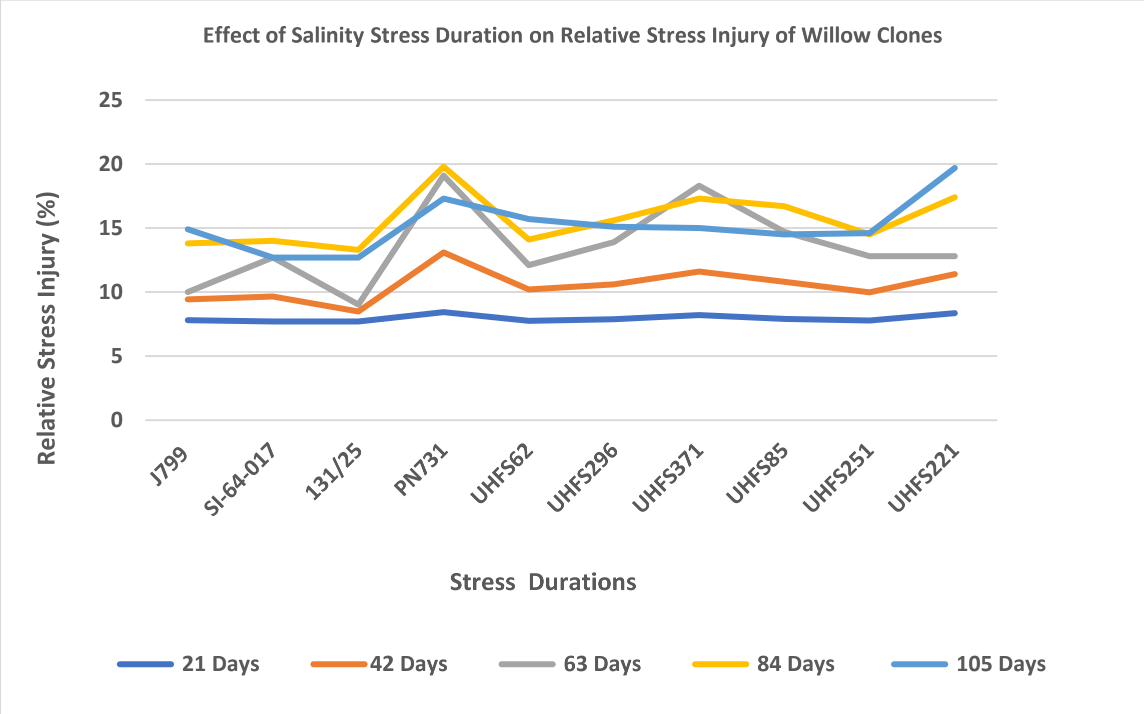

Figure 5: Relative stress injury (RSI, %) of willow (*Salix* spp.) clones under salinity stress at five durations (21, 42, 63, 84, and 105 days).

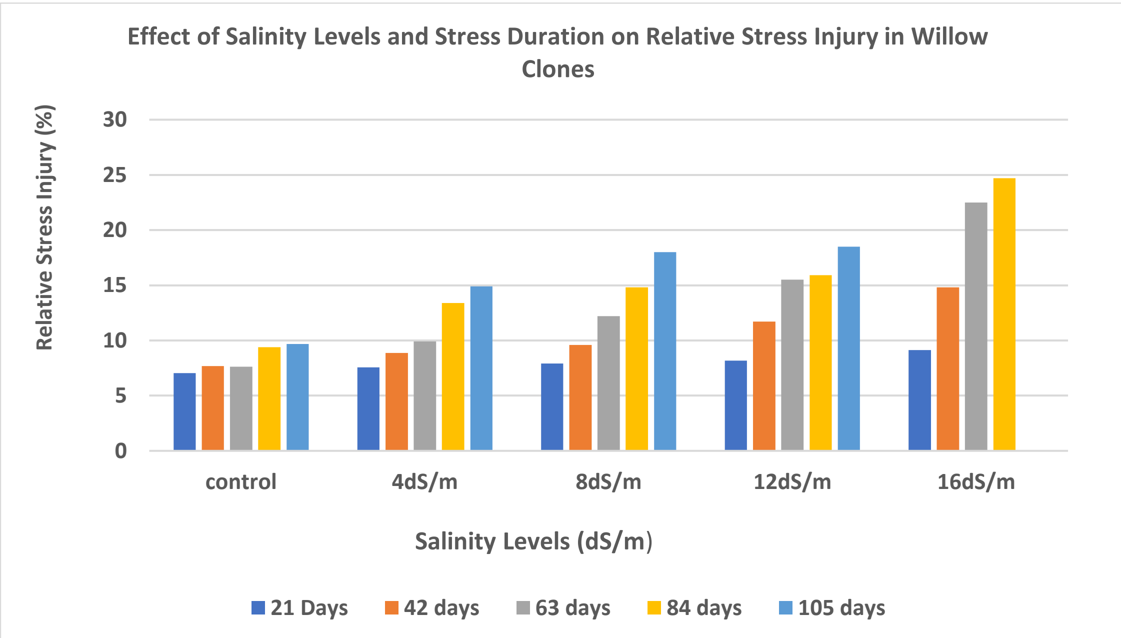

Figure 6: Effect of salinity levels (0, 4, 8, 12, and 16 dS m^{-1}) and stress durations (21, 42, 63, 84, and 105 days) on relative stress injury (RSI, %) in willow (*Salix* spp.) clones.

3.2.3 Water Potential (Ψ_w)

Leaf water potential became more negative with increasing salinity and longer exposure (Figure 7), confirming osmotic stress. Variation among clones was evident across salinity treatments (Figure 8). Clone 131/25 consistently showed fewer negative values, indicating better maintenance of water status than PN731.

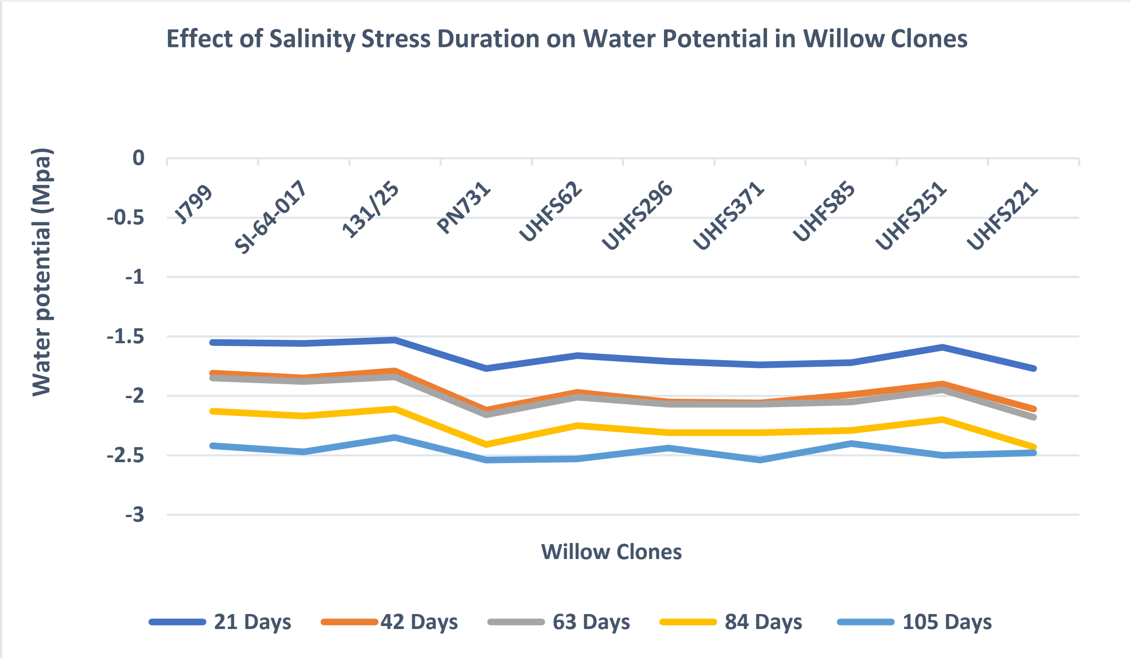

Figure 7: Effect of salinity stress duration (21, 42, 63, 84, and 105 days) on water potential (MPa) in ten willow (*Salix* spp.) clones

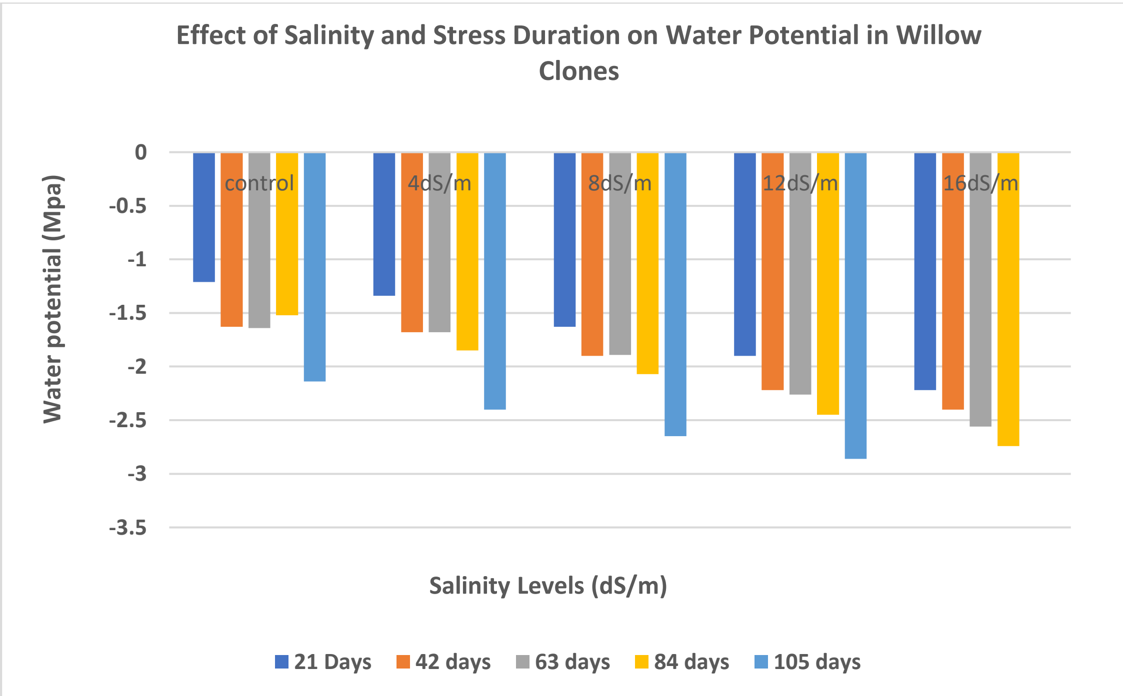

Figure 8: Water potential (MPa) of willow (*Salix* spp.) clones under increasing salinity levels (0, 4, 8, 12, and 16 dS m⁻¹) measured across five stress durations (21, 42, 63, 84, and 105 days)

3.2.4 Osmotic Potential (Ψ_s)

Osmotic potential also decreased over time and with increasing salinity (Figures 9 and 10), indicating osmotic adjustment. Clone 131/25 demonstrated the most significant adjustment capacity compared to PN731 and UHFS221.

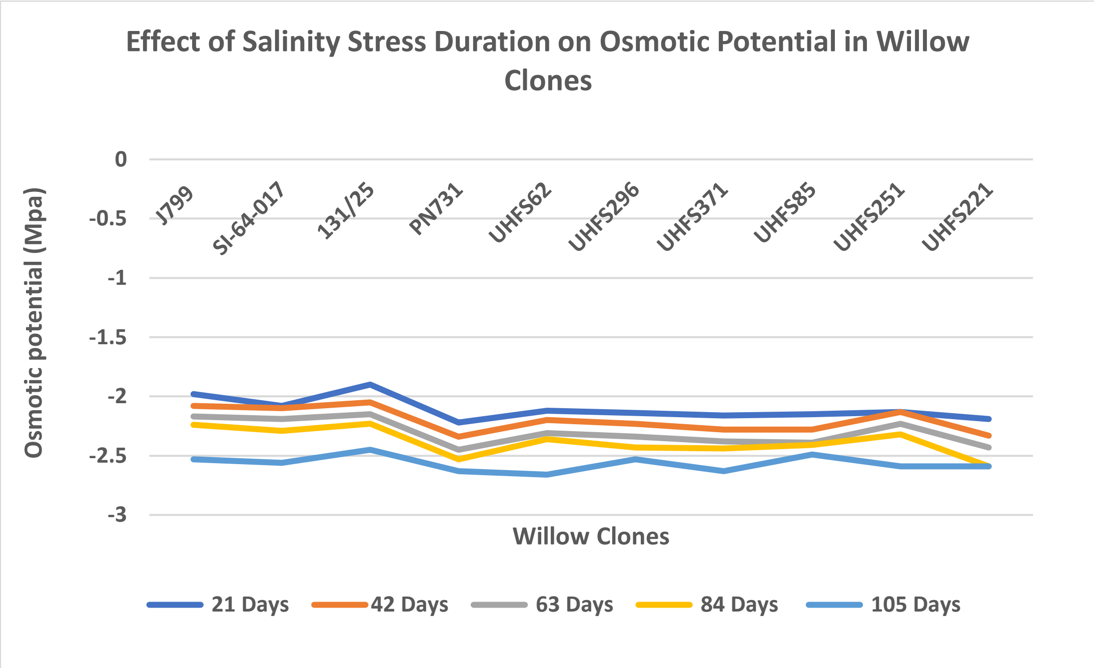

Figure 9: Osmotic potential (MPa) of willow clones under salinity stress at five durations (21, 42, 63, 84, and 105 days)

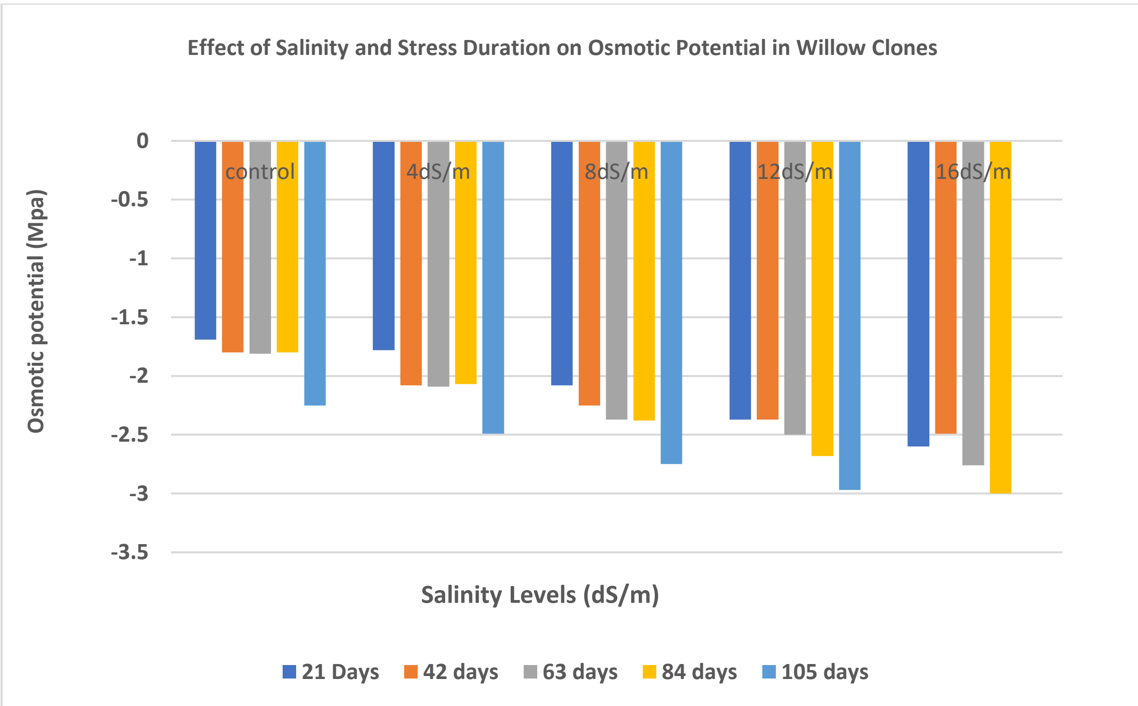

Figure 10: Osmotic potential (MPa) of willow clones at increasing salinity levels (0, 4, 8, 12, and 16 dS/m) over five stress durations (21, 42, 63, 84, and 105 days)

3.3 *Biochemical Responses*

Proline accumulation increased significantly under salinity stress over time (Figure 11) and at higher salinity levels (Figure 12). Tolerant clones such as 131/25 showed the highest proline levels, indicating enhanced Osmo protection

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3.3.1 Proline Content

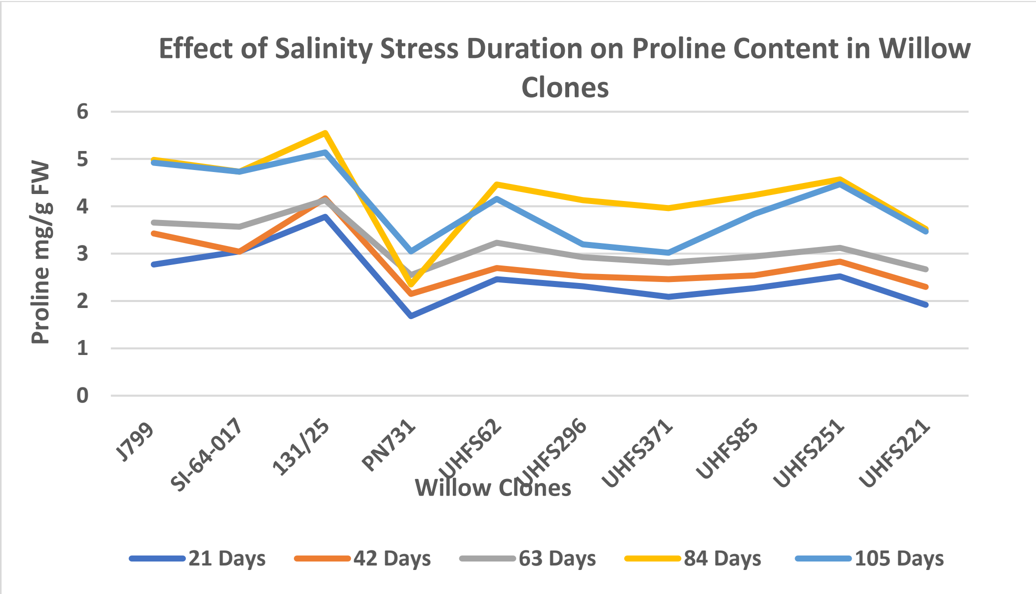

Figure 11: Proline content (mg g⁻¹ fresh weight) in willow clones subjected to salinity stress for five durations (21, 42, 63, 84, and 105 days).

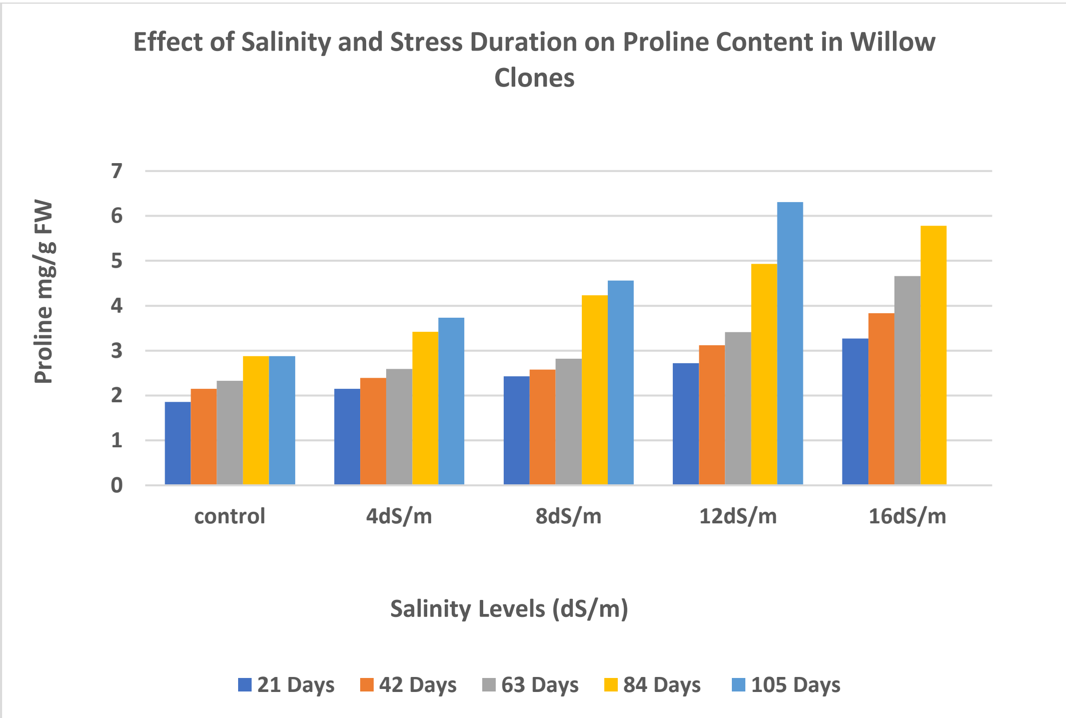

Figure 12: Proline content (mg g⁻¹ fresh weight) in willow clones under increasing salinity levels (0, 4, 8, 12, and 16 dS m⁻¹) across five stress durations (21, 42, 63, 84, and 105 days).

3.3.2 Lipid Peroxidation (MDA Content)

MDA content, a marker of oxidative damage, increased with stress duration (Figure 13) and salinity level (Figure 14). Sensitive clones such as PN731 exhibited the highest MDA concentrations, while 131/25 maintained the lowest levels.

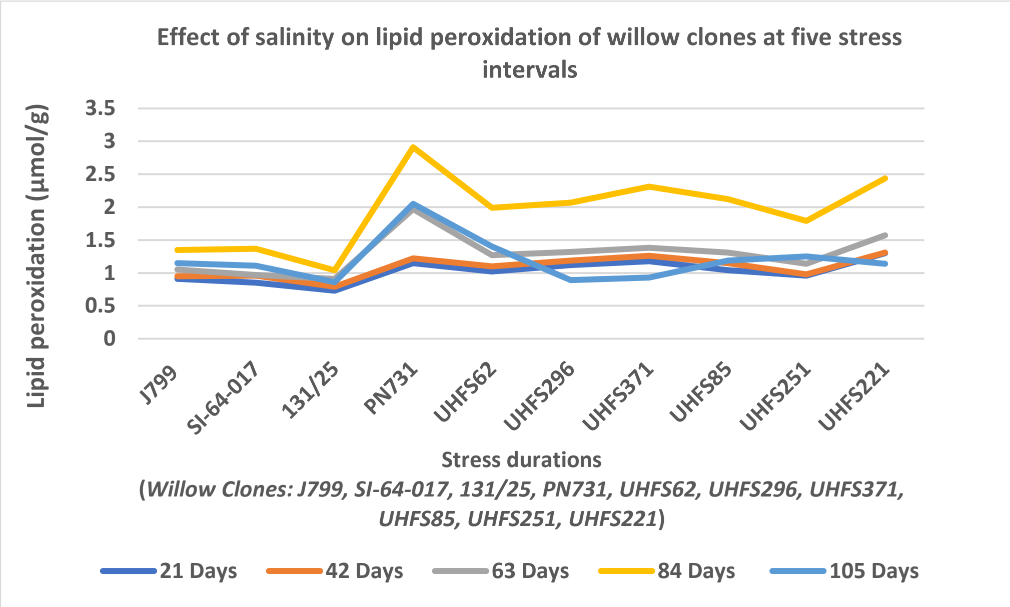

Figure 13: Lipid peroxidation (MDA content) in willow clones subjected to salinity stress at five-time intervals (21, 42, 63, 84, and 105 days)

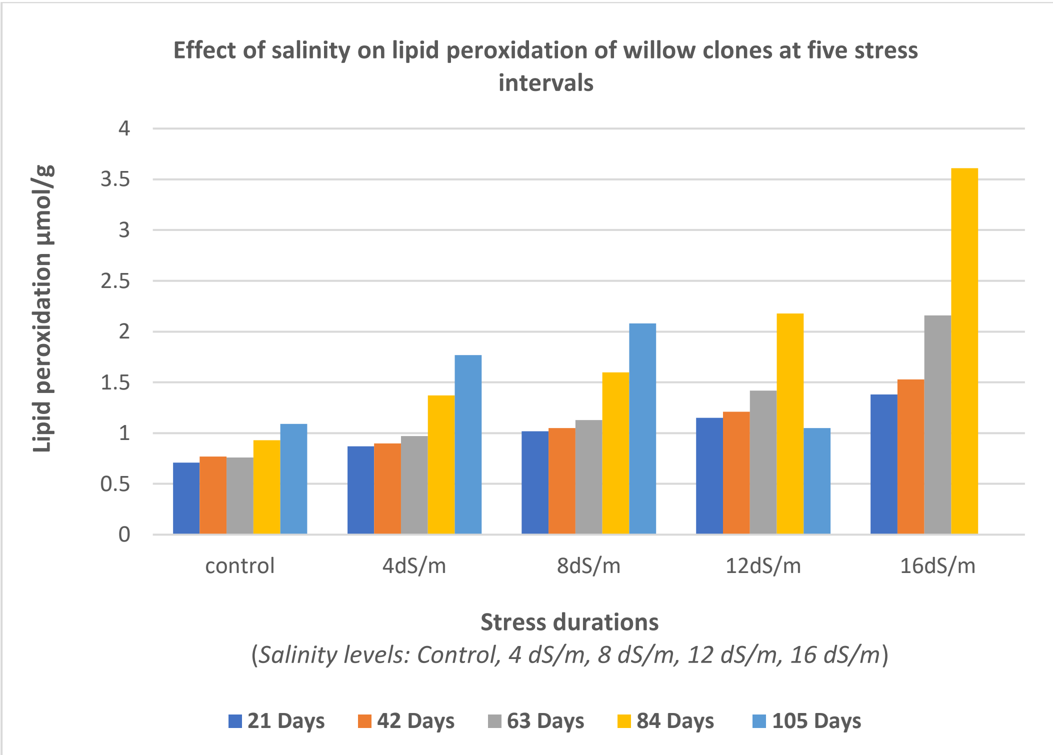

Figure 14: Lipid peroxidation responses of willow clones under different salinity levels, assessed at five consecutive time intervals

3.4 *Antioxidant Enzyme Activity*

3.4.1 Superoxide Dismutase (SOD)

SOD activity increased with salinity duration (Figure 15) and salinity levels (Figure 16), with tolerant clones exhibiting higher enzymatic activity, indicating improved oxidative stress mitigation.

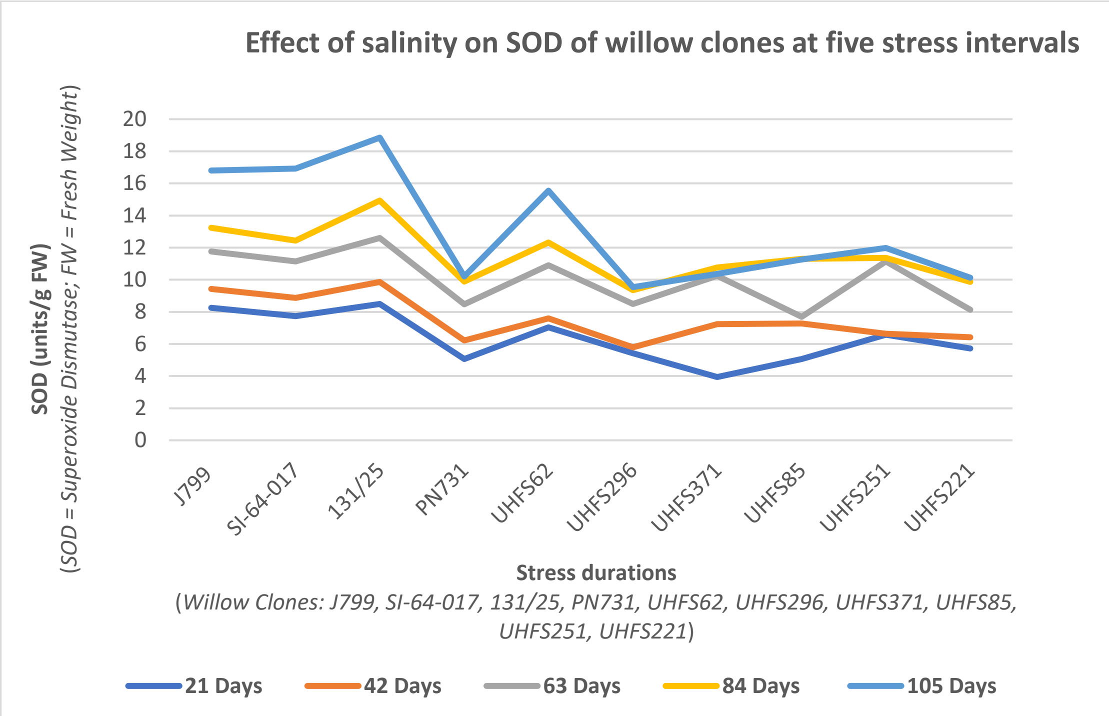

Figure 15: Superoxide dismutase (SOD) activity in willow clones subjected to salinity stress measured at five time points

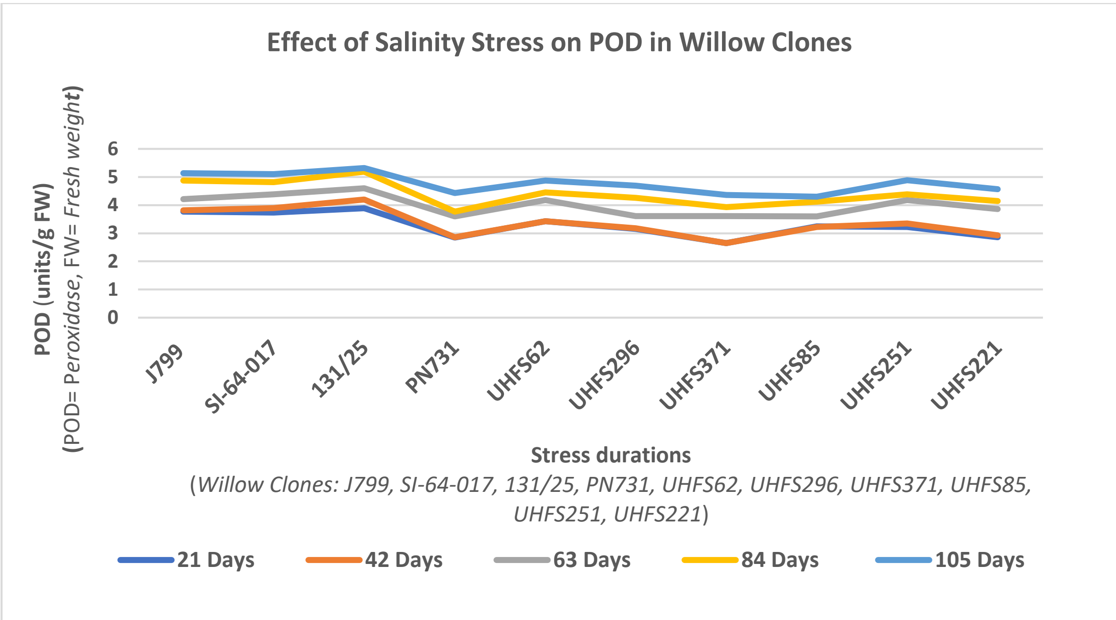

Figure 16: Superoxide dismutase (SOD) activity in willow clones under varying salinity levels measured across five time points

3.4.2 Peroxidase (POD)

POD activity also increased under salinity stress (Figure 17), with variations among clones (Figure 18) following a pattern similar to SOD activity.

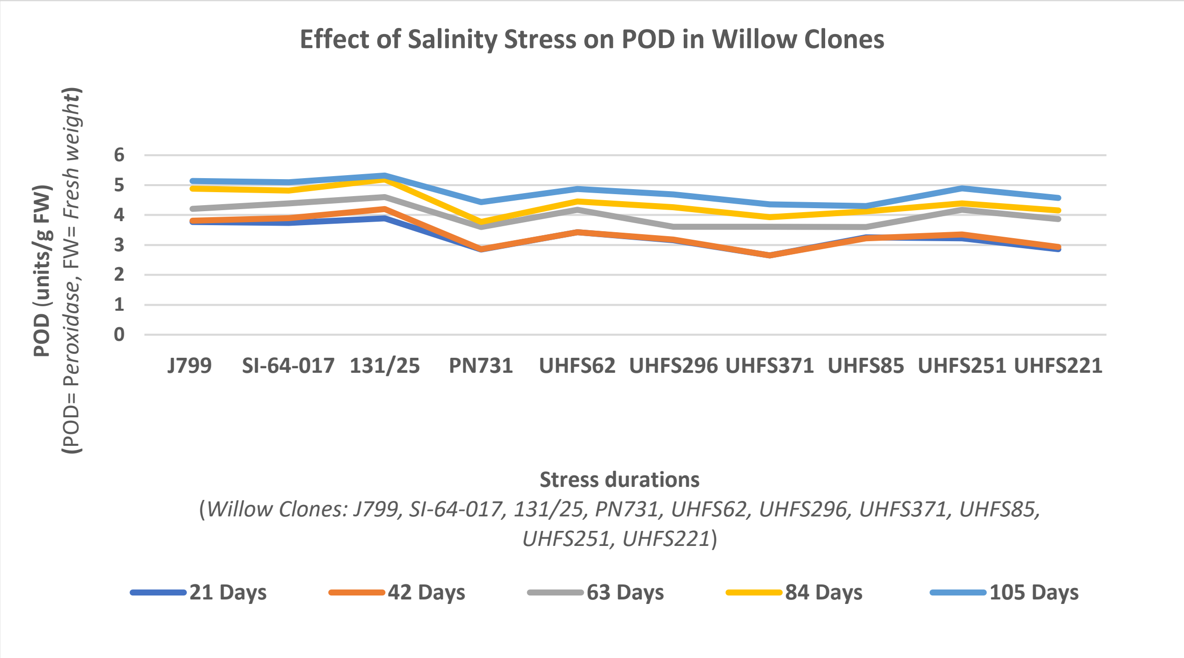

Figure 17: Peroxidase activity in ten willow clones under different salinity stress conditions

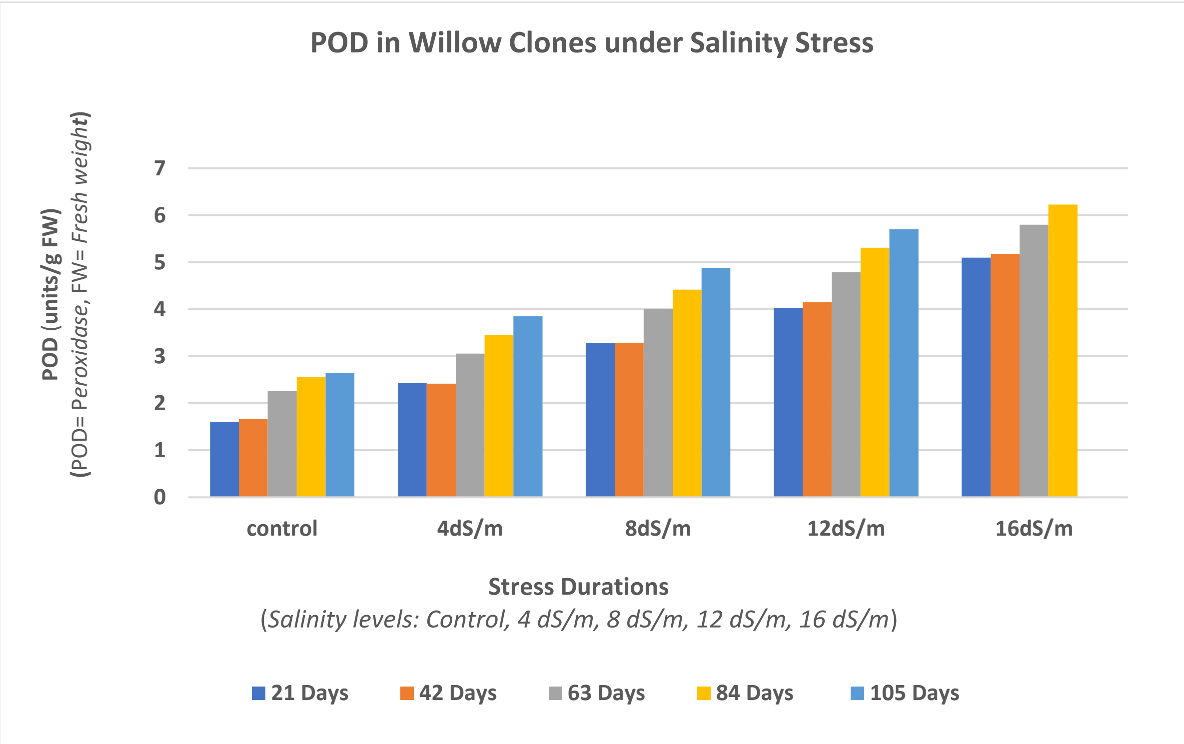

Figure 18: Variation in peroxidase activity among willow clones under salinity stress

3.5 *Chlorophyll Content*

Chlorophyll a, chlorophyll b, and total chlorophyll levels declined significantly under salinity stress (Figure 19), with greater pigment loss at higher salinity levels and longer durations of exposure (Figure 20). Tolerant clones, such as 131/25, retained higher chlorophyll levels compared to sensitive clones, like PN731.

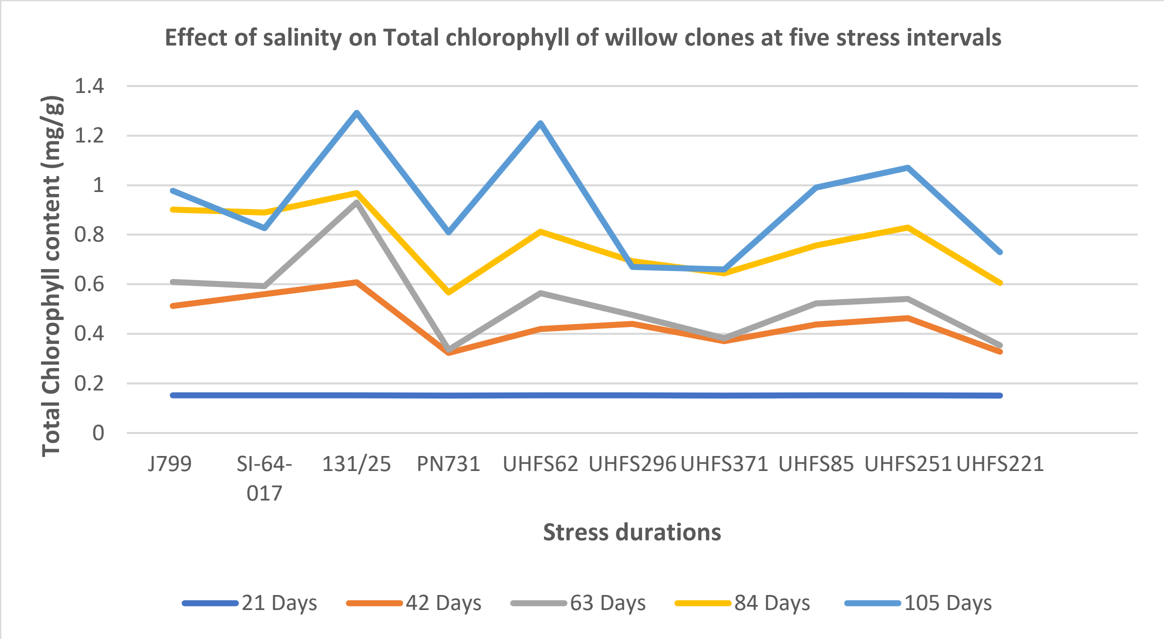

Figure 19: Total chlorophyll content in ten willow clones under salinity stress

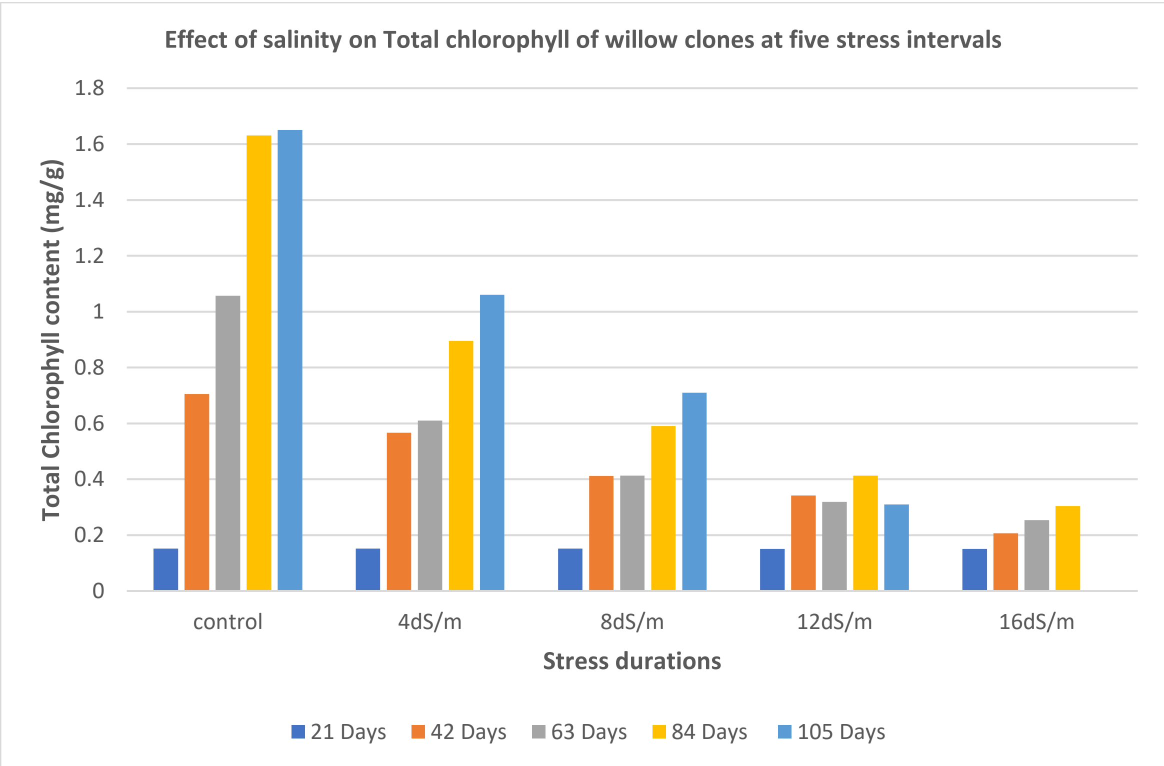

Figure 20: Variation in total chlorophyll content among willow clones under salinity stress

3.6 Correlation Analysis

Pearson's correlation coefficients among physiological, biochemical, and antioxidant traits were calculated and presented as a heat map (Figure 21). Positive correlations were observed among RWC, water potential, osmotic potential, proline content, SOD, POD, and growth parameters, while RSI, MDA content, and Na+/K+ ratio were negatively associated with growth and survival.

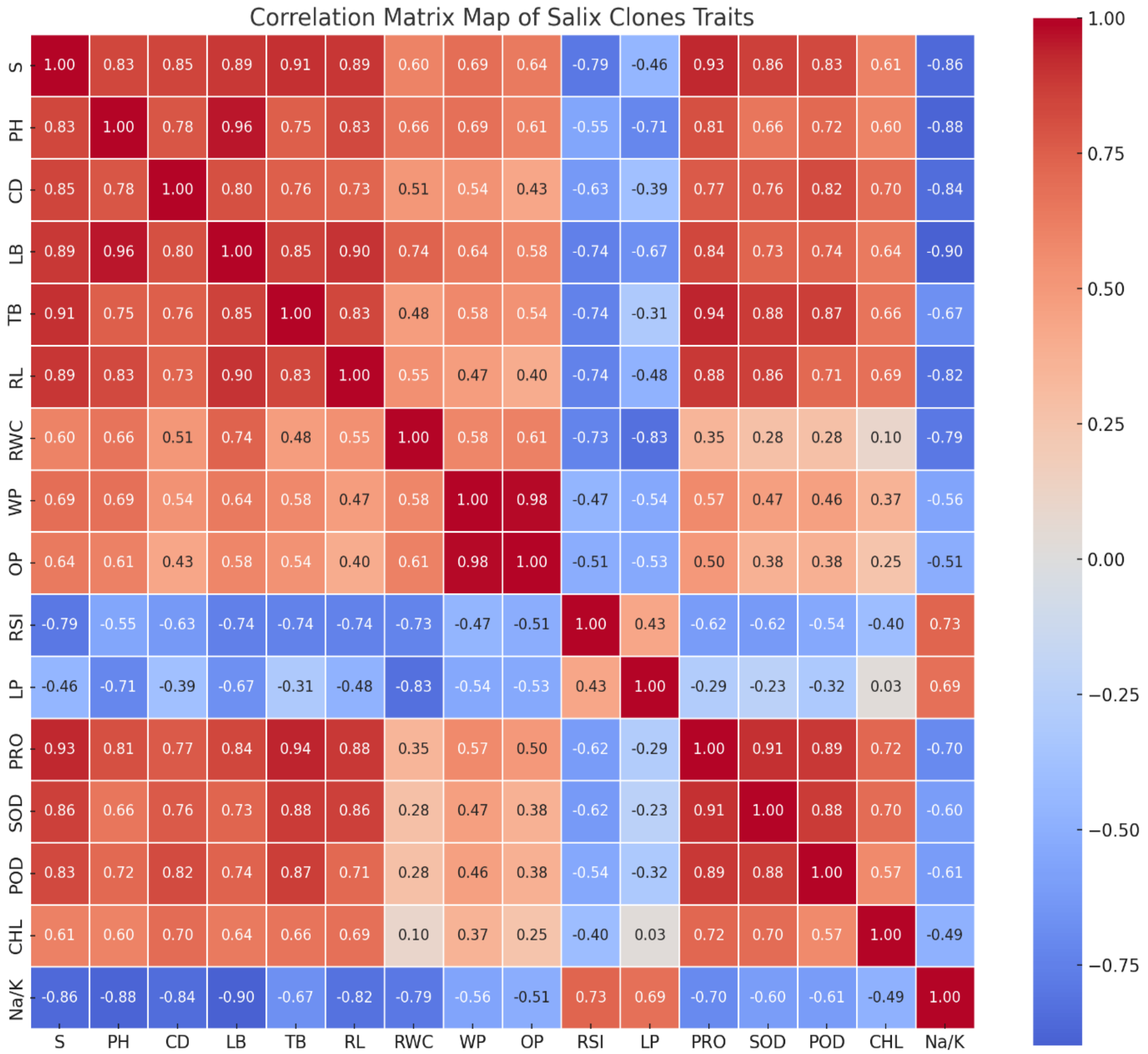

Figure 21: Heat map of Pearson's correlation coefficients among physiological, biochemical, and antioxidant traits in ten willow (*Salix* spp.) clones under salinity stress.

Positive correlations are shown in blue and negative correlations in red, with color intensity proportional to the magnitude of r. Data are pooled across all salinity stress intervals (0–16 dS m^{-1} NaCl) and clones. Significant correlations are indicated by $p < 0.05$ (*), $p < 0.01$ (**). Trait abbreviations: RWC, relative water content; RSI, relative stress injury; WP, water potential; OP, osmotic potential; PRO, proline; MDA, lipid peroxidation; SOD, superoxide dismutase; POD, peroxidase; CHL, total chlorophyll.

4.1 *Plant Growth and Water Relations under Salinity Stress*

Salinity stress significantly reduced growth parameters, including plant height and collar diameter, with the most notable decrease observed after prolonged exposure (105 days) at the highest salinity level (16 dS/m). These results align with earlier studies on *Salix* and related woody species, which report cumulative growth inhibition under salt stress[11,12]. Physiological measurements revealed consistent reductions in relative water content (RWC), water potential (Ψ_w), and osmotic potential (Ψ_s), indicating increased osmotic stress and impaired water uptake. Clone 131/25 maintained superior water status across all stress durations and salinity levels, with higher RWC and less negative water potentials compared to sensitive clones such as PN731. This suggests effective osmotic adjustment mechanisms, likely involving solute accumulation and ion compartmentalisation, in line with previous findings in *Populus* and *Salix* species[13]. Conversely, clones PN731 and UHFS221 demonstrated weaker osmotic regulation and experienced greater water deficit under salinity stress.

4.2 *Membrane Stability and Stress Injury*

Relative stress injury (RSI), a marker of membrane damage caused by ionic toxicity, increased proportionally with salinity intensity and exposure duration. The highest RSI was observed in PN731, indicating significant membrane disruption, while clone 131/25 maintained considerably lower RSI values, reflecting enhanced membrane stability under salt stress. The sharp rise in RSI beyond 8 dS/m suggests a threshold for membrane injury, consistent with previous observations[14,15]. These findings confirm RSI as a reliable physiological indicator for screening salinity tolerance in willow clones.

4.3 *Osmo protective Role of Proline*

Proline, a vital Osmo protectant and reactive oxygen species scavenger, accumulated gradually in all clones as salinity and exposure time increased. The tolerant clone 131/25 exhibited the highest proline levels, indicating effective osmotic adjustment and cellular protection, whereas sensitive clones, such as PN731 and UHFS85, showed comparatively lower accumulation. This dose-dependent proline response aligns with studies in *Salix viminalis* and other woody species[16,17], emphasising its key role in stress tolerance through osmotic regulation and protein stabilization.

4.4 *Oxidative Stress and Lipid Peroxidation*

Lipid peroxidation, assessed through malondialdehyde (MDA) content, increased significantly under saline conditions, indicating heightened oxidative damage to membranes. Sensitive clones, such as PN731 and UHFS221, displayed higher MDA levels, while 131/25 had the lowest, suggesting it possesses superior antioxidant defences. MDA levels escalated sharply beyond 12 dS/m, implying a threshold where oxidative injury worsens. These findings align with previous studies that associate salinity-induced ROS production with lipid peroxidation as a marker of oxidative stress[18,19].

4.5 *Antioxidant Enzyme Activities*

Activities of superoxide dismutase (SOD) and peroxidase (POD) increased with salinity intensity and duration across all clones, emphasising their role in ROS detoxification and cellular protection. Clone 131/25 consistently exhibited the highest enzyme activities, followed by J799 and SI-64-017, indicating a strong enzymatic defence system. Clones with lower activities, including UHFS296, UHFS371, and UHFS85, showed increased oxidative damage, reinforcing the link between antioxidant capacity and salinity tolerance[20,21].

4.6 *Photosynthetic Pigment Stability*

Salinity stress caused significant reductions in chlorophyll a, chlorophyll b, and total chlorophyll, especially in sensitive clones. Clone 131/25 maintained the highest chlorophyll levels, indicating better photosynthetic performance under stress. The loss of chlorophyll was associated with pigment degradation through chlorophyllase activation and damage to the photosystem, consistent with previous studies[22,11]. Differences became statistically significant after 42 days of stress, with moderate salinity (4 dS/m) showing minimal pigment loss and severe salinity (16 dS/m) resulting in the greatest reductions.

4.7 *Trait Correlations and Implications for Breeding*

Pearson's correlation analysis showed positive relationships among growth and physiological traits, including RWC, water potential, proline content, and antioxidant enzyme activities, while stress injury markers (RSI, MDA) were negatively correlated with growth. These patterns confirm that using combined physiological and biochemical parameters is a reliable way to assess salinity tolerance. The consistent superior performance of clone 131/25 across these traits highlights its potential for cultivation in salt-affected areas and its value as a donor genotype in breeding programmes aimed at improving salt tolerance in willow.

5. Conclusion

This study thoroughly evaluated the physiological and biochemical responses of ten willow (*Salix* spp.) clones under increasing salinity in semi-arid conditions. Significant genotypic variation in salt tolerance was observed across key parameters, including relative water content (RWC), water potential (Ψw), osmotic potential (Ψs), proline accumulation, lipid peroxidation (MDA), and antioxidant enzyme activities (SOD, POD). Clones 131/25, J799, and SI-64-017 consistently demonstrated superior performance across all salinity levels and exposure durations. These genotypes maintained better cellular hydration and water status, showed efficient osmotic adjustment, accumulated higher proline levels, experienced less oxidative membrane damage, and exhibited increased antioxidant enzyme activity—collectively supporting their strong tolerance to salinity stress. The identified clones have significant potential for practical applications, such as agroforestry in salt-affected and water-scarce regions, biomass and renewable energy production on marginal lands, and ecological restoration of degraded, saline landscapes. Their adoption can improve ecosystem resilience, promote sustainable land management, and contribute to climate-smart agriculture in semi-arid, salt-affected areas.

Acknowledgments

The authors sincerely thank the Director of the ICAR–Central Soil Salinity Research Institute (CSSRI), Karnal, Haryana, for providing the necessary facilities and institutional support for this research. Neha and T.N. Manohara are especially grateful to the Group Coordinator (Research) and the Director of the Institute of Wood Science and Technology (IWST), Bengaluru, for their ongoing support and encouragement.

Author Contributions

NS: Conceptualization and research design; data acquisition, analysis, and interpretation; statistical analysis; manuscript preparation; and manuscript review and editing. **TNM**: Manuscript review, editing, and final corrections. All authors have read and approved the final version of the manuscript.

Funding

This research was supported by Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana, through a merit stipend awarded to Neha Saini as part of her Ph.D. programme.

Data Availability Statement

The raw data underpinning this article's conclusions can be obtained from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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