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Physiological insights into salinity-induced oxidative stress and its impact on photosynthetic efficiency and chlorophyll content in *Taxus wallichiana* Zucc. (Himalayan Yew)

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Abstract
Soil salinization is a major abiotic stress that limits plant growth, photosynthetic performance, and survival, particularly in ecologically and pharmacologically important species such as *Taxus wallichiana* Zucc. (Himalayan yew). This study evaluated the physiological and biochemical responses of T. wallichiana seedlings exposed to 150 mM NaCl under controlled conditions. Salinity stress induced pronounced morphological symptoms, including leaf chlorosis, wilting, and stunted growth. Chlorophyll fluorescence measurements revealed significant reductions in maximum photochemical efficiency (Fv/Fm), effective quantum yield (ΦPSII), and electron transport rate (ETR), indicating impaired photosystem II activity. Concurrently, chlorophyll a, chlorophyll b, and total chlorophyll contents declined, reflecting accelerated pigment degradation and constrained photosynthetic capacity. Biochemical assays demonstrated elevated hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) levels, indicating enhanced reactive oxygen species (ROS) accumulation and lipid peroxidation. Histochemical analyses using NBT and DAB staining corroborated these findings, showing intensified superoxide and H_2O_2 accumulation in salt-stressed tissues. Collectively, these results indicate that 150 mM NaCl disrupts photosynthetic efficiency and redox homeostasis in *T. wallichiana*, leading to oxidative damage and growth inhibition. This study provides a baseline for further investigation into antioxidant defense mechanisms, osmoprotective strategies, and ion homeostasis in this valuable gymnosperm species.

Keywords: Salinity stress, *Taxus wallichiana*, photosystem II, chlorophyll degradation, oxidative stress, reactive oxygen species

Introduction

Salinity stress, primarily resulting from elevated NaCl concentrations in soils, is a pervasive abiotic factor that adversely affects plant growth and productivity worldwide. This stress manifests through osmotic imbalance, ionic toxicity, and oxidative damage, collectively impairing physiological functions and leading to reduced crop yields and ecosystem stability (Munns and Tester, 2008; Acosta-Motos *et al*., 2017) [1, 17]. In plants, salinity-induced osmotic stress reduces water uptake, leading to turgor loss and stomatal closure, which in turn limits CO_2 assimilation and photosynthetic efficiency (Munns and Tester, 2008) [17]. Simultaneously, the accumulation of toxic ions such as Na^+ and Cl^- disrupts cellular ion homeostasis, affecting enzyme activities and membrane integrity (Zhou *et al*., 2024). Furthermore, salinity stress induces the overproduction of reactive oxygen species (ROS), including superoxide radicals ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2), leading to oxidative stress that damages cellular components such as lipids, proteins, and nucleic acids (Wang *et al*., 2024) [25]. Photosynthesis is particularly sensitive to salinity stress. Elevated Na^+ concentrations can interfere with the photosynthetic electron transport chain, reducing the efficiency of photosystem II (PSII) and impairing the electron transport rate (ETR) (Lu *et al*., 2023). This disruption leads to a decrease in the maximum photochemical efficiency (Fv/Fm) and effective quantum yield (ΦPSII), indicating compromised photosynthetic performance (Wang *et al*., 2024) [25]. Additionally, salinity stress accelerates the degradation of chlorophyll pigments, further diminishing the plant's photosynthetic capacity (Wang *et al*., 2024) [25]. *Taxus wallichiana* Zucc., commonly known as Himalayan yew, is a conifer species native to the eastern Himalayas. Due to its ecological significance and medicinal properties, understanding its response to salinity stress is crucial. While research on salinity stress in gymnosperms is limited, studies on other plant species have demonstrated that salinity-induced oxidative stress leads to increased levels of MDA and H_2O_2, indicating enhanced lipid peroxidation and cellular damage (Lu *et al*., 2023; Wang *et al*., 2024) [25]. This study aims to investigate the physiological responses of *T. wallichiana* to salinity stress by assessing changes in photosynthetic efficiency, chlorophyll content, and oxidative stress markers under controlled conditions. The findings will provide valuable insights into the mechanisms underlying salinity tolerance in this species and contribute to the broader understanding of stress physiology in gymnosperms.

Materials and Methods

1. Plant materials collection and stress treatments

Uniformly aged, approximately 1.5-year-old *T. wallichiana* saplings were collected from Rupa village (27.4445° N, 92.48066° E) in the West Kameng district of Arunachal Pradesh, India. The saplings were carefully transplanted into 15 cm pots containing garden soil and acclimated under controlled growth chamber conditions (16 h light/8 h dark photoperiod, 200 µmol m^{-2} s^{-1} photosynthetic photon flux density, day/night temperatures of 25 °C/20 °C, and 65% relative humidity). The experimental setup comprised two randomized treatment groups, each with three biological replicates. After acclimatization, plants were placed in pots filled with clean soil that was washed with distilled water and brought to a moist, even state; the drainage water was

checked until low salt levels were seen, and a simple paste extract was used to confirm low starting conductivity. Two groups were set: control and saline treatment. For the treatment, a 150 mM NaCl solution was made with distilled water and added evenly to each pot of the replicates to reach and keep the planned salt level, while controls received the same amount of distilled water only. Pots were kept in a random layout under the same light and temperature. Soil moisture was kept near the usual level to avoid sudden shocks, and conductivity was checked from time to time to make sure salt levels stayed steady. Sampling was done when morphological symptoms were appeard.

2. Determination of chlorophyll content and chlorophyll fluorescence

For total chlorophyll, 100 mg of leaves were incubated in 3 mL of 80% acetone in the dark for 48 hours, using 80% acetone as the blank for readings at 663 nm and 645 nm; concentrations were then calculated with the standard equation and reported as µg mL^{-1} fresh weight, following Kapoor and Pande (2015) [12].

$$\text{Chlorophyll a} = [(12.7 \times A663) - (2.63 \times A645)]\ V/\ (W \times 1000)$$

$$\text{Chlorophyll b} = [(22.9 \times A645) - (4.48 \times A663)]\ V/\ (W \times 1000)$$

$$\text{Total Chlorophyll} = [(20.2 \times A645) + (8.02 \times A663)]\ V/\ (W \times 1000)$$

Chlorophyll fluorescence was measured using a PAM fluorometer (OPTI-SCIENCE OS1p) following the instrument manual. Before readings, plants were dark-adapted for 30 minutes to reset photosystems. The recorded parameters included maximum PSII efficiency (Fv/Fm), electron transport rate (ETR), and effective quantum yield (YII); here, Fv is variable fluorescence and Fm is maximum fluorescence in the dark-adapted state (Devi *et al*., 2020) [5].

3. Assessment of oxidative stress markers

Hydrogen peroxide (H_2O_2) content was quantified by measuring absorbance at 480 nm following the approach described by Sagisaka (1976). Lipid peroxidation was assessed by determining malondialdehyde (MDA) content, recording absorbance at 532 nm and correcting for non-specific turbidity by subtracting readings at 600 nm, as outlined by Heath and Packer (1968).

4. Histochemical detection of oxidative markers

Histochemical detection of oxidative markers was performed according to the protocol of Roy *et al*., 2024 [20]. In situ superoxide was visualized by NBT staining. Stressed and control leaves were vacuum-infiltrated in 50 mM potassium phosphate buffer (pH 7.8) containing 0.1% NBT and 10 mM sodium azide, then cleared in a bleaching solution for 12 hours to remove chlorophyll, and examined microscopically for purple formazan deposits. Hydrogen peroxide (H_2O_2) was detected using DAB staining. Leaves from both groups were vacuum-infiltrated in DAB solution for 1 hour, washed, bleached for 12 hours, and observed under a microscope for the characteristic brown precipitate.

5. Statistical Analysis

Data analyses were conducted using OriginPro 2024 (OriginLab Corporation, Northampton, MA, USA) and R software. Data are presented as the mean ± standard error (SE) of three independent experiments, with each experiment involving multiple plant replicates. Analysis of variance (ANOVA) was employed to determine significant differences between treatments. Significance levels are indicated as follows: ns (not significant), * ($P < 0.05$), ** ($P < 0.01$) and *** ($P< 0.001$).

Results

1. Comparative assesment of morphological stress symptoms

Symptomes were appeared after six days of treatment. Treatment induced pronounced morphological alterations in *T. wallichiana*. seedlings compared to the control plants. As shown in Figure 3.1, control seedlings exhibited vigorous growth with healthy, turgid, and green foliage, indicating normal physiological functioning under non-stress conditions. In contrast, plants exposed to saline conditions displayed evident symptoms of stress, including leaf chlorosis, wilting, and reduced overall vigour. The saline-treated seedlings also showed diminished leaf density and slight stunting in shoot growth. These visible morphological impairments suggest that salinity adversely affected the plant's water status and photosynthetic efficiency, reflecting the onset of osmotic and ionic stress typical under high salt conditions.

Fig 1: Morphological appearance of Taxus spp. seedlings under control and salinity stress conditions. Control plant (left) exhibits normal growth with healthy green foliage, whereas the saline-treated plant (right) shows visible stress symptoms including leaf chlorosis and wilting.

2. Analysis of chlorophyll fluorescence and chlorophyll content

Salinity treatment caused a clear decline in photosynthetic performance relative to the control, as reflected by consistent reductions across all chlorophyll fluorescence parameters and pigment pools (Figure 3.2). Morphologically, saline-treated *T. wallichiana* saplings exhibited visible stress symptoms including leaf wilting and chlorosis, whereas control plants maintained healthy green foliage and normal growth.

Specifically, the maximum photochemical efficiency of PSII (Fv/Fm) was significantly lower under saline conditions, indicating a loss of PSII functionality consistent with stress-induced downregulation of reaction centers. The effective quantum yield of PSII (YII) also decreased markedly in the saline group, demonstrating that a smaller fraction of absorbed light energy was utilized for photochemistry during actinic illumination. In line with these trends, the relative electron transport rate (ETR) was substantially reduced under salinity, reflecting restricted electron flow through the photosynthetic chain.

Pigment analysis further confirmed the decline in photosynthetic capacity. Both chlorophyll a and chlorophyll b contents decreased significantly in saline-treated leaves, and total chlorophyll (TC) exhibited a marked reduction compared with the control.

These reductions suggest that salinity impairs pigment biosynthesis and accelerates chlorophyll degradation, collectively constraining photosynthetic efficiency and overall plant vitality under salt stress.

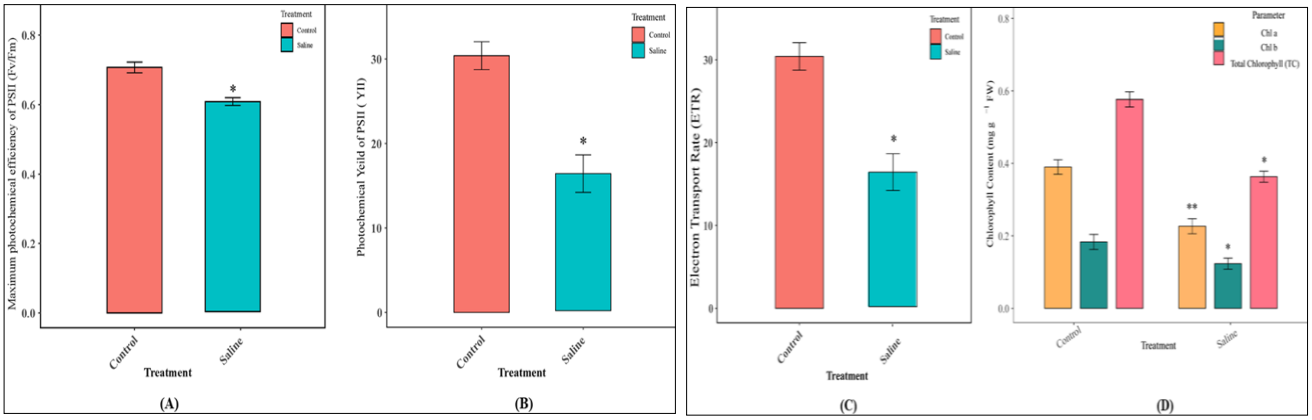

Fig 2: Effects of salinity stress on photosynthetic performance and pigment content in *T. wallichiana* leaves. (A) Maximum photochemical efficiency of PSII (Fv/Fm), (B) Photochemical yield of PSII (Y(II)), (C) Electron transport rate (ETR), and (D) Chlorophyll content (Chl a, Chl b, and total chlorophyll, TC) under control and saline conditions. Data represent mean ± SD (n = 3). Asterisks indicate statistically significant differences compared to the control (* < 0.05; **< 0.01).

3. Assessment of oxidative stress

Salinity triggered pronounced oxidative stress in *T. wallichiana* saplings, as evidenced by substantial increases in ROS accumulation and membrane lipid peroxidation relative to the control (Figure 3.3). Hydrogen peroxide (H_2O_2) content rose sharply in response to saline treatment, with a highly significant elevation indicating excessive ROS buildup within leaf tissues. This surge in H_2O_2 suggests disruption of cellular redox homeostasis and an imbalance between ROS generation and detoxification capacity under salt stress.

In parallel, the level of malondialdehyde (MDA)-a key indicator of lipid peroxidation, was markedly higher in saline-exposed samples than in controls, signifying enhanced oxidative degradation of membrane lipids. The elevated MDA content reflects extensive damage to cellular membranes and compromised structural integrity induced by salinity stress. Overall, these findings confirm that salt exposure intensifies oxidative pressure in Taxus seedlings, leading to ROS accumulation and lipid peroxidation, both of which are hallmarks of stress-induced cellular injury.

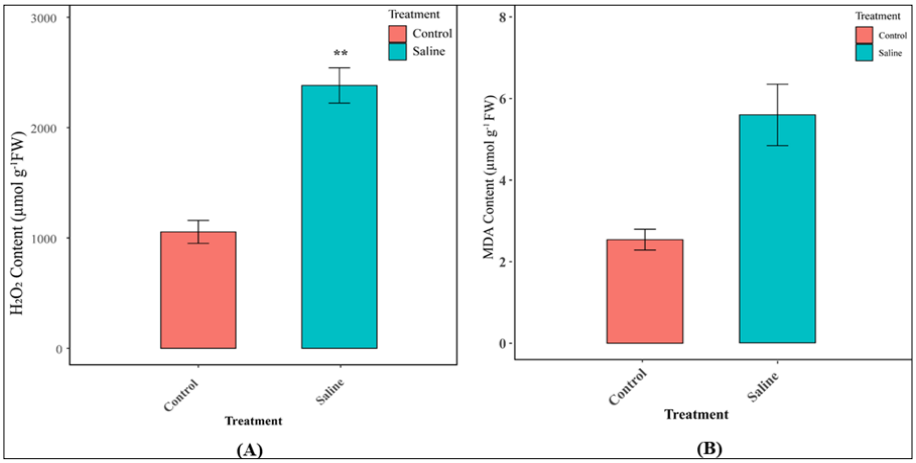

Fig 3: Effects of salinity stress on oxidative stress indicators in *T. wallichiana* leaves. (A) Hydrogen peroxide (H_2O_2) content and (B) malondialdehyde (MDA) content under control and saline conditions. Data are presented as mean ± SD (n = 3). Asterisks indicate statistically significant differences compared to the control ($p < 0.01$).

4. Histochemical detection of stress markers

Histochemical staining further substantiated the biochemical evidence of oxidative stress induced by salinity in Taxus spp. seedlings (photoplate 3.4) Leaves subjected to saline treatment exhibited markedly stronger nitro blue tetrazolium (NBT) staining than the controls, characterized by dense blue formazan deposits distributed along the leaf lamina. This intense coloration signifies enhanced in situ accumulation of superoxide radicals ($O_2^{\bullet-}$) under salt stress.

Similarly, 3,3′-diaminobenzidine (DAB) staining produced prominent brown precipitates in the saline-treated leaves, indicating substantial hydrogen peroxide (H_2O_2) accumulation and the formation of oxidative hotspots within the tissue.

The concurrent increase in NBT and DAB staining intensity corroborates the quantitative findings of elevated ROS and lipid peroxidation, reinforcing that salinity provokes extensive oxidative perturbations at both cellular and tissue

levels. Collectively, these histochemical and biochemical results demonstrate that salt stress disrupts redox equilibrium, leading to excessive ROS generation and consequent oxidative damage in Taxus foliage.

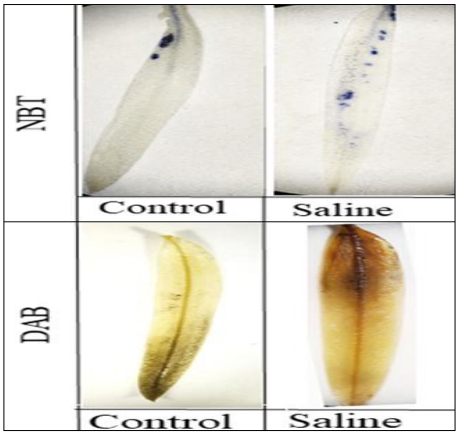

Fig 4: Representative histochemical detection of reactive oxygen species in leaves under control and saline conditions using NBT and DAB staining, showing stronger blue formazan deposits (superoxide, NBT) and brown polymerization products (hydrogen peroxide, DAB) in saline-treated tissue compared to control, indicative of salt-induced ROS accumulation in *T. wallichiana.*

Pearson's Correlation Analysis

Under salinity stress, *T. wallichiana* displayed a pronounced linkage between oxidative load and impaired photosynthetic function, as revealed by the correlation matrix (Figure X) connecting biochemical damage markers with photophysiological traits. Hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) were strongly and negatively correlated with PSII effective quantum yield (YII), electron transport rate (ETR), maximum photochemical efficiency (Fv/Fm), and chlorophyll a, chlorophyll b, and total chlorophyll content. This indicates that elevated reactive oxygen species and lipid peroxidation coincided with diminished photochemistry and pigment depletion. Conversely, Y (II), ETR, and Fv/Fm were tightly and positively interrelated and closely associated with chlorophyll pools, highlighting the role of pigment integrity in supporting PSII performance. Together, these correlations depict a salt-induced cascade in *T. wallichiana*, where ROS accumulation and membrane damage drive pigment loss and PSII dysfunction-hallmarks of photoinhibition and reduced light-harvesting capacity under ionic-osmotic stress

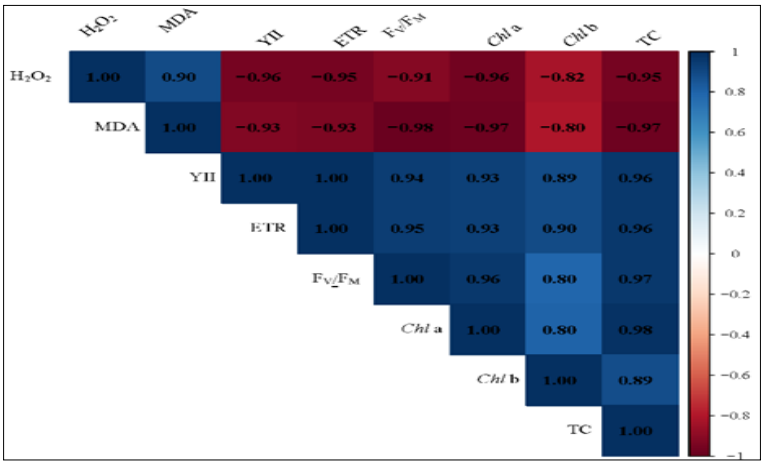

Fig 5: Pearson's correlation heatmap of oxidative stress markers (H_2O_2, MDA), photosynthetic performance parameters (YII, ETR, Fv/Fm), and chlorophyll pigments (*Chl* a, *Chl* b, TC), with tile values indicating Pearson's r and the colour scale spanning −1 to +1.

Discussion

Salinity stress imposes multifaceted constraints on plant systems, primarily through osmotic imbalance, ion toxicity, and oxidative perturbations that collectively impair growth and photosynthetic efficiency (Munns and Tester, 2008; Acosta-Motos *et al*., 2017) [1, 17]. In the present study, *T. wallichiana* seedlings subjected to saline conditions exhibited distinct morphological impairments, including chlorosis, wilting, and growth retardation, in contrast to the healthy and vigorous control plants. Such visual symptoms are characteristic of salt-induced water deficit and ion accumulation in leaf tissues, leading to reduced cell turgor and stomatal closure (Shabala and Cuin, 2008) [22]. These changes ultimately constrain carbon assimilation and energy balance, as reported in several woody and coniferous species exposed to salinity stress (Gupta & Huang, 2014) [9]. The marked reductions in chlorophyll fluorescence parameters-namely maximum photochemical efficiency (Fv/Fm), effective quantum yield (YII), and electron transport rate (ETR)-further indicate photoinhibition and compromised photosystem II (PSII) performance under saline conditions. A decline in Fv/Fm is a widely accepted indicator of PSII damage and impaired reaction center stability (Maxwell and Johnson, 2000; Kalaji *et al*., 2016) [11, 14]. The observed decreases in Y (II) and ETR reflect reduced electron flow through the thylakoid membrane, leading to limited ATP and NADPH generation necessary for carbon fixation (Atta *et al*., 2023) [3, 4]. Parallel declines in chlorophyll a, chlorophyll b, and total chlorophyll suggest inhibited pigment biosynthesis or accelerated degradation, consistent with oxidative bleaching and chlorophyllase activation reported under salinity stress (Ashraf and Harris, 2013; Sudhir and Murthy, 2004) [2, 23]. These findings collectively demonstrate that salinity impairs both photochemical efficiency and pigment stability in Taxus seedlings. Enhanced oxidative stress was evident from the substantial increases in H_2O_2 and MDA contents observed under saline treatment. Elevated H_2O_2 reflects excessive ROS accumulation resulting from disrupted electron transport in chloroplasts and mitochondria, while higher MDA levels indicate enhanced lipid peroxidation and membrane injury (Gill and Tuteja, 2010; Hasanuzzaman *et*

al., 2020) [8]. Such ROS over accumulation is a typical hallmark of salt stress and often correlates with reduced antioxidant capacity or enzymatic inefficiency (Mittler, 2017) [15]. Histochemical assays corroborated these biochemical findings. The intense blue formazan deposits in NBT-stained leaves and pronounced brown coloration in DAB-stained tissues confirmed enhanced superoxide ($O_2^{\bullet-}$) and H_2O_2 accumulation in situ, signifying oxidative hotspots and cellular redox imbalance. These observations are consistent with earlier reports showing that salt exposure increases ROS localization within leaf mesophyll and vascular tissues (Foyer and Noctor, 2016; Sarker and Oba, 2018) [7, 21]. Collectively, these morphological, physiological, and biochemical responses underscore the sensitivity of Taxus spp. to saline stress. The results indicate that salinity disrupts photosynthetic electron transport, enhances pigment degradation, and intensifies ROS-mediated oxidative damage, culminating in reduced growth and vitality. Further investigations focusing on antioxidant enzyme dynamics and osmolyte accumulation could elucidate potential adaptive responses or tolerance thresholds in Taxus under saline environments.

Conclusion

The present study demonstrates that salinity exerts a substantial inhibitory effect on the growth, photosynthetic efficiency, and redox balance of *Taxus wallichiana* seedlings. Morphological deterioration, characterized by chlorosis and wilting, was accompanied by significant declines in chlorophyll fluorescence parameters (Fv/Fm, YII, ETR) and pigment content, indicating impaired PSII activity and reduced photosynthetic competence. Concurrently, elevated levels of hydrogen peroxide and malondialdehyde, supported by intensified NBT and DAB staining, revealed severe oxidative stress and membrane damage under saline conditions. Collectively, these findings highlight that salt stress disrupts both photochemical and antioxidative homeostasis in Taxus, leading to oxidative injury and growth suppression. The study provides a physiological basis for understanding salt sensitivity in Taxus spp. and underscores the importance of exploring antioxidant regulation, ion homeostasis, and osmoprotective mechanisms in future research to enhance salinity tolerance in this economically and pharmacologically valuable genus.

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