Exogenous Salicylic Acid Reverses Water Splitting and Electron Transport Activity of Leaves and Triggers the Transition to Flowering in *Phlomis tuberosa* Under Salt Stress

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Abstract

To investigate the protective role of salicylic acid (SA) in alleviating salt-induced damage in *Phlomis tuberosa* plants. Fifty-day-old seedlings were exposed to salt stress by irrigating 40 mM sodium chloride solution. Salt stress significantly inhibited plant growth by diminishing shoot and root dry weights, plant height, and relative water content (RWC). This growth inhibition was primarily associated with excessive Na^+ accumulation in plant shoots. However, root-application of SA at 0.1 mM mitigated the toxic effects of salt-induced damage by inhibiting Na^+ translocation to leaves, resulting in significant improvements in growth-related attributes, including dry mass. Salt stress markedly affected the chlorophyll *a* fluorescence transient (OJIP) curve, as indicated by an increase in the O-J phase coupled with reduced maximum quantum yield (F_v/F_m) and oxygen-evolving complex efficiency of PSII (F_v/F_o). Conversely, SA supplementation reversed these effects by improving JIP-test parameters, water- splitting acitivity, and electron transport within PSII. Furthermore, pretreatment with salicylic acid under salt stress conditions markedly enhanced the activities of antioxidative enzymes compared with those in the salinity alone treatment. Notably, SA enhanced flowering in *P. tuberosa* regardless of stress, and the positive correlation between nitric oxide (NO) levels and flower number suggested a role for NO in salicylic acidmediated floral transition. Overall, by enhancing carotenoids levels, reducing reactive oxygen species (ROS) accumulation, preserving thylakoid membrane integrity, improving the balance and functionality between PSI and PSII and optimizing energy fluxes; SA alleviated the detrimental effects of salt stress on photosynthesis and mitigated stress-induced damage.

Keywords: Ionic homeostasis, OJIP transient, Oxidative stress, *Phlomis tuberosa*, Photosynthesis, Salicylic acid, Salinity

Introduction

Among the abiotic stresses, salinity is a significant stressor that severely restricts plant growth and development, thereby reducing crop yield (Munns and Gilliham, 2015; Liu et al., 2024). Under saline con-

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ditions, the excessive uptake of Na^+ and Cl^- reduces the K^+/Na^+ ratio in plant tissues and induces osmotic stress, leading to ionic toxicity and nutritional imbalances in plants (Isayenkov and Maathuis, 2019; Mukhopadhyay et al., 2021; Hryvusevich et al., 2021). One of the common consequences of this ionic toxicity and nutritional imbalance is the overproduction of reactive oxygen species (ROS), which causes damage to cellular membranes, stimulates tissue necrosis, and decreases plant vigor (Hasanuzzaman et al., 2021; İbrahimova et al., 2021). Under these conditions, excessive ROS accumulation reduces light-capturing efficiency by accelerating chlorophyll (Chl) degradation, impair membrane integrity and photosynthesis-related proteins (Miller et al., 2010), compromises the structural and functional integrity of photosystem II (PSII), and inhibit photochemical activity and electron transport chains (ETC), and consequently, ultimately reducing photosynthetic capacity and crop yield (Raihan et al., 2025).

To mitigate the excessive production of ROS and minimize oxidative damage to the photosynthetic apparatus, plants have broadly evolved several adaptive strategies, including the maintenance of osmotic balance, regulation of mineral homeostasis, activation of enzymatic and non-enzymatic antioxidants systems, (e.g., carotenoids, glutathione, ascorbic acid and phenolic compounds), synthesis of phytohormones, generation of nitric oxide, and accumulation of compatible solutes (Isayenkov and Maathuis, 2019; Ramasamy and Mahawar, 2023). Since these endogenous mechanisms provide only partial protection against the detrimental effects of salt stress, considerable progress has been made in recent years toward alleviating salt-induced toxicity and enhancing plant tolerance to salinity through the exogenous application of protective compounds, including proline (Koc et al., 2024), nitric oxide (Wang et al., 2024), jasmonic acid (Ali et al., 2022), salicylic acid (SA) (Kumar et al., 2024), and melatonin (Kaya et al., 2022). Among these protective compounds, exogenous SA is known to play an important role in modulating the antioxidant defense responses and mitigating the negative effects of ROS, thereby enhancing plants tolerance to challenging adverse environmental conditions, including heavy metal toxicity (Maghsoudi et al., 2020), water deficit (Kareem et al., 2019), salinity (Yadav et al., 2020), and heat stress (Arif et al., 2020).

SA supplementation at appropriate concentrations has been reported to enhance plant growth and improve stress tolerance to salt stress in various plant species (Hayat et al., 2010; Jayakannan et al., 2015; Barwal et al., 2024), including wheat (Hafeez et al., 2024), soybean (Farhangi-Abriz and Ghassemi-Golezani, 2018), and *Egletes viscosa* (Batista et al., 2019). Exogenous SA enhances plant tolerance to salt stress by increasing the activities of antioxidant enzymes, including catalase (CAT), peroxidase (POX), superoxide dismutase (SOD), glutathione S-transferase, glutathione reductase, and glutathione peroxidase (Ahanger et al., 2020; Barwal et al., 2024; Liu et al., 2024). It may also promote the biosynthesis or accumulation of key plant growth hormones, such as auxins, gibberellins, and abscisic acid (Sharma

et al., 2020; Kashif et al., 2024), increasing the concentration of antioxidants, including proline, glycine betaine, flavonoids, and alkaloids (Alizadeh et al., 2024; Barwal et al., 2023) and improving Na^+ and K^+ balance (Jayakannan et al., 2015; Batista et al., 2019; Kumar et al., 2024).

Furthermore, under salinity stress, exogenous SA enhances photosynthesis by stabilizing Chl (Lotfi et al., 2020; Islam et al., 2023), improving stomatal conductance, and protecting PSII efficiency from ROS damage (Ma et al., 2017; Raihan et al., 2025). These mechanisms help plants for increasing the salt tolerance.

Given that the involvement of salicylic acid (SA) in floral development has not been fully characterized, the present study examined how exogenous SA and NaCl influence the onset of flowering in *P. tuberosa*. We aimed to clarify whether SA contributes to the acceleration of flowering under both optimal and saline conditions. Earlier reports have suggested that SA may modulate floral induction even in the absence of environmental stress (Martínez et al., 2004; Wada and Takeno 2013). Additional findings indicate that SA plays an important role in the regulation of flowering initiation in certain plant species. For instance, stressassociated increases in SA levels have been reported to promote early flowering in *Arabidopsis* and *Lemna paucicostata*, partly through suppression of the floral repressor *FLOWERING LOCUS C* (*FLC*) (Wada et al., 2010). Furthermore, nitric oxide (NO) is known to interact with redoxregulated signaling networks and has been proposed to influence the expression or activity of key flowering regulators (Hou et al., 2025). SAinduced NO generation may therefore contribute to activating molecular mechanisms underlying the floral transition. Accordingly, the present work investigated the potential involvement of NO in SAmediated control of flowering in *Phlomis tuberosa*.

Phlomis tuberosa belongs to the *Lamiaceae* family and is cultivated for its medicinal value. *P. tuberosa* is capable of surviving in extreme environmental conditions, including highaltitude and arid or semiarid habitats characterized by intense ultraviolet radiation and recurrent droughts can constrain its growth and yield (Ghasemifar et al., 2022). Despite its documented tolerance to various environmental stresses, the effects of salinity on *P. tuberosa* growth and productivity remain poorly investigated. To the best of our knowledge, no studies have investigated whether exogenous SA enhances salt stress tolerance in *P. tuberosa* . Hence, the mechanisms by which this growth regulator may enhance salt-stress tolerance in *P. tuberosa* are still not fully understood. The current study aimed to determine whether application mitigates salinity stress by improving photochemical performance. To address this question, we investigated in some detail the biochemical mechanisms by which SA affects the plant growth, PSII functioning, ionic homeostasis, and antioxidative system in salt-treated *P. tuberosa* plants. Unlike previous studies, particular attention was given to fluorescence-based parameters to elucidate how SA modulates PSII photochemical performance and contributes to the mitigation of salt stress in medicinal *P. tuberosa*.

The findings of this study are expected to provide valuable ecological and economic strategies for enhancing plant resilience in saline environments.

Materials and methods

Plant material and treatments

Seeds of *Phlomis tuberosa* were colleted from Mishoudagh, near the city of Marand, 65 km south of Tabriz (45°38' E, 38°22' N) at an elevation of 1800 m, East Azarbaijan Province, northwestern Iran . Healthy and uniform seeds were surface-sterilized with a 5% sodium hypochlorite (NaOCl) solution for 5 min and then rinsed thoroughly with distilled water. Ten-day-old seedlings were transferred to Hoagland nutrient solution (Johnson et al., 1957) and were pre-cultured for 34 days before the treatments were initiated. The plants were grown in a nutrient solution containing: 6 mM KNO_3, 4 mM $Ca(NO_3)_2$, 2 mM $NH_4H_2PO_4$, 1 mM $MgSO_4$, 50 µM H_3BO_3, 2 µM $MnSO_4$, 2 µM $ZnSO_4$, 0.5 µM $CuSO_4$, 0.5 µM H_2MoO_4 and 0.02 mM $FeSO_4$-EDTA. Salicylic acid (SA) was supplied through the nutrient medium at a final concentration of 0.1 mM with the pH adjusted to 5.6–5.8, and the entire solution was replaced every 7 days. The concentration and mode of SA application method of SA were determined through a preliminary dose–response experiment. *P. tuberosa* seedlings were treated withconcentrations of SA, including 0.05, 0.1, 0.5, 1, 5, and 10 mM, applied either as a foliar spray or to the root zone. Based on growth responses, 0.1 mM SA applied to the root zone was selected for subsequent experimentsbecause it produced a significantly great increase in seedling growth than foliar application or the other concentrations tested. At 49 days after germination, 40 mM NaCl were added to the nutrient solution described above. The salt concentration was selected based on the effect of different NaCl concentrations, including 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 mM on seedling growth in a preliminary study. The seedling growth was reduced by approximately 50% compared with the control and this concentration was therefore selected as a semi-lethal concentration of salinity for further experiments (data not shown), at 40 mM. Plants were maintained in a controlled with temperatures of 30-35/18-22 ºC, respectively, under a 16 h light/8 h dark photoperiod. , The relative humidity maintained at 40-45 % and a daily photon flux density of approximately 400-450 µmol m^{-2} s^{-1}.

Determination of plant biomass and leaf relative water content

Fully expanded and mature leaves were collected for the measurement of physiological and biochemical parameters. Shoots and roots were carefully harvested to determine their dry weight (DW) and subsequently dried at 70 ºC for 48 h . For subsequent analyses, the samples were immediately immersed in liquid nitrogen and maintained under these conditions until analysis. Relative water content (RWC) and percentage (%) of yield loss were determined according to the the method described by Habibi (2020).

Determination of proline

Proline was determined according to the method described by Bates et al., (1973). Leaf samples from each group were homog-

enized in 3% (w/v) sulphosalicylic acid at 4 °C, and the homogenates were centrifuged at 3,000 × g for 20 min. Approximately 2 ml of the extract was transferred to a test tube, followed by an addition of 2 ml of glacial acetic acid and ninhydrin reagent. The tubes were cooled on crushed ice, and the absorbance of the resulting red-color complex was measured at 520 nm using a UV-visible spectrophotometer, with toluene serving as the blank. A standard curve was prepared using proline (Sigma-Aldrich) as the reference standard.

Determination of Na and K contents

All samples were dry-ashed at 550 °C for 8 h, and the resulting dry residues were mixed with 0.5 M HCl and brought to a final volume with double-distilled water. Following acid digestion, the samples were transferred to a hot plate maintained at 120 °C and heated for an additional 1 h. The digested samples were subsequently diluted to a final volume of 50 mL with double-distilled water to prior to analysis. Then, The concentrations of Na and K were subsequently determined by inductively coupled plasma-atomic emission spectrometry (ICP-AES, INTEGRA XL2, GBC; Australia).

Determination of total chlorophylls and carotenoids

The concentrations of chlorophyll *a*, chlorophyll *b*, and total carotenoids in leaf tissues were determined following pigment extraction in chilled acetone. The Samples were incubated in darkness at 4 °C for 24 h to ensure complete extraction of the pigments, following the procedure described by Lichtenthaler and Wellburn (1985).

Chlorophyll a fluorescence measurement

Chlorophyll a fluorescence parameters were measured daily between 09:00 and 11:00 husing a Pocket-PEA chlorophyll fluorimeter (Plant Efficiency Analyser, Hansatech Instruments Ltd., UK). Prior to measurement, the leaves were dark-adapted for at least 30 min. The JIP-test analysis was used to quantify a range of phenomenological and biophysical parameters associated with the functions of photosystem II (PSII) and photosystem I (PSI). Analysis of the polyphasic chlorophyll fluorescence rise (OJIP curve) provided insights into photosynthetic electron fluxes (Strasser et al., 2004; Kumar et al., 2020). The JIP-test is based on the polyphasic rise in fast chlorophyll a florescence and is widely used for investigating the correlation between light dependent reactions and chlorophyll a florescence ChlF. The O–J phase of the fluorescence rise reflects the closure of afraction of the PSII reaction centers associated with the reduction of the primary electron acceptor QA. The J–I phase corresponds primarily to the reduction of the plastoquinone (PQ) pool, whereas the I–P phase is typically associated with the reduction of electron acceptors, on the PSI acceptors on the PSI acceptor side, including $NADP^+$. Specific parameters reflecting functional and structural alterations in PSII were calculated according to protocols (Kalaji et al., 2011), as ascribed in the subsequent sections.

F_o: Minimum fluorescence, when all PSII reaction centers (RC) are open.

F_m: Maximum fluorescence, when all PSII reaction centers are closed.

F_v: Variable fluorescence.

F_v/F_m: Maximum quantum yield of PSII.

F_v/F_o: Efficiency of the oxygen-evolving complex on the donor side of the PSII.

F_m/F_o: Maximum fluorescence normalized by minimum fluorescence.

V_j: Relative variable fluorescence at 2 ms (J-step), that refers to the number of closed RCs relative to the total number of RCs.

V_i: Relative variable fluorescence at 30 ms (I-step), that reflects the ability of PSI and its acceptors to oxidize reduced plastoquinone.

N: Turnover number: number of QA reduction events

S_m: Normalized total complementary area above the OJIP transient (reflecting multiple-turnover QA reduction events) or total electron carriers per RC.

ABS/RC: Absorption flux per RC; that reflects the proportion between chlorophyll *a* molecule amounts in fluorescence-emitting antenna complexes and the active reaction centers.

TR_o/RC: Trapped energy flux per RC.

ET_o/RC: Electron transport flux per RC.

DI_o/RC: Dissipated energy flux per RC.

ABS/CS: Absorption flux per CS; represents the amount of photon energy absorbed by the antenna associated with active and inactive reaction centres of PSII.

TR_o/CS_m: Trapped energy flux per CS.

ET_o/CS_m: Electron transport flux per CS.

DI_o/CS_m: Dissipated energy flux per CS.

RC/C_m: Amount of active PSII RCs per CS.

$ABS/CS_m = \approx F_o$: Absorbed photon flux per excited PSII cross section at time zero.

φP_o: Maximum quantum yield of primary photochemistry*;* that indicates the probability of trapping the energy of absorbed photons by PSII reaction centers.

φE_o: Quantum yield for the reduction of end acceptors of PSI per photon absorbed.

ψE_o: Probability (at time 0) that trapped exciton moves an electron into the electron transport chain beyond QA.

PI_{abs}: The performance index.

Assay of antioxidative enzymes and related metabolites

Enzyme activity was assayed in leaves harvested at midday. The activities of superoxidase dismutase (SOD; EC 1.15.1.1), catalase (CAT; EC 1.11.1.6), and ascorbate peroxidase (APX; EC 1.11.1.11) were determined according to the methods described by Habibi (2020). SOD activity was determined using the nitro blue tetrazolium (NBT) photoreduction method described by Giannopolitis and Ries (1977), using a reaction mixture containing 0.1 mM EDTA, 50 mM Na_2CO_3 pH 10.2, 13 mM methionine, 63 μM nitroblue tetrazolium chloride (NBT), 13 μM riboflavin. CAT activity was determined following a protocol adapted from Simon et al. (1974) using the H_2O_2 as substrate. CAT activity was determined from the decrease in absorbance at 240 nm, which reflects the decomposition of hydrogen peroxide (H_2O_2). APX activity was assayed by monitoring the oxidation of ascorbate using a modified reaction mixture containing 50 mM phosphate buffer (pH 7), 0.2 mM EDTA, 0.5 mM ascorbic acid, and 50 μg BSA, as detailed by Boominathan and Doran (2002). The rate of lipid peroxidation was estimated based on the concentration of malondialdehyde (MDA) formed in a reaction mixture containing thiobarbituric acid (TBA, Sigma) at 532 nm. MDA concentra-

tion was quantified using a standard curve prepared with 1,1,3,3-tetraethoxypropane (Sigma-Aldrich). H_2O_2 content was determined according to the method of Velikova et al. (2000) using a standard curve of H_2O_2 (Sigma-Aldrich).

Determination of NO concentration

Nitric oxide(NO) concentration was determined using the modified method described by Wu et al. (2016). Fresh leaf sample of a known weight were homogenized in ice-cold 50 mM acetic acid buffer (pH 3.6) containing 4% Zn diacetate. Absorbance was measured at 540 nm, and NO concentration was calculated using a standard curve prepared with known concentrations of $NaNO_2$.

Statistical analysis

The experiments were conducted using a completely randomized design using four independent replicates. Data were analyzed using one-way analysis of variance (ANOVA) in Sigma Stat version 3.5. Treatment means were compared using Tukey's honestly significant differences among treatments (HSD). test at a significance level of $P< 0.05$. Data are presented as means±-standard deviation (SD) in all Figures and Tables, with different letters indicate statistically significant differences among treatments. Spearman rankorder correlation analysis was performed using SigmaStat version 3.5 to measure the relationship between the number of flowers and NO levels, based on 15 independent replicates. Chlorophyll florescence data were assessed using the PEA Plus software, version 1.10.

Results

The root application of SA improved the growth parameters under salt stress

Measurement of the growth parameters in *P. tuberosa* plants under salt stress showed that salinity significantly reduced shoot fresh and dry weight, root dry weight, and plants height (Table 1). Exogenous application of SA improved significant morphological parameters , including shoot fresh and dry weights . However, SA application did not significantly affect root dry weight and plant height parameters in salt-stressed plants..

Plants treated with NaCl exhibited a significant reduction in relative water content (RWC) compared with the control group (Table 2). In contrast, plants treated with both NaCl and SA maintained RWC levels similar to those of the control plants, highlighting the positive role of SA in maintaining plant water status under salt stress.

Table 1. Effect of SA on fresh and dry weight and tap root and stem length of *P. tuberosa* under NaCl stress. Values are mean ± SD (n = 4); different letters indicate significant differences ($p < 0.05$, Tukey's test)

Treatment	Shoot FW	Root FW	Shoot DW	Root DW	Plants height	Root length
Control	5.10±0.32 [a]	3.44±0.22 [a]	0.85±0.10 [a]	0.62±0.10 [a]	23.2±2.32 [a]	6.53±0.73 [b]
NaCl	3.42±0.30 [b]	3.24±0.33 [a]	0.58±0.13 [b]	0.41±0.08 [b]	17.5±.1.89 [b]	6.10±0.08 [b]
SA	5.27±0.28 [a]	3.54±0.37 [a]	0.89±0.12 [a]	0.66±0.09 [a]	24.1±3.02 [a]	8.75±1.02 [a]
SA+NaCl	4.92±0.24 [a]	3.09±0.26 [a]	0.84±0.11 [a]	0.42±0.07 [b]	18.3±1.26 [b]	7.99±0.07 [a]

Furthermore, salt stress increased proline content compared with the control treatment. Salt-treatment significantly increased Na^+ concentrations both in shoots and roots compared with the control plants. However, the application of SA to the root zone under salt stress reduced shoot Na^+ concentration by 32%. (Table 2). Under salinity stress, shoot K^+ concentration decreased compared with the control. However, SA application under NaCl stress treatment alleviated this reduction, improving shoot K by 27% relative to NaCl-stressed plants (Table 2). However, root K was not significantly influenced by SA application in stressed plants.

SA mitigated the adverse effects salt stress on the PSII function

While after 15 days of salt stress, *P. tuberosa* plants showed a significant decrease in chlorophyll *a* contents, plants exposed to only salt stress could maintain chlorophyll *b* and carotenoids contents at relatively high levels (Fig. 1). However, the decreases in chlorophyll *a* contents in the leaves of stressed plants were significantly mitigated by SA application. Carotenoids contents were not significantly affected by exposure to salinity alone, however, the application of SA raised this parameter in plants under salt stress. Although we detected a clear decrease in the maximum quantum yield (F_v/F_m) of leaves after 15 days of exposure to salt stress (Fig. 2), the application of SA mitigated this reduction, improving F_v/F_m relative to stressed plants. Exposure to salinity alone caused a marked decline in the oxygen-evolving complex efficiency of PSII (F_v/F_o) and the performance index of photosystems (PI_{abs}), however, the application of SA raised these parameters in plants under salt stress.

According to the fast chlorophyll *a* fluorescence (OJIP) kinetics, fluorescence dropped sharply after 15 days of salt stress, indicating reductions in the I-P phases compared to control plants. However, the O-J phase showed a marked increase in fluorescence intensity relative to control plants (Fig. 3). In contrast, SA application under NaCl stress effectively suppressed this O-J phase of the fluorescence rise.

A sharp increase in V_j (relative variable fluorescence at J step) was also detected in leaves of salt-stressed *P. tuberosa* plants(-Fig. 4). In addition, exposure salinity alone resulted in significant increase in several specific energy fluxes, including absorption flux per reaction center (ABS/RC), trapped energy flux per reaction center (TR_o/RC)

Table 2. Effect of SA on RWC, proline, and element content in *P. tuberosa* under NaCl stress. Values are mean ± SD (n = 4); different letters indicate significant differences ($p < 0.05$, Tukey's test)

Treatment	RWC (%)	Proline	Shoot Na	Root Na	Shoot K	Root K
Control	86.7±5.5 ^{a}	10.2±1.2 ^{c}	15.3±2.0 ^{b}	25.3±2.7 ^{b}	74.3±6.8 ^{a}	65.9±6.2 ^{ab}
NaCl	70.3±4.1 ^{b}	16.3±1.7 ^{b}	25.6±2.3 ^{a}	38.4±3.5 ^{a}	40.6±6.5 ^{c}	52.3±4.7 ^{b}
SA	87.2±5.8 ^{a}	11.0±1.4 ^{c}	14.6±1.5 ^{b}	24.7±3.6 ^{b}	72.6±4.6 ^{a}	69.2±4.9 ^{a}
SA+NaCl	83.2±3.9 ^{ab}	19.5±1.3 ^{a}	17.3±1.9 ^{b}	35.8±5.2 ^{a}	55.6±5.1 ^{b}	60.1±9.5 ^{ab}

and electron transport flux per reaction center (ET_o/RC) compared with the control (Fig. 4). These changes reveal substantial deviations from the control values, as reflected by the greater distances from the control profile in the spider plot (Fig. 4). Conversely, the phenomenological energy flux model identified PSII as a critical site of affected by salt stress . This stress resulted in decreases in several phenomenological energy fluxes, including absorption flux per cross-section (ABS/CS_m), trapped energy flux per cross-section (TR_o/CS_m), electron transport flux per cross-section (ET_o/CS_m), and the reduction flux beyond PSI (RE_o/CS_m) under salt stress compared with the control. These changes in JIP-test parameters represented a considerable impact on the structural and functional integrity of the photosynthetic apparatus under salt stress. In addition, salt stress markedly affected the quantum yield of primary photochemistry F_v/F_m (φP_o), the quantum yield for electron transport (φE_o), () and the efficiency/probability that an elec-

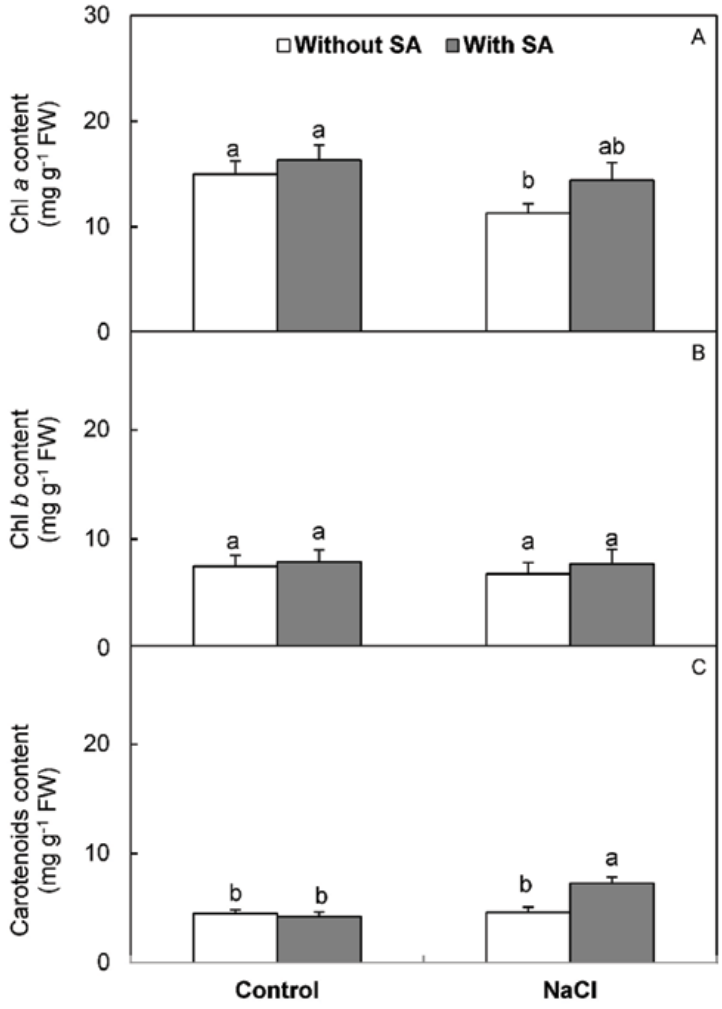


Fig. 1. Effect of SA on chlorophyll *a*, chlorophyll *b*, and carotenoid contents in *P. tuberosa* leaves under NaCl stress. Values are mean ± SD (n = 4). Different letters indicate significant differences ($p < 0.05$, Tukey's test).

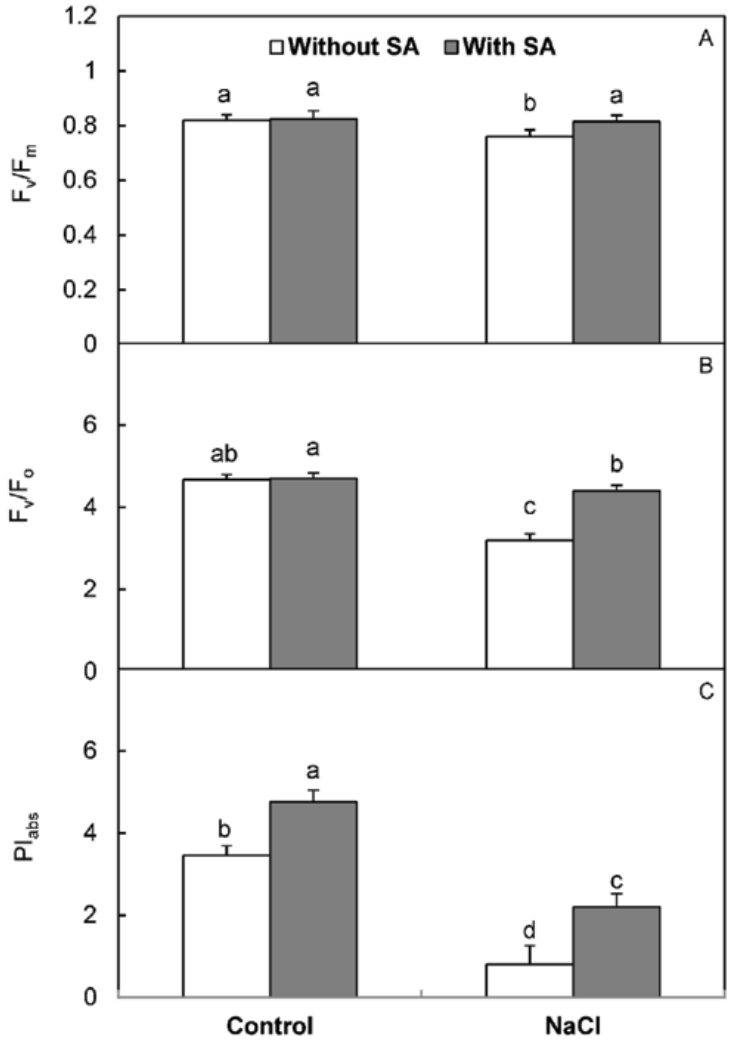


Fig. 2. Effect of SA on Fv/Fm, Fv/Fo, and PIabs in *P. tuberosa* leaves under NaCl stress. Values are mean ± SD (n = 4). Different letters indicate significant differences ($p < 0.05$, Tukey's test).

Table 3. Effect of SA on Chl *a*, Chl *b*, Car (Carotenoids), F_v/F_m, F_v/F_o and PI_{abs} in *P. tuberosa* under NaCl stress. Values are mean ± SD (n = 4); different letters indicate significant differences ($p < 0.05$, Tukey's test)

Treatment	Chl *a*	Chl *b*	Car	F_v/F_m	F_v/F_o	PI_{abs}
Control	15.1±1.23 ^{a}	7.6±0.99 ^{a}	4.51±0.36 ^{b}	0.82±0.02 ^{b}	4.6±0.12 ^{a}	3.46±0.23 ^{ab}
NaCl	11.3±0.89 ^{b}	6.8±1.05^{a}	4.62±0.47 ^{b}	0.76±0.025 ^{a}	3.2±0.15 ^{c}	0.81±0.45 ^{b}
SA	16.3±1.42 ^{a}	7.9±1.10 ^{a}	4.23±0.45 ^{b}	0.83±0.03 ^{b}	4.7±0.14 ^{a}	4.76±0.28 ^{a}
SA+NaCl	14.4±1.65 ^{ab}	7.71±1.35 ^{a}	7.25±0.55^{a}	0.81±0.024 ^{a}	4.4±0.13 ^{b}	2.22±0.33 ^{ab}

tron is transferred beyond Q^A (ψE_0)) (Fig. 4). However, SA s pretreatmentsignificantly alleviated these stress-induced changes, as indicated by reductions in F_o, ABS/RC, TR_o/RC and ET_o/RC, compared with plants exposed to salt stress alone. Thus, SA application under salt stress effectively modulated these parameters toward values closer to control. In addition, ABS/CS_m, TR_o/CS_m, ET_o/CS_m and RE_o/CS_m were significantly improved in plants treated with both NaCl and SA, highlighting the positive role of SA in maintaining photosystem function under salt stress.

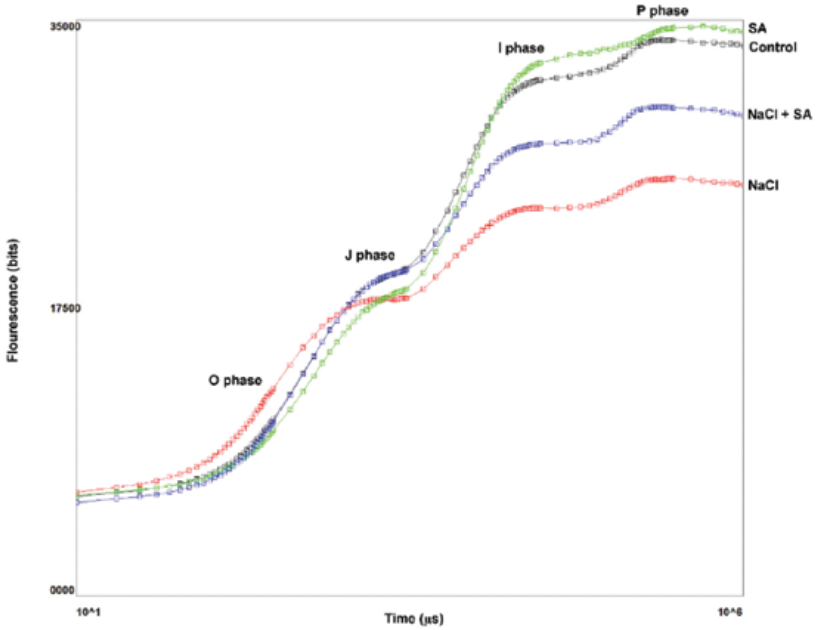


Fig. 3. Effect of SA application on the fast chlorophyll a fluorescence transient kinetics of *P. tuberosa* under NaCl stress

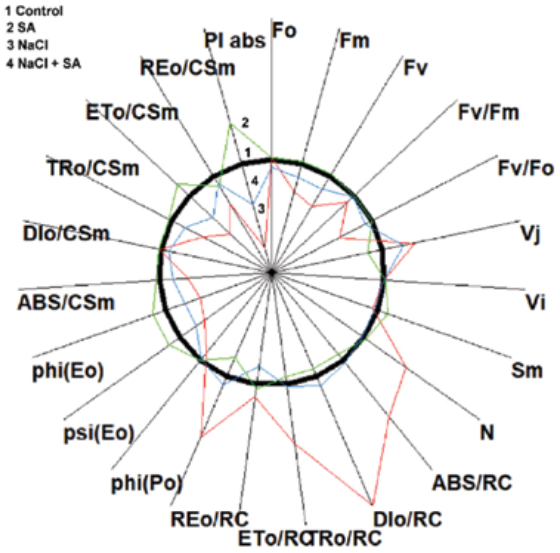


Fig. 4. Radar plots showing various technical fluorescence parameters of *P. tuberosa* plants affected by SA under salt stress.

SA at 0.1 mM was an alleviant for the oxidative stress effects caused by salinity

Significant changes in the activities of antioxidant enzymes, including APX and CAT, were observed under salinity alone treatment (Fig. 5).

Furthermore, the exogenous application of SA under salt stress conditions markedly enhanced the activities of APX, SOD, and CAT compared with those in the salinity alone treatment. Interestingly, plants treated with SA and salinity combination exhibited significantly higher in both NO and H_2O_2 content than the control (Fig. 6). Under salt stress, MDA content increased compared with the control, however, this increases in the leaves of salt-stressed *P. tuberosa* plants was significantly prevented by SA application at 0.1 mM.

SA application induces flowering of P. tu-

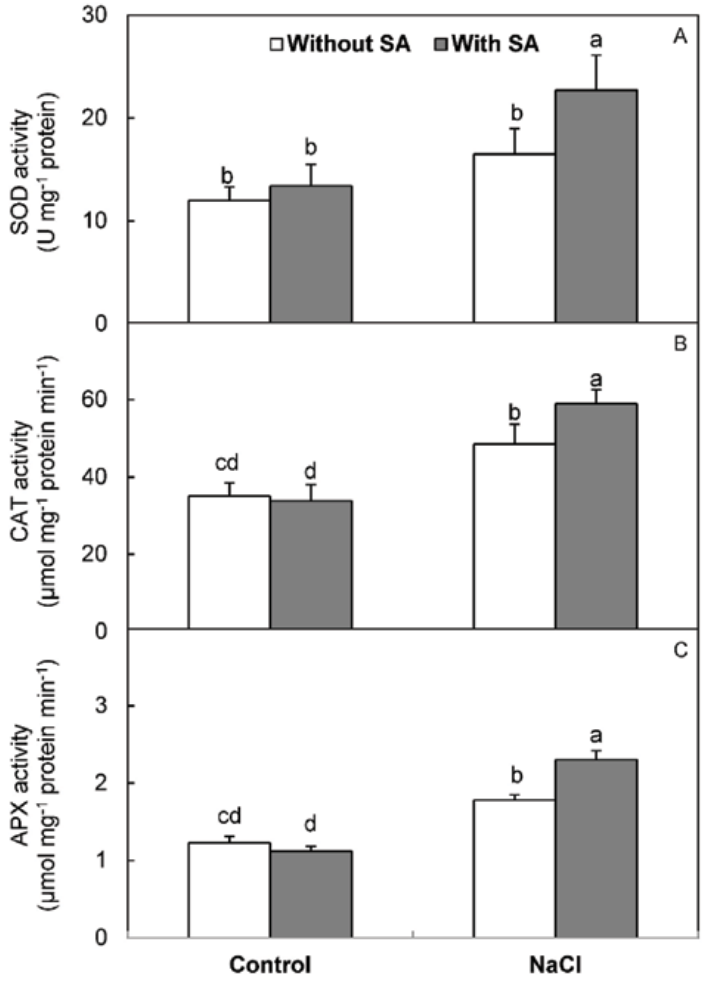


Fig. 5. Effect of SA on SOD, CAT, and APX activities in *P. tuberosa* leaves under NaCl stress. Bars with the same letter are not significantly different ($p < 0.05$, Tukey's test). Values are mean ± SD (n = 4).

berosa under both stress and non-stress conditions

Although the number of flowers was not affected by salt stress, salicylic acid (SA) treatment significantly increased flower number under both saltstress and nonstress conditions. Our results indicated that while exogenous SA application under control conditions did not significantly alter endogenous NO levels, it led to a pronounced accumulation of NO under salinity stress, suggesting that the SA-mediated enhancement of the NO signal is triggered under stressful conditions. In addition, a positive correlation was observed between flower number and nitric oxide (NO) levels in *P. tuberosa* plants subjected to SA alone ($r = 0.52$, $p < 0.05$) or exposed to SA and NaCl combination ($r = 0.86$, $p < 0.01$) (Fig. 7).

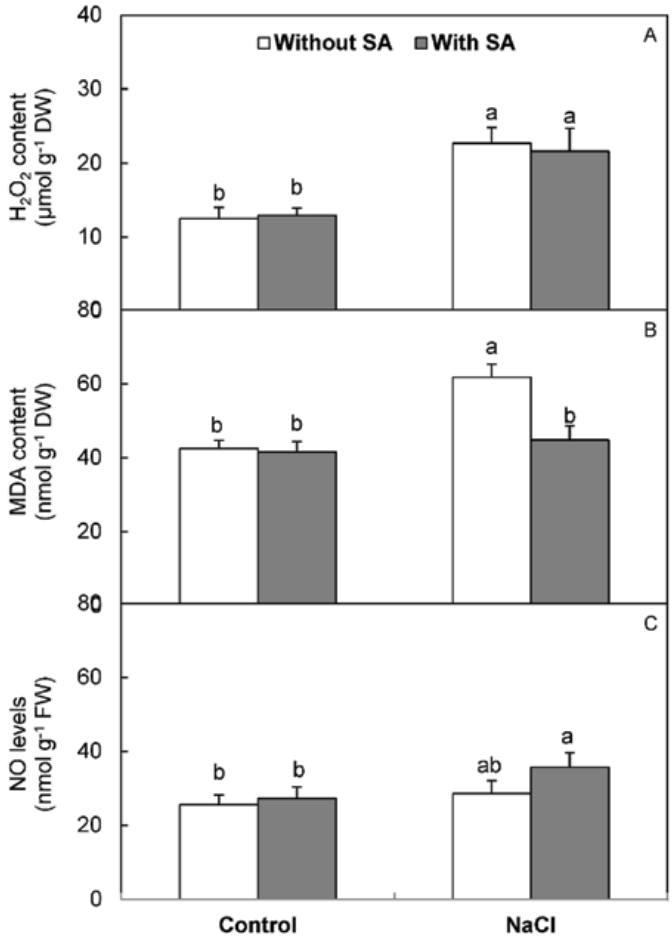


Fig. 6. Effect of SA application on the content of hydrogen peroxide (H_2O_2), maloendialdehyde (MDA), and nitric oxide (NO) in *P. tuberosa* leaves under NaCl-stressed conditions. Bars indicated with the same letter are not significantly different ($p < 0.05$, Tukey test). Values are the mean ± SD (n = 4).

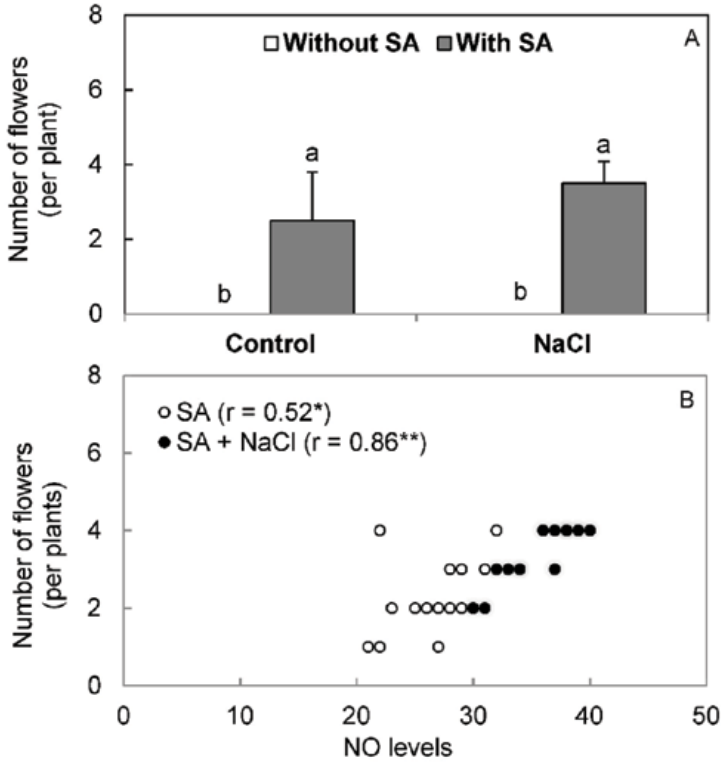


Fig. 7. Effect of SA application on the number of flowers per plant in *P. tuberosa* under NaCl-stressed conditions (A). Correlation between the number of flowers and NO levels (B) recorded in *P. tuberosa* plants exposed to SA or SA + NaCl. **p* < 0.05; ***p* < 0.01; n.s., not significant.

Discussion

Salinity induces hyperosmotic and hyperionic conditions, which lead to excessive ROS production, ultimately resulting in stunted plant growth and development (Chaves et al., 2009). The present study revealed that salt stress significantly inhibited plant growth, which is primarily related to excessive ion accumulation in plant cells coupled with decreased uptake of essential nutrients and water, which together restrict cell division and development (Acosta-Motos et al., 2015). Similar reductions in biomass and shorter shoots under salt stress have been shown in various plant species, including maize (Raza et al., 2019), barley (Akhter et al., 2023), wheat (Genc et al., 2019), and black gram (Ahmad et al., 2024). In this study, the results revealed that under salt-stressed conditions, the K^+ content decreased, and the Na^+ content increased in the *P. tuberosa* leaves. Increased Na^+can also interfere with the uptake and homeostasis of other nutrients (Wakeel et al., 2011; Guo et al., 2019) and cause ionic imbalance (Roshdy et al., 2021), which can may disrupt plasma membrane integrity in this species. SA alleviated salt stress by reducing excessive Na^+

translocation to leaves through the transpiration stream, thereby enhancing plant growth (X et al., 2025). Furthermore, the application of SA in *P. tuberosa* plants was capable of increasing shoot K^+ content, and lowering Na^+/K^+ ratio, which maintained the integrity of the cell membranes from oxidative damage (El-Hawary et al., 2023). Additionally, a significant improvement in proline content was detected in leaves of salt-stressed *P. tuberosa* plants, however, exogenous SA application under NaCl stress further increased proline. Similar results were reported by Liu et al., (2016), who showed that SA application further increased the contents of proline in the leaves of NaCl-treated *Nitraria tangutorum* seedlings. Furthermore, our results demonstrated that SA significantly improves water status under salt stress, as evidenced by maintaining the leaf RWC. This observed increase in leaf RWC may be attributed to SA's role in enhancing water use efficiency and protecting cellular hydration. In conclusion, the current study's findings were consistent with previous reports on *Egletes viscosa* (Batista et al., 2019) and *Olea europeae* (Methenni et al., 2018) in which SA application significantly alleviated the detrimental effects of salt stress on plant growth. In the present study, chlorophyll *a* concentration was markedly decreased in salt-stressed *P. tuberosa* plants, highlighting that salt stress may have impaired chlorophyll biosynthesis of leaf pigments and accelerated their degradation. In fact, salt stress damages chloroplasts and reduce the chlorophyll content, thereby impeding the photosynthesis capacity (Chen et al., 2021; Khalvandi et al., 2021). Additionally, This reduction in leaf pigment contents may be associated with the Na^+ accumulation, K^+depletion , and subsequent lipid peroxidation within leaf cells when exposed to salinity (Taïbi et al., 2016; Nieves-Cordones et al., 2019). In our research, carotenoids concentrations were substantially greater in plants treated with NaCl+SA than in those treated with NaCl alone. This increase suggests that SA application stimulates carotenoids biosynthesis and reduces carotenoids degradation under salt stress, possibly because of diminished oxidative stress (Farhangi-Abriz and Ghassemi-Golezani 2018). This increased carotenoids content may contribute to maintaining PSII efficiency under salt stress (Habibi 2021) and may play a major role in photoprotection (Zhang et al., 2025). In this study, we also employed OJIP transients, which provides indicators of photosynthetic electron transport rate and the efficacy of light energy capture in the PSII, to evaluate PSII performance in plants treated with salt stress and SA treatment. Findings revealed that salt stress led to a more rapid elevation in fluorescence during the O−J phase, suggesting impaired re-oxidation of QA and its increased accumulation, which may reflect decreased efficiency of electron transport beyond QA (Kalaji et al., 2016). In addition, salt stress significantly increased F_o consistent with previous findings reported by Che et al., (2022) and Li et al., (2020). These finding showed that the increase in F_o may be associated with stress-induced inhibition of PSII reaction centers, which can restrict electron transfer from QA to QB and reduce the efficacy of energy trapping within PSII. The quantum yield of primary photochem-

istry F_v/F_m, a key chlorophyll fluorescence parameter, was lower in salt-stressed *P. tuberosa* plants, indicating decreased photosynthetic capacity. This reduction may have lead to greater susceptibility to oxidative stress (Mohagheghian et al., 2025). The F_v/F_o parameter reflects the performance of the water-splitting complex on the donor side of PSII (Schreiber et al., 1995). In this study, the decline in the F_v/F_o ratio under salt stress indicated an inhibition of osmotic water uptake(Fricke and Peters 2002) and disruption of the electron transport and photophosphorylation processes (Pereira et al., 2000). In addition, the decreased PI_{abs} in the salt stress treatment group revealed a reduced efficiency in the number of functional reaction centers and the ability to channel and convey electrons to the electron transport chain (Kalaji et al., 2011). SA supplementation diminished ABS/RC and improved φP_o and φE_o in the salt-stressed *P. tuberosa* plants, highlighting more efficient electron transfer from antennae to RCs (Li et al., 2022). Additionally, salt stress significantly increased Vj and N levels as compared with the control , reflecting increased electron transport through QA reduction and enhanced energy fluxes associated with chlorophyll fluorescence transients. These changes likely indicated the plant's physiological adjustments to cope with stress and meet the elevated energy demands (Akhter et al., 2021). SA supplementation notably reduced these parameters, suggesting that SA suplementation helped stabilizing electron transfer processes and restoring balance to chlorophyll fluorescence transients (Li et al., 2022). Reductions in the phenomenological energy flux parameters, including ABS/CS_m, TR_o/CS_m, ET_o/CS_m, DI_o/CS_m and RE_o/CS_m revealed that PSII was strongly affected by salt stress compared with the control. These reductions may be associated with the conversion of active PSII reaction centers into inactive centers, thereby impairing energy trapping and electron transport within PSII (Chen et al., 2021). Similar results were reported in tomato leaves by Chen et al., (2021) demonstrating the universal impact of salt stress on photosynthetic machinery. Therefore, these findings highlighted the protective role of SA in improving photosynthesis and sustaining productivity under saline conditions.

In addition to the non-enzymatic antioxidant system, the enzymatic antioxidant system including SOD, CAT and APX, is another defense strategy in plants against stress by scavenging excessive ROS (Mahawar and Shekhawat 2019; Mahawar et al., 2024). SOD is typically recognized as the primary antioxidant enzyme that converts superoxide radicals into H_2O_2, a compound subsequently neutralized into H_2O and O_2 by CAT and APX (Khalvandi et al., 2021). APX is a pivotal enzyme within the AsA-GSH cycle, performing the detoxification of H_2O_2 to H_2O by using AsA as an electron donor (Leng et al., 2021). Our research showed increased APX and CAT activities in *P. tuberosa* plants under NaCl stress, corroborating studies on various plant species, including tomato (Faizan et al., 2021), hybrid P*ennisetum* (Li et al., 2020), rice (Koc et al., 2024) and wheat (Khalvandi et al., 2021) and saffron (*Crocus sativus* L.) (Niknam et al., 2025). Our research also revealed that

the activities of SOD, APX and CAT were markedly enhanced upon SA supplementation. This positive effect of SA on the activities of antioxidative enzymes was similar to that observed in salt-stressed Chinese cabbage (Kaya et al., 2020) and wheat (El-Hawary et al., 2023) plants. Under salt stress treatment, increased H_2O_2 and MDA levels exhibited over-accumulation of ROS, which induced significant oxidative damage to cellular biomolecules (Foyer et al., 2020; Liu et al., 2024). The significant increase in ROS accumulation in the leaves of the stressed *P. tuberosa* plants, which was consistent with the decrease in F_v/F_m, highlighting that the reduction of photochemical activity closely attributed to the marked variations in oxidative status. Similarly, were previously demonstrated by. This finding also aligns with previous reports of Azzabi et al., (2012), which the relationships between oxidative status and a decrease in F_v/F_m was observed. In contrast, when the *P. tuberosa* seedings was primed with SA, MDA levels decreased in NaCl-treated plants. These results revealed that SA can play a protective role in diminishing ROS accumulation and diminishing membrane oxidation damage. Similar reduction in MDA values have been observed in tobacco seedlings subjected to SA treatment under salt stress (Song et al., 2025) further corroborating our findings. Interestingly, in this study, endogenous NO content of SA-treated plants exhibited a significant increase which was only detected under salinity stress, revealing that SA affects NO accumulation under stress conditions. Regarding to relationships of SA and NO, several studies have highlighted that SA acts upstream of NO to enhance antioxidant biosynthesis and mitigate oxidative damage caused by environmental stresses, including salt stress in cotton seedlings (Dong et al., 2015), drought stress in wheat (Kolupaev et al., 2018), and heat stress in *Lablab purpureus* L (Rai et al., 2020). However, to determine the synergistic effects of NO and SA in boosting antioxidant defense mechanisms and mitigating NaCl stress damages, will require to be further analysed.

Exogenous SA promoted flowering in *P. tuberosa* under both saline and non-saline conditions, indicating that its effect on floral transition was not strictly stress-dependent. This observation was consistent with earlier reports showing that SA can regulate flowering under both normal and stress conditions (Cleland et al., 1982; Martínez et al., 2004; Wada and Takeno 2013). The positive correlation between flower number and endogenous NO further suggested a potential involvement of NO in SA-related flowering regulation, although the present data support an associative rather than definitive mechanistic role (Hou et al., 2025).

Conclusion

The present study showed that salt stress significantly inhibited plant growth by reducing shoot dry biomass, which was primarily attributed to excessive Na^+ accumulation in plant cells coupled with reduced uptake of K^+ and water. However, SA application reversed the toxic effects of salt-induced damages by inhibiting excessive Na^+ translocation to leaves, resulting in a significant increase in growth-related attributes. The significant increase in ROS

accumulation in the leaves of the stressed *P. tuberosa* plants, which was consistent with the decrease in F_v/F_m, highlighting that the reduction of photochemical activity closely attributed to the marked variations in oxidative status. In contrast, when the *P. tuberosa* plants was primed with SA, MDA levels decreased in NaCl-treated plants. Our findings suggested a potential synergistic or interactive role of NO in SA-mediated antioxidant defense mechanisms and mitigating NaCl stress damages. SA application promoted flowering in *P. tuberosa* irrespective of salinity. Moreover, the positive correlation between NO levels and flower number suggested that NO may function as a signaling component in SAmediated regulation of the floral transition. Our results revealed that SA application can play a protective role in reducing ROS accumulation and diminishing membrane oxidation damage resulting in significant photosynthetic improvements.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

References

Abdullaev, F., Pirogova, P., Vodeneev, V. and Sherstneva, O. (2024) 'Chlorophyll fluorescence in wheat breeding for heat and drought tolerance', *Plants*, 13(19), p.2778. https://doi.org/10.3390/plants13192778.

Acosta-Motos, J.R., Ortuño, M.F., Bernal-Vicente, A., Diaz-Vivancos, P., Sanchez-Blanco, M.J. and Hernandez, J.A. (2015) 'Physiological and biochemical mechanisms of', *Eugenia myrtifolia* L. plants in response to NaCl stress. *Planta*, 242(4), pp.829–846. https://doi.org/10.1007/s00425-015-2330-0.

Ahanger, M.A., Aziz, U., Alsahli, A.A., AlYemeni, M.N. and Ahmad, P. (2020) 'Influence of exogenous salicylic acid and nitric oxide on growth, photosynthesis and ascorbate–glutathione cycle in salt-stressed', *Vigna angularis*. *Biomolecules*, 10(1), p.42. https://doi.org/10.3390/biom10010042.

Ahmad, W., Ijaz, M., Zafar-ul-Hye, M., Tahir, M., Khan, M.A. and Ahmad, I. (2024) 'Silicon-mediated improvement in drought and salinity stress tolerance of black gram (', *Vigna mungo* L.). *ACS Omega*, 9(35), pp.37231–37242. https://doi.org/10.1021/acsomega.4c05342.

Akhter, M.S., Hossain, M.A., Hasanuzzaman, M. and Fujita, M. (2023) 'Silicon supplement improves growth, yield and antioxidant defense system in barley under salt stress', *Journal of Soil Science and Plant Nutrition*, 23(2), pp.1694–1712. https://doi.org/10.1007/s42729-023-01234-5.

Akhter, S.M., Andersen, M.N. and Liu, F. (2021) 'Influence of salinity stress on photosystem II efficiency and electron

transport in barley genotypes probed by chlorophyll', *a* fluorescence. *Journal of King Saud University - Science*, 33(1), p.101239. https://doi.org/10.1016/j.jksus.2020.101239.

Ali, L., Asghar, H.N., Khan, M.Y., Saleem, M.F. and Hussain, S. (2022) 'Growth, photosynthesis and antioxidant enzyme modulation in broccoli under salinity stress', *South African Journal of Botany*, 148, pp.104–111. https://doi.org/10.1016/j.sajb.2022.04.010.

Alizadeh, M.M., Gerami, M., Majidian, P. and Ghorbani, H.R. (2024) 'Potential application of biochar and salicylic acid to alleviate salt stress in soybean', *Heliyon*, 10(4), p.e26677. https://doi.org/10.1016/j.heliyon.2024.e26677.

Arif, Y., Sami, F., Siddiqui, H., Bajguz, A. and Hayat, S. (2020) 'Salicylic acid in relation to other phytohormones: a study towards understanding its role in plant defense signaling under environmental challenges', *Environmental and Experimental Botany*, 175, p.104040. https://doi.org/10.1016/j.envexpbot.2020.104040.

Azzabi, G., Pinnola, A., Betterle, N., Bassi, R. and Alboresi, A. (2012) 'Enhancement of non-photochemical quenching as an adaptive strategy of the moss', *Physcomitrella patens* to cope with salt stress. *Plant and Cell Physiology*, 53(10), pp.1815–1825. https://doi.org/10.1093/pcp/pcs121.

Barwal, S.K., Singh, P., Kumar, A., Yadav, S. and Singh, V.P. (2024) 'Mechanistic insights of salicylic acid–mediated salt stress tolerance in maize seedlings', *Heliyon*, 10(16), p.e34486. https://doi.org/10.1016/j.heliyon.2024.e34486.

Bates, L.S., Waldren, R.P. and Teare, I.D., 1973. Rapid determination of free proline for water-stress studies. *Plant and Soil*, 39(1), pp.205–207. https://doi.org/10.1007/BF00018060.

Batista, V.C.V., do Amaral, M.N., Riffel, A., Auler, P.A. and Moraes, D.M. (2019) 'Salicylic acid modulates primary and secondary metabolism to alleviate salt-induced photosynthesis impairment in', *Egletes viscosa*. *Environmental and Experimental Botany*, 167, p.103870. https://doi.org/10.1016/j.envexpbot.2019.103870.

Boominathan, R. and Doran, P.M. (2002) 'Ni-induced oxidative stress in roots of the Ni hyperaccumulator,', *Alyssum bertolonii*. *New Phytologist*, 156(2), pp.202–205. https://doi.org/10.1046/j.1469-8137.2002.00506.x.

Chaves, M.M., Flexas, J. and Pinheiro, C., 2009. Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. *Annals of Botany*, 103(4), pp.551–560. https://doi.org/10.1093/aob/mcn125.

Che, Y., Xu, X., Wang, Y., Wang, H. and Zhao, H. (2022) 'Potassium application mitigates salt stress impacts by alleviating proton diffusion in thylakoids and maintaining photosynthetic efficiency', *Environmental and Experimental Botany*, 194, p.104708. https://doi.org/10.1016/j.envexpbot.2021.104708.

Chen, L., Zhao, Y., Xu, S., Zhang, Z., Xu, Y., Zhang, J. and Chong, K. (2021) 'Melatonin promotes seed germination

under salt stress by regulating ABA and GA balance in cotton (', *Gossypium hirsutum* L.). *Plant Physiology and Biochemistry*, 162, pp.506–516. https://doi.org/10.1016/j.plaphy.2021.03.016.

Chen, X., Wang, Y., Li, J., Jiang, A. and Cheng, Y. (2021) 'Ascorbic acid-induced photosynthetic adaptability to salt stress monitored by OJIP chlorophyll fluorescence transient analysis', *Frontiers in Plant Science*, 12, p.594400. https://doi.org/10.3389/fpls.2021.594400.

Cleland, C.F., Tanaka, O. and Feldman, L.J., 1982. Influence of plant growth substances and salicylic acid on flowering and growth in the Lemnaceae (duckweeds). *Aquatic Botany*, 13, pp.3–20. https://doi.org/10.1016/0304-3770(82)90036-190036-1).

Dong, Y.J., He, M.R., Wang, Z.L., Chen, W.F., Hou, J., Qiu, X.K. and Zhang, J.W. (2015) 'Interaction of nitric oxide and salicylic acid alleviates salt stress in cotton seedlings', *Journal of Soil Science and Plant Nutrition*, 15(2), pp.561–573. https://doi.org/10.4067/S0718-95162015005000041.

El-Hawary, M.M., Hashem, O.S.M. and Hasanuzzaman, M. (2023) 'Seed application with ascorbic acid and salicylic acid mitigates salt stress in wheat', *Agronomy*, 13(2), p.493. https://doi.org/10.3390/agronomy13020493.

Faizan, M., Faraz, A., Yusuf, M., Khan, S.T. and Hayat, S. (2021) 'Zinc oxide nanoparticles induce salt tolerance by enhancing photosynthetic performance and antioxidant system in tomato', *Plant Physiology and Biochemistry*, 161, pp.122–130. https://doi.org/10.1016/j.plaphy.2021.02.015.

Farhangi-Abriz, S. and Ghassemi-Golezani, K., 2018. How can salicylic acid and jasmonic acid mitigate salt toxicity in soybean plants? *Ecotoxicology and Environmental Safety*, 147, pp.1010–1016. https://doi.org/10.1016/j.ecoenv.2017.09.070.

Foyer, C.H., Kyndt, T. and Hancock, R.D. (2020) 'Vitamin C in plants: novel concepts, new perspectives, and potential applications', *Antioxidants & Redox Signaling*, 32(7), pp.463–485. https://doi.org/10.1089/ars.2019.7812.

Genc, Y., Oldach, K., Verbyla, A.P., Lott, G., Hassan, M., Tester, M., Wallwork, H. and McDonald, G.K. (2019) 'Sodium exclusion and salt tolerance in bread wheat', *Frontiers in Plant Science*, 10, p.1280. https://doi.org/10.3389/fpls.2019.01280.

Ghasemifar, E., Habibi, G. and Bakhshi-Khaniki, G. (2022) 'Pretreatment with salicylic acid and nitric oxide mitigated silver nanoparticles toxicity and enhanced their removal in medicinal', *Phlomis tuberosa* plants. *Acta Biologica Cracoviensia Series Botanica*, 64(2), pp.35–48. https://doi.org/10.24425/abcsb.2022.140302.

Guo, Q., Liu, L. and Barkla, B.J. (2019) 'Membrane lipid remodeling in response to salinity', *International Journal of Molecular Sciences*, 20(17), p.4264. https://doi.org/10.3390/ijms20174264.

Habibi, G. (2020) 'Comparison of CAM expression, photochemistry and antioxidant responses in', *Sedum album*

and *Portulaca oleracea* under combined stress. *Physiologia Plantarum*, 170(4), pp.550–568. https://doi.org/10.1111/ppl.13193.

Hafeez, M.B., Zahra, N., Khan, Z.A., Raza, A., Ahmad, S., Khan, A. and Farooq, M. (2024) 'Plant growth-promoting rhizobacteria improve photosynthetic performance and antioxidant defense system under salinity stress', *Plant Stress*, 12, p.100480. https://doi.org/10.1016/j.stress.2024.100480.

Hasanuzzaman, M., Bhuyan, M.H.M.B., Zulfiqar, F., Raza, A., Mohsin, S.M., Mahmud, J.A., Fujita, M. and Fotopoulos, V. (2021) 'Reactive oxygen species and antioxidant defense in plants under abiotic stress', *International Journal of Molecular Sciences*, 22(17), p.9326. https://doi.org/10.3390/ijms22179326.

Hasanuzzaman, M., Shabala, L., Brodribb, T.J., Zhou, M. and Shabala, S. (2017) 'Screening methods as proxies for drought tolerance in barley', *Functional Plant Biology*, 44(3), pp.253–266. https://doi.org/10.1071/FP16202.

Hayat, Q., Hayat, S., Irfan, M. and Ahmad, A., 2010. Effect of exogenous salicylic acid under changing environment: a review. *Environmental and Experimental Botany*, 68(1), pp.14–25. https://doi.org/10.1016/j.envexpbot.2009.08.005.

He, Y., Zhu, Z., Yang, J., Ni, X. and Zhu, B., 2009. Grafting increases the salt tolerance of tomato by improving photosynthesis and antioxidant enzyme activities. *Environmental and Experimental Botany*, 66(2), pp.270–278. https://doi.org/10.1016/j.envexpbot.2009.02.005.

Hou, X., Shi, M., Zhang, Z., Yao, Y., Li, Y., Li, C., Yu, W., Wang, C. and Liao, W. (2025) 'DNA demethylation is involved in nitric oxide-induced flowering in tomato', *Journal of Integrative Agriculture*, 24, pp.1769–1785. https://doi.org/10.1016/j.jia.2024.03.076.

Hryvusevich, P., Nekrasov, V., Dunaeva, E., Baluška, F. and Shabala, S. (2021) 'Sodium influx and potassium efflux in sunflower roots under high salinity', *Frontiers in Plant Science*, 11, p.2181. https://doi.org/10.3389/fpls.2020.613936.

Isayenkov, S.V. and Maathuis, F.J.M. (2019) 'Plant salinity stress: many unanswered questions remain', *Frontiers in Plant Science*, 10, p.80. https://doi.org/10.3389/fpls.2019.00080.

Johnson, C.M., Stout, P.R., Broyer, T.C. and Carlton, A.B. (1957) 'Comparative chlorine requirements of different plant species', *Plant and Soil*, 8(4), pp.337–353. https://doi.org/10.1007/BF01398944.

Kalaji, H.M., Govindjee, Bosa, K., Kościelniak, J. and Żuk-Gołaszewska, K. (2011) 'Effects of salt stress on photosystem II efficiency and CO_2 assimilation of barley', *Environmental and Experimental Botany*, 73, pp.64–72. https://doi.org/10.1016/j.envexpbot.2010.10.009.

Kalaji, H.M., Jajoo, A., Oukarroum, A., Brestic, M., Zivcak, M., Samborska, I.A., Cetner, M.D., Łukasik, I., Goltsev, V. and Ladle, R.J. (2016) 'Chlorophyll', *a* fluorescence as a tool to monitor physiological status of plants

under abiotic stress conditions. *Acta Physiologiae Plantarum*, 38, p.102. https://doi.org/10.1007/s11738-016-2113-y.

Kareem, F., Rihan, H. and Fuller, M.P. (2019) 'The effect of salicylic acid on drought tolerance in wheat (', *Triticum aestivum* L.). *Journal of Crop Science and Biotechnology*, 22(1), pp.37–45. https://doi.org/10.1007/s12892-018-0014-3.

Kashif, M.H., Hussain, S., Raza, A., Ahmad, S., Shahzad, B. and Farooq, M. (2024) 'Salicylic acid-mediated alleviation of salt stress in', *Asarum sieboldii* Miq. *Chemosphere*, 362, p.142604. https://doi.org/10.1016/j.chemosphere.2024.142604.

Kaya, C., Ashraf, M., Alyemeni, M.N., Corpas, F.J. and Ahmad, P. (2020) 'Nitric oxide-mediated enhancement of the ascorbate–glutathione cycle under arsenic toxicity in maize plants', *Journal of Hazardous Materials*, 399, p.123020. https://doi.org/10.1016/j.jhazmat.2020.123020.

Kaya, C., Ashraf, M., Corpas, F.J. and Ahmad, P. (2022) 'Melatonin and salicylic acid regulate nitrogen metabolism and detoxify arsenic toxicity in maize', *Environmental Pollution*, 297, p.118727. https://doi.org/10.1016/j.envpol.2021.118727.

Kaya, C., Ugurlar, F., Ashraf, M., Corpas, F.J. and Ahmad, P. (2020) 'Endogenous nitric oxide in salicylic acid-induced salinity tolerance of pepper plants', *Plant Physiology and Biochemistry*, 147, pp.10–20. https://doi.org/10.1016/j.plaphy.2019.11.038.

Kaya, C., Ugurlar, F., Ashraf, M., Corpas, F.J. and Ahmad, P. (2023) 'Synergistic effects of melatonin and salicylic acid in improving drought tolerance of tomato plants', *Scientia Horticulturae*, 321, p.112368. https://doi.org/10.1016/j.scienta.2023.112368.

Khalvandi, M., Siosemardeh, A., Roohi, E. and Keramati, S. (2021) 'Salicylic acid alleviates drought stress in winter wheat by regulating photosynthetic capacity and antioxidant defense', *Heliyon*, 7, p.e05908. https://doi.org/10.1016/j.heliyon.2021.e05908.

Koc, Y.E., Aycan, M. and Mitsui, T. (2024) 'Exogenous proline improves salinity tolerance by enhancing antioxidant defense and photosynthetic performance in rice', *Plant Physiology and Biochemistry*, 214, p.108914. https://doi.org/10.1016/j.plaphy.2024.108914.

Kolupaev, Y.E., Yastreb, T.O. and Obozny, A.I., 2018. Combined effect of salicylic acid and nitric oxide donor on antioxidant enzymes and heat resistance of wheat seedlings under drought stress. *Applied Biochemistry and Microbiology*, 54, pp.418–424. https://doi.org/10.1134/S0003683818040120.

Kumar, D., Singh, H., Raj, S. and Soni, V., 2020. Chlorophyll *a* fluorescence kinetics of mung bean (*Vigna radiata* L.) grown under artificial continuous light. *Biochemistry and Biophysics Reports*, 24, p.100813. https://doi.org/10.1016/j.bbrep.2020.100813.

Leng, X., Wang, P., Wang, C., Zhu, X. and Li, X. (2021) 'Genome-wide identification

and characterization of the ascorbate peroxidase gene family in', *Populus trichocarpa*. *Genes*, 12(3), p.334. https://doi.org/10.3390/genes12030334.

Li, G., Peng, X., Wei, L. and Kang, G. (2013) 'Salicylic acid increases antioxidant capacity in wheat leaves under salt stress by enhancing glutathione and ascorbate accumulation', *Gene*, 529(2), pp.321–325. https://doi.org/10.1016/j.gene.2013.08.023.

Li, L., Song, Y., Guan, C., Li, Y. and Chen, X. (2022) 'Silicon ameliorates salt stress by regulating photosynthesis and antioxidant metabolism in cotton', *Antioxidants*, 11(8), p.1520. https://doi.org/10.3390/antiox11081520.

Li, P., Zhu, Y., Song, X. and Song, F. (2020) 'Effects of salinity and drought stress on growth, photosynthesis and antioxidant metabolism of hybrid', *Pennisetum*. *Plant Physiology and Biochemistry*, 155, pp.93–104. https://doi.org/10.1016/j.plaphy.2020.07.003.

Li, X., Zhang, L., Ahammed, G.J., Li, Z., Wei, J., Shen, C., Yan, P., Zhang, L., Han, W. and Fu, J. (2019) 'Salicylic acid acts upstream of nitric oxide in regulating flavonoid biosynthesis and abiotic stress tolerance in tea plants', *Environmental and Experimental Botany*, 161, pp.367–374. https://doi.org/10.1016/j.envexpbot.2019.01.012.

Lichtenthaler, H. and Wellburn, A.R. (1985) 'Determination of total carotenoids and chlorophylls a and b of leaf in different solvents', *Biochemical Society Transactions*, 11, pp.591–592. https://doi.org/10.1042/bst0110591.

Liu, W., Zhang, Y., Yuan, X., Xuan, Y., Gao, Y. and Yan, Y. (2016) 'Exogenous salicylic acid improves salinity tolerance of', *Nitraria tangutorum* by enhancing antioxidant capacity. *Russian Journal of Plant Physiology*, 63(1), pp.132–142. https://doi.org/10.1134/S1021443716010101.

Liu, Y., Zhang, L., Liu, B., Wu, Y., Sun, Y. and Guo, S. (2024) 'Biochar improves soil properties and growth of', *Melissa officinalis* under salt stress. *Scientia Horticulturae*, 338, p.113704. https://doi.org/10.1016/j.scienta.2024.113704.

Maghsoudi, K., Arvin, M.J. and Ashraf, M., 2020. Mitigation of arsenic toxicity in wheat by salicylic acid. *Journal of Soil Science and Plant Nutrition*, 20, pp.577–588. https://doi.org/10.1007/s42729-019-00146-6.

Mahawar, L. and Shekhawat, G.S. (2019) 'Heme oxygenase 1 mediated mitigation of NaCl-induced oxidative stress in', *Eruca sativa*. *Physiology and Molecular Biology of Plants*, 25(4), pp.895–904. https://doi.org/10.1007/s12298-019-00672-1.

Mahawar, L., Sharma, P. and Shekhawat, G.S., 2024. Copper oxide and zinc oxide nanoparticles influence photosynthesis and antioxidant defense in *Raphanus sativus* under salinity stress. *Plant Physiology and Biochemistry*, 206, p.108281. https://doi.org/10.1016/j.plaphy.2023.108281.

Martínez, C., Pons, E., Prats, G. and León, J., 2004. Salicylic acid regulates flowering time and links defense responses and reproductive development. *The Plant*

Journal, 37(2), pp.209–217. https://doi.org/10.1046/j.1365-313X.2003.01980.x.

Methenni, K., Balti, R., Chehab, H., Ouled-Messaoud, M. and Trabelsi, N. (2018) 'Salicylic acid and calcium alleviate salinity effects on growth and physiological traits of olive', *Scientia Horticulturae*, 233, pp.349–358. https://doi.org/10.1016/j.scienta.2018.01.046.

Miller, G., Suzuki, N., Ciftci-Yilmaz, S. and Mittler, R. (2010) 'Reactive oxygen species homeostasis and signalling during drought and salinity stresses', *Plant, Cell & Environment*, 33(4), pp.453–467. https://doi.org/10.1111/j.1365-3040.2009.02083.x.

Mukhopadhyay, R., Sarkar, B., Ghosh, S., Mondal, S., Tripathi, D.K., Pandey, S., Corpas, F.J., Khan, M.I. and Ahmad, A., 2021. Role of salicylic acid in enhancing plant tolerance to salt stress. *Plant Physiology and Biochemistry*, 164, pp.196–212. https://doi.org/10.1016/j.plaphy.2021.04.024.

Munns, R. and Gilliham, M. (2015) 'Salinity tolerance of crops – what is the cost?', *New Phytologist*, 208(3), pp.668–673. https://doi.org/10.1111/nph.13519.

Munns, R. and Tester, M. (2008) 'Mechanisms of salinity tolerance', *Annual Review of Plant Biology*, 59, pp.651–681. https://doi.org/10.1146/annurev.arplant.59.032607.092911.

Nazar, R., Iqbal, N., Syeed, S. and Khan, N.A., 2011. Salicylic acid alleviates the adverse effects of salt stress by improving photosynthesis and antioxidant metabolism in mustard. *Plant Physiology and Biochemistry*, 49(2), pp.123–131. https://doi.org/10.1016/j.plaphy.2011.01.003.

Nikolaeva, M.K., Maevskaya, S.N., Shugaev, A.G. and Bukhov, N.G. (2010) 'Effect of drought on chlorophyll fluorescence parameters and antioxidant activities in wheat leaves', *Russian Journal of Plant Physiology*, 57(1), pp.87–95. https://doi.org/10.1134/S1021443710010130.

Noreen, S., Fatima, N., Akhtar, S., Hussain, K. and Ashraf, M. (2021) 'Regulation of photosynthetic efficiency, ion homeostasis, and antioxidant defense by salicylic acid in rice under salt stress', *Journal of Plant Growth Regulation*, 40(4), pp.1753–1767. https://doi.org/10.1007/s00344-020-10245-y.

Pandey, P., Srivastava, R.K. and R.S., D., 2024. Alleviatory role of ascorbic acid and proline on salt-induced oxidative stress in wheat seedlings. *Biologia Plantarum*, 68, pp.417–428. Available at: https://doi.org/10.32615/bp.2024.035.

Porcel, R. and Ruiz-Lozano, J.M. (2004) 'Arbuscular mycorrhizal influence on leaf water potential, solute accumulation, and oxidative stress in soybean plants subjected to drought stress', *Journal of Experimental Botany*, 55(403), pp.1743–1750. https://doi.org/10.1093/jxb/erh188.

Qiu, Z., Guo, J., Zhang, F., Sun, L. and Li, C. (2014) 'Improved salt tolerance through overexpression of a chloroplast-localized small heat shock protein in', *Arabidopsis thaliana*. *Plant Physiology and Biochemistry*, 80, pp.47–53. https://doi.org/10.1016/j.plaphy.2014.03.025.

Rady, M.M. and Hemida, K.A. (2016)

'Salicylic acid increases salt tolerance and improves antioxidant systems in maize', *Environmental Science and Pollution Research*, 23(8), pp.7013–7022. https://doi.org/10.1007/s11356-015-5910-7.

Rady, M.M., Taha, R.S., Mahdi, A.H., Metwally, I.H. and Salem, M.F. (2023) 'Ascorbic acid enhances salt tolerance in barley by boosting antioxidant defense and maintaining photosynthetic efficiency', *Agronomy*, 13(7), p.1765. https://doi.org/10.3390/agronomy13071765.

Rao, M., Ahmed, S., Younis, U., Raza, A., Mumtaz, S., Jatoi, F.A., Anwar, S., Alharby, H.F. and Bamagoos, A.A. (2024) 'Interactive effects of salicylic acid and zinc oxide nanoparticles on salinity tolerance in tomato', *Chemosphere*, 345, p.140567. https://doi.org/10.1016/j.chemosphere.2023.140567.

Rizwan, M., Ali, B., Qayyum, A., Ok, Y.S., Adrees, M., Ibrahim, M., Zia-ur-Rehman, M., Farooq, Q. and Abbas, F. (2016) 'Mechanisms of salinity tolerance in plants: a review', *Environmental Science and Pollution Research*, 23(9), pp.8697–8710. https://doi.org/10.1007/s11356-016-6470-9.

Sarker, U. and Oba, S. (2018) 'Salinity stress and red amaranth: oxidative stress response and antioxidant defense', *Scientia Horticulturae*, 239, pp.314–322. https://doi.org/10.1016/j.scienta.2018.05.058.

Sayfzadeh, S., Ahmadi, A. and Shikhi, S. (2024) 'Salicylic acid improves chlorophyll fluorescence, photosynthesis and antioxidant response of maize under salt stress', *Plant Stress*, 12, p.100512. https://doi.org/10.1016/j.stress.2024.100512.

Shahverdi, M.A., Omidi, H., Azizinezhad, R. and Taleei, A. (2017) 'Salicylic acid-mediated effects on photosynthesis, osmolytes and antioxidative enzymes in salt-stressed rapeseed', *Acta Physiologiae Plantarum*, 39, p.275. https://doi.org/10.1007/s11738-017-2565-y.

Sharma, A., Sidhu, G.P.S., Araniti, F., Bali, A.S., Shahzad, B., Tripathi, D.K., Brestic, M., Skalicky, M. and Landi, M. (2020) 'Exogenous application of salicylic acid improves salinity tolerance in plants: a meta-analysis', *Ecotoxicology and Environmental Safety*, 196, p.110537. https://doi.org/10.1016/j.ecoenv.2020.110537.

Shemi, R., Wang, R., Peng, Y., Liu, D., Meng, Y., Sun, Y., Li, K., Chen, S., Xiao, F. and Ma, X. (2020) 'Silencing of MAPK genes enhances salinity tolerance in rice seedlings via boosting ROS scavenging and osmotic adjustment', *International Journal of Molecular Sciences*, 21(9), p.3220. https://doi.org/10.3390/ijms21093220.

Simon, L.M., Fatrai, Z., Jonas, D.E. and Matkovics, B. (1974) 'Study of peroxide metabolism enzymes during the development of', *Phaseolus vulgaris*. *Biochemie und Physiologie der Pflanzen*, 166, pp.387–392.

Song, X., Chen, J., Xu, C., Cai, X., Song, W., Chang, A., Zhang, Y. and Luo, C. (2025) 'Physiological and molecular mechanisms of exogenous salicylic acid in enhancing salt tolerance in tobacco seedlings by regulating

antioxidant defence system and gene expression', *Frontiers in Plant Science*, 16, p.1545865. https://doi.org/10.3389/fpls.2025.1545865.

Strasser, R.J., Tsimilli-Michael, M. and Srivastava, A. (2004) 'Analysis of the chlorophyll', *a* fluorescence transient. In: Papageorgiou, G.C. and Govindjee, eds. *Chlorophyll a fluorescence: a signature of photosynthesis*. Dordrecht: Springer, pp.321–362.

Tavallali, V., Karimi, S., Rahemi, M., Ramezanian, A. and Eshghi, S. (2010) 'Role of salicylic acid and nitric oxide in salt tolerance of', *Salvia officinalis*. *Acta Physiologiae Plantarum*, 32, pp.263–270. https://doi.org/10.1007/s11738-009-0403-1.

Turkan, I. and Demiral, T., 2009. Recent developments in understanding salinity tolerance. *Environmental and Experimental Botany*, 67(1), pp.2–9. https://doi.org/10.1016/j.envexpbot.2009.05.008.

Velikova, V., Yordanov, I. and Edreva, A. (2000) 'Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines', *Plant Science*, 151(1), pp.59–66.

Wada, K.C. and Takeno, K., 2013. Salicylic acid-mediated stress-induced flowering. In: Hayat, S., Ahmad, A. and Alyemeni, M.N., eds. *Salicylic acid: plant growth and development*. Dordrecht: Springer Netherlands, pp.163–182.

Wada, K.C., Yamada, M., Shiraya, T. and Takeno, K. (2010) 'Salicylic acid and the flowering gene FLOWERING LOCUS T homolog are involved in poor-nutrition-stress-induced flowering of', *Pharbitis nil*. *Journal of Plant Physiology*, 167(5), pp.447–452. https://doi.org/10.1016/j.jplph.2009.07.009.

Wang, X., Su, L., Wang, X., Zhang, Q. and Guo, Y. (2024) 'Melatonin improves salt tolerance in', *Brassica napus* by modulating photosynthesis and ROS homeostasis. *Plant Physiology and Biochemistry*, 214, p.108922. https://doi.org/10.1016/j.plaphy.2024.108922.

Wu, Q., Su, N., Zhang, X., Liu, Y., Cui, J. and Liang, Y. (2016) 'Hydrogen peroxide, nitric oxide and UV RESISTANCE LOCUS interact to mediate UV-B-induced anthocyanin biosynthesis in radish sprouts', *Scientific Reports*, 6(1), p.29164.

Xiao, X., Wang, Y., Qiu, Q., Zhang, M. and Ma, X. (2022) 'Salicylic acid enhances salt tolerance in potato by improving ion homeostasis and antioxidant defense', *Plant Physiology and Biochemistry*, 187, pp.140–151. https://doi.org/10.1016/j.plaphy.2022.08.020.

Xu, J., Tian, Y., Li, F., Li, P., Gao, Y. and Chen, W. (2016) 'Exogenous ascorbic acid improves photosynthesis and antioxidant capacity in tomato plants under salinity stress', *Biologia Plantarum*, 60(1), pp.181–188. https://doi.org/10.1007/s10535-015-0577-0.

Yadav, K., Singh, N. and Verma, P. (2021) 'Salicylic acid-induced modulation of photosynthesis and oxidative stress tolerance in', *Triticum aestivum* under salt stress. *Physiology and Molecular Biology of Plants*, 27(6), pp.1263–1275.

https://doi.org/10.1007/s12298-021-00987-0.

Yin, J., Jia, J., Lian, Z., Liang, X., Xin, G., Liu, W. and Li, T. (2019) 'Effects of salinity stress on chlorophyll fluorescence and photosynthetic performance in soybean', *Agronomy*, 9(12), p.655. https://doi.org/10.3390/agronomy9100655.

Zarei, M., Shabani, L., Sabzalian, M.R. and Fazeli, A. (2016) 'Salicylic acid regulates secondary metabolite production and antioxidant capacity in', *Stevia rebaudiana* under salinity stress. *Plant Physiology and Biochemistry*, 100, pp.67–75. https://doi.org/10.1016/j.plaphy.2016.01.015.

Zhang, J., Jia, W., Yang, J. and Ismail, A.M. (2006) 'Role of ABA in integrating plant responses to drought and salinity stresses', *Field Crops Research*, 97(1), pp.111–119. https://doi.org/10.1016/j.fcr.2005.08.018.

Zhang, L., Gao, M., Zhang, L., Li, B. and Han, M. (2024) 'Salicylic acid enhances salt tolerance in maize through improved photosynthesis and redox regulation', *Environmental and Experimental Botany*, 212, p.105555. https://doi.org/10.1016/j.envexpbot.2023.105555.