Enhancing salt tolerance in a hybrid poplar *(Populus nigra x maximowiczii)* through exogenous putrescine application: Growth, physiological, and biochemical responses

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Abstract

Introduction: Polyamines regulate plant growth and development under normal conditions and protect plants from a multitude of biotic and abiotic stresses. However, little information is available about how polyamines improve salt tolerance.

Methods: This study was conducted to investigate whether exogenous polyamine application has the potential to maintain poplar growth and yield under a short duration of salt stress. We analyzed the trends in growth and physiological and biochemical responses of hybrid poplar (*Populus nigra x maximowiczii*, clone NM6) plants to salt stress by foliar application of putrescine (a diamine). Leaves of young greenhouse-grown plants were sprayed with 1 mM putrescine on several days following one-time application of 100 mM and 200 mM NaCl via roots. For measurement of growth, plant height, stem diameter, and leaf number for each plant were recorded throughout the experiment. Studies on the physiological and biochemical trends included gas exchange, total chlorophyll, carotenoids, soluble sugars and proteins, amino acids, polyamines, and relative water content in leaves.

Results: Putrescine spray on the salt-treated plants caused a significant increase in chlorophyll a & b, carotenoids, and soluble proteins on a fresh weight basis. Putrescine application caused a significant increase in fructose, glucose, and galactose in all plants, but putrescine spray had a variable impact on the sucrose content of 100 mM NaCl-treated plants. Based on metabolic responses, plants treated with 100 mM NaCl fared better when sprayed with putrescine than those treated with 200 mM NaCl.

Discussion: In conclusion, the results indicate that putrescine did reverse the effects of salt in many ways, and there were trends indicating that sprayed putrescine promotes salt tolerance in young poplar plants.

1 Introduction

Polyamines (PAs) are emerging as critical regulators of plant adaptation to abiotic stress (Minocha et al., 2014, Alcázar et al., 2006, Blázquez, 2024, Atabayeva et al., 2020, Zhao et al., 2024b, Pál et al., 2018, Tabur et al., 2024), particularly in mitigating the detrimental effects of salt stress on growth and metabolism (Borromeo et al., 2023, Antoniou et al., 2021, Raziq et al., 2022, Shao et al., 2022). Polyamines like putrescine (Put), spermidine (Spd), and spermine (Spm) are derived from various amino acids (AAs), and influence fundamental processes such as seed germination, photosynthesis, root architecture, and stress signaling (Alcázar et al., 2010, Pál et al., 2021, Pál et al., 2018, Blázquez, 2024). While extensive studies have explored PA functions in several model crop species (Wuddineh et al., 2017, Sundararajan et al., 2021, Pál et al., 2025, Imran et al., 2021, Antoniou et al., 2021, Borromeo et al., 2023), their role in woody plants- particularly fast-growing poplars, remains unexplored despite their significant ecological and economic importance (Zalesny Jr et al., 2019). Woody plants, including poplars, face unique challenges in research due to their long-life cycles and complex anatomical structures, and ethical considerations in genetic studies (Neale and Kremer, 2011, Chen et al., 2021). Understanding the stress adaptation mechanisms in woody perennial plants is crucial for sustainable forestry and climate resilience, especially in the face of global warming and increasing soil salinity.

Abiotic stresses such as salinity, drought, extreme temperatures, heavy metals impose several constraints on plant growth and productivity (Cramer et al., 2011, Parihar et al., 2015, Zhang et al., 2020a, Zhang et al., 2023, Kundu, 2023). Salt stress in particular, disrupts water uptake, ion homeostasis, photosynthesis, and carbon (C) and nitrogen (N) metabolism, leading to reduced growth, delayed development, and premature senescence (Hanson et al., 2016, Sun et al., 2022, Bargmann et al., 2009, Dinneny, 2015, Calhoun et al., 2023, Haak et al., 2017). The accumulation of chloride ions further exacerbates these effects by interfering with nutrient absorption, impacting chlorophyll content, root-shoot ratio, and reproductive development (Cramer et al., 2011, Amiour et al., 2012, Parihar et al., 2015, Zhang et al., 2020a, Kumar et al., 2023, Magallon and Dinneny, 2019). Azimi et al. (2021) reported that N deficiency is a major consequence of salt stress, reducing leaf area and inducing chlorosis, which hinders plant productivity. While N fertilization is a common approach to mitigate these effects, excessive application of N contributes to environmental hazards such as water pollution and algal blooms, necessitating sustainable stress mitigation strategies (Li et al., 2021, Zhang et al., 2020a).

Polyamines have been recognized as key regulators of abiotic stress responses, accumulating in plants subjected to salinity, heavy metals, drought, and temperature extremes (Alcázar et al., 2010, Mohapatra et al., 2010, Pál et al., 2018, Malik et al., 2022, Wang et al., 2024b, Kundu, 2023). They play a vital role in modulating carbon metabolism, stabilizing membrane integrity, scavenging reactive oxygen species (ROS), and regulating osmotic balance (Majumdar et al., 2013, Pál et al., 2021, Pál et al., 2025). Polyamines can also enhance N assimilation, thereby improving chlorophyll synthesis and overall plant growth (Alcázar et al., 2010, Tamang et al., 2021). Several studies have demonstrated that exogenous PA application improves plant resilience by modulating hormone signaling like abscisic acid, gene expression (Paul et al., 2018), and enzymatic responses under salt stress and others (Borromeo et al., 2023, Xiong et al.,

2018, Wang et al., 2024b). Seed priming with PAs has been reported to enhance photosynthetic pigments, proline accumulation, root growth and biomass production in crops such as tomato (Borromeo et al., 2023), rice (Mahmud et al., 2024, Zhang et al., 2024), rapeseed (ElSayed et al., 2022), and soybean (Sheteiwy et al., 2021) under salinity stress. Additionally, exogenous PA treatments have been shown to increase soluble sugars, proteins, and amino acid accumulation, enhancing abiotic stress tolerance in economically significant plants like grapevine, ginseng, rice (Sundararajan et al., 2021), and tomato (Raziq et al., 2022). Recent studies have also shown that exogenous spermidine (Spd) treatment increased resistance to fusariosis in flax by suppression of PA metabolism (Augustyniak et al., 2025). However, despite these promising findings, studies in woody perennials remain limited, particularly in fast-growing trees like poplars.

Poplars (*Populus* spp.) are among the fastest-growing perennial tree species, widely distributed across North America, China and India (Dickmann, 2001, Hawkins et al., 2003, Plomion et al., 2016, Zalesny Jr et al., 2019, Buell et al., 2023); and serve as key resources for sustainable forestry, bioenergy production, and lumber industry (Yi et al., 2022). Poplars exhibit notable stress tolerance, including resistance to salt and heavy metal contamination (Zalesny Jr et al., 2019, Lin et al., 2023), making them ideal candidates for genetic and physiological research (Guerra et al., 2011). Several varieties of poplars have shown potential to be grown in marginal sandy lands (Ghezehei et al., 2021). Advances in 'omics' technologies and the sequencing of poplar genome (Ma et al., 2019) have enabled deeper insights into their stress response pathways. Notably, transgenic studies in hybrid poplars have identified genes like *NAC13*, which enable salt tolerance (Zhang et al., 2019). Beyond traditional transcriptomics, the emergence of single-cell RNA sequencing (scRNA-seq) has revolutionized poplar research by providing high-resolution gene expression profiles of vascular system and wood plasticity under drought stress (Gomez-Soto et al., 2025, Conde et al., 2022). Additionally, some studies have applied scRNA-seq to dissect root adaptations to soil compaction and salt stress, revealing cell-type-specific regulatory mechanisms (Zhu et al., 2025, Liu et al., Li et al., 2024). Recent computational advances, such as orthologous marker genes groups (OMGs), have further enabled cross-species comparison of conserved cell types, expanding the potential for identifying PA-mediated functions across plant taxa at single-cell resolution (Chau et al., 2025). Although not employed in this study, such approaches offer promising avenues for future research on PA specific stress responses in woody perennials. Understanding how PAs contribute to salt stress tolerance at tissue- or cell-specific resolution could bridge a critical knowledge gap in tree physiology and enhance strategies for improving stress resilience in forestry species.

Among poplar hybrids, *Populus nigra x maximowiczii* (NM6 clone) is widely cultivated in North America due to its fast growth, high biomass yield, asexual propagation capacity, and genetic stability (Labrecque and Teodorescu, 2005, Guerra et al., 2011). Zalesny Jr et al. (2019) also reported that the NM6 clone was more salt-tolerant than other willow and poplar species. Yet no studies have investigated the physiological and biochemical effects of exogenous PA application in mitigating salt stress in NM6 poplars. Given the increasing soil salinity issues affecting forestry and agricultural productivity, understanding how PA treatments can enhance stress tolerance in NM6 poplars is of scientific as well as economic significance.

To address this research gap, we investigated the role of exogenous PA application in conferring salt stress tolerance in young NM6 poplars. Using a controlled greenhouse experiment, we assessed the physiological and biochemical responses of NM6 poplar plants

to NaCl-induced stress and examined whether foliar-application of Put could alleviate its adverse effects. Specifically, we aimed to answer the following research questions: 1. How does NaCl stress affect the physiological and metabolic processes in NM6 poplar plants? 2. Can exogenous application of Put mitigate NaCl-induced stress; and if so, through which biochemical and physiological pathways? We hypothesize that Put treatment will enhance salt stress tolerance in NM6 poplars by improving plant growth, photosynthetic efficiency, and metabolic stability (soluble sugars, AAs, and PAs) thereby promoting growth and stress resilience. This study provides novel insights into PA-induced stress mitigation strategies in woody plants and expands current knowledge on sustainable approaches to enhance abiotic stress resilience in forestry species.

2 Materials and methods

2.1 Plant Material and Growth Conditions

This study utilized young cuttings of hybrid poplar (*Populus nigra x maximowiczii* - NM6) plant, collected from a 5-year-old, healthy tree at the University of New Hampshire (UNH) Kingman Farm. The cuttings were rooted and maintained at the UNH MacFarlane greenhouse for one growing season of (mid-April to late June) before the initiation of experiment.

During the initial growth phase, cuttings (~15 cm in height, 0.6 cm stem diameter) were kept under mist for two weeks in 17.78 cm grow tubes containing PRO-MIX soil with Mycorrhizae**.** The grow tubes were placed in trays to retain excess water, ensuring consistent soil moisture, and plants were watered regularly. After 2 months, well-established plants (~30–38 cm in height with 9–10 leaves) were selected and transferred to 33 cm pots filled with a 1:1 mixture of vermiculite and perlite**.**

The experiments were conducted from July to September 2021 in a controlled greenhouse environment with natural sunlight and a 16-hour photoperiod**.** The temperature in the greenhouse ranged between 22°C and 24°C**,** with a relative humidity of 70%. Plants were irrigated and fertilized twice daily using an automated drip-line irrigation system with Jack's Pure Water LX - Professional fertilizer (J.R. Peters Inc., https://www.jrpeters.com/17-4-17-pure-water-lx). Each plant received 200 mL of water at 08:00 AM and 2:00 PM**,** with additional watering in the evening on with water, if needed.

2.2 Experimental Design

A nonrandomized block design was used in this study, ensuring that plants remained in fixed positions throughout the experiment. Salt treatment with sodium chloride (NaCl) was initiated 21 days after transplanting the cuttings into 33 cm pots, when the plants were approximately 3 months old from the initial collection of cuttings. A total of 36 plants were assigned to 6 treatment groups (n = 6 per treatment): treatment A (control- no NaCl, no Put), treatment B (100 mM NaCl), treatment C (200 mM NaCl), treatment D (control + 1 mM Put foliar spray), treatment E (100 mM NaCl + 1 mM Put foliar spray), and treatment F (200 mM NaCl + 1 mM Put foliar spray).

Putrescine was purchased from Sigma-Aldrich as Put dihydrochloride (≥98% purity, molecular weight = 161.07). A 0.5% Silwet solution was added as a surfactant to enhance the penetration of the 1 mM Put solution. To ensure uniform salt application, drip irrigation was halted 18 hours before salt treatment**.** Salt solutions were applied via root irrigation by pouring 200 mL of NaCl solution per pot at the time of treatment initiation (Day 0) and again 6 hours later. Drip irrigation resumed 6 hours after the final salt application**.** Saucers were placed beneath the pots to prevent water leakage.

For treatments receiving Put (D, E, and F), 1 mM Put foliar spray was applied immediately after salt treatment**.** Each plant received approximately 30 mL of Put per spray session, applied only to the leaves while the soil was carefully covered with plastic sheets to prevent contamination. After spraying, excess solution was manually tapped off the leaves**.** The foliar spray was applied on days 0, 3, 6, and 13.

Leaf samples (~6th leaf from the top) were collected on days 3, 6, 13, and 20 for physiological and biochemical analyses.

2.3 Sample Collection

Plant samples including the remaining leaves and root tissues**,** were collected at various time points before and after treatment application for physiological and biochemical analyses.

2.3a Leaf Sampling

Zero-time leaf discs were collected 1 day prior to stress treatments (day 0), which served as a baseline measurement. Sampling was performed from 1representative plant per treatment group. Leaves were washed with fresh water, pat-dried between 2 layers of paper towels, and subsequently collected using specific extraction solutions or buffers (Supplemental Table 1). For post-treatment collections, the fully expanded 6th leaf from the top was sampled. Leaf discs (6.35 mm diameter) were obtained using a standard paper punch and stored in the appropriate solvents or buffers as per day 0 collection.

Physiological traits like relative water content (RWC) and chlorophyll content were measured using leaf discs. Gas exchange measurements were taken with LICOR-6400 directly on the plant leaves. Biochemical assays like soluble sugars, PAs, AAs, and total protein were done with leaf discs. All samples (except leaf discs for biomass measurements) were stored at -20 ºC until further analyses.

2.3b Roots Sampling

Root samples were collected at the time of harvesting. To ensure cleanliness, roots were thoroughly washed under running tap water to remove residual vermiculite and perlite. Fine secondary roots (2–3 mm in diameter) were carefully snipped from the main root using sterilized scissors. Approximately 100 mg of fresh root tissue was immediately placed into 2 mL microfuge tubes containing respective extraction solutions (Supplemental Table 1) for further biochemical analyses, including PA and AA quantification.

2.4 Plant Growth Parameters

Plant growth rate was assessed based on changes in height and stem diameter over time. Measurements were recorded on days 0, 15, 30, and 45 post-treatments with salt. Stem basal diameter was measured using a digital caliper, with a consistent reference point marked near the base of the stem to ensure accuracy across measurement intervals. Growth in height and stem diameter was expressed as a percentage increase using the following formulas:

Increase in height (%) = [(Height on next day - height on previous day)/ Height of day 1] * 100

Increase in diameter (%) = [Diameter on next day – diameter on previous day)/ Diameter of day 1] * 100

2.5 Soluble Sugars

Soluble sugars were quantified following a modified protocol from Blagden et al. (2022). Leaf discs (50 ± 2 mg FW) was incubated at 65°C for 30 min in 1 mL of 80% ethanol. The extract was allowed to cool to room temperature for 5 min, followed by vortexing at medium speed for 2 min. After centrifugation at 13,000 × g for 8 min, the supernatant was filtered into an autosampler vial using a 0.45 µm nylon syringe filter (Pall Corp., Port Washington, NY) fitted onto a 3 mL syringe (Becton, Dickinson and Company, Franklin Lakes, NJ).

Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC) was used for sugar quantification based on external standard curves. The mobile phase consisted of 80% acetonitrile (ACN) and 20% double-distilled water (ddH□O), with a flow rate of 2 mL min□¹. The injection

volume was 40 µL into a 200 µL loop, and separation was performed using a Phenomenex Luna-NH□ analytical column **(**5 µm, 100 Å, 250 × 4.6 mm, #00G-4378-EO**)**.
A total of 11 sugars were quantified: xylose, arabinose, fructose, mannose, glucose, galactose, sucrose, trehalose, rhamnose monohydrate, maltose monohydrate, and raffinose pentahydrate. Detection was performed using a Shimadzu RID-10A refractive index detector (RID) set at 30°C (Shimadzu Scientific Instruments Inc., Columbia, MD). The total run time was 15 min, including column washing and stabilization between injections. The column temperature was maintained at 25°C throughout the analysis.
Chromatographic data were processed using Perkin Elmer TotalChrom software (version 6.2.1). Peaks were identified by matching retention times with known sugar standards. An 8-point external standard curve (3 mg mL□¹) was generated for individual sugar quantification **(**Supplemental Table 2). Unresolved sugar peaks were quantified as combined concentrations based on their summed peak areas, including xylose + arabinose, glucose + galactose, and trehalose + maltose.

2.6 Polyamines and Amino acids

For the quantification of different AAs and PAs, approximately 40±2 mg fresh leaf discs were collected in 5% perchloric acid ($HCLO_4$) at a ratio of 1:25 (w:v) in 2 mL microfuge tubes. The samples were freeze-thawed three times and processed for dansylation following a modified protocol from Minocha et al. (1994), (Minocha and Long, 2004) and (McDermot et al., 2020). After the final thawing, the samples were vortexed at high speed for 2 min and centrifuged at 14,000 × g for 8 min.
For each sample and external standards, 20 µL of an internal standards mix containing 0.05 mM α-methyl-DL phenylalanine (for AAs) and 0.05 mm heptane diamine (for PAs), both dissolved in 5% $HCLO_4$, was added to each tube. Subsequently, 100 µL of the freeze-thawed extract was combined with 100 µL of 2.691 M sodium carbonate and 100 µL of freshly prepared dansyl chloride (20 mg/mL in acetone). The mixture was vortexed and incubated at 60°C for 30 min. After incubation, the microfuge tubes were cooled at room temperature for 3 min, followed by the addition of 45 µL glacial acetic acid to terminate the reaction. Acetone was evaporated using a speed-vac for 10 min, and 1735 µL methanol was added to the mixture.
The methanol extract was filtered using a 0.45 µm nylon syringe filter fitted onto a 3 mL syringe before transferring the solution into autosampler vials. Separation of AAs and PAs was performed using a 15 cm column (Phenomenex Synergi Hydro-RP 80 Å, LC Column 150 x 4.6 mm, 4 µm). Quantification was achieved using a fluorescence detector (Series 200 PerkinElmer) set at 340 nm for excitation and 510 nm for emission. Relative quantification of AAs and PAs was performed using an external standard curve (Supplemental Table 3). Chromatographic data were analyzed using Perkin Elmer TotalChrom software (version 6.2.1), incorporating a multiplication factor within the software to determine the concentration of each component in nmol g□¹ FW of tissue.

2.7 Total Soluble Proteins

Total soluble protein content was quantified following a modified protocol from Minocha et al. (2019). Fresh leaf discs (50 ± 2 mg FW) were extracted in a freshly prepared 100 mM Tris buffer (pH 8.0) containing 20 mM MgCl□, 10 mM NaHCO□, 1 mM ethylenediaminetetraacetic acid (EDTA), and 10% (v/v) glycerol. The extraction was performed using a three-cycle freeze-thaw method.

The extract was centrifuged at 13,000 × g for 5 min, and the supernatant was used for total soluble protein quantification following the Bradford (1976) method. The analysis was conducted using Bio-Rad protein assay dye reagent (Bio-Rad Laboratories, Hercules, CA), with bovine serum albumin (BSA) as the standard. Absorbance was recorded at 595 nm using a spectrophotometer. A standard curve (0.1–0.5 mg/mL) was generated to quantify protein concentrations in the samples.

2.8 Relative Water Content

Relative water content (RWC) was determined by measuring the (FW) of the leaf tissue immediately after collection. For DW, the leaf samples were incubated in an oven at 70°C for 48 hours. The samples were reweighed after 24 hours to confirm that no residual moisture remained. Relative water content was calculated using the following formula:

$$RWC (\%) = [(FW - DW)/FW] * 100$$

2.9 Chlorophyll Content

Chlorophyll content was quantified following the method described by Minocha et al. (2009) and McDermot et al. (2020). Two leaf discs (8–13 mg FW) were transferred into 2 mL microfuge tubes containing 1 mL of 95% ethanol. The tubes were incubated in a water bath at 60°C for 16 hours, wrapped with aluminum foil to prevent chlorophyll degradation.

Following incubation, the samples were vortexed for 1 min, centrifuged at 13,000 × g for 5 min, and absorbance readings were recorded at A□□□, A□□□, and A□□□ nm using a spectrophotometer. The total chlorophyll, chlorophyll a, chlorophyll b, and carotenoid contents were calculated using the equations described by Lichtenthaler (1987):

1. Total chlorophyll (a + b) = $[(22.24(A_{649}) + 5.24(A_{664})) * \text{volume of ethanol(mL)}]/\text{FW of tissue (mg)}$
2. Chlorophyll a $=[(13.36(A_{664}) - 5.19(A_{649})) * \text{volume of ethanol (mL)}]/ \text{FW of tissue (mg)}$
3. Chlorophyll b $= [(27.43(A_{649}) - 8.12(A_{664}) * \text{volume of ethanol (mL)}]/ \text{FW of tissue (mg)}$
4. Total carotenoids $=[(4.8(A_{470}) - 12.7(A_{649}) + 3.65(A_{664}) * \text{volume of ethanol (mL)}]/ \text{FW of tissue (mg)}$

2.10 Leaf Gas Exchange

Gas exchange parameters, including photosynthetic rate (P_n), transpiration rate (E), and stomatal conductance (g_s) were measured on a fully expanded 6th leaf from the apex for each plant. Measurements were taken on 3–4 plants per treatment using a portable photosynthesis system (LI-6400/XT, Li-COR Biosciences, Lincoln, NE, USA).

Gas exchange was recorded between 09:00 and 12:00 under a photosynthetic photon flux density (PPFD) of 1000 µmol m□² s□¹ provided by a red-blue LED light source (6 cm² chamber). Airflow was maintained at 500 µmol s□¹, and reference CO□ concentrations were set to 400 µmol mol□¹. The block temperature was maintained at 25°C. Leaf humidity was not actively controlled but ranged between 50–60% during measurements.

2.11 Statistical Analysis

All statistical analyses were conducted with JMP Pro 18 (www.jmp.com/en_us/home.html). Data were analyzed using a two-way analysis of variance (ANOVA) to assess statistically significant differences between treatments within each time point. Post-hoc comparisons were performed using Tukey's test, with statistical significance set at $p < 0.05$. Graphical figures of

the data were generated using the ggplot2 package, and both heatmap and correlation plots were produced with the pheatmap and corrplot packages in RStudio (R version 4.4.1).

3 Results

3.1 Effects of Put spray on Plant Growth and Morphological Symptoms

Changes in height and stem diameter were measured for various days till 45 days after salt treatment. Figure 1 (A, B) indicates that the combination of NaCl with Put enhances growth in terms of both stem length and diameter compared to NaCl treatments alone. Our data suggest that Put had a growth-promoting effect on plants under salt stress, leading to better overall growth metrics. Morphological symptoms in the salt-treated plants began to appear 13 days after salt exposure. Leaf morphology analysis **(**Fig. 1C) under salt stress revealed that 100 mM NaCl and 200 mM NaCl caused more severe chlorosis and necrosis compared to the control (A). However, the application of 1 mM Put reduced these stress symptoms in salt-treated plants. Putrescine-treated leaves displayed a healthier appearance, with markedly less chlorosis and necrosis, highlighting its protective effect under salt-induced damage.

3.2 Effects of Put spray on Gas exchange, chlorophyll and total soluble protein

Leaf gas exchange was measured in the 6th fully matured leaf at 7 and 35 days after NaCl treatment. On day 35, g_s was significantly lower in control plants compared to 100 mM NaCl-treated plants under Put spray (Fig. 2A). Transpiration rate (E) was significantly lower in control plants compared to100 mM NaCl treated plants under Put at the same time point (Fig. 2D). Net photosynthesis (Pn) remained consistently higher under salt stress vs. control, though differences were not statistically significant (Supplementary Fig. 1C). Carotenoid was significantly higher in Put-sprayed plants *vs.* unsprayed for 200 mM NaCl on the 3rd day (Fig 2B). Total soluble protein was significantly lower under 100 mM NaCl compared to control on 13th day (Fig 2D). Relative water content (RWC) ranged between 70-75%, however there was no significant changes on the RWC under salt stress on any days (Fig. 2C). Fresh weight/dry weight was significantly higher in salt treated plants compared to Put-sprayed control plants on 13th day (Fig. 2F). These results indicate that Put application mitigates the adverse effects of salinity on gas exchange and pigment stability, while modulating carotenoid, protein FW/DW content in a stress- and time-dependent manner.

3.3 Soluble sugar content

Soluble sugar content was measured in the 6th fully matured leaf on several days after NaCl treatment. Fructose content significantly increased under Put-sprayed 100 mM NaCl *vs.* unsprayed at 6th day (Fig. 3A). Fructose was significantly higher in 200 mM NaCl treated plants compared to control and 100 mM NaCl treated plants on the 6th day. Glucose and galactose were non-separable in our HPLC system; hence are represented together. Glucose+galactose levels were significantly lower in 200 mM NaCl treated plants compared to Put sprayed control plants on the 6th day (Fig. 3B). Glucose + galactose levels were significantly lower in 200 mM NaCl-treated plants at 6th and 13th day compared to Put-sprayed control plants. Sucrose levels were significantly lower in Put-sprayed plants *vs.* unsprayed for 100 mM NaCl on the 6th day (Fig. 3C). Sucrose was significantly higher for Put-sprayed 200 mM NaCl plants on the 13th day. Sucrose was also significantly higher in Put-sprayed plants than those unsprayed for control and 100 mM NaCl on the 13th day. Our results also showed that sucrose contents were also significantly higher in 100 mM NaCl treated plants compared to the control plants on 20th day. These findings suggest Put may help regulate soluble sugar accumulation under salt stress in a time- and sugar-dependent manner.

3.4 Amino acids and Polyamines content in leaves and roots

Amino acids and PAs were measured in the 6th fully matured leaf on several days after NaCl treatment. Among all the AAs, Arg+Thr+Gly and Gln were the most dominant. On day 3, 200 mM NaCl treated plants had significantly higher levels of Gln, Ser, and GABA compared to all other treatments (Fig. 4). Put-sprayed plants under 200 mM NaCl treatment, had significantly higher Gln, Ser and Orn on day 6 (Fig. 4A, B, 5A). Additionally, Put-sprayed plants under 100 mM NaCl had significantly higher Glu and Asp compared to control plants on day 6 (Fig 5B, C). Serine was also significantly higher on Put-sprayed, salt treated plants on day 13. Alanine, Pro, Arg+Thr+Gly, Phe+Cys, and Ile was significantly higher in unsprayed *vs.* sprayed 200 mM NaCl treated plants on day 3 (Fig. 6A, B, Supplementary Fig. 1, 2). Alanine was also significantly higher in 100 mM NaCl plants compared to control on day 6. Our results also showed that Pro was significantly lower on day 6 in Put-sprayed, 200 mM NaCl-treated plants (Fig. 6B). Histidine was significantly higher in Put-sprayed, 200 mM NaCl-treated plants compared to other treatments on day 6 (Fig. 6B). Foliar spray of Put significantly altered endogenous Put and Spm content under 200 mM NaCl on day 3 (Fig. 7A). However, PA levels varied under salt treatment on other days, but the differences were not significantly different (Fig. 7B, C). These findings suggest that Put application influences AA and PA accumulation under salt stress in a time- and dose dependent manner.

Amino acids and PAs content of the roots were analyzed (with HPLC) only at 21 days after NaCl treatment at the termination of the study. Salt significantly increased Arg+Thr+Gly, Gln, Orn, Phe+Cys, and Ser accumulation (in both concentrations of NaCl) compared to the control (Fig. 8A, B). Lysine was significantly lower in 200 mM NaCl compared to control plants (Supplementary Fig 4A). Among PAs, Put was significantly increased under salt stress, while Spd was significantly reduced in salt exposured roots (Fig. 8C). Spermine was undetected in salt treated roots. Our results also showed that total PA content in roots were lower in 100 mM NaCl-treated plants compared to both the control and 200 mM NaCl-treated plants. These results strongly suggest that salt stress influences AAs and PAs accumulation.

3.5 Combined analysis of polyamine metabolic cycle in roots

To better understand the metabolic coordination among AAs and PAs in roots under salt stress, we conducted a correlation analysis based on their relative contents across control, 100 mM NaCl, and 200 mM NaCl treatments (Fig. 9A). The heatmap revealed 2 major metabolite clusters: first cluster was comprised with Gln, Pro, Ser, GABA, Put and another dominated by Spm, Spd, and Ala. Glutamine showed strong positive correlations with Ala, Ser, and Arg+Thr+Gly, but was significantly negatively correlated with Spd and Lys ($p < 0.05$). Similarly, Gly, Ser, and GABA were significantly positively correlated with Gln (Fig. 9B). These findings suggest that increasing salt concentrations may shift nitrogen metabolism in roots, reducing AA accumulation while enhancing PA synthesis, possibly as part of a salt stress adaptation response.

4 Discussion

Soil salinity is a growing global threat to agriculture and forestry, significantly reducing crop yields and limiting the availability of arable land. Salt stress adversely affects plants by limiting growth, water uptake, inducing toxic ion accumulation, and triggering oxidative stress (Hasanuzzaman et al., 2013, Dinneny, 2015, Safdar et al., 2019, Sun et al., 2022, Rady et al., 2023), which hampers plant growth and productivity (Sun et al., 2022, Liu et al., 2023, Rodríguez Coca et al., 2023, Zhang et al., 2023). The primary symptoms of salinity stress are stunted plant growth, leaf burning, chlorosis, leaf rolling, reduced water uptake, nutrient deficiency, stomatal closure, photosynthesis inhibition through reduced leaf area, and altered metabolism (Hasanuzzaman et al., 2013, de Oliveira et al., 2013, Sun et al., 2022, Calhoun et al., 2023). Plants tolerate salt by reprogramming their physiological, molecular, and biochemical responses (Gupta and Huang, 2014, Yang and Guo, 2018, Raza et al., 2022, Wang et al., 2024a, Liu et al., 2025). Different salinity levels have varying effects on plants, and the physiological and biochemical responses may also differ in different plants (Ding et al., 2010, Nguyen et al., 2018, Zhao et al., 2021, Sun et al., 2022). It has been studied by Ding et al. (2010) that salt-tolerant plants like *P. euphratica* Oliv had high up-regulation of antioxidant enzymes compared to salt-sensitive *P. popularis 35-44*. It has also been found by Zalesny Jr et al. (2019) that salt-tolerant *P. nigra X maximowiczii* **(**NM2 and NM6 hybrid clones) had superior mineral uptake capacity from high-salinity soils than in low-salinity soils. Seed priming or exogenous treatment with various hormones or nitrogenous compounds may mitigate salt stress damage in plants when they grow up (Ma et al., 2012, Srivastava et al., 2021, Zafar et al., 2022, Eisa et al., 2023, Zhang et al., 2024).

Polyamines like Put, Spd have been widely studied for their protective roles in alleviating abiotic stress in crop plants (Nahar et al., 2016, Xiong et al., 2018, Alcázar et al., 2020, Islam et al., 2021, Ma et al., 2022, Blázquez, 2024, Zhang et al., 2024). High tolerance to salt stress due to foliar Put application has been reported in many plant species (Supplemental Table 4). Despite the growing interest in PAs as potential biostimulants, their role in salt stress tolerance in woody perennials, particularly poplar, remains largely unexplored. In this study, we investigated the effects of exogenous Put application on hybrid poplar under salt stress, aiming to elucidate its potential as a non-transgenic, physiological intervention for enhancing stress resilience.

4.1 Effect of putrescine on physiological parameters

In this study, visible stress symptoms, such as leaf tip burn, chlorosis, and browning, began to appear in NM6 poplar plants after 13 days of exposure to 100 mM NaCl. Interestingly, 200 mM NaCl-treated plants exhibited milder visual symptoms, with less leaf burn and chlorosis. Plants treated with Put alone developed brown spots near the leaf tips but maintained darker green pigmentation overall. However, Put-sprayed plants under salt stress exhibited more severe symptoms, with signs of salt-burn appearing in the middle parts of the leaves. In addition to leaf symptoms, salt-treated plants also exhibited slower growth and gradual leaf wilting, similar to previous findings in salt-stressed cotton, microalgae and poplar (Cao et al., 2020, Calhoun et al., 2023, Hu et al., 2023, Zhao et al., 2024a). In our study, Put-sprayed 100 mM NaCl treated plants demonstrated the most vigorous growth among all treatment groups, suggesting a possible protective or growth-promoting effect of Put under moderate salt stress.

Abiotic stress is known to induce long-term changes in gene expression, contributing to the development of molecular stress memory (Wang et al., 2014, Georgii et al., 2019, Tikhomirova

et al., 2022, Liu et al., 2022). Wang et al. (2025) reported that *Populus alba X Populus glandulosa* exhibited higher photochemical activity under 100 and 200 mM NaCl stress in later period. A similar pattern was observed in our study, where NM6 poplars exhibited higher net photosynthesis rates after 35 days of salt exposure compared to the early (7-day) stress period. Furthermore, after 35 days, Put-sprayed plants showed higher stomatal conductance and transpiration rates than unsprayed controls under salt stress, aligning with previous observations in *Camellia sinensis* and *Cucumis sativus* by Xiong et al. (2018) and Shen et al. (2019). These findings suggest that Put may enhance physiological resilience over time by modulating long-term stress responses and photosynthetic recovery. Salt stress also affected the total soluble protein content. We observed a significant reduction in protein levels under salt, likely due to increased protein degradation or decreased synthesis, which could account for the concurrent rise in free amino acid pools. However, Put spray under 100 mM NaCl restored protein levels, while under 200 mM NaCl, sprayed plants had lower protein content than unsprayed counterparts. Putrescine treatment did not affect protein levels in non-stressed plants, indicating its stress-dependent influence. Relative water content (RWC) also increased after 13 days of salt exposure, though the Put spray did not affect RWC under stress. This suggests that changes in soluble protein are more likely due to active metabolic regulation than water availability. Similarly, the FW/DW ratio increased under salt, reflecting possible osmotic adjustment.

4.2 Effect of putrescine on soluble sugars

Liang et al. (2018), Liu et al. (2022) and Zhang et al. (2024) reported that one of the most common adaptive responses to abiotic stress in plants such as *Arabidopsis thaliana*, *Populus euphratica*, *Morus multicaulis* and *Oryza sativa* is the accumulation of soluble sugars. This osmotic adjustment is a well-documented mechanism in various species including Tartary buckwheat, where sugars act as key osmolytes. In our study, we observed a significant increase in fructose and sucrose levels following 6 days of salt stress, consistent with these findings. In contrast, glucose + galactose levels were significantly reduced, indicating a differential modulation of carbohydrate metabolism under salt exposure. These shifts may be part of a stress-induced self-regulatory mechanism, wherein sugars contribute to osmotic balance, protect membrane stability, and support energy supply for metabolic adaptation. It has been previously established that such osmolyte accumulation also aids in maintaining high intracellular K^+ and favorable Na^+/K^+ ratios, while preventing dehydration and oxidative damage (Liu et al., 2022, Azeem et al., 2023). Moreover, Put-sprayed NM6 plants exhibited significantly enhanced sugar accumulation under salt stress compared to unsprayed controls. This aligns with earlier findings in *Cucumis sativus* by Yuan et al. (2015), suggesting that exogenous PAs may regulate carbon metabolism under salt stress through crosstalk between the PA and sugar synthesis pathways. Therefore, our results indicate that Put may contribute to sugar-mediated salt tolerance in poplar by enhancing osmotic adjustment and supporting cellular metabolism under stress.

4.3 Effect of putrescine on amino acids and polyamines

Polyamines (PAs) are small nitrogen-rich compounds known to mitigate salt-induced damage in plants through diverse biochemical and physiological mechanisms (Alcázar et al., 2010, Minocha et al., 2014, Saha et al., 2015, Choudhary et al., 2023). Under salt stress, plants often accumulate high levels of PAs, which help reduce lipid peroxidation, stabilize membrane integrity and ionic homeostasis, and regulate stress-responsive genes (Minocha et al., 2014, Alcázar et al., 2020).

In this study, salt stress induced time-specific increases in Put and Spm content in the leaves, especially under 100 mM NaCl, while Spd levels decreased over time. The accumulation increased under salt in the roots, whereas Spd levels declined, and Spm was not detected. These responses are consistent with prior studies showing that PA profiles under salt stress are species-specific and dynamically regulated (Zhang et al., 2020b, Huo et al., 2020, Baniasadi et al., 2018, Ghalati et al., 2020, Shu et al., 2015). The importance of Spm in stress adaptation is further supported by studies in *Arabidopsis*, where Spm synthase mutants were more salt-sensitive (Marco et al., 2019).

Polyamine catabolism is tightly linked to amino acid (AA) metabolism through shared intermediates and signaling molecules (Kundu, 2023, Blázquez, 2024). Several studies have shown that PAs act as stress messengers influencing the accumulation of stress-related amino acids such as GABA, Ala, and Pro (Gupta et al., 2013, Marco et al., 2019, Singh and Roychoudhury, 2020, Srivastava et al., 2021, Shao et al., 2022, Verslues et al., 2023). In our study, Glu, Ser, Ala, His, Arg+Thr+Gly, Gln, GABA, and Pro increased in the leaves following salt exposure, particularly after 6 days, indicating a time-dependent AA response. Putrescine spray under salt stress further influenced AA profiles. In the roots, salt treatment led to elevated levels of Gln, Arg+Thr+Gly, Phe+Cys, Orn, His, Ser, and Pro. The accumulation of these AAs may reflect a metabolic shift supporting osmoprotection and nitrogen storage in poplar under salt stress (Srivastava et al., 2021, Verslues et al., 2023, Wilhelmi et al., 2025). A notable decline in Lys in roots under 200 mM NaCl may be attributed to the translocation of these AAs to aerial tissues, as previously reported in *Camellia sinensis* (Hijaz et al., 2018). Interestingly, Gln was positively correlated with GABA, Gly, and Serine in roots, suggesting that enhanced Gln biosynthesis under salt stress enhance precursors for producing other AAs. Notably, the interaction between AA metabolism and the TCA cycle may contribute to enhanced salt tolerance, as Ali et al. (2019) suggested, providing a metabolic basis for the coordinated changes observed in our study.

5 Conclusions

This study demonstrates that *Populus nigra* × *maximowiczii* (clone NM6) exhibits adaptive physiological and metabolic responses under salt stress, particularly when treated with exogenous Put. Under 100 mM NaCl: a level that reflects marginal salinity; Put-treated plants showed higher growth, enhanced conductivity and transpiration rates, changes in soluble protein, sugars, and AAs level. These results suggest improved osmotic adjustment and metabolic stability, contributing to greater salt stress resilience in NM6 poplar. With these results under moderate salinity, NM6 poplar shows promise as an alternative species for marginally saline soils, mainly when supported by low-cost, non-transgenic interventions such as Put application. Due to the unique role of deep-rooting system, soil-binding capacity, and faster growth, NM6 offers significant potential for land restoration, sustainable silviculture, and bioenergy production in marginal sandy land. With the advent of newer technologies and help of artificial intelligence, integrating physiological treatments with genetic improvements could further enhance their role in saline land management in future.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contribution

SK, RM and SC conceived and designed the experiment. SK, MW, MG, RM, SL collected the samples. RM supervised and provided resources for HPLC. SK, SL, analyzed the data. SK wrote the manuscript. SM and RM supervised the results and analysis. SM supervised and edited the draft manuscript.

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Generative AI statement

The author(s) declare that some Generative AI was used for checking grammatical statement for the creation of this manuscript.

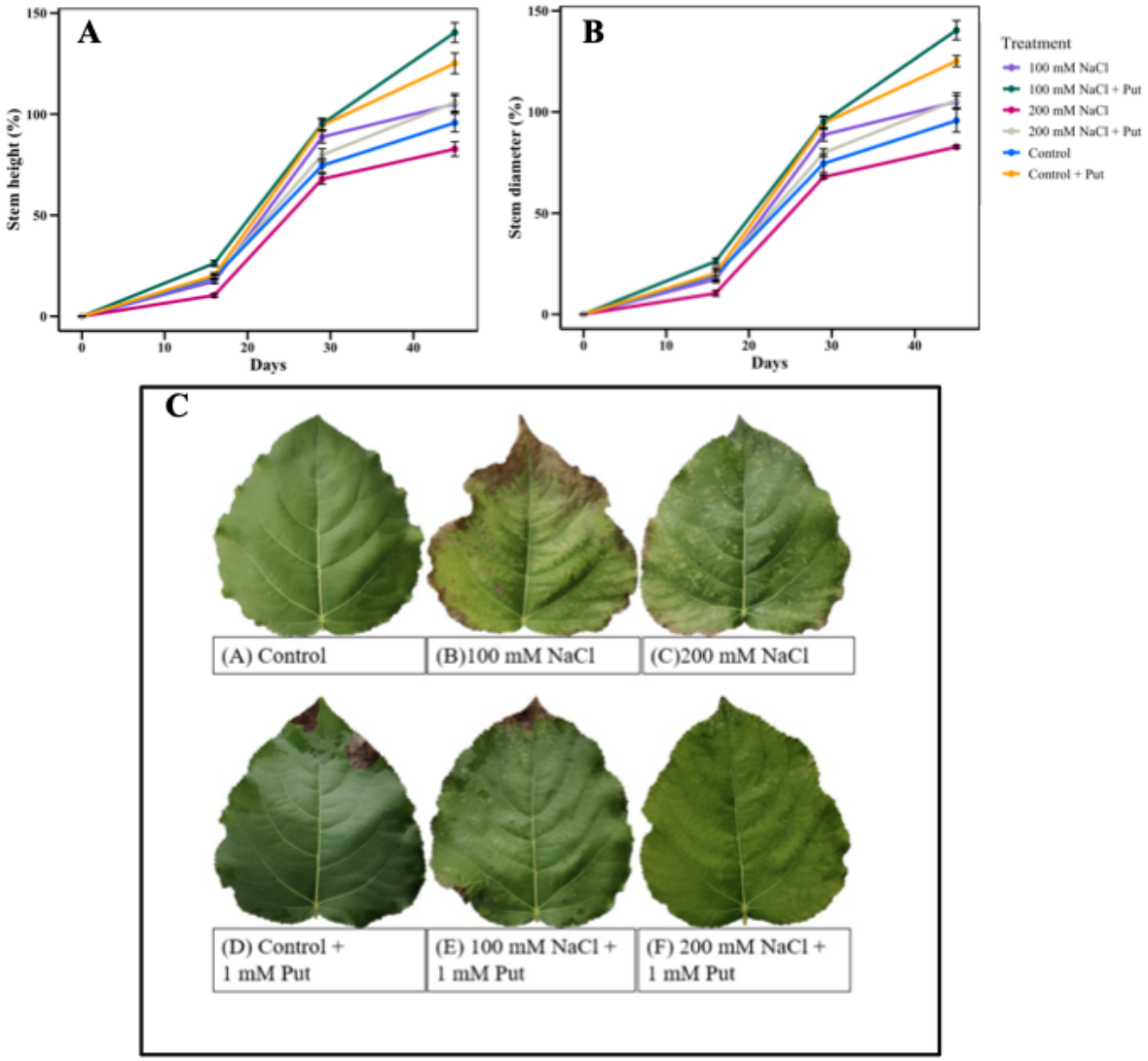


Figure 1: The effect of two different concentrations of NaCl (± Putrescine spray) on the growth and morphology of hybrid poplar NM6 plants. (A, B) Average stem length and stem diameter changes over 45 days of salt treatment. (C) Morphological symptoms of leaves under different treatments**:** (A) Control, (B) 100 mM NaCl, (C) 200 mM NaCl, (D) Control + 1 mM Put, (E) 100 mM NaCl + 1 mM Put, (F) 200 mM NaCl + 1 mM Put. Data represent mean ± SE (n = 6).

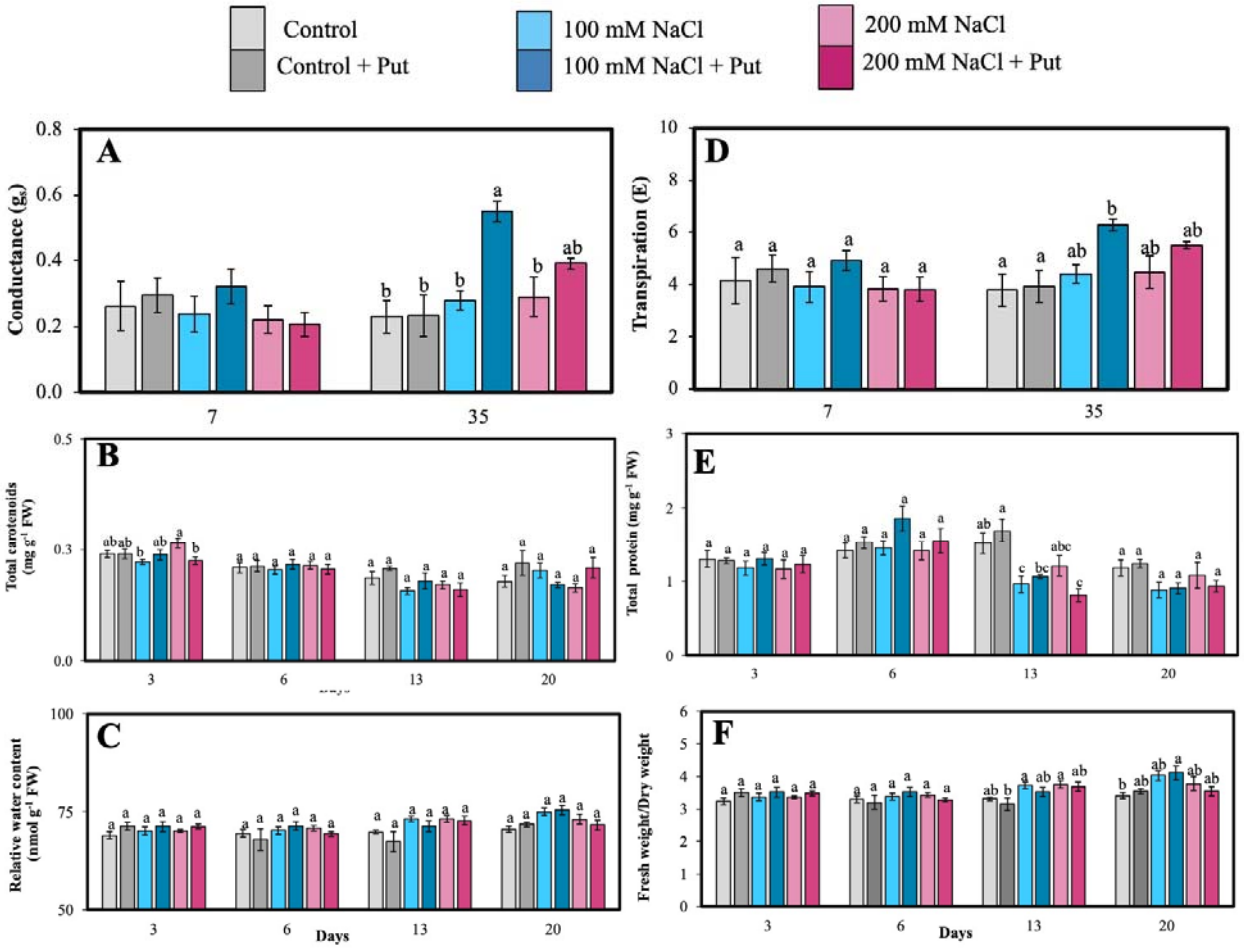


Figure 2. Effect of two different concentrations of NaCl (± Putrescine spray) on physiological accumulation in hybrid poplar NM6 leaves over time. (**A**) Conductance, (**B**) Carotenoid, (**C**) Relative water content, (**D**) Transpiration, (**E**) Protein, and **(F)** Fresh weight/dry weight measured at several days after salt treatment. Different letters indicate statistically significant differences ($p < 0.05$) among treatments. Data represent mean ± SE (n = 3, 5).

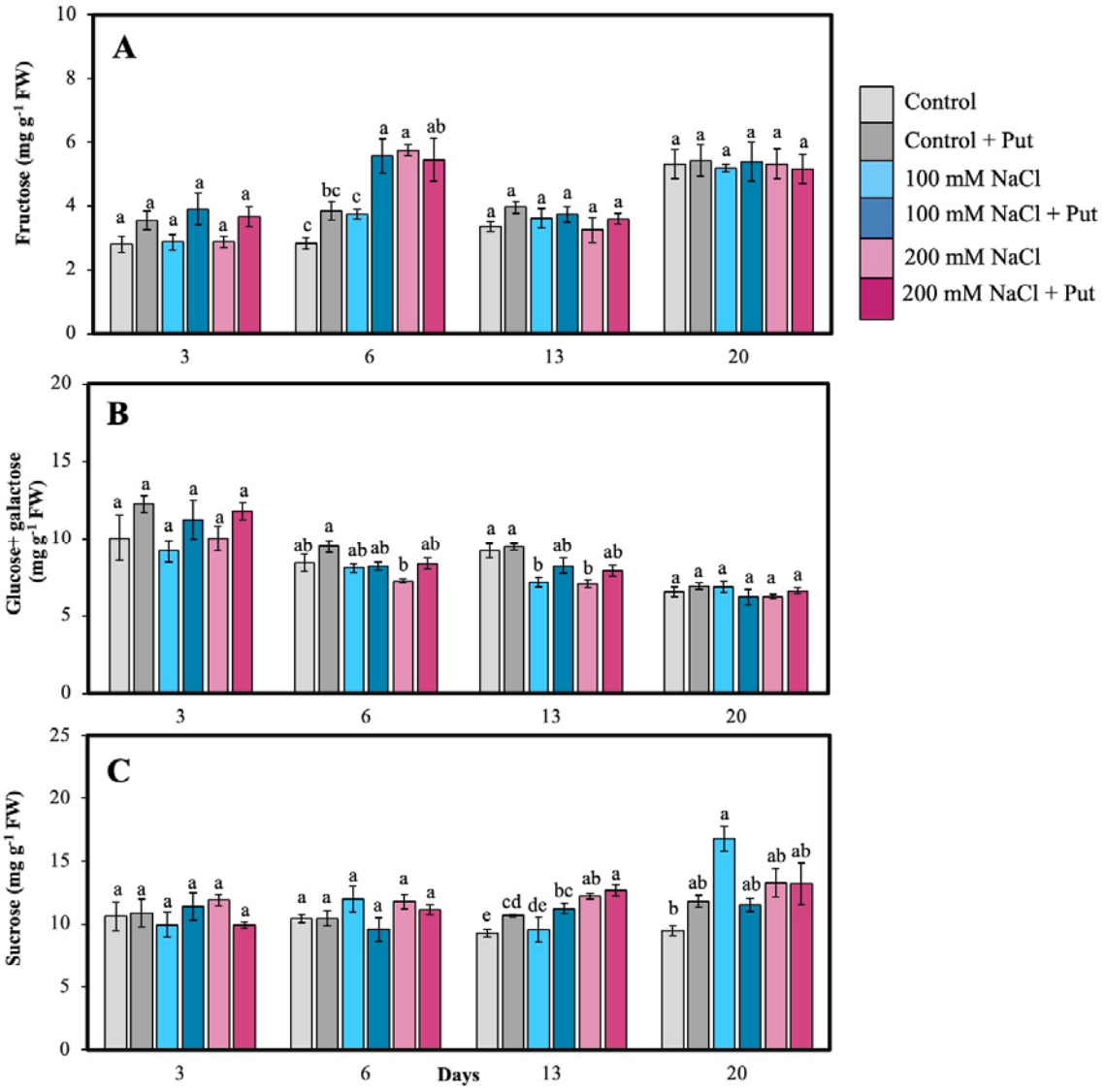


Figure 3: Effect of two different concentrations of NaCl (± Putrescine spray) on soluble sugar accumulation in hybrid poplar NM6 leaves over time. (**A**) Fructose, (**B**) Glucose + Galactose, and (**C**)Sucrose measured at 3, 6, 13, and 20 days after salt treatment. Different letters indicate statistically significant differences ($p < 0.05$) among treatments. Data represent mean ± SE (n = 5).

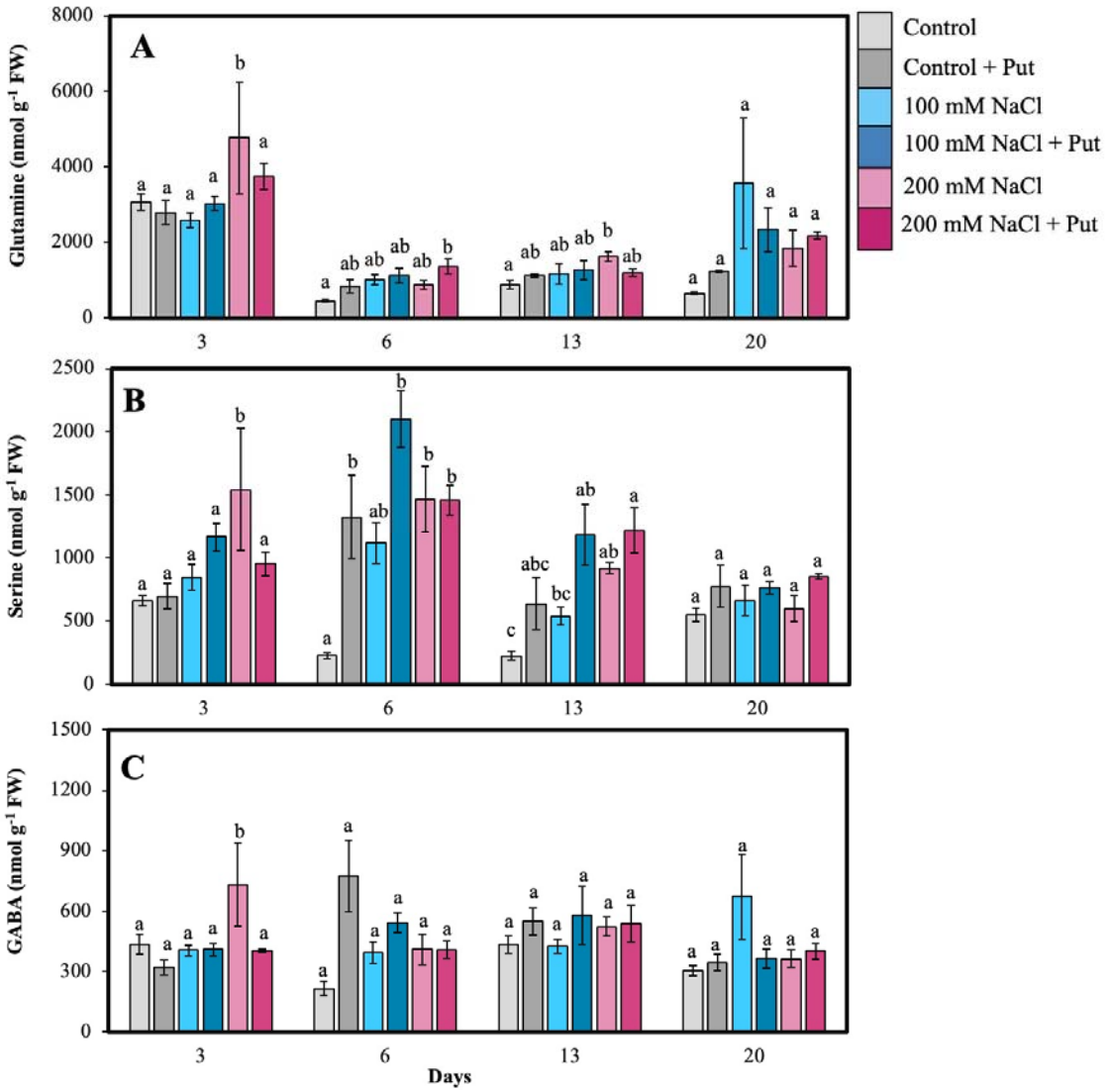


Figure 4: Effect of two different concentrations of NaCl (± Putrescine spray) on amino acids accumulation in hybrid poplar NM6 leaves over time. (**A**) Glutamine, (**B**) Serine, and (**C**) GABA measured at 3, 6, 13, and 20 days after salt treatment. Different letters indicate statistically significant differences ($p < 0.05$) among treatments. Data represent mean ± SE ($n = 5$).

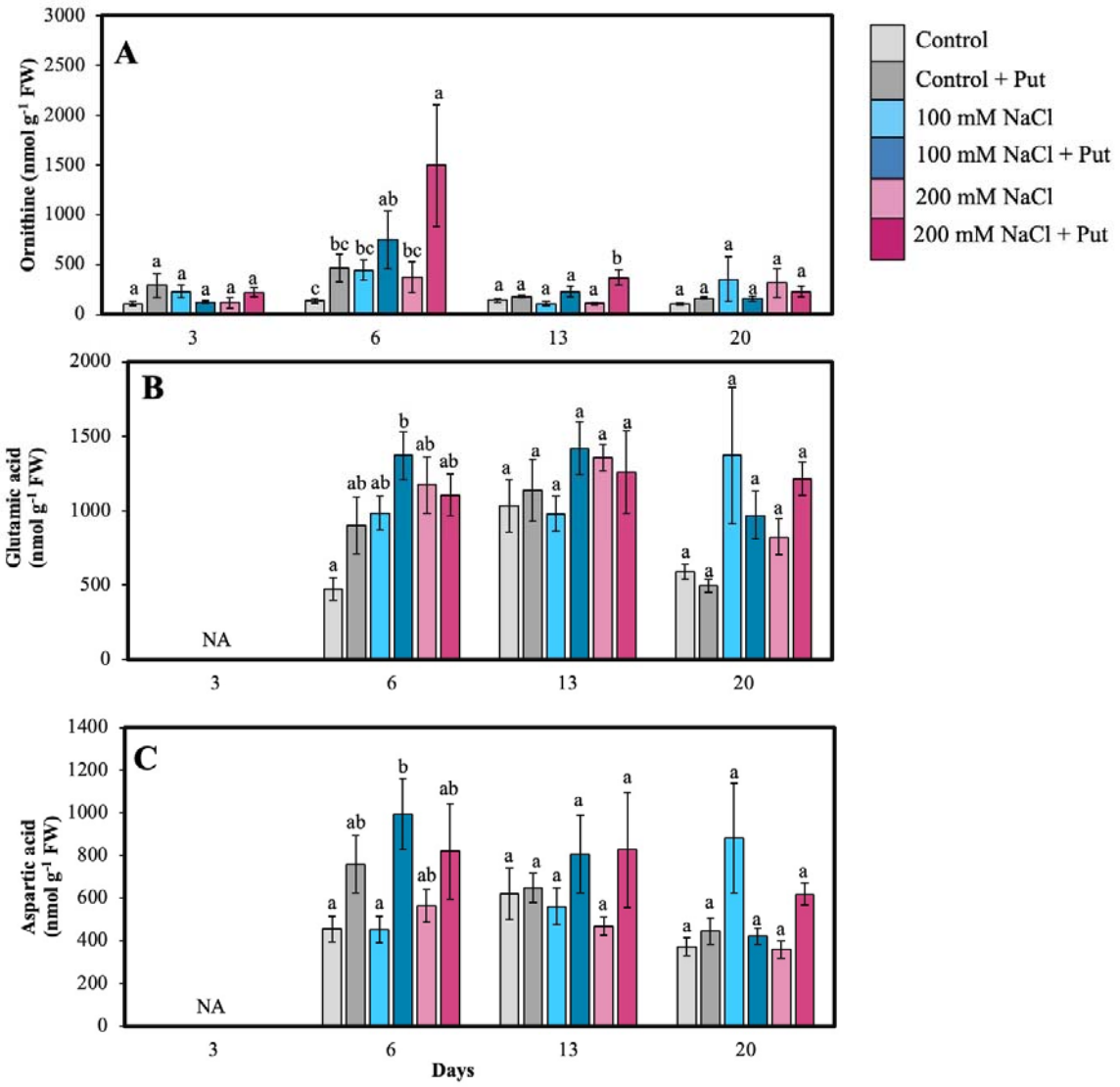


Figure 5: Effect of two different concentrations of NaCl (± Putrescine spray) on amino acids and polyamines accumulation in hybrid poplar NM6 leaves over time. (**A**) Ornithine levels, (**B**) Log Glutamic acid levels, and (**C**) Aspartic acid levels measured at 3, 6, 13, and 20 days after salt treatment. Different letters indicate statistically significant differences ($p < 0.05$) among treatments. Data represent mean ± SE (n = 5).

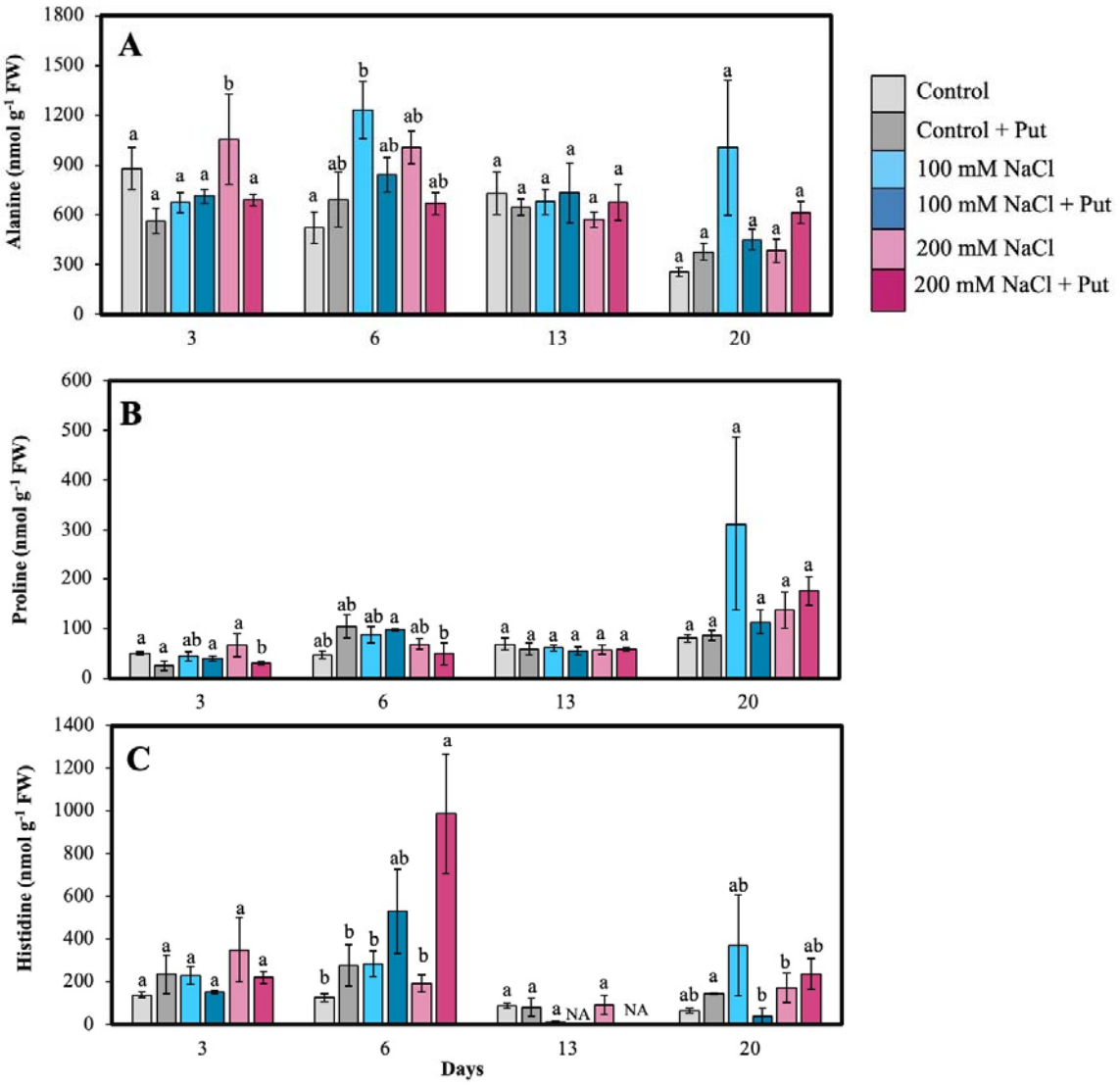


Figure 6: Effect of two different concentrations of NaCl (± Putrescine spray) on amino acids and polyamines accumulation in hybrid poplar NM6 leaves over time. (**A**) Alanine levels, (**B**) Proline levels, and (**C**) Histidine levels measured at 3, 6, 13, and 20 days after salt treatment. Different letters indicate statistically significant differences ($p < 0.05$) among treatments. Data represent mean ± SE (n = 5).

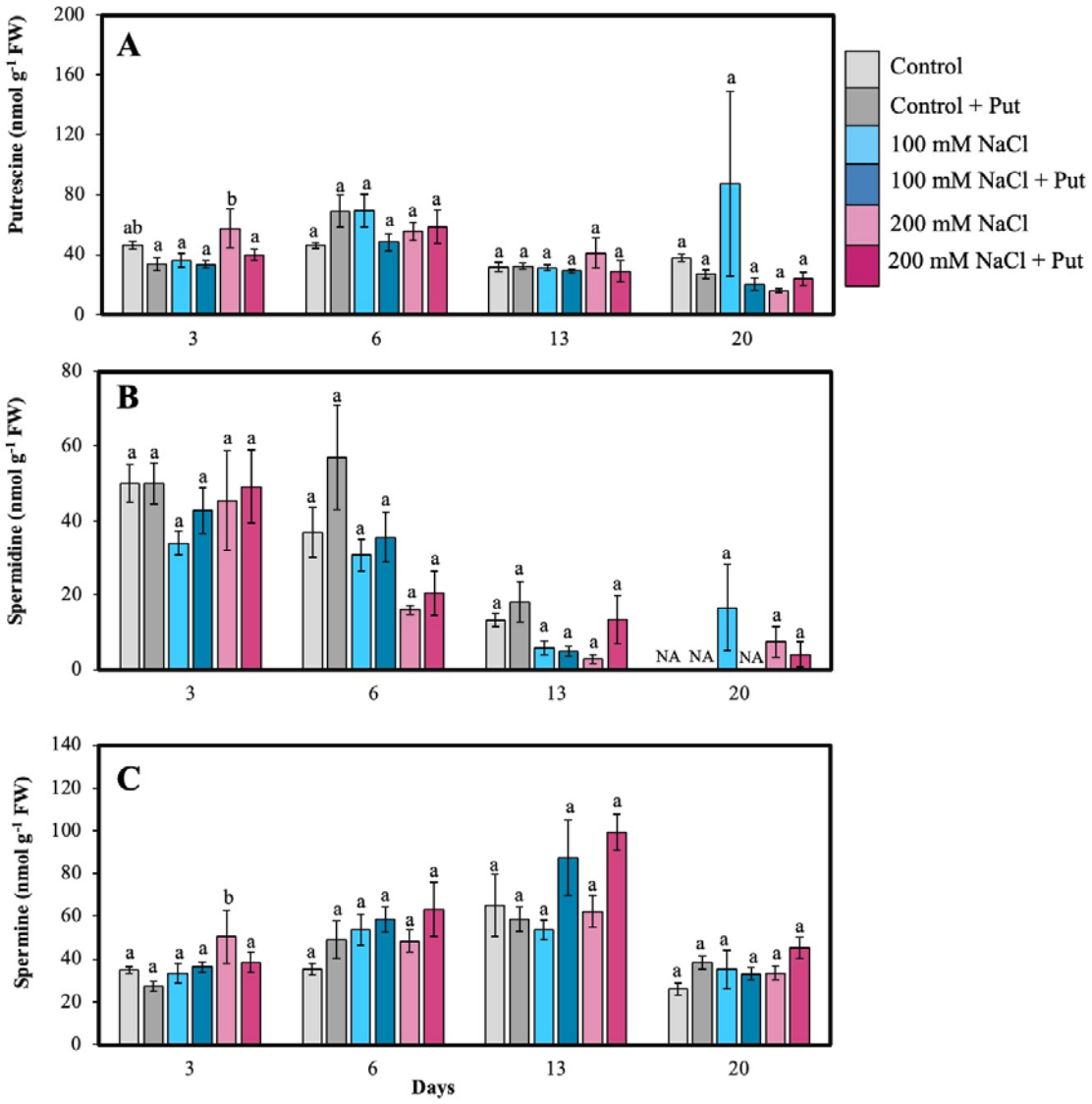


Figure 7: Effect of two different concentrations of NaCl (± Putrescine spray) on amino acids and polyamines accumulation in hybrid poplar NM6 leaves over time. (**A**) Putrescine levels, (**B**) Spermidine levels, and (**C**) Spermine levels measured at 3, 6, 13, and 20 days after salt treatment. Different letters indicate statistically significant differences ($p < 0.05$) among treatments. Data represent mean ± SE (n = 5).

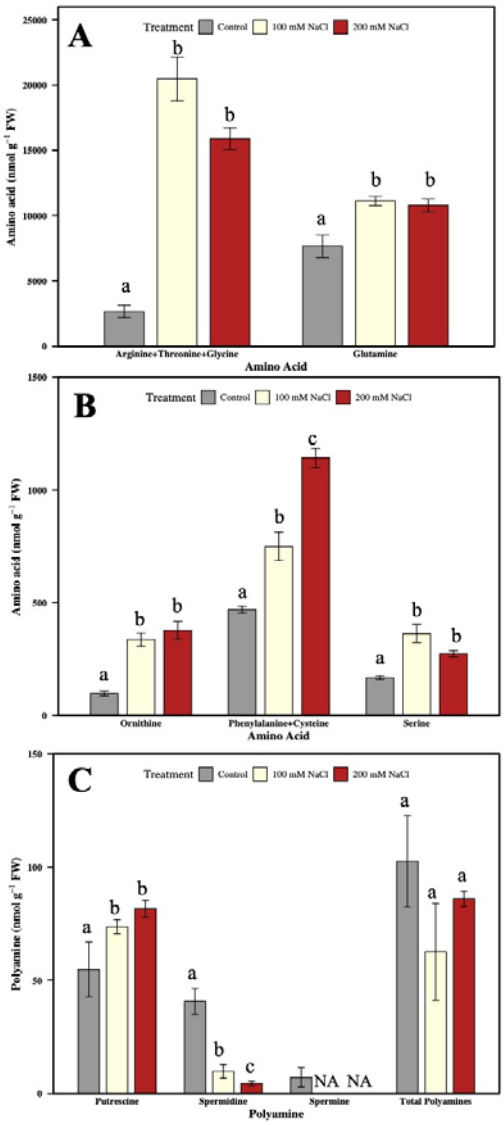


Figure 8: Effect of two different concentrations of NaCl on amino acids and polyamines accumulation in hybrid poplar NM6 roots. (**A, B**) Amino acids levels, (**C**) Polyamines levels measured at 21 days after salt treatment. Different letters indicate statistically significant differences ($p < 0.05$) among treatments. Data represent mean ± SE ($n = 4$).

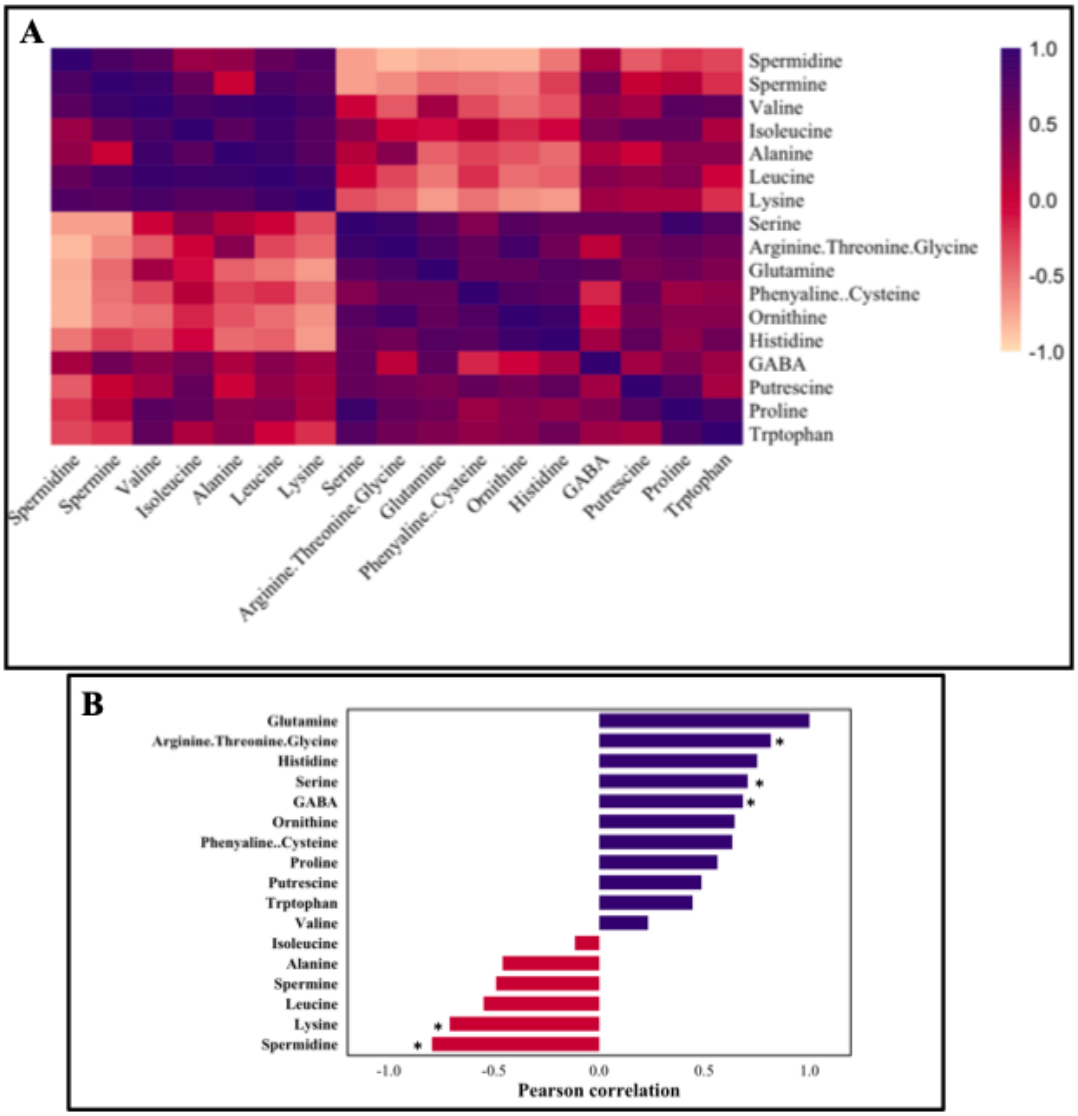
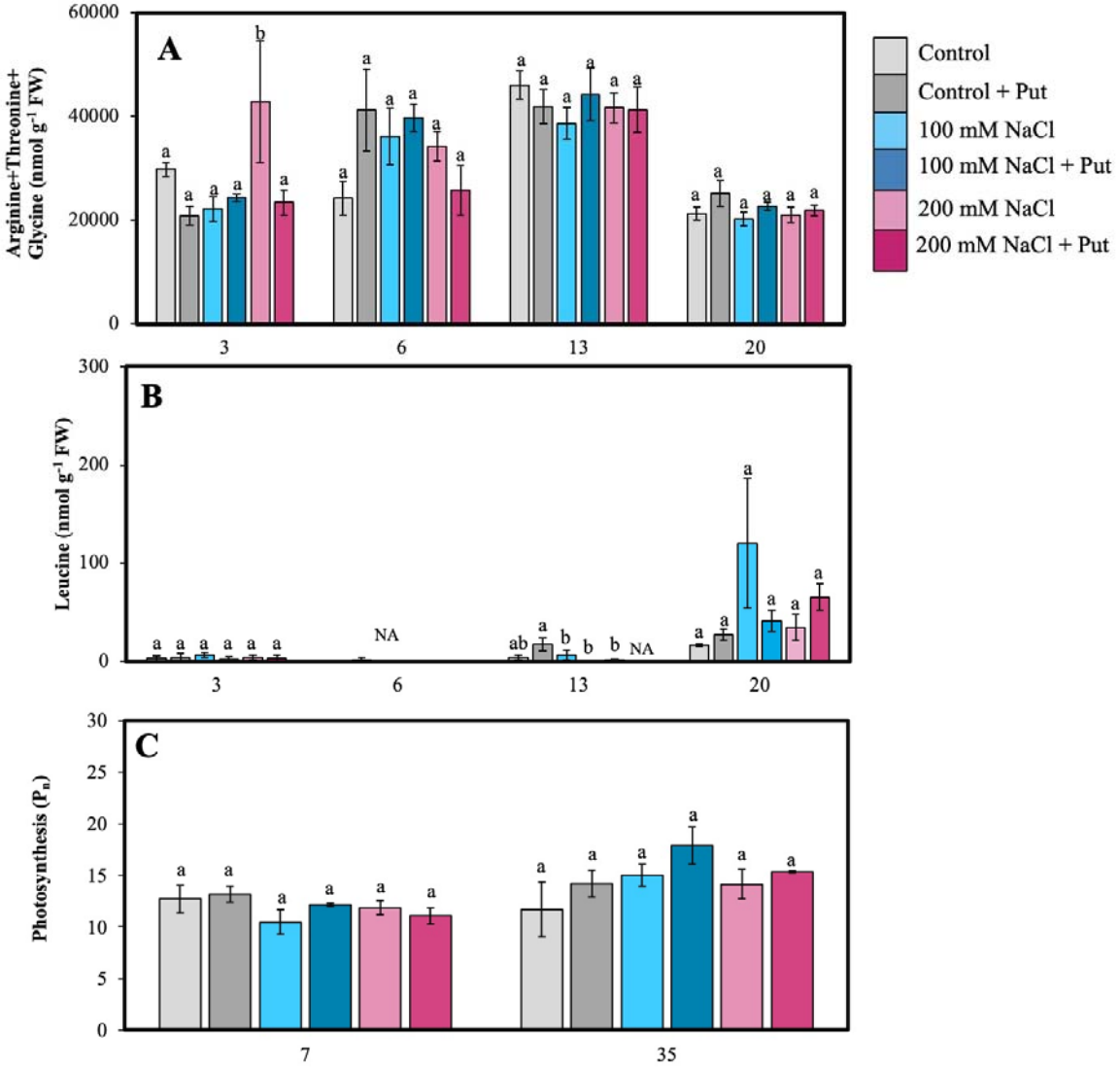
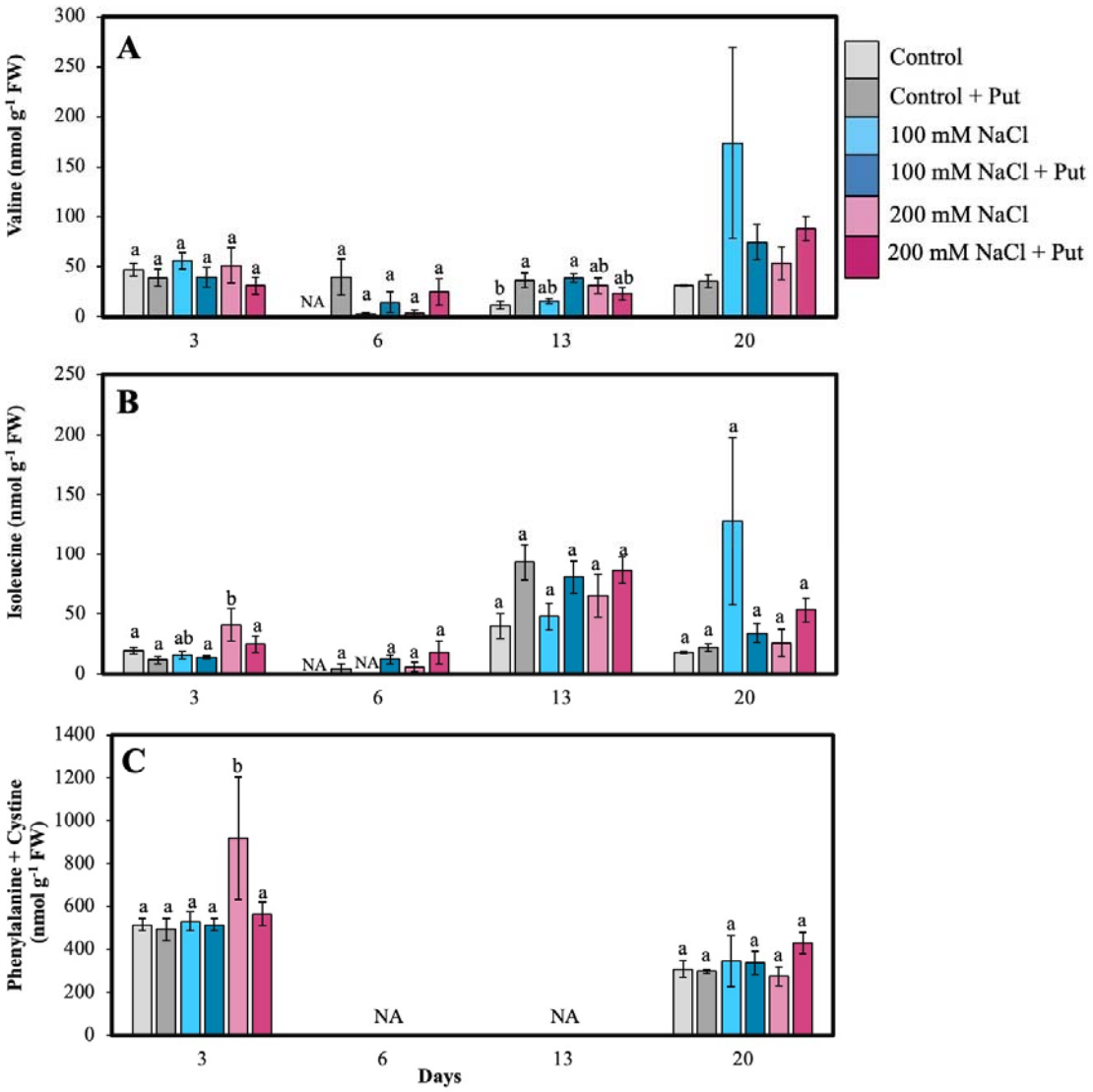


Figure 9. (A) Correlation coefficients of amino acids and polyamines analyzed in the poplar roots treated with two different concentrations of salt. (B) Pattern correlation analysis with Glutamine. The graph reports the significant features detected and ordered according to their correlation coefficient when correlated with Glutamine. The purple color represents a positive correlation, and the red color represents a negative correlation. Correlation distance- Pearson r. Correlation significant at *$p < 0.05$.

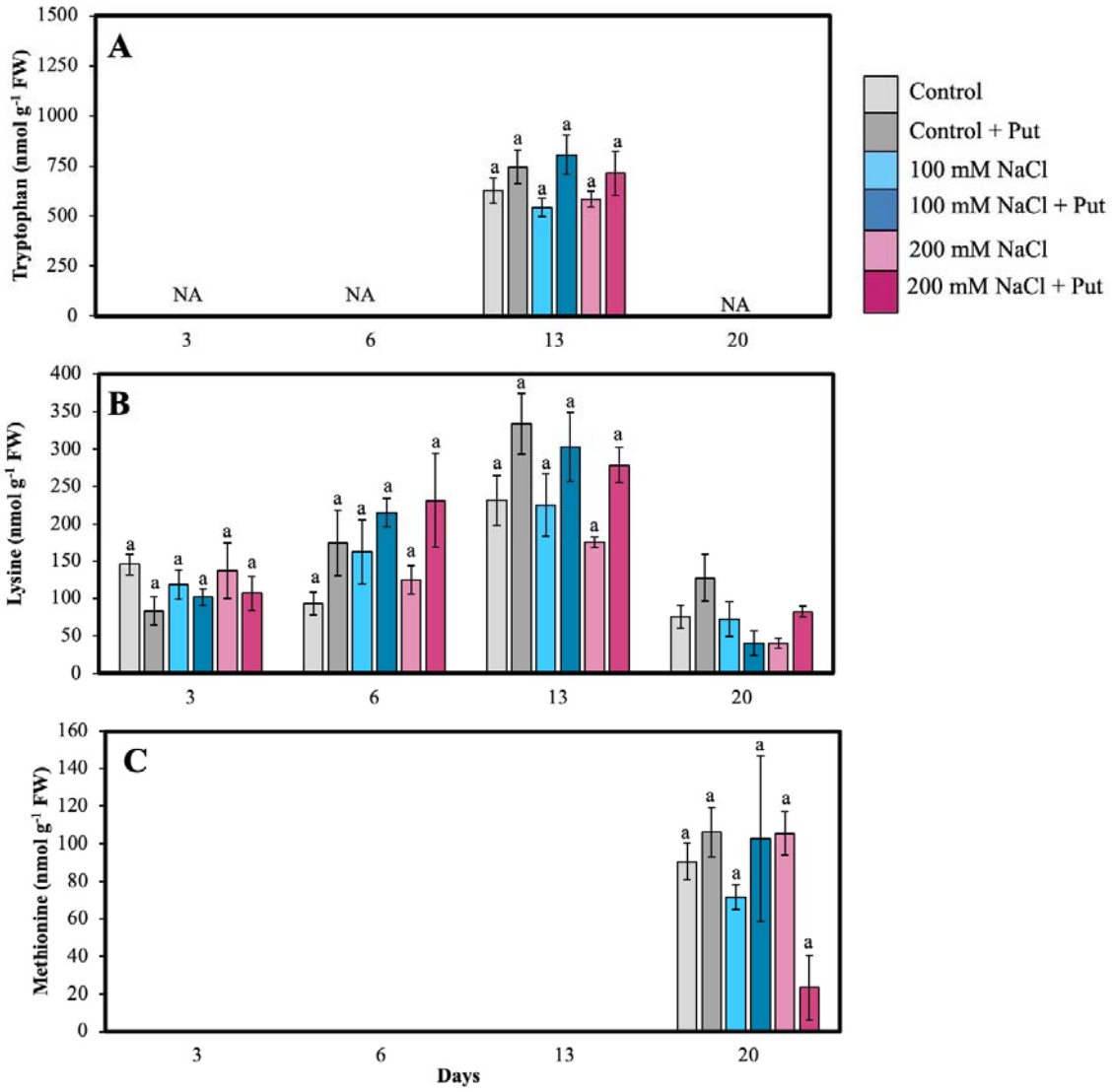
Supplementary Figures



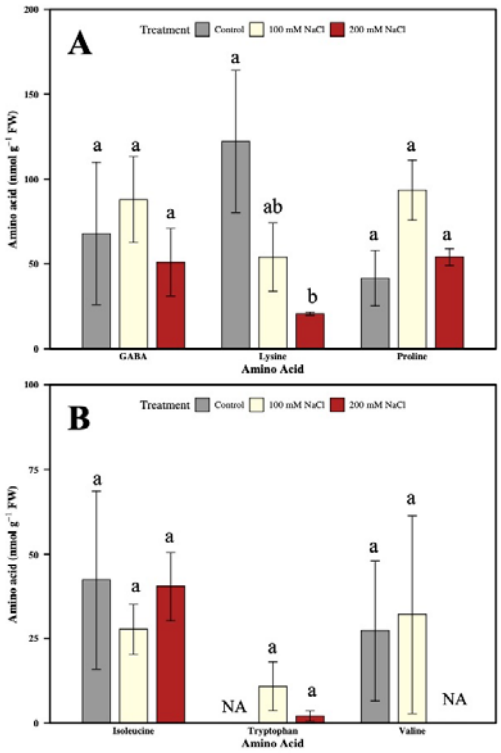
Supplementary Figure 1: Effect of two different concentrations of NaCl (± Putrescine spray) on amino acids and photosynthesis accumulation in hybrid poplar NM6 leaves over time. (**A**) Arginine+Threonine+Glycine levels, (**B**) Leucine levels, and (**C**) Photosynthesis levels measured at several days after salt treatment. Different letters indicate statistically significant differences ($p < 0.05$) among treatments. Data represent mean ± SE (n = 5).



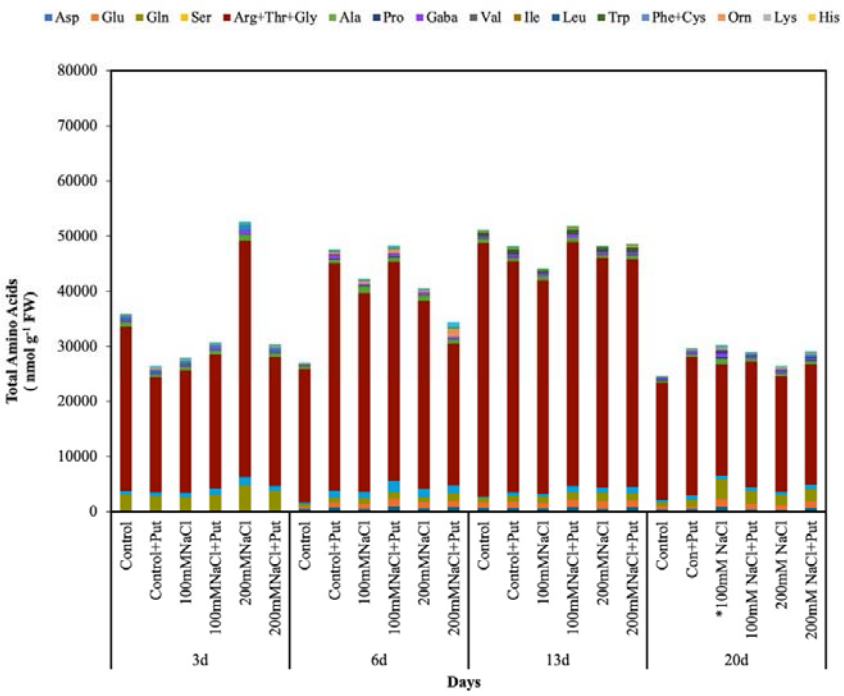
Supplementary Figure 2: Effect of two different concentrations of NaCl (± Putrescine spray) on amino acids and polyamines accumulation in hybrid poplar NM6 leaves over time. (**A**) Valine levels, and (**B**) Isoleucine levels, and **(C)** Phenylalanine+Cystine levels measured at several days after salt treatment. Different letters indicate statistically significant differences ($p < 0.05$) among treatments. Data represent mean ± SE (n = 5).



Supplementary Figure 3: Effect of two different concentrations of NaCl (± Putrescine spray) on amino acids and polyamines accumulation in hybrid poplar NM6 leaves over time. (**A**) Tryptophan levels, (**B**) Lysine levels, and (**C**) Methionine levels measured at several days after salt treatment. Different letters indicate statistically significant differences ($p < 0.05$) among treatments. Data represent mean ± SE ($n = 5$).



Supplementary Figure 4: Effect of two different concentrations of NaCl (± Putrescine spray) on amino acids accumulation in hybrid poplar NM6 roots over time. (**A**) GABA, Lysine, Proline levels, and (**B**) Isoleucine, Tryptophan, Valine levels, measured on day 21 after salt treatment. Different letters indicate statistically significant differences ($p < 0.05$) among treatments. Data represent mean ± SE (n = 4).



Supplementary Figure 5: Effect of two concentrations of NaCl (± Putrescine spray) on total amino acid accumulation in hybrid poplar NM6 leaves over time. Stacked bars show the concentrations (nmol g□¹ FW) of individual amino acids measured at 3, 6, 13, and 20 days after salt treatment.

References

ALCÁZAR, R., ALTABELLA, T., MARCO, F., BORTOLOTTI, C., REYMOND, M., KONCZ, C., CARRASCO, P. & TIBURCIO, A. F. 2010. Polyamines: molecules with regulatory functions in plant abiotic stress tolerance. *Planta,* 231**,** 1237-1249.

ALCÁZAR, R., BUENO, M. & TIBURCIO, A. F. 2020. Polyamines: Small amines with large effects on plant abiotic stress tolerance. *Cells,* 9**,** 2373.

ALCÁZAR, R., MARCO, F., CUEVAS, J. C., PATRON, M., FERRANDO, A., CARRASCO, P., TIBURCIO, A. F. & ALTABELLA, T. 2006. Involvement of polyamines in plant response to abiotic stress. *Biotechnology letters,* 28**,** 1867-1876.

AMIOUR, N., IMBAUD, S., CLÉMENT, G., AGIER, N., ZIVY, M., VALOT, B., BALLIAU, T., ARMENGAUD, P., QUILLERÉ, I. & CAÑAS, R. 2012. The use of metabolomics integrated with transcriptomic and proteomic studies for identifying key steps involved in the control of nitrogen metabolism in crops such as maize. *Journal of Experimental Botany,* 63**,** 5017-5033.

ANTONIOU, C., ZARZA, X., GOHARI, G., PANAHIRAD, S., FILIPPOU, P., TIBURCIO, A. F. & FOTOPOULOS, V. 2021. Involvement of polyamine metabolism in the response of Medicago truncatula genotypes to salt stress. *Plants,* 10**,** 269.

ATABAYEVA, S., MINOCHA, R., MINOCHA, S., RAKHYMGOZHINA, A., NABIEVA, A., NURMAHANOVA, A., ҚENZHEBAYEVA, S., ALYBAYEVA, R. & ASRANDINA, S. 2020. Response of plants to cadmium stress. *International Journal of Biology and Chemistry,* 13**,** 109-117.

AUGUSTYNIAK, B., WOJTASIK, W., SAWUŁA, A., BURGBERGER, M. & KULMA, A. 2025. Spermidine treatment limits the development of the fungus in flax shoots by suppressing polyamine metabolism and balanced defence reactions, thus increasing flax resistance to fusariosis. *Frontiers in Plant Science,* 16**,** 1561203.

AZEEM, M., PIRJAN, K., QASIM, M., MAHMOOD, A., JAVED, T., MUHAMMAD, H., YANG, S., DONG, R., ALI, B. & RAHIMI, M. 2023. Salinity stress improves antioxidant potential by modulating physio-biochemical responses in Moringa oleifera Lam. *Scientific Reports,* 13**,** 1-17.

AZIMI, S., KAUR, T. & GANDHI, T. K. 2021. A deep learning approach to measure stress level in plants due to Nitrogen deficiency. *Measurement,* 173**,** 108650.

BANIASADI, F., SAFFARI, V. R. & MOUD, A. A. M. 2018. Physiological and growth responses of Calendula officinalis L. plants to the interaction effects of polyamines and salt stress. *Scientia Horticulturae,* 234**,** 312-317.

BARGMANN, B. O., LAXALT, A. M., RIET, B. T., VAN SCHOOTEN, B., MERQUIOL, E., TESTERINK, C., HARING, M. A., BARTELS, D. & MUNNIK, T. 2009. Multiple PLDs required for high salinity and water deficit tolerance in plants. *Plant and Cell Physiology,* 50**,** 78-89.

BLAGDEN, M., HARRISON, J. L., MINOCHA, R., SANDERS-DEMOTT, R., LONG, S. & TEMPLER, P. H. 2022. Climate change influences foliar nutrition and metabolism of red maple (Acer rubrum) trees in a northern hardwood forest. *Ecosphere,* 13**,** e03859.

BLÁZQUEZ, M. A. 2024. Polyamines: Their role in plant development and stress. *Annual review of plant biology,* 75.

BORROMEO, I., DOMENICI, F., DEL GALLO, M. & FORNI, C. 2023. Role of polyamines in the response to salt stress of tomato. *Plants,* 12**,** 1855.

BUELL, C. R., DARDICK, C., PARROTT, W., SCHMITZ, R. J., SHIH, P. M., TSAI, C.-J. & URBANOWICZ, B. 2023. Engineering custom morpho-and chemotypes of Populus for sustainable production of biofuels, bioproducts, and biomaterials. *Frontiers in Plant Science,* 14**,** 1288826.

CALHOUN, S., KAMEL, B., EDMUNDSON, S., HOLGUIN, O., MACH, P., MCKIE-KRISBERG, Z., BAUMGART, L., BLABY, I., BOWEN, B. & CHEN, C. 2023. A Multi-omic Characterization of the Physiological Responses to Salt Stress in Scenedesmus obliquus UTEX393.

CAO, J.-F., HUANG, J.-Q., LIU, X., HUANG, C.-C., ZHENG, Z.-S., ZHANG, X.-F., SHANGGUAN, X.-X., WANG, L.-J., ZHANG, Y.-G. & WENDEL, J. F. 2020. Genome-wide characterization of the GRF family and their roles in response to salt stress in Gossypium. *BMC genomics,* 21**,** 1-16.

CHAU, T. N., TIMILSENA, P. R., BATHALA, S. P., KUNDU, S., BARGMANN, B. O. & LI, S. 2025. Orthologous marker groups reveal broad cell identity conservation across plant single-cell transcriptomes. *Nature Communications,* 16**,** 201.

CHEN, Y., TONG, S., JIANG, Y., AI, F., FENG, Y., ZHANG, J., GONG, J., QIN, J., ZHANG, Y. & ZHU, Y. 2021. Transcriptional landscape of highly lignified poplar stems at single-cell resolution. *Genome biology,* 22**,** 1-22.

CHOUDHARY, S., WANI, K. I., NAEEM, M., KHAN, M. M. A. & AFTAB, T. 2023. Cellular responses, osmotic adjustments, and role of osmolytes in providing salt stress resilience in higher plants: polyamines and nitric oxide crosstalk. *Journal of Plant Growth Regulation,* 42**,** 539-553.

CONDE, D., TRIOZZI, P. M., PEREIRA, W. J., SCHMIDT, H. W., BALMANT, K. M., KNAACK, S. A., REDONDO-LÓPEZ, A., ROY, S., DERVINIS, C. & KIRST, M. 2022. Single-nuclei transcriptome analysis of the shoot apex vascular system differentiation in Populus. *Development,* 149**,** dev200632.

CRAMER, G. R., URANO, K., DELROT, S., PEZZOTTI, M. & SHINOZAKI, K. 2011. Effects of abiotic stress on plants: a systems biology perspective. *BMC plant biology,* 11**,** 1-14.

DE OLIVEIRA, A. B., ALENCAR, N. L. M. & GOMES-FILHO, E. 2013. Comparison between the water and salt stress effects on plant growth and development. *Responses of organisms to water stress,* 4**,** 67-94.

DICKMANN, D. 2001. *Poplar culture in north America*, NRC Research Press.

DING, M., HOU, P., SHEN, X., WANG, M., DENG, S., SUN, J., XIAO, F., WANG, R., ZHOU, X. & LU, C. 2010. Salt-induced expression of genes related to Na+/K+ and ROS homeostasis in leaves of salt-resistant and salt-sensitive poplar species. *Plant molecular biology,* 73**,** 251-269.

DINNENY, J. R. 2015. Traversing organizational scales in plant salt-stress responses. *Current Opinion in Plant Biology,* 23**,** 70-75.

EISA, E. A., HONFI, P., TILLY-MÁNDY, A. & MIRMAZLOUM, I. 2023. Exogenous Melatonin Application Induced Morpho-Physiological and Biochemical Regulations Conferring Salt Tolerance in Ranunculus asiaticus L. *Horticulturae,* 9**,** 228.

ELSAYED, A. I., MOHAMED, A. H., RAFUDEEN, M. S., OMAR, A. A., AWAD, M. F. & MANSOUR, E. 2022. Polyamines mitigate the destructive impacts of salinity stress by enhancing photosynthetic capacity, antioxidant defense system and upregulation of calvin cycle-related genes in rapeseed (Brassica napus L.). *Saudi Journal of Biological Sciences,* 29**,** 3675-3686.

GEORGII, E., KUGLER, K., PFEIFER, M., VANZO, E., BLOCK, K., DOMAGALSKA, M. A., JUD, W., ABDELGAWAD, H., ASARD, H. & REINHARDT, R. 2019. The systems architecture of molecular memory in poplar after abiotic stress. *The Plant Cell,* 31**,** 346-367.

GHALATI, R. E., SHAMILI, M. & HOMAEI, A. 2020. Effect of putrescine on biochemical and physiological characteristics of guava (Psidium guajava L.) seedlings under salt stress. *Scientia Horticulturae,* 261**,** 108961.

GHEZEHEI, S. B., EWALD, A. L., HAZEL, D. W., ZALESNY JR, R. S. & NICHOLS, E. G. 2021. Productivity and profitability of poplars on fertile and marginal sandy soils under different density and fertilization treatments. *Forests,* 12**,** 869.

GOMEZ-SOTO, D., PEREIRA, W. J., PIEDRABUENA-DIAZ, A., DERVINIS, C., KIRST, M., ALLONA, I., PERALES, M. & CONDE, D. 2025. Single-nuclei transcriptomics revealed auxin-driven mechanisms of wood plasticity and severe drought tolerance in poplar. *bioRxiv***,** 2025.01. 08.631930.

GUERRA, F., GAINZA, F., PÉREZ, R. & ZAMUDIO, F. 2011. Phytoremediation of heavy metals using poplars (Populus spp.): a glimpse of the plant responses to copper, cadmium and zinc stress. *Handbook of phytoremediation. Nova Science, New York***,** 387-413.

GUPTA, B. & HUANG, B. 2014. Mechanism of salinity tolerance in plants: physiological, biochemical, and molecular characterization. *International journal of genomics,* 2014.

GUPTA, K., DEY, A. & GUPTA, B. 2013. Plant polyamines in abiotic stress responses. *Acta physiologiae plantarum,* 35**,** 2015-2036.

HAAK, D. C., FUKAO, T., GRENE, R., HUA, Z., IVANOV, R., PERRELLA, G. & LI, S. 2017. Multilevel regulation of abiotic stress responses in plants. *Frontiers in plant science,* 8**,** 1564.

HANSON, A. D., BEAUDOIN, G. A., MCCARTY, D. R. & GREGORY III, J. F. 2016. Does abiotic stress cause functional B vitamin deficiency in plants? *Plant Physiology,* 172**,** 2082-2097.

HASANUZZAMAN, M., NAHAR, K. & FUJITA, M. 2013. Plant response to salt stress and role of exogenous protectants to mitigate salt-induced damages. *Ecophysiology and responses of plants under salt stress***,** 25-87.

HAWKINS, S., LEPLÉ, J.-C., CORNU, D., JOUANIN, L. & PILATE, G. 2003. Stability of transgene expression in poplar: a model forest tree species. *Annals of forest science,* 60**,** 427-438.

HIJAZ, F., NEHELA, Y. & KILLINY, N. 2018. Application of gamma-aminobutyric acid increased the level of phytohormones in Citrus sinensis. *Planta,* 248**,** 909-918.

HU, J., ZOU, S., HUANG, J., HUAN, X., JIN, X., ZHOU, L., ZHAO, K., HAN, Y. & WANG, S. 2023. PagMYB151 facilitates proline accumulation to enhance salt tolerance of poplar. *BMC genomics,* 24**,** 345.

HUO, L., GUO, Z., WANG, P., ZHANG, Z., JIA, X., SUN, Y., SUN, X., GONG, X. & MA, F. 2020. MdATG8i functions positively in apple salt tolerance by maintaining photosynthetic ability and increasing the accumulation of arginine and polyamines. *Environmental and Experimental Botany,* 172**,** 103989.

IMRAN, M., AAQIL KHAN, M., SHAHZAD, R., BILAL, S., KHAN, M., YUN, B.-W., KHAN, A. L. & LEE, I.-J. 2021. Melatonin ameliorates thermotolerance in soybean seedling through balancing redox homeostasis and modulating antioxidant defense, phytohormones and polyamines biosynthesis. *Molecules,* 26**,** 5116.

ISLAM, M. J., RYU, B. R., AZAD, M. O. K., RAHMAN, M. H., RANA, M. S., LIM, J.-D. & LIM, Y.-S. 2021. Exogenous putrescine enhances salt tolerance and ginsenosides content in Korean ginseng (Panax ginseng Meyer) sprouts. *Plants,* 10**,** 1313.

KUMAR, A., BEHERA, I., LANGTHASA, M. & PRAKASHNAROJU, S. 2023. Effect of plant growth-promoting rhizobacteria on alleviating salinity stress in plants: A review. *Journal of Plant Nutrition,* 46**,** 2525-2550.

KUNDU, S. 2023. *Physiological and Biochemical Responses to Salt and Cadmium Stress and It's Amelioration by Exogenous Application of Polyamines in Hybrid Poplar (Populus n igra x maximowiczii, Clone NM6)*, University of New Hampshire.

LABRECQUE, M. & TEODORESCU, T. I. 2005. Field performance and biomass production of 12 willow and poplar clones in short-rotation coppice in southern Quebec (Canada). *Biomass and Bioenergy,* 29**,** 1-9.

LI, M., PETRIE, M. D., TARIQ, A. & ZENG, F. 2021. Response of nodulation, nitrogen fixation to salt stress in a desert legume Alhagi sparsifolia. *Environmental and Experimental Botany,* 183**,** 104348.

LI, P., LIU, Q., WEI, Y., XING, C., XU, Z., DING, F., LIU, Y., LU, Q., HU, N. & WANG, T. 2024. Transcriptional landscape of cotton roots in response to salt stress at single-cell resolution. *Plant Communications,* 5.

LIANG, W., MA, X., WAN, P. & LIU, L. 2018. Plant salt-tolerance mechanism: A review. *Biochemical and biophysical research communications,* 495**,** 286-291.

LICHTENTHALER, H. K. 1987. [34] Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. *Methods in enzymology.* Elsevier.

LIN, T., LU, Q., ZHENG, Z., LI, S., LI, S., LIU, Y., ZHU, T., CHEN, L., YANG, C. & HAN, S. 2023. Soil cadmium stress affects the phyllosphere microbiome and associated pathogen resistance differently in male and female poplars. *Journal of Experimental Botany***,** erad034.

LIU, H., TODD, J. L. & LUO, H. 2023. Turfgrass Salinity Stress and Tolerance—A Review. *Plants,* 12**,** 925.

LIU, L., CAO, X., ZHAI, Z., MA, S., TIAN, Y. & CHENG, J. 2022. Direct evidence of drought stress memory in mulberry from a physiological perspective: Antioxidative, osmotic and phytohormonal regulations. *Plant Physiology and Biochemistry,* 186**,** 76-87.

LIU, Q., KANG, J., DU, L., LIU, Z., LIANG, H., WANG, K., HE, H., ZHANG, X., WANG, Q. & HONG, Y. Single-cell multiome reveals root hair-specific responses to salt stress. *New Phytologist*.

LIU, Q., KANG, J., DU, L., LIU, Z., LIANG, H., WANG, K., HE, H., ZHANG, X., WANG, Q. & HONG, Y. 2025. Single-cell multiome reveals root hair-specific responses to salt stress. *New Phytologist*.

MA, J., WAN, D., DUAN, B., BAI, X., BAI, Q., CHEN, N. & MA, T. 2019. Genome sequence and genetic transformation of a widely distributed and cultivated poplar. *Plant biotechnology journal,* 17**,** 451-460.

MA, L., LI, Y., YU, C., WANG, Y., LI, X., LI, N., CHEN, Q. & BU, N. 2012. Alleviation of exogenous oligochitosan on wheat seedlings growth under salt stress. *Protoplasma,* 249**,** 393-399.

MA, S., ZHOU, X., JAHAN, M. S., GUO, S., TIAN, M., ZHOU, R., LIU, H., FENG, B. & SHU, S. 2022. Putrescine regulates stomatal opening of cucumber leaves under salt stress via the H2O2-mediated signaling pathway. *Plant Physiology and Biochemistry,* 170**,** 87-97.

MAGALLON, K. J. & DINNENY, J. R. 2019. Environmental stress: Salinity ruins a plant's day in the sun. *Current Biology,* 29**,** R360-R362.

MAHMUD, M. R., ISLAM, M. A., HU, Q., ZHANG, X., WANG, W., XU, N., YANG, C. & LIU, J. 2024. Salt stress-induced polyamine biosynthesis contributes to blast resistance in rice. *Phytopathology Research,* 6**,** 1-16.

MAJUMDAR, R., SHAO, L., MINOCHA, R., LONG, S. & MINOCHA, S. C. 2013. Ornithine: the overlooked molecule in the regulation of polyamine metabolism3. *Plant and Cell Physiology,* 54**,** 990-1004.

MALIK, A., YADAV, P. & SINGH, S. 2022. Role of polyamines in heavy metal stressed plants. *Plant Physiology Reports***,** 1-15.

MARCO, F., BUSÓ, E., LAFUENTE, T. & CARRASCO, P. 2019. Spermine confers stress resilience by modulating abscisic acid biosynthesis and stress responses in Arabidopsis plants. *Frontiers in Plant Science,* 10**,** 972.

MCDERMOT, C. R., MINOCHA, R., D'AMICO III, V., LONG, S. & TRAMMELL, T. L. 2020. Red maple (Acer rubrum L.) trees demonstrate acclimation to urban conditions in deciduous forests embedded in cities. *PLoS one,* 15**,** e0236313.

MINOCHA, R. & LONG, S. 2004. Simultaneous separation and quantitation of amino acids and polyamines of forest tree tissues and cell cultures within a single high-performance liquid chromatography run using dansyl derivatization. *Journal of Chromatography A,* 1035**,** 63-73.

MINOCHA, R., LONG, S., TURLAPATI, S. A. & FERNANDEZ, I. 2019. Dynamic species-specific metabolic changes in the trees exposed to chronic N+ S additions at the Bear Brook Watershed in Maine, USA. *Annals of Forest Science,* 76**,** 1-13.

MINOCHA, R., MAJUMDAR, R. & MINOCHA, S. C. 2014. Polyamines and abiotic stress in plants: a complex relationship. *Frontiers in plant science,* 5**,** 175.

MINOCHA, R., SHORTLE, W. C., LONG, S. L. & MINOCHA, S. C. 1994. A rapid and reliable procedure for extraction of cellular polyamines and inorganic ions from plant tissues. *Journal of Plant Growth Regulation,* 13**,** 187-193.

MOHAPATRA, S., CHERRY, S., MINOCHA, R., MAJUMDAR, R., THANGAVEL, P., LONG, S. & MINOCHA, S. C. 2010. The response of high and low polyamine-producing cell lines to aluminum and calcium stress. *Plant Physiology and Biochemistry,* 48**,** 612-620.

NAHAR, K., HASANUZZAMAN, M., RAHMAN, A., ALAM, M. M., MAHMUD, J.-A., SUZUKI, T. & FUJITA, M. 2016. Polyamines confer salt tolerance in mung bean (Vigna radiata L.) by reducing sodium uptake, improving nutrient homeostasis, antioxidant defense, and methylglyoxal detoxification systems. *Frontiers in Plant Science,* 7**,** 1104.

NEALE, D. B. & KREMER, A. 2011. Forest tree genomics: growing resources and applications. *Nature Reviews Genetics,* 12**,** 111-122.

NGUYEN, H. C., LIN, K. H., HO, S. L., CHIANG, C. M. & YANG, C. M. 2018. Enhancing the abiotic stress tolerance of plants: from chemical treatment to biotechnological approaches. *Physiologia plantarum,* 164**,** 452-466.

PÁL, M., RAHMAN, A., HAMOW, K. Á., NAGY, K., JANDA, T., DERNOVICS, M. & SZALAI, G. 2025. Genotype-specific and light dependence of polyamine uptake and metabolism in wheat plants. *Plant Physiology and Biochemistry***,** 109659.

PÁL, M., SZALAI, G., GONDOR, O. K. & JANDA, T. 2021. Unfinished story of polyamines: Role of conjugation, transport and light-related regulation in the polyamine metabolism in plants. *Plant Science,* 308**,** 110923.

PÁL, M., TAJTI, J., SZALAI, G., PEEVA, V., VÉGH, B. & JANDA, T. 2018. Interaction of polyamines, abscisic acid and proline under osmotic stress in the leaves of wheat plants. *Scientific reports,* 8**,** 12839.

PARIHAR, P., SINGH, S., SINGH, R., SINGH, V. P. & PRASAD, S. M. 2015. Effect of salinity stress on plants and its tolerance strategies: a review. *Environmental science and pollution research,* 22**,** 4056-4075.

PAUL, S., BANERJEE, A. & ROYCHOUDHURY, A. 2018. Role of polyamines in mediating antioxidant defense and epigenetic regulation in plants exposed to heavy metal toxicity. *Plants Under Metal and Metalloid Stress: Responses, Tolerance and Remediation***,** 229-247.

PLOMION, C., BASTIEN, C., BOGEAT-TRIBOULOT, M.-B., BOUFFIER, L., DÉJARDIN, A., DUPLESSIS, S., FADY, B., HEUERTZ, M., LE GAC, A.-L. & LE PROVOST, G. 2016. Forest tree genomics: 10 achievements from the past 10 years and future prospects. *Annals of Forest Science,* 73**,** 77-103.

RADY, M. M., MOSSA, A.-T. H., YOUSSOF, A. M., OSMAN, A. S., AHMED, S. M. & MOHAMED, I. A. 2023. Exploring the reinforcing effect of nano-potassium on the antioxidant defense system reflecting the increased yield and quality of salt-stressed squash plants. *Scientia Horticulturae,* 308**,** 111609.

RAZA, A., TABASSUM, J., FAKHAR, A. Z., SHARIF, R., CHEN, H., ZHANG, C., JU, L., FOTOPOULOS, V., SIDDIQUE, K. H. & SINGH, R. K. 2022. Smart reprograming of plants against salinity stress using modern biotechnological tools. *Critical Reviews in Biotechnology***,** 1-28.

RAZIQ, A., DIN, A. M. U., ANWAR, S., WANG, Y., JAHAN, M. S., HE, M., LING, C. G., SUN, J., SHU, S. & GUO, S. 2022. Exogenous spermidine modulates polyamine metabolism and improves stress responsive mechanisms to protect tomato seedlings against salt stress. *Plant Physiology and Biochemistry,* 187**,** 1-10.

RODRÍGUEZ COCA, L. I., GARCÍA GONZÁLEZ, M. T., GIL UNDAY, Z., JIMÉNEZ HERNÁNDEZ, J., RODRÍGUEZ JÁUREGUI, M. M. & FERNÁNDEZ CANCIO, Y. 2023. Effects of Sodium Salinity on Rice (Oryza sativa L.) Cultivation: A Review. *Sustainability,* 15**,** 1804.

SAFDAR, H., AMIN, A., SHAFIQ, Y., ALI, A., YASIN, R., SHOUKAT, A., HUSSAN, M. U. & SARWAR, M. I. 2019. A review: Impact of salinity on plant growth. *Nat. Sci,* 17**,** 34-40.

SAHA, J., BRAUER, E. K., SENGUPTA, A., POPESCU, S. C., GUPTA, K. & GUPTA, B. 2015. Polyamines as redox homeostasis regulators during salt stress in plants. *Frontiers in Environmental Science,* 3**,** 21.

SHAO, J., HUANG, K., BATOOL, M., IDREES, F., AFZAL, R., HAROON, M., NOUSHAHI, H. A., WU, W., HU, Q. & LU, X. 2022. Versatile roles of polyamines in improving abiotic stress tolerance of plants. *Frontiers in Plant Science,* 13**,** 1003155.

SHEN, J.-L., WANG, Y., SHU, S., JAHAN, M. S., ZHONG, M., WU, J.-Q., SUN, J. & GUO, S.-R. 2019. Exogenous putrescine regulates leaf starch overaccumulation in cucumber under salt stress. *Scientia horticulturae,* 253**,** 99-110.

SHETEIWY, M. S., SHAO, H., QI, W., DALY, P., SHARMA, A., SHAGHALEH, H., HAMOUD, Y. A., EL-ESAWI, M. A., PAN, R. & WAN, Q. 2021. Seed priming and foliar application with jasmonic acid enhance salinity stress tolerance of soybean (Glycine max L.) seedlings. *Journal of the Science of Food and Agriculture,* 101**,** 2027-2041.

SHU, S., YUAN, Y., CHEN, J., SUN, J., ZHANG, W., TANG, Y., ZHONG, M. & GUO, S. 2015. The role of putrescine in the regulation of proteins and fatty acids of thylakoid membranes under salt stress. *Scientific reports,* 5**,** 1-16.

SINGH, A. & ROYCHOUDHURY, A. 2020. Role of γ-aminobutyric acid in the mitigation of abiotic stress in plants. *Protective chemical agents in the amelioration of plant abiotic stress: biochemical and molecular perspectives***,** 413-423.

SRIVASTAVA, V., MISHRA, S., CHOWDHARY, A. A., LHAMO, S. & MEHROTRA, S. 2021. The γ-aminobutyric acid (GABA) towards abiotic stress tolerance. *Compatible solutes engineering for crop plants facing climate change***,** 171-187.

SUN, Y., OH, D.-H., DUAN, L., RAMACHANDRAN, P., RAMIREZ, A., BARTLETT, A., TRAN, K.-N., WANG, G., DASSANAYAKE, M. & DINNENY, J. R. 2022. Divergence in the ABA gene regulatory network underlies differential growth control. *Nature plants,* 8**,** 549-560.

SUNDARARAJAN, S., SIVAKUMAR, H. P., NAYEEM, S., RAJENDRAN, V., SUBIRAMANI, S. & RAMALINGAM, S. 2021. Influence of exogenous polyamines on somatic embryogenesis and regeneration of fresh and long-term cultures of three elite indica rice cultivars. *Cereal Research Communications,* 49**,** 245-253.

TABUR, S., OZMEN, S. & ONEY-BIROL, S. 2024. Promoter role of putrescine for molecular and biochemical processes under drought stress in barley. *Scientific Reports,* 14**,** 19202.

TAMANG, B. G., LI, S., RAJASUNDARAM, D., LAMICHHANE, S. & FUKAO, T. 2021. Overlapping and stress-specific transcriptomic and hormonal responses to flooding and drought in soybean. *The Plant Journal,* 107**,** 100-117.

TIKHOMIROVA, T. S., KRUTOVSKY, K. V. & SHESTIBRATOV, K. A. 2022. Molecular traits for adaptation to drought and salt stress in birch, oak and poplar species. *Forests,* 14**,** 7.

VERSLUES, P. E., BAILEY-SERRES, J., BRODERSEN, C., BUCKLEY, T. N., CONTI, L., CHRISTMANN, A., DINNENY, J. R., GRILL, E., HAYES, S. & HECKMAN, R.

W. 2023. Burning questions for a warming and changing world: 15 unknowns in plant abiotic stress. *The Plant Cell,* 35**,** 67-108.

WANG, G., RYU, K. H., DINNENY, A., CARLSON, J., GOODSTEIN, D., LEE, J., OH, D.-H., OLIVA, M., LISTER, R. & DINNENY, J. R. 2024a. Diversification of gene expression across extremophytes and stress-sensitive species in the Brassicaceae. *bioRxiv***,** 2024.06. 21.599952.

WANG, J., DING, C., CUI, C., SONG, J., JI, G., SUN, N., QI, S., LI, J., XU, Z. & ZHANG, H. 2025. Physiological and molecular responses of poplar to salt stress and functional analysis of PagGRXC9 to salt tolerance. *Tree Physiology,* 45**,** tpaf039.

WANG, W., KANG, W., SHI, S. & LIU, L. 2024b. Physiological and metabolomic analyses reveal the mechanism by which exogenous spermine improves drought resistance in alfalfa leaves (Medicago sativa L.). *Frontiers in Plant Science,* 15**,** 1466493.

WANG, X., VIGNJEVIC, M., JIANG, D., JACOBSEN, S. & WOLLENWEBER, B. 2014. Improved tolerance to drought stress after anthesis due to priming before anthesis in wheat (Triticum aestivum L.) var. Vinjett. *Journal of experimental botany,* 65**,** 6441-6456.

WILHELMI, M. D. M. R., MANEEJANTRA, N., BALASUBRAMANIAN, V. K., PURVINE, S. O., WILLIAMS, S., DIFAZIO, S., STEWART JR, C. N., AHKAMI, A. H. & BLUMWALD, E. 2025. Salinity-Induced Photorespiration in Populus Vascular Tissues Facilitate Nitrogen Reallocation. *Plant, Cell & Environment,* 48**,** 781-791.

WUDDINEH, W., MINOCHA, R. & MINOCHA, S. C. 2017. Polyamines in the context of metabolic networks. *Polyamines: methods and protocols***,** 1-23.

XIONG, F., LIAO, J., MA, Y., WANG, Y., FANG, W. & ZHU, X. 2018. The protective effect of exogenous putrescine in the response of tea plants (Camellia sinensis) to salt stress. *HortScience,* 53**,** 1640-1646.

YANG, Y. & GUO, Y. 2018. Unraveling salt stress signaling in plants. *Journal of integrative plant biology,* 60**,** 796-804.

YI, L., WU, M., YU, F., SONG, Q., ZHAO, Z., LIAO, L. & TONG, J. 2022. Enhanced cadmium phytoremediation capacity of poplar is associated with increased biomass and Cd accumulation under nitrogen deposition conditions. *Ecotoxicology and Environmental Safety,* 246**,** 114154.

YUAN, Y., ZHONG, M., SHU, S., DU, N., HE, L., YUAN, L., SUN, J. & GUO, S. 2015. Effects of exogenous putrescine on leaf anatomy and carbohydrate metabolism in cucumber (Cucumis sativus L.) under salt stress. *Journal of Plant Growth Regulation,* 34**,** 451-464.

ZAFAR, S., PERVEEN, S., KAMRAN KHAN, M., SHAHEEN, M. R., HUSSAIN, R., SARWAR, N., RASHID, S., NAFEES, M., FARID, G. & ALAMRI, S. 2022. Effect of zinc nanoparticles seed priming and foliar application on the growth and physio-biochemical indices of spinach (Spinacia oleracea L.) under salt stress. *PLoS One,* 17**,** e0263194.

ZALESNY JR, R. S., STANGE, C. M. & BIRR, B. A. 2019. Survival, height growth, and phytoextraction potential of hybrid poplar and Russian Olive (Elaeagnus Angustifolia L.) established on soils varying in salinity in North Dakota, USA. *Forests,* 10**,** 672.

ZHANG, H., ZHAO, Y. & ZHU, J.-K. 2020a. Thriving under stress: how plants balance growth and the stress response. *Developmental Cell,* 55**,** 529-543.

ZHANG, K., KHAN, M. N., KHAN, Z., LUO, T., ZHANG, B., BI, J., HU, L. & LUO, L. 2024. Seed priming with ascorbic acid and spermidine regulated auxin biosynthesis to promote root growth of rice under drought stress. *Frontiers in Plant Science,* 15**,** 1482930.

ZHANG, X., CHENG, Z., ZHAO, K., YAO, W., SUN, X., JIANG, T. & ZHOU, B. 2019. Functional characterization of poplar NAC13 gene in salt tolerance. *Plant Science,* 281**,** 1-8.

ZHANG, X., HE, P., GUO, R., HUANG, K. & HUANG, X. 2023. Effects of salt stress on root morphology, carbon and nitrogen metabolism, and yield of Tartary buckwheat. *Scientific Reports,* 13**,** 12483.

ZHANG, Y., YAO, Q., SHI, Y., LI, X., HOU, L., XING, G. & AHAMMED, G. J. 2020b. Elevated CO2 improves antioxidant capacity, ion homeostasis, and polyamine metabolism in tomato seedlings under Ca (NO3) 2-induced salt stress. *Scientia Horticulturae,* 273**,** 109644.

ZHAO, K., FAN, G., YAO, W., CHENG, Z., ZHOU, B. & JIANG, T. 2024a. PagMYB73 enhances salt stress tolerance by regulating reactive oxygen species scavenging and osmotic maintenance in poplar. *Industrial Crops and Products,* 208**,** 117893.

ZHAO, S., ZHANG, Q., LIU, M., ZHOU, H., MA, C. & WANG, P. 2021. Regulation of plant responses to salt stress. *International Journal of Molecular Sciences,* 22**,** 4609.

ZHAO, X., WANG, S., GUO, F. & XIA, P. 2024b. Genome-wide identification of polyamine metabolism and ethylene synthesis genes in Chenopodium quinoa Willd. and their responses to low-temperature stress. *BMC genomics,* 25**,** 370.

ZHU, M., HSU, C.-W., PERALTA OGOREK, L. L., TAYLOR, I. W., LA CAVERA, S., OLIVEIRA, D. M., VERMA, L., MEHRA, P., MIJAR, M. & SADANANDOM, A. 2025. Single-cell transcriptomics reveal how root tissues adapt to soil stress. *Nature***,** 1-9.