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ORIGINAL PAPER

Multifaceted Impact of Exogenous Salicylic Acid on *Vicia Faba* L. Under Salt Stress: Plant Growth, Water Status, and Photosynthetic Performance (OJIP Fluorescence)

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Abstract

Purpose This study investigated the effect of salt stress on growth, water status, and photosynthetic activity in faba bean plants and the role of salicylic acid (SA) in mitigating the harmful effects of salt stress.

Methods Faba bean plants were subjected to different levels of salt stress (0, 90, 120, and 150 mM NaCl) and salicylic acid (0, 0.5, and 1 mM SA). Salt and SA treatments were applied starting from the seedling stage and continued for two months.

Results Results show that salt stress significantly affects the different studied parameters. Salinity strongly decreases the plant weight (fresh and dry) and the plant water status (Leaf Water Potential (LWP), Stomatal conductance (gs), Relative water content (RWC). The analysis of the Photosystem II (PSII) function disruption indicates that salt stress induced an electron transport inhibition at the donor side of the PSII due to the Oxygen-Evolving Complex (OEC) inactivation (positive-K-band) and reduced the PSII unit's energetic connectivity (positive-L-band). The evaluation of the rate reduction of the end electron acceptor at the Photosystem I (PSI) side revealed that salt stress resulted in gradual decreases in the reduction rates. Nevertheless, the exogenous application of salicylic acid (SA) allowed plants to maintain a high weight/length value with a significant improvement in plant water status. Chlorophyll-*a* fluorescence analysis shows that SA application improved at the donor side of electron transport (lower intensity of the K and L-band), with a larger pool size under the combination of 0.5 mM SA and high salt stress levels applications.

Conclusion These results suggest that the salt stress significantly affects the PSII by the inactivation of the OEC and decreasing the PSII unit's connectivity. However, SA had a beneficial effect on the PSII and PSI salt stress tolerance in *Vicia faba* L.

Keywords Salt stress · Salicylic acid · Water potential · Photosystem II (PSII) · Chlorophyll fluorescence (OJIP)

1 Introduction

Salinity represents one of the most significant abiotic stressors constraining global agricultural productivity. Recent FAO (2024) data indicate that 1.4 billion hectares (over 10% of the world's arable land) currently experience salinity stress, with projections suggesting an additional one billion hectares may become affected through secondary salinization processes driven by climate change and unsustainable agricultural practices. Salt stress can affect all growing phases, including germination, seedling emergence, and vegetative stages, affecting the whole crop (Anaya et al. 2017; Lamnai et al. 2021, 2022). Salt stress induces multifaceted physiological disruptions that collectively impair plant growth and development. Primary effects include osmotic stress through decreased water potential and stomatal

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closure (Van Zelm et al. 2020; Zhang et al. 2022), followed by ionic imbalance characterized by Na + accumulation and K + depletion. This Na+/K + perturbation disrupts enzyme activation and membrane potential maintenance (Dawood et al. 2022). Subsequently, salt stress triggers Na + toxicity, leading to chlorophyll degradation and photosynthetic inhibition through both stomatal limitation and direct damage to photosystem II (PSII) reaction centers (Abdulmajeed et al. 2022; Boorboori and Li 2025).

Salt stress triggers stomatal closure, reducing CO_2 assimilation and impairing electron transport, which suppresses photosynthetic capacity (Soliman et al. 2024; Zhang et al. 2023). Additionally, salinity stress decreases the Fv/Fm ratio (indicating PSII photoinhibition) and elevates non-photochemical quenching (NPQ) as protective mechanisms against excess energy (Boorboori and Li 2025; Saravi et al. 2022). In addition, abiotic stress constrains the electron transport chain and reduces the number of open PSII reaction centers, thereby compromising photochemical efficiency (Choudhury and Moulick 2023; Fghire et al. 2015; Zhang and Sharkey 2009).

The chlorophyll *a* fluorescence presents a non-destructive method to evaluate and detect photosynthetic activity efficiency by giving information on the electrons transfer between PSII components (Akhter et al. 2021; Fghire et al. 2015). The OJIP chlorophyll fluorescence transient captures the sequential reduction of photosynthetic electron carriers during the light-induced polyphasic rise from initial (O) to peak fluorescence (P), with intermediate J and I steps. The O-J phase reflects Q_A reduction at PSII, while J-I represents electron transfer to Q_B and subsequent plastoquinone (PQ) pool reduction via the cytochrome b_6f complex and plastocyanin (PC). The I-P phase indicates the final reduction of PSI terminal acceptors (ferredoxin and $NADP^+$), completing the electron transport chain (Brestic and Allakhverdiev 2022; Chen et al. 2024). Each phase serves as a diagnostic marker: O-J for PSII donor-side integrity, J-I for intersystem electron transport efficiency, and I-P for PSI acceptor-side capacity. After that, OJIP analysis can be considered a powerful technique for studying the PSII structural stability and energy fluxes between the PSII components (Akhter et al. 2021).

Moreover, the introduced SA in *Vigna angularis* had a beneficial influence on plant tolerance to salinity-induced oxidative stress. Reflected by a limited peroxidation compound and enhanced enzymatic and non-enzymatic antioxidant activity (Ahanger et al. 2019). Several research works have revealed the positive effect of SA on chlorophylls and carotenoid levels, plant water status, stomatal conductance, and photosynthetic electrons transfer under stress conditions (Ahanger et al. 2019; Lotfi et al. 2020). Chlorophyll a fluorescence analysis revealed that salicylic acid (SA) application significantly enhanced photosynthetic efficiency in salt-stressed mung bean plants (Lotfi et al. 2020; Ogunsiji et al. 2023) and melon plant (Fatima et al. 2023) and in soursop (da Silva et al. 2022a; Silva et al. 2022b). SA mitigates salinity impacts by improving water balance, physiological processes, and key metabolic pathways, thereby sustaining plant growth under stress (Khoshbakht et al. 2018). Elucidating such tolerance mechanisms could inform strategies to optimize crop productivity through targeted growth regulation.

Exogenous salicylic acid (SA) enhanced salinity tolerance in *Vigna angularis* by reducing oxidative damage through lower lipid peroxidation and elevated antioxidant activity (Ahanger et al. 2019). SA treatment preserved photosynthetic function by maintaining chlorophyll content, stomatal conductance, and electron transport efficiency (Lotfi et al. 2020).

This study employs an innovative combination of chlorophyll fluorescence kinetics (OJIP analysis) and physiological traits to investigate salicylic acid (SA) mediated salt tolerance in faba bean (*Vicia faba* L.). We hypothesize that SA enhances salinity resilience through two synergistic mechanisms: (1) stabilization of PSII function (OEC integrity and electron transport efficiency, quantified via JIP-test parameters), and (2) activation of antioxidant defenses. In this study, we hypothesize SA enhances salinity resilience by simultaneously: (1) stabilizing PSII function through OEC protection and electron transport efficiency, (2) optimizing stomatal regulation and water homeostasis, and (3) preserving photosynthetic capacity to sustain growth. This unique tripartite methodology will establish quantitative relationships between photochemical efficiency, water-use dynamics, and whole-plant performance under salt stress. Our integrated approach uniquely links photosynthetic performance (OJIP transient), stomatal regulation, relative water content (RWC), and growth metrics to establish SA's holistic protective role in legumes under salt stress.

2 Materials and Methods

2.1 Plant Material and Growth Conditions

Faba beans (*Vicia faba* L.) cv "Reina Mora" seedlings were grown in a greenhouse with controlled conditions: average temperature (day/night) of 25.8 ± 2.5 °C/14.2 ± 1.5 °C, relative humidity ranging from 38.8 to 79.4%, and 11 h of sunshine. Intact seeds, which were homogeneous, identical in size and color, and free from wrinkles, were chosen and then disinfected for 1 min in 70% (v/v) ethanol and then soaked in 20% (v/v) commercial bleach for 10 min. Seeds were rinsed several times with sterile distilled water. After that, the seeds were sowed in sterilized peat in an incubator at 28 °C. After obtaining two true leaves, the seedlings were transplanted to pots (20 cm in diameter and 30 cm high) filled with sterilized sand and peat at a 2:1 ratio. We applied salt stress using four levels of saline irrigation with sodium chloride (NaCl): 0 mM (control), 90 mM, 120 mM, and 150 mM. We combined these salt stress levels with three concentrations of salicylic acid (SA): 0 mM, 0.5 mM, and 1 mM. The treatments were arranged as follows:

- Control: No salt stress (0 mM NaCl) and no SA (0 mM).
- 90 N, 120 N, 150 N: Salt stress levels of 90 mM, 120 mM, and 150 mM NaCl, respectively, without SA.
- 90 N/0.5SA, 120 N/0.5SA, 150 N/0.5SA: Salt stress levels of 90 mM, 120 mM, and 150 mM NaCl, respectively, combined with 0.5 mM SA.
- 90 N/1SA, 120 N/1SA, 150 N/1SA: Salt stress levels of 90 mM, 120 mM, and 150 mM NaCl, respectively, combined with 1 mM SA.

Table 1 summarizes the experimental treatments, including NaCl salinity levels (0-150 mM) and SA concentrations (0–1 mM).

We arranged the pots in a completely randomized design, treating each pot as one replicate, with ten pots per treatment. We irrigated the pots once a week with half-strength Hoagland solution. During the two-month salt treatment period, we watered the pots with the corresponding treatments every three days, and applied distilled water every two weeks to prevent salt accumulation. The plants irrigated with distilled water were taken as a control. All Analysis were done at the end of experiments (After 2 months of experience).

2.2 Plant Growth Parameters

To assess salinity and SA effects on faba bean growth, we harvested the plants at the end of the experiment (after two months). We measured shoot and root length followed by fresh weight determination using an analytical balance (±0.0001 g). We then measured dry weight after oven-drying the samples at 80 °C for 48 h in a forced-air oven until they reached constant mass.

Table 1 Different treatments of salt stress (sodium chloride, NaCl) in combination with Salicylic acid (SA) studied in this experiment

Salicylic acid (SA)	Salinity (NaCl)			
	0 mM	90 mM	120 mM	150 mM
0 mM	Control	90 N	120 N	150 N
0.5 mM	0.5SA	90 N/0.5SA	120 N/0.5SA	150 N/0.5SA
1 mM	1SA	90 N/1SA	120 N/1SA	150 N/1SA

2.3 Leaf Water Potential Measurement

Plant water status Pre-Dawn Leaf Water Potential (Ψleaf) was measured using a Scholander pressure chamber (SKPD 1400, Skye Instruments, Powys, UK). For each treatment, four representative plants were selected, and newly matured leaves were excised at the petiole base using a razor blade. Samples were immediately placed in the humidified chamber, and balancing pressure was recorded when xylem sap first appeared at the cut surface. Measurements were completed within 15 s of excision to minimize errors.

2.4 Stomatal Conductance Measurement

Stomatal conductance (gs; mmol m^{-2} s^{-1}) was measured on sunny days at midday using a portable porometer (Leaf Porometer, Decagon Device, Inc., Washington, USA). Prior to measurements, the device was calibrated using the manufacturer's reference plate. For each treatment, six replicate measurements were taken from the distal third of two uppermost leaves, with the sensor head oriented perpendicular to incident sunlight. All measurements were conducted on clear days with stable photosynthetic photon flux density.

2.5 Relative Water Content Determination

We determined leaf relative water content (RWC) using the standard gravimetric method. Fresh weight (FW) was measured immediately after leaf excision. Samples were then hydrated in distilled water at 4 °C for 12 h in darkness to obtain turgid weight (TW), followed by drying at 80 °C for 48 h until constant mass to measure dry weight (DW).

We calculated RWC using the formula:

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

Where FW is the fresh weight, DW is the dry weight, and TW is the turgid weight of the leaf.

2.6 Leaf Surface

Photos of fresh detached leaves were analyzed using the Mesirum-pro software (V.2) to determine the leaf surface.

We calibrated and processed the images following the user manual instructions, which involved setting a scale based on a known reference length and selecting leaf areas by color alignment to accurately measure surface size. This method ensured precise and reproducible leaf surface measurements.

2.7 Measurement of Chlorophyll *a* Fluorescence

Chlorophyll fluorescence measurements were performed two months after sowing using a portable chlorophyll-a fluorometer (Plant Efficiency Analyzer, Handy PEA, Hansatech Instruments, King's Lynn, UK). Before the fluorescence measurements, the leaf samples were dark-adapted for 20 min. Then dark-adapted leaves were exposed to a saturating red-light pulse (3500 µmol photons m-^{2}s-^{1}) over an area of 4 mm diameter. The fluorescence signal was recorded in a time scan from 50 µs to 1s (Strasser and Strasser 1995). The data were transferred with handy PEA v1.30 software and treated according to the equations of the JIP-test parameters (Table 2) (Strasser et al. 2004).

The fluorescence transient thus generated, plotted on a logarithmic time scale, clearly shows intermediate steps, named OJIP, from the basal level O to the last step P, where F_P= F_m, maximal fluorescence. At the first step O the emitted fluorescence F_0 is proportional to the fraction of open reaction centers (RC). F_m was reached when the excitation intensity is high enough to ensure the closure of all RCs of PSII, namely when all the Q_A are entirely reduced. Different normalizations as relative variable fluorescence were performed at OJ, OI, and IP curves phases. The calculation formula of parameters and description are listed in Table 2.

OJIP parameters and their meaning: Minimum fluorescence recorded in the dark-adapted sample is labeled F_O. Maximum fluorescence is labeled as $F_{m,}$ and F_t is the fluorescence signal, recorded at time t after the beginning of illumination with PAR.

2.8 Statistical Analysis

The experiments were carried out with a completely randomized design. All data were statistically analyzed by ANOVA using CoStat software. Values are means of four replicates; the means were separated with the least significant difference (LSD) test at ($p < 0.05$).

3 Results

3.1 Plant Growth Parameters

The growth parameters of faba bean plants cultivated under different salt stress and SA are displayed in Table 3. The analysis of variance shows that the growth parameters were significantly ($p < 0.001$) influenced by salt stress, SA, and their combination (Table 4). However, SA treatment did not significantly affect root length.

The fresh shoot weight (SFW), as displayed in Table 3, was in decrease with increased salinity, and we have noted reductions of 40%, 52%, and 76% for 90 N, 120 N, and 150 N, respectively, in comparison to the control. The SA application allows an amelioration of SFW where we have noted an increase in SFW of about 37% and 86% for 150 N/0.5 SA and 150 N/1SA, respectively, compared to

Table 2 Summary of formulas and description of the chlorophyll a fluorescence OJIP curves parameters used in this study (Strasser et al. 2010)

Technical fluorescence parameters	
F_t	Fluorescence at time t after onset of actinic illumination
$F_0 = F_{50µs}$	Minimal fluorescence, when all PSII RCs are open
$F_L = F_{150µs}$	Fluorescence intensity at the L-step (150 µs) of OJIP
$F_K = F_{300µs}$	Fluorescence intensity at the K-step (300 µs) of OJIP
$F_J = F_{2ms}$	Fluorescence intensity at the J-step (2 ms) of OJIP
$F_I = F_{30ms}$	Fluorescence intensity at the I-step (30 ms) of OJIP
$F_P = F_m$	Maximal recorded fluorescence intensity, at the peak P of OJIP
$F_v = F_t - F_0$	Variable fluorescence at time t
F_m	Time (ms) to reach the maximal fluorescence intensity F_M
$V_t = (F_t - F_0)/(F_m - F_0)$	Relative variable fluorescence at time t
$V_K = (F_K - F_0)/(F_m - F_0)$	Relative variable fluorescence at the K-step
$V_J = (F_J - F_0)/(F_m - F_0)$	Relative variable fluorescence at the J-step
$W_t = (F_t - F_0)/(F_J - F_0)$	Relative variable fluorescence Fv to the amplitude FJ – FO
$W_L = W_{150µs} = (F_{150µs} - F_0)/(F_J - F_0)$	Relative variable fluorescence at the L-step to the amplitude F_J
$W_K = W_{300µs} = (F_{300µs} - F_0)/(F_J - F_0)$	Relative variable fluorescence at the K-step to the amplitude F_J
$M_0 = 4\ (F_{300µs} - F_0)/(F_M - F_0)$	Approximated initial slope (in ms^{-1}) of the fluorescence transient normalized on the maximal variable fluorescence F_V
$W_{OK} = (F_t - F_0)/(F_K - F_0)$	Ratio of variable fluorescence F_t-F_0 to the amplitude F_K-F_0
$W_{OJ} = (F_t - F_0)/(F_J - F_0)$	Ratio of variable fluorescence F_t-F_0 to the amplitude F_J-F_0
$W_{OI} = (F_t - F_0)/(F_I - F_0)$	Ratio of variable fluorescence F_t-F_0 to the amplitude F_I-F_0

In subscript "0" (or "o" when written after another subscript) indicates that the parameter refers to the onset of illumination, when all RCs are assumed to be open

Table 3 Growth parameters of faba bean plant grown under four salt stress levels (0, 90, 120 and 150 mM NaCl) combined to Salicylic acid (SA) (0, 0.5 and 1 mM SA). Data are means ($n = 4$), values with different letters are significantly different ($p < 0.05$) according to the LSD test

	Shoot fresh weight (SFW) (g)	Root fresh weight (RFW) (g)	Shoot dry weight (SDW) (g)	Root dry weight (RDW) (g)	Shoot length (SL) (cm)	Root length (RL) (cm)
Control	20.5 ± 1.7 a	15.0 ± 3.5 a	5.1 ± 0.4 a	3.4 ± 0.9 a	70.8 ± 5 a	32.0 ± 2.8 a
0.5SA	17.8 ± 0.8 b	11.0 ± 0.4 b	4.4 ± 0.2 b	2.5 ± 0.4 b	58.5 ± 1.3 b	27.8 ± 0.5 b
1SA	16.1 ± 0.7 c	9.1 ± 0.5 c	4.0 ± 0.2 c	1.9 ± 0.2 c	54.8 ± 0.9 bc	26.5 ± 0.6 bc
90 N	12.2 ± 0.5 f	6.9 ± 0.2 de	3.0 ± 0.1 f	1.4 ± 0.2 de	49.5 ± 3.0 de	23.0 ± 1.2 e
90 N/0.5SA	14.7 ± 0.2 d	7.7 ± 0.3 cd	3.6 ± 0.1 d	1.5 ± 0.23 cd	52.5 ± 1.0 cd	25.3 ± 0.5 cd
90 N/1SA	13.5 ± 0.7 e	7.2 ± 0.1 de	3.4 ± 0.2 e	1.5 ± 0.1 cd	49.5 ± 0.6 de	24.3 ± 0.5 de
120 N	9.9 ± 0.2 gh	5.4 ± 0.1 fgh	2.5 ± 0.1 gh	1.1 ± 0.1 defg	40.3 ± 0.5 gh	19.5 ± 0.6 gf
120 N/0.5SA	10.7 ± 0.4 g	6.5 ± 0.2 def	2.7 ± 0.1 g	1.4 ± 0.1 de	46 ± 1.2 ef	21.0 ± 0.0 f
120 N/1SA	10.2 ± 0.2 g	6.0 ± 0.2 efg	2.5 ± 0.1 g	1.3 ± 0.2 def	44 ± 0.8 fg	20.0 ± 0.0 f
150 N	4.9 ± 0.3 j	3.9 ± 0.3 h	1.2 ± 0.1 j	0.9 ± 0.1 g	25.8 ± 6.1 j	13.8 ± 1.7 i
150 N/0.5SA	6.7 ± 0.9 i	4.6 ± 0.3 gh	1.7 ± 0.2 i	1.0 ± 0.1 efg	35.78 ± 2.9 i	15.8 ± 0.5 h
150 N/1SA	9.1 ± 0.3 h	5.0 ± 0.1 gh	2.3 ± 0.1 h	0.9 ± 0.1 fg	39.5 ± 0.6 hi	18.0 ± 0.0 g

Table 4 Statistical analysis of variance of growth and physiological parameters of faba bean grown under salt stress combined with Salicylic Acid

	Salt stress	Salicylic acid	Salt stress*Salicylic acid
DF	3	2	6
SFW	***	*	***
RFW	***	***	***
SDW	***	***	***
RDW	***	***	***
SL	***	***	***
RL	***	ns	***
gs	***	*	***
LWP	***	***	***
RWC	***	***	***
LS	***	***	***

*** highly significant ($p < 0.001$); ** high significance ($p < 0.01$); * significant ($p < 0.05$); ns: no significance difference

Degrees of Freedom (DF), Shoot fresh weight (SFW), Root fresh weight (RFW), Shoot dry weight (SDW), Root dry weight (RDW), Shoot length (SL), Root length (RL)

150 N treatment. The comparison of means reveals the no-significance difference between 120 N and 150 N/1 SA and between treatments under 120 N with or without SA.

The shoot dry weight (SDW) results, as presented in Table 3, display the same tendency of decreasing with increasing salt stress. The reductions of SDW in comparison to the control were 41%, 51%, and 76% for 90 N, 120 N, and 150 N treatments, respectively. In comparison to each respective salt treatment, the SA application proved an increase in SDW. These increases were 20%, 8%, and 42% for 90 N/0.5SA, 120 N/0.5SA, and 150 N/0.5SA, respectively. Application of 1 mM SA under high salt stress level (150 N) reveals a significant increase of 92%. The comparison of means (Table 3) reveals no significant difference between treatments under 120 mM of NaCl and between 120 N and 150 N/1SA.

Shoot length (SL), as presented in Table 3, was significantly decreased under salt stress from 63.25 cm recorded under control to 27.25 cm recorded under the highest salt stress treatment (150 N). They corresponded to an amount of 64% of reduction in comparison to control. Less reductions in comparison to the control were noted under two other treatments with 30% and 43% for 90 N and 120 N, respectively. Compared to their respective salt stress level, exogenous treatment with 0.5 mM SA induced increases of SL by 6%, 14%, and 39% for 90 N/0.5SA, 120 N/0.5SA, and 150 N/0.5SA treatments, respectively. An increase of 53% was noted under 150 N/1 SA with the application of 1 mM SA. The comparison of means (Table 3) indicates no significant difference between 150 N/1 SA and 120 N and between 120 N/0.5SA, 90 N/1SA, and 90 N.

Table 3 displays the variation in fresh root weight (RFW) under different salt and SA treatments. As presented in Table 4, the statistical analysis shows a significant difference between most treatments. Further, we have noted a significant decrease of RFW under salt stress by 54%, 64%, and 74% for 90 N, 120 N, and 150 N, respectively. However, applying 0.5 mM SA in combination with salinity allows plants to increase their RFW by 12%, 20%, and 18% under 90 N, 120 N, and 150 N treatments, respectively. A significant increase (28%) was noted under 150 N/1 SA treatment by applying 1 mM SA. Thereafter, no significant difference between 150 N/1SA, 150 N/0.5SA, 120 N, and 120 N/1SA was noted (Table 3).

As shown in Table 3, the salt stress treatments significantly decreased the dry root weight (DRW). These reductions reached 59%, 68%, and 74% for 90 N, 120 N, and 150 N, respectively, compared to the control. A beneficial effect of SA is demonstrated in combination with salt stress. Elsewhere, the increasing amount was 7%, 27%, and 11% under the effect of 0.5 mM SA, respectively to 90 N/0.5SA, 120 N/0.5SA, and 150 N/0.5SA. Mean comparison indicates no significant difference between all treatments under 90 N and 120 N with and without SA; and between 120 N, 120/1SA, 150 N/0.5SA, and 150 N/0.5SA.

The root length (RL) was affected significantly ($p<0.001$) by salt stress, where the SA alone had no significant effect on RL (Table 4). Further, RL was reduced from 31.5 cm recorded under control to 14.3 cm recorded under 150 N treatment, with an amount of 57% reduction, followed by 39% and 28% of reduction recorded respectively under 120 N and 90 N in comparison with the control. The application of 0.5 mM SA induced an increase of RL by 10%, 8%, and 14% under 90 N/0.5SA, 120 N/0.5SA, and 150 N/0.5SA, respectively. The application of 1 mM allows an increase of 30% under 150 N/1SA treatment. Mean comparison indicates no significant difference inside SA treatments of each salt stress treatment (120 N and 90 N and control).

3.2 Plant Physiological Parameters

3.2.1 Stomatal Conductance

The analysis of variance demonstrates that stomatal conductance was highly ($p<0.001$) influenced by salt stress and the combination of salt stress and SA. This last one influenced the gs at ($p<0.05$). Table 5 displays the negative effect of salt stress on gs, and we have noted a reduction of 52%, 75%, and 92% under 90 N, 120 N, and 150 N, respectively, compared to the control. It is apparent from this Table 5 an increase under salt stress treatments combined with 0.5 mM SA by 32%, 67%, and 39% to reach respectively a gs value of 44.53, 29.5, and 8.2 mmol (H_2O). m^{-2}. s^{-1} under 90 N/0.5SA, 120 N/0.5SA, and 150 N/0.5SA treatments, respectively. However, the application of 1 mM SA had a lower ratio of increases under 90 N and 120 N, but under 150 N shows a higher percentage of increases by 107%. Mean comparison shows that there was a significant ($p<0.05$) effect between treatments except between 90 N and 120 N/0.5SA; and between 120 N, 120 N/1SA, and 150 N/1SA.

3.2.2 Leaf Water Potential (LWP)

The LWP was decreased with increased salinity levels (Table 5). Under control conditions, the LWP was at -0.22 MPa. However, this value reaches −0.4, -0.59, and −0.82 MPa for 90 N, 120 N, and 150 N treatments, respectively. As presented in Table 4, the analysis of variance reveals that salt stress, SA, and their combination had a highly significant effect ($p<0.001$) on LWP. Compared to the respective salt treatment, the combination of salt stress with SA increased LWP respectively under 0.5 and 1 mM SA by 25% and 20% under 90 N; 17% and 31% under 120 N, and by 9% and 26% under 150 N. The comparison of means shows that there is a significant ($P<0.05$) difference between all studied treatments (Table 5).

3.2.3 Relative Water Content (RWC)

The RWC was significantly ($p<0.001$) influenced by the salt stress, SA, and their combination, as revealed in Table 4. The comparison of means indicates a significant difference ($p<0.05$) between all treatments (Table 5). Under unstressed conditions, the RWC was upper than 80%. However, the salt stress application induced a gradient of decrease of RWC, and we have noted reductions of 11%, 24%, and 28% for 90 N, 120 N, and 150 N, respectively. SA application under different stress treatments increases the RWC by 10%, 5%, 6%, and 7% for 120 N/0.5SA, 150 N/0.5SA, 120 N/1 SA, and 150 N/1 SA, respectively, in comparison to the respective salt stress levels.

Table 5 Physiological parameters of faba bean plant grown under four salt stress levels (0, 90, 120 and 150 mM NaCl) combined to salicylic acid (0, 0.5 and 1 mM SA). Data are means ($n=4$), values with different letters are significantly different ($p<0.05$) according to the LSD test

	Stomatal conductance (gs) (mmol. s-1. m-2)	Leaf water potential (LWP) (MPa)	Relative water content (RWC) (%)	Leaf surface area (LS) (cm^2)
Control	69.9 ± 12.8 a	-0.22 ± 0.01 a	0.83 ± 0.012 a	428.2 ± 17.8 a
0.5SA	58.0 ± 3.2 b	-0.24 ± 0.01 b	0.80 ± 0.00 b	378.7 ± 14.2 b
1SA	53.0 ± 3.4 b	-0.29 ± 0.01 c	0.79 ± 0.001 c	336.8 ± 15.9 c
90 N	33.7 ± 1.1 de	-0.4 ± 0.004 c	0.74 ± 0.001 f	227.0 ± 4.6 f
90 N/0.5SA	44.5 ± 1.5 c	-0.3 ± 0.002 e	0.77 ± 0.001 d	293.3 ± 23.6 d
90 N/1SA	36.6 ± 1.7 d	-0.32 ± 0.01 d	0.76 ± 0.002 e	253.7 ± 12.5 e
120 N	17.7 ± 2.6 gh	-0.59 ± 0.01 e	0.63 ± 0.002 j	137.0 ± 9.7 i
120 N/0.5SA	29.5 ± 1.2 e	-0.49 ± 0.01 g	0.69 ± 0.002 g	195.0 ± 15.4 g
120 N/1SA	21.8 ± 1.2 fg	-0.41 ± 0.01 f	0.67 ± 0.001 h	163.0 ± 8.75 h
150 N	5.9 ± 0.1 i	-0.82 ± 0.01 h	0.60 ± 0.006 k	74.2 ± 5.5 k
150 N/0.5SA	8.2 ± 2.1 gh	-0.75 ± 0.01 i	0.63 ± 0.003 j	96.5 ± 5.8 j
150 N/1SA	12.2 ± 0.7 hi	-0.61 ± 0.01 j	0.64 ± 0.007 i	113.8 ± 5.7 j

3.2.4 Leaf Surface (LS)

Table 5 shows the plant's leaf surface (LS) responses to different salinity treatments. The analysis of variance indicates that all studied factors and combinations influenced the LS significantly at the levels of $p<0.001$. Besides, the comparison of means shows a significant ($p<0.05$) difference between all treatments. From the data in Table 5, it is apparent that LS decreased with the increases in salt stress from 428 cm² recorded under the control conditions to 227, 136, and 74 cm² recorded under 90 N, 120 N 150 N treatments, respectively. Consequently, the LS loss of 47%, 68%, and 83% is revealed in the respective order. Nevertheless, SA application in combination with salt stress improves the LS by inducing increases of around 29%, 42%, 30%, and 53% for 90 N/0.5SA, 120 N/0.5SA, 150 N/0.5SA, and 150 N/1SA, respectively, in comparison to the respective salt stress levels.

3.3 OJIP Curves

3.3.1 Chlorophyll Fluorescence

As shown in Fig. 1, the chlorophyll fluorescence rises kinetics present different responses to salinity treatments (0; 90; 120; 150 mM NaCl) in combination with different SA levels (0; 0.5 and 1 mM SA). As shown in Fig. 1, the fast induction fluorescence rises were changed depending on the salinity and the SA application levels. Moreover, the F_0 increased significantly by increasing the salinity and SA concentration. Under 150 N and 150 N/1SA treatments, fluorescence rise shows the O-J-I-P polyphasic curve deformation. At about 300 µs, a new "K" step appeared, exhibiting a gradual transformation of the OJIP fluorescence rise kinetics into OKJIP. When plants were exposed to the strong salinity stress (Fig. 1), we have notedd a decrease in the maximal

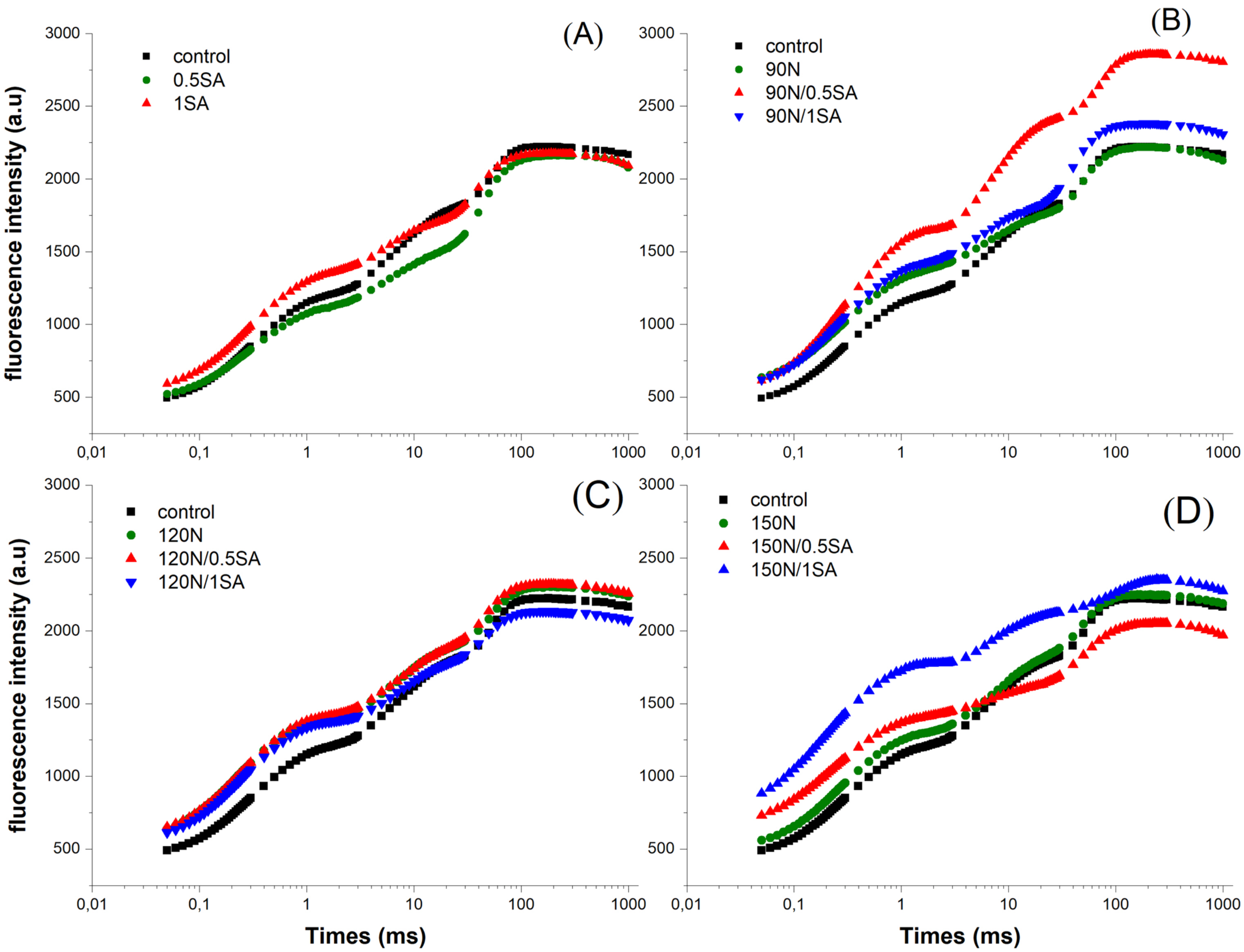

Fig. 1 OJIP transient exhibited by faba bean leaves after dark adaptation. After a pulse of continuous red light for one second of 3500 µmol photons m^{-2} s^{-1}. Values are means ($n=4$) **A**: treatment of salicylic acid (0; 0.5 and 1 mM); **B**: 90 mM NaCl in combination with different concentrations of SA; **C**: 120 mM NaCl in combination with different concentrations of SA; **D**: 150 mM NaCl in combination with different concentrations of SA

fluorescence (P) level, the intensity of the variable fluorescence, and the K step induction.

3.3.2 Relative Variable Fluorescence and Difference of Relative Variable Fluorescence

To further study the effect of SA and salt stress on the OJIP rise kinetics, a normalization between F_0 and F_M was made for all treatment curves, then presented in a logarithmic time scale as relative variable fluorescence (RVF) $V_t = (F_t\text{-}F_0)/(F_M\text{-}F_0)$ and $\Delta V=V_{t\ (treated)}-V_{t\ (control)}$ (Figs. 2 and 3, respectively). This normalization allows the revelation of hidden information and bands. Dependent on the salt stress treatments, the V_t and ΔV present a positive K peak and a negative I peak due to the change in the fluorescence rise of *Vicia faba* L. (Fig. 3). The V_t and ΔV curve comparison presents that the 150 N treatment shows higher K and ΔK peaks (300 µs).

3.3.3 L-band

To compare the effect of SA and the salinity stress, we fulfilled other normalization and subtractions on the fluorescence rise kinetics for the reflected events in the OK, OJ, OI, and IP phases (Fig. 4). Figure 4 presents the normalization between the O-step and k-step (300 µs) as $W_{OK}=(F_t\text{–}F_0)/(F_k\text{-}F_0)$ and the subtraction between the salt stress treatments and the control $\Delta W_{OK}=W_{OK\ (treated)}\text{-}W_{OK\ (control)}$ in a linear time scale. This subtraction revealed at 150 µs a new hidden band (L-band) between the O and K steps. As indicated by Strasser et al. (2004), the state of the PSII unit's grouping or energetic connectivity can be indicated by the L-band. Therefore the L-band is higher when grouping probability or the connectivity is lower. Based on this fact and as indicated by Fig. 4, the salinity stress, even moderate stress (90 N), resulted in lower connectivity of PSII units. However, the application of SA improved the connectivity

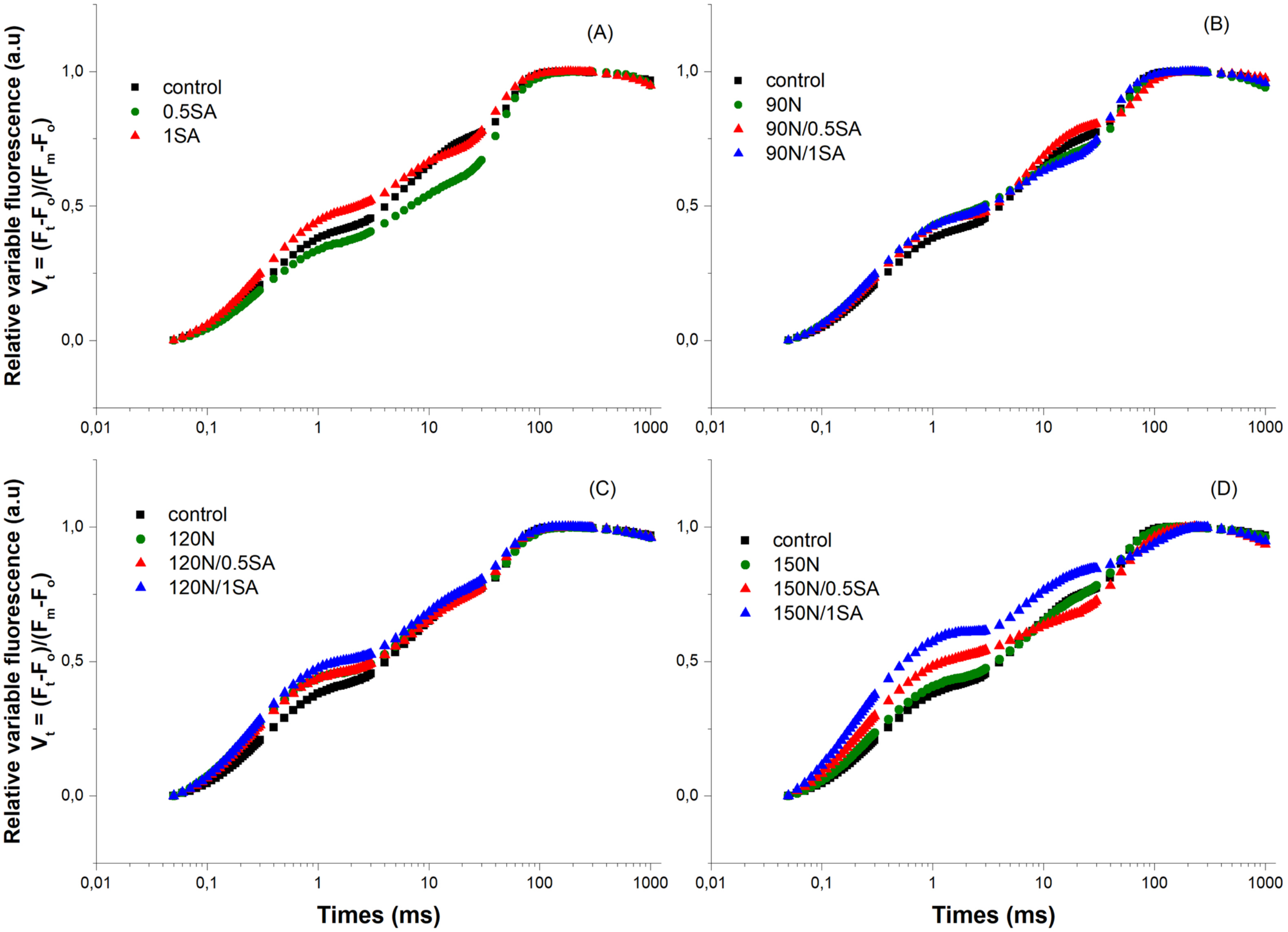

Fig. 2 OJIP curve of relative variable fluorescence $[V_t= (F_t\text{–}F_0)/(F_M\text{–}F_0)]$; after double normalization between $F_0=0$ and $F_M=1$ of the fluorescence rise of faba bean leaves at different treatments. Values are means ($n=4$). **A**: treatment of salicylic acid (0; 0.5 and 1 mM); **B**: 9 0 mM NaCl in combination with different concentrations of SA; **C**; 120 mM NaCl in combination with different concentrations of SA; **D**: 150 mM NaCl in combination with different concentrations of SA

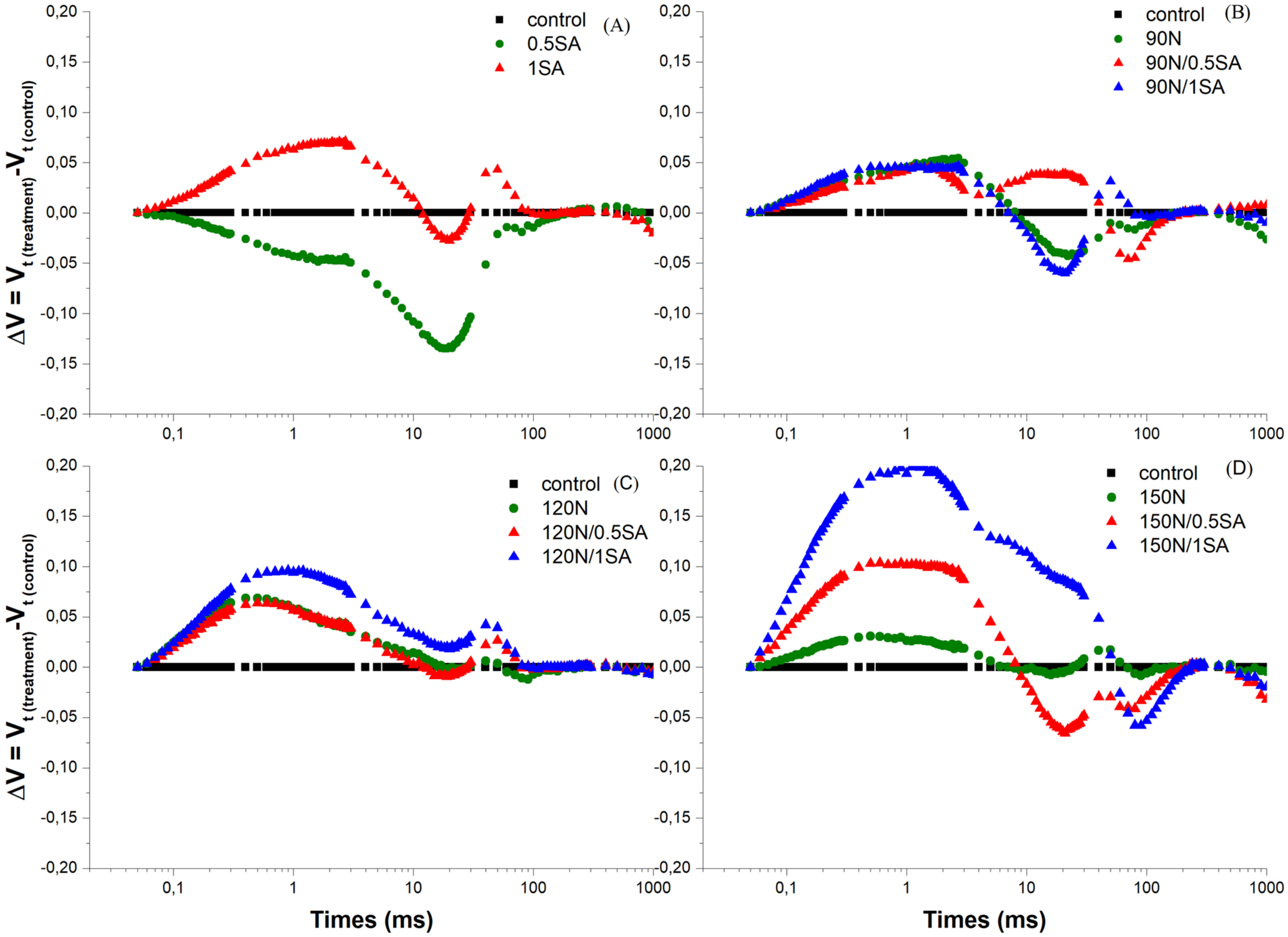

Fig. 3 Difference of relative variable fluorescence [$\Delta V = V_{t\ (treatment)} - V_{t\ (control)}$] on the logarithmic time scale of faba bean leaves at different treatments. Values are means (*n*=4). **A**: treatment of salicylic acid (0; 0.5 and 1 mM); **B**: 90 mM NaCl in combination with different concentrations of SA; **C**; 120 mM NaCl in combination with different concentrations of SA; **D**: 150 mM NaCl in combination with different concentrations of SA

of the PSII units, except for 150 N, where we have noted lower connectivity under SA treatment.

3.3.4 K-band

Figure 5 presents the double normalization by F_0 (50 µs) and FJ (2 ms) as $W_{OJ} = (F_t - F_0)/(F_J - F_0)$ and the subtraction between the salt stress treatments and the control as $\Delta W_{OJ} = W_{OJ\ (treated)} - W_{OJ\ (control)}$. The Fig. 5 plots reveal that the k-step is not clearly elicited in the fluorescence rise under salinity stress. However, a clearer positive K-band was exhibited in the different kinetics ΔW_{OJ}, which was salinity stress level dependent. Different salinity stress resulted in a lower intensity of the K peak in combination with SA. The K peak increases indicate an OEC center's inactivation. Thereafter, comparing different kinetics ΔW_{OJ} indicates that salt stress provoked a higher inactivation of OEC centers under higher stress and negligible inactivation for medium stress levels.

3.3.5 I-P Phase of OJIP Chl a Fluorescence

As presented in Figs. 6 and 7, the evaluation of the salinity effect on the IP phase was performed using two different normalization procedures. The first is a double normalization by F_0 (50 µs) and I (30 ms) as $W_{OI} = (F_t - F_0)/(F_I - F_0)$ (only $W_{OI}>1$ is presented). The second normalization between I-(30 ms) and P-step as $W_{IP} = (F_t - F_I)/(F_P - F_I)$ and plotted in a linear time scale for the range of 30–200 ms.

The electron flow from PQH_2 to the end electron acceptors at the acceptor side of PSI is reflected by the maximal amplitude of the fluorescence rise for each curve of W_{OI}. In comparison to the control, there was a different effect of salt stress on the electron flow. However, the electron flow has a marked SA-dependent increase/decrease (depending

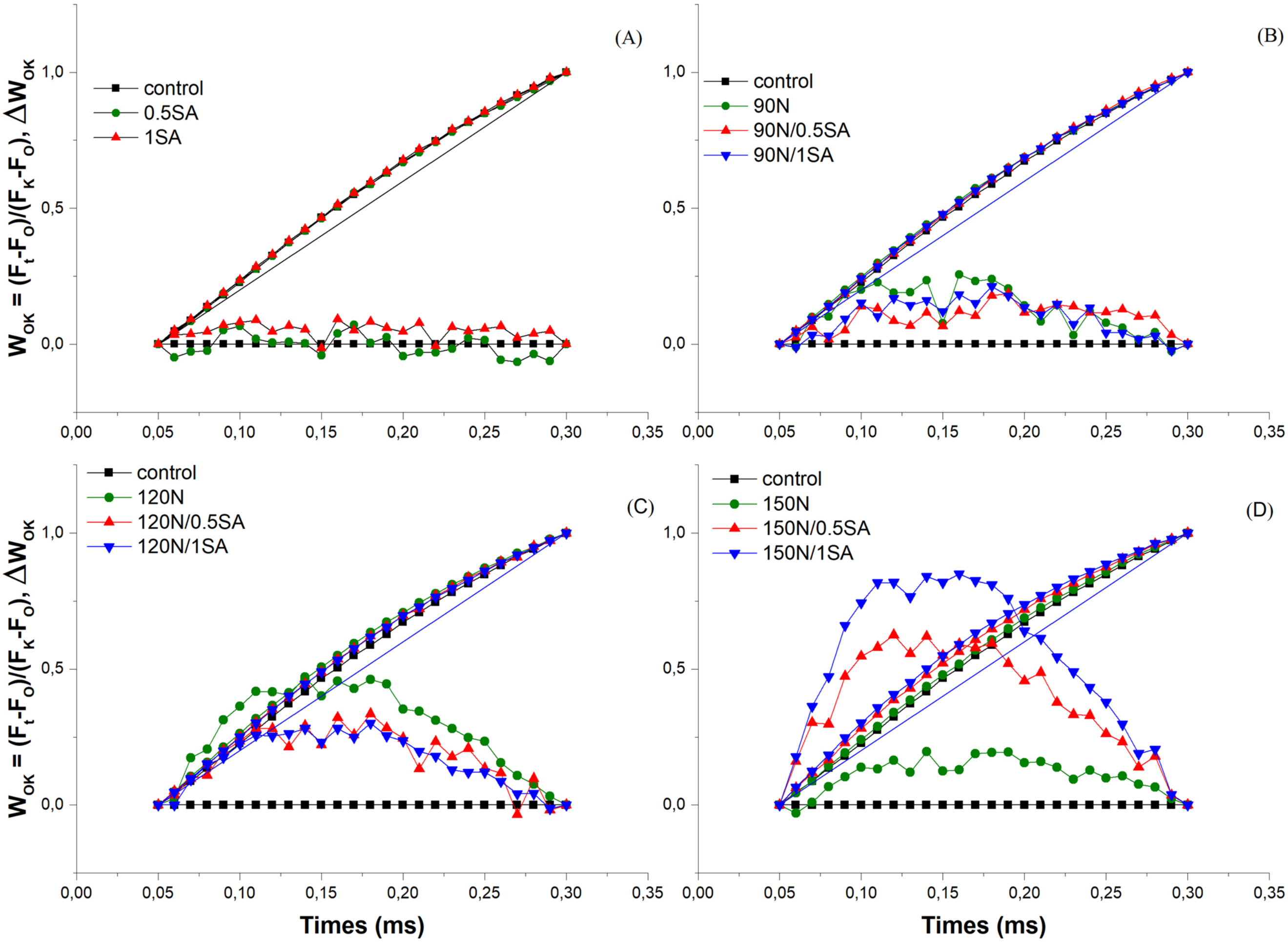

Fig. 4 Responses of the L-band of *Vicia faba* L. leaves to salt stress and salicylic acid treatments. The fluorescence rises kinetics normalized by F_0 and F_K as $W_{OK}= (F_t - F_0)/(F_K - F_0)$, and the difference kinetics $\Delta W_{OK} = W_{OK}$ (treated) - W_{OK} (control) were represented. Values are means ($n=4$). **A**: treatment of salicylic acid (0; 0.5 and 1 mM); **B**: 90 mM NaCl in combination with different concentrations of SA; **C**; 120 mM NaCl in combination with different concentrations of SA; **D**: 150 mM NaCl in combination with different concentrations of SA

on the SA levels and the salinity combination levels). At the 0.5SA, the electron flow increases with high levels of salinity concentration (> 120 mM). However, the amplitude of the W_{OI} was lower at salinity treatment combined with 1 mM SA than in control as a result of the injury of the photosynthetic apparatus.

Figure 7 presents the normalization between I-step and P-step. Allowing to evaluate the rate of the end electron acceptors reduction in the PSI under the different treatments. The higher (or lower) reduction rate is reflected by a lower (or greater) value of the time point corresponding to the point of W_{IP}= 0.5. As compared to the control, salt stress resulted in a gradual decrease in the reduction rate of the PSI end electron acceptors reflected by the increase of half times. This lower conduction rate was very apparent under the 150 N/1SA treatment reaching late halftime (> 80 ms) than the control.

4 Discussion

Salt stress is a significant global challenge, adversely affecting plant growth and productivity at various developmental stages (Anaya et al. 2018; Lamnai et al. 2021; Lee et al. 2010). This research aims to identify the possible positive impact of salicylic acid on the growth, physiological and photosynthetic parameters of the faba bean plant grown under salt stress. Our results demonstrated that salt stress significantly reduced both fresh and dry weight ($p<0.001$) by inducing a severe loss of plant weight. This growth reduction was accompanied by declines in photosynthetic parameters, such as chlorophyll fluorescence (OJIP), and water status, suggesting that impaired photosynthetic efficiency and water balance are closely associated with the observed growth inhibition. Similar findings have been reported in tomato and basil, where salt stress caused reductions in shoot and root dry mass, leaf area, and photosynthetic rates

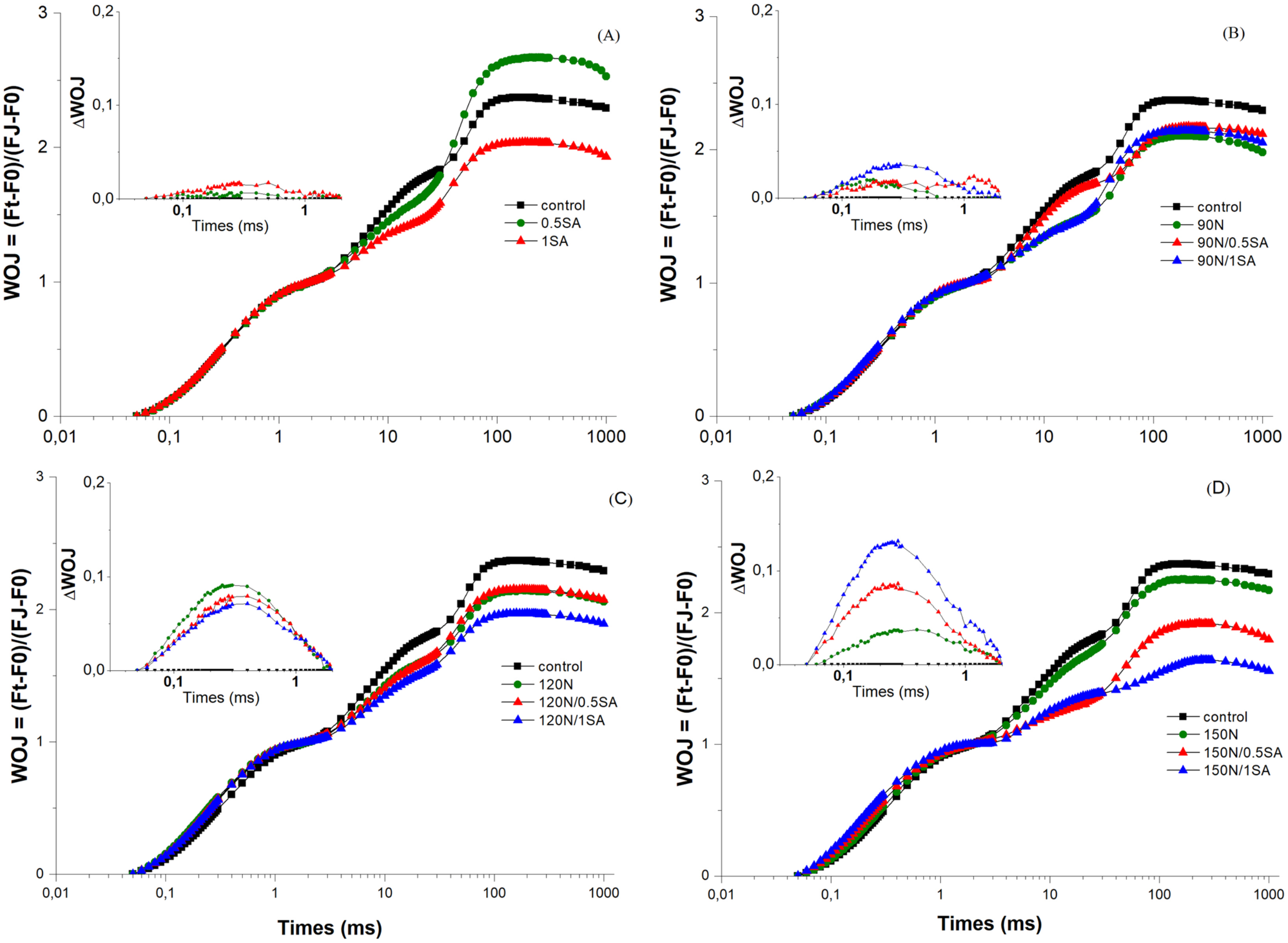

Fig. 5 Responses of the K-band of *Vicia faba* L. leaves to salt stress with salicylic acid treatment. (A) The fluorescence rises kinetics normalized by F0 and FJ were expressed as $W_{OJ}= (F_t – F_0)/(F_J–F_0)$ and (B) the difference kinetics ΔW_{OJ} = W_{OJ} (treated) - W_{OJ} (control). Values are means (n=4). **A**: treatment of salicylic acid (0; 0.5 and 1 mM); **B**: 90 mM NaCl in combination with different concentrations of SA; **C**: 120 mM NaCl in combination with different concentrations of SA; **D**: 150 mM NaCl in combination with different concentrations of SA

(Mimouni et al. 2016; Silva et al. 2022a, b, 2023). Similarly, SA application under no stress conditions induced significant reductions in growth parameters. SA's growth inhibition under non stressed conditions likely reflects its role as a defense primer inducing ROS and activating stress response pathways that divert resources from growth (Mulaudzi et al. 2023), as supported by findings in *Brassica napus* and *Dianthus superbus* under SA treatment (Dawood et al. 2023; Ma et al. 2017). However, exogenous SA application enhanced plant salt stress tolerance, as evidenced by maintained a high weight/length value. These findings align with previous reports in *Medicago sativa* (Palma et al. 2013), strawberry (Lamnai et al. 2021), and alkaline-stressed cucumber seedlings (Nie et al. 2018), where SA similarly improved weight under salt stress. Notably, reduced growth parameters can be considered a strategy to avoid stressful conditions and reduce their impacts (Rollins et al. 2013). Salt stress resulted in net photosynthesis decreasing due to stomatal closing leading to reduced uptake and use of water and nutrients. Salt stress caused a decrease in net photosynthesis due to stomatal closure, resulting in reduced water and nutrient uptake. However, SA promotes plant growth by stimulating root growth to increase water and nutrient uptake capacity and by promoting leaf photosynthetic capacity to accumulate more carbohydrates (Miao et al. 2023). In addition, this improvement can be attributed to the stimulatory effect of SA on Rubisco activity by maintaining higher activity and increasing stimulatory pigment contents (Alam et al. 2022). Miao et al. (2023) reported a strong correlation between plant growth and leaf photosynthetic parameters. The plant water status was evaluated using the leaf water potential (LWP), stomatal conductance (gs), and the relative water content (RWC). The LWP reflects the status of bound water to plant tissues regarding the water flow induced by the evaporative demand at the leaves governed by the law of the tension-cohesion in the soil-plant-atmosphere (Zhang

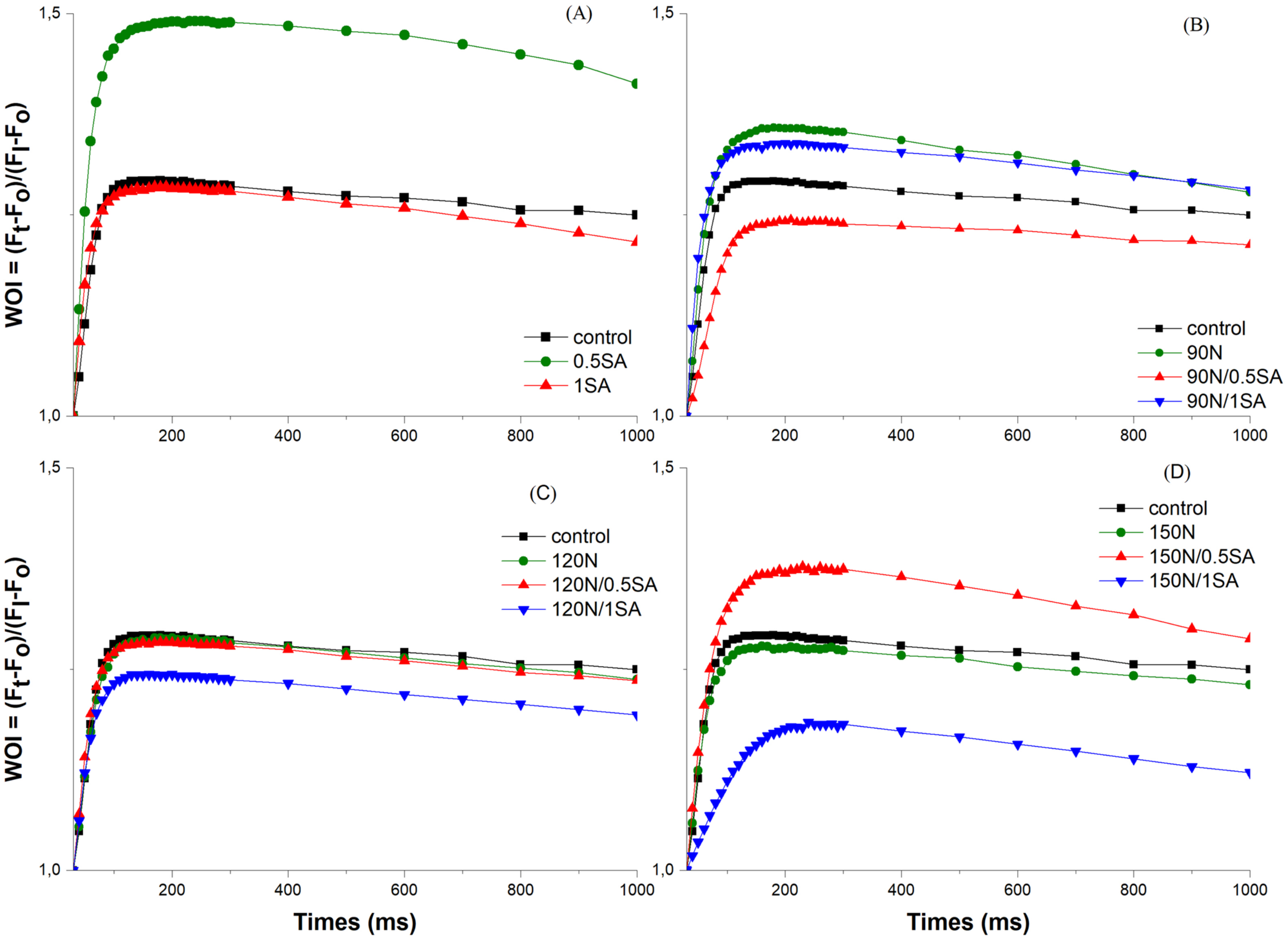

Fig. 6 Changes in the I-P phase of OJIP Chlorophyll a fluorescence of *Vicia faba* L. leaves under salt stress with salicylic acid treatment. The fluorescence rise normalized by F_0 and F_I as $W_{OI} = (F_t - F_0)/(F_I - F_0)$ ($W_{OI}>1$), Values are means ($n=4$). **A**: treatment of salicylic acid (0; 0.5 and 1 mM); **B**: 90 mM NaCl in combination with different concentrations of SA; **C**; 120 mM NaCl in combination with different concentrations of SA; **D**: 150 mM NaCl in combination with different concentrations of SA

and Huang 2021). Although, our results indicated, leaf water potential (Ψ_{leaf}) decreased progressively with increasing salt stress (90 to 150 mM NaCl), SA application further reduced LWP (Ψleaf) compared to salt-stressed alone (Table 5). This suggests SA actively regulates stomatal behavior to enhance water-use efficiency under salinity, rather than passively intensifying osmotic stress. Those results are in agreement with those found in *Brassica juncea* L. (rapeseed) grown under salt stress (Hayat et al. 2012) and mustard grown under drought stress (Nazar et al. 2015), which indicated that SA alleviates the negative effect of salt stress and improves the LWP. Many research works indicate in different plants that SA increases the accumulation of the osmolytes (proline, soluble protein, glycine betaine, and sugar) to mitigates cells' water potential for avoid dehydration and thereafter play a protective role effect against the salt stress (Anaya et al. 2017; Farhangi-Abriz and Ghassemi-Golezani 2018; Lamnai et al. 2021).

The decrease in the LWP leads to improved leaf water status by improving cell osmotic potential adjustment and preventing the leaves from dehydration under abiotic stress (Issa Ali et al. 2019; Lamnai et al. 2021). In this experiment, relative water content (RWC), a key indicator of plant water status, decreased significantly under salt stress ($p<0.001$). However, treatment with salicylic acid (SA) significantly improved RWC values ($p<0.001$). These results are in agreement with those of Hafez et al. (2020), who reported that SA application increased RWC in wheat plants grown on saline and sodic soils. In addition, Dawood et al. (2022) showed that foliar application of SA increased drought and salinity tolerance in faba bean by improving RWC. Taken together, these studies highlight the role of SA in maintaining plant water balance under stress conditions. Research shows that application of salicylic acid (SA) improves relative water content (RWC) in salt-stressed strawberry plants by addressing ionic toxicity (Lamnai et al. 2021).

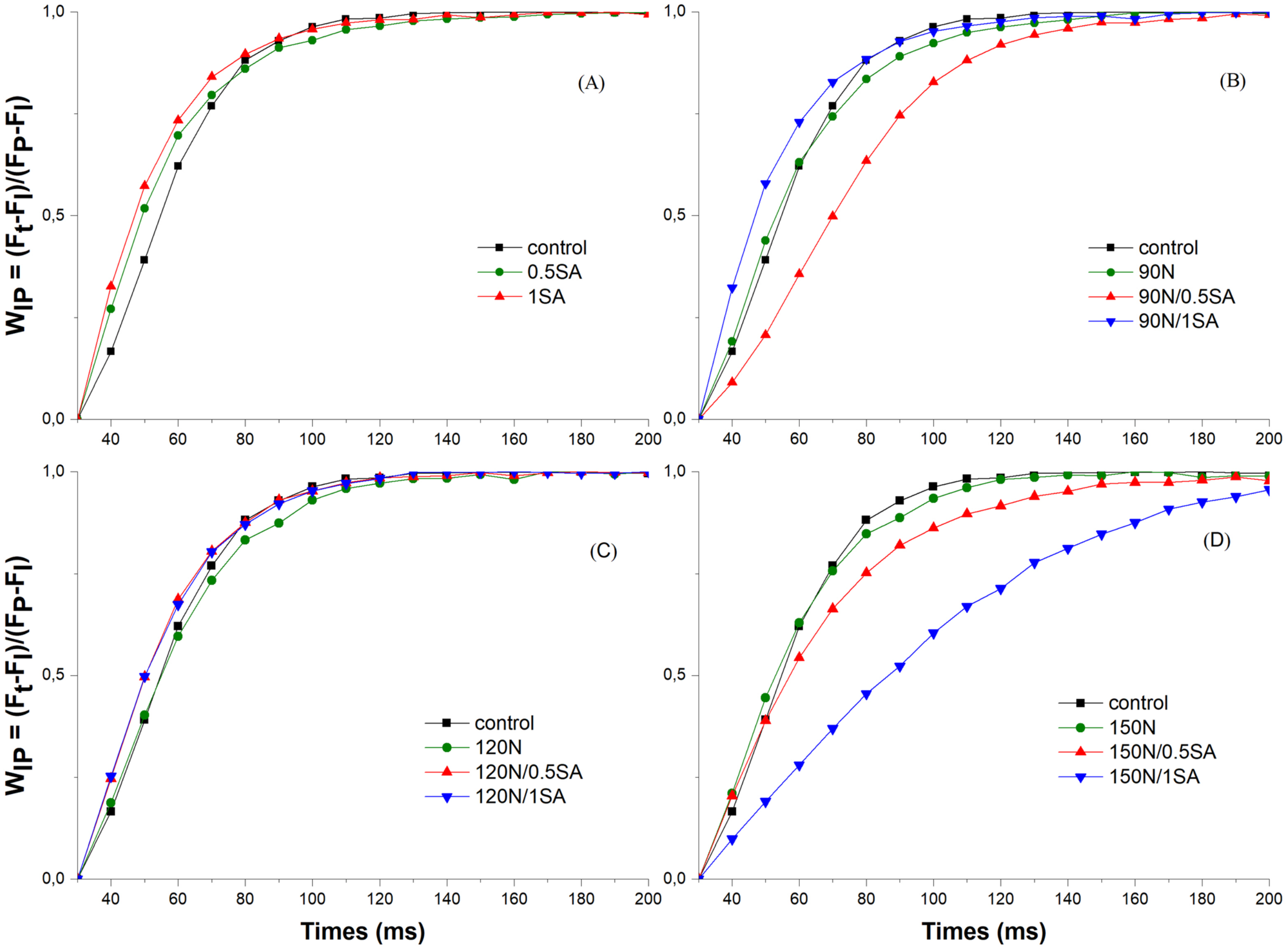

Fig. 7 I-P phase changes in the OJIP Chlorophyll a fluorescence rises of *Vicia faba* L. leaves under SA and salt treatments applications. The fluorescence rises kinetics normalized by F_I and F_P as $W_{IP} = (F_t - F_I)/(F_P - F_I)$ were plotted in the 30–300 ms time range (linear scale). Values are means ($n=4$). **A**: treatment of salicylic acid (0; 0.5 and 1 mM); **B**: 90 mM NaCl in combination with different concentrations of SA; **C**; 120 mM NaCl in combination with different concentrations of SA; **D**: 150 mM NaCl in combination with different concentrations of SA

Elsewhere, Salicylic acid (SA) mitigated salt stress in faba bean by enhancing photosynthetic efficiency, preserving leaf water potential (LWP), and increasing relative water content (RWC) key adaptive responses to salinity (Layachi and Kechrid 2023; Mimouni et al. 2016). However, salt stress triggered early stomatal closure, reducing stomatal conductance. While this conserved tissue hydration, it also limited CO_2 uptake, impairing photosynthesis and growth. These results align with prior studies demonstrating SA's role in alleviating salt-induced photosynthetic decline and improving water status, ultimately supporting better growth under saline conditions (Silva et al. 2022a, b, 2023; Yang et al. 2023).

Salt stress increases salt toxicity through increasing Na^+ accumulation in plant cells. Further, root water uptake decreases, and in consequence, the leave cell's water content is reduced (Farhangi-Abriz and Ghassemi-Golezani 2018). SA counteracts these effects through two mechanisms, first by modulation of Na^+ transporter expression to limit cytosolic sodium accumulation, and second by activation of H^+ pumps to maintain favourable ion gradients (Dawood et al. 2023; Nazar et al. 2011; Sheteiwy et al. 2022). This regulatory function preserves cellular water status despite osmotic stress.

Moreover, it was reported that SA influences the production of abscisic acid (ABA) and its content in the leaves and therefore moderates the stomata aperture (Liu et al. 2022; Wang et al. 2018). The stomatal conductance was significantly ($p<0.001$) affected by salt stress in the present work. Then presents decreases with the salt increasing levels due to the stomata closing to minimize transpiration and further the water loss. However, exogenous SA application induces an increase in gs. Similar results have been revealed that SA can increase stomatal conductance under salt stress in wheat plants (Hafez et al. 2020), strawberries (Lamnai et al. 2021), and faba bean (Anaya et al. 2017). Stomatal closure aperture limits water loss but decreases CO_2 uptake and further induces an inhibition in the photosynthetic activity (Nazar et al. 2015). The use of the exogenous SA application improves the leaf water status under salt stress by moderating osmotic adjustment and restricting stomatal conductance reduction, which will positively maintain photosynthetic activity and reduce damage. In this case, in the present study, SA may have improved CO2 uptake and hence photosynthesis as it improved plant water status and increased stomatal aperture. Therefore, it is natural that assimilation and hence mass increase would occur. Leaf surface area findings support this idea.

The fast Chlorophyll *a* fluorescence OJIP transient is an interesting tool to evaluate the photosynthetic organisms' performance and physiological vitality by assessing electron transport activity (Strasser et al. 2004). Fluorescence emitted mainly by the Chlorophyll *a* molecules of the PSII antenna is served to monitor consecutive steps of excitation energy transformation (Strasser et al. 2004). The Q_A reduction is related directly to the variable part of the fluorescence. During the photo-induced reduction of quinone acceptors Q_A, the variable part is always increasing (Duysens and Sweers 1963). However, the signal of fluorescence is sensitive to the whole electron-transfer process; further, the later stage of energy transformation determines the level of Q_A reduction.

In this study, the polyphasic chlorophyll *a* fluorescence transient (OJIP) was measured to evaluate the effects of salt stress and salicylic acid (SA) on the photochemical efficiency of PSII. The OJIP transient (Fig. 1) showed reduced fluorescence intensity with increasing NaCl concentration. Fluorescence analysis revealed that salt stress disrupted PSII function by affecting the oxygen-evolving complex (OEC) and reducing PSII connectivity. However, SA application had a beneficial effect on photochemical efficiency. Elevated salt concentrations caused a significant increase in minimal fluorescence (F_0) and a significant decrease in both variable fluorescence (F_v) and maximal fluorescence (F_m). However, SA application mitigated these effects by increase all cited parameters (Fig. 1), also by maintaining OEC integrity and improving electron transport efficiency, thereby mitigating photosynthetic damage (Nazar et al. 2015; Pai and Sharma 2024). These findings are consistent with studies in mustard and tomato, where SA increased photosynthetic efficiency and biomass under salinity stress (Arruda et al. 2023; Mimouni et al. 2016). The decrease in fluorescence at J, I, and P can be explained by two reasons, first, by electron transport inhibition at the donor side of the PSII, which results in the accumulation of $P680^+$, and second due to a decrease in the pool size of plastoquinone. On the reducing side of PSII, the electron acceptors' plastoquinone pool size can be estimated by the area between F_0 and F_M over the fluorescence induction curve. The blockage of the electron transfer from RC to the quinone pool reduces this area dramatically. Compared to control to determine the leaves', the area over the fluorescence curve was not significantly decreased under salt treatment (data not shown). Therefore, we supposed that salinity does not inhibit the electron transfer rates at the acceptor side of PSII. Elsewhere, the increase of the area with the combination of the SA suggests that SA promotes the electron transfer rate. The OEC injury is widely studied by the K-step fluorescence and W_K; the increase in these parameters is considered a specific indicator of PSII donor photo-inhibition (De Ronde et al. 2004; Strasser 1997; Tóth et al. 2005). In the present study, a clear K-band with a peak at about 0.3 ms was induced by salinity stress. However, the application of SA results in a lower K peak (Figs. 3 and 5). A similar salt-induced K-band was also observed in perennial ryegrass (Dąbrowski et al. 2016) and maize (Kan et al. 2017). These observations indicate that salt stress destroys the OEC and impairs the electron transfer capacity on the donor side of PSII. Since the OEC is involved in the photo-oxidation of water during the light reactions of photosynthesis, we speculated that the oxidation of water was impaired in stressed *Vicia faba* L. Elsewhere, the application of SA may play a role in the alleviation of the negative effect of salinity stress by protecting the OEC.

A positive L-band was also induced by salinity stress in *Vicia faba* L. leaves (Fig. 3). A similar salt-induced L-band was also observed in perennial ryegrass(Dąbrowski et al., 2016) and maize (Kan et al. 2017). A positive L-band indicates (according to JIP-test and the grouping concept) the reduction of energy exchanged between independent PSII units or that units are less grouped (Strasser et al. 2004). The stable confirmation is the grouped one; therefore, the PSII units had lost their stability and had become more ungrouped and fragile under salinity treatment. However, the application of SA ameliorates the energetic connectivity. A similar salt-induced decrease in the energetic connectivity between the PSII units was observed in wheat (Mehta et al. 2010) and maize (Kan et al. 2017).

In order to evaluate the reduction rate of PSI, two different standardization procedures were performed. Figure 6 shows that the electron flow to the end acceptors' at the PSI side was decreased due to the salt stress application. Similar results were reported in perennial ryegrass (Dąbrowski et al. 2016), maize (Kan et al. 2017), and wheat (Mehta et al. 2010) under salt stress. Overall, 0.5 mM SA demonstrated significant potential as an eco-friendly strategy to enhance faba bean productivity in saline soils, offering a promising solution for sustainable agriculture in salt-affected regions.

5 Conclusion

This study shows that salt stress severely impairs growth, photosynthetic activity, and PSII function in faba bean (*Vicia faba* L.) by inactivating the oxygen-evolving complex (OEC) and reducing PSII connectivity. However, applying 0.5 mM salicylic acid (SA) effectively alleviated these effects by enhancing growth, improving leaf water potential (LWP) and relative water content (RWC), and preserving photosynthetic efficiency. SA protected the OEC, increased electron transport rates, and minimized damage to both PSII and PSI. Fluorescence analysis revealed that salt stress inhibited electron transport at the PSII donor side, decreased the reduction rate, and reduced the pool size of PSII end acceptors, associating these effects with OEC centers inactivation (positive K band) and decreased of energetic connectivity of PSII units (positive L band). SA application alleviated these disturbances by protecting the OEC and improving the reduction rate of PSI end acceptors. Among the concentrations tested, 0.5 mM SA produced the greatest improvements in growth and photosynthesis under salt stress, suggesting its potential as a practical and environmentally friendly solution for sustainable faba bean cultivation in saline soils.

Author Contributions F. Anaya: Conceptualization, Methodology, Investigation, Data curation, Writing - original draft; R. Fghire: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing - review & editing; S. Wahbi: Methodology and Validation; I.S. Carvalho: Resources, Validation, review; K. Loutfi: Conceptualization, Validation, review and Project administration.

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Declarations

Conflict of Interest Authors declare no conflict of interest.

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