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*Original Paper*

# Exogenous application of salicylic acid mitigates salt stress in rice seedlings by regulating plant water status and preventing oxidative damage

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**Abstract**

Salicylic acid (SA) is a hormone participating in the acclimation of plants to biotic and abiotic stresses, including salinity. We investigated the possible underlying mechanism of mitigating salt stress by SA using NaCl-treated rice plants sprayed twice with exogenous SA at different concentrations. SA application resulted in increased growth, relative water content, proline accumulation, the quantum efficiency of photosynthesis and activity of superoxide dismutase in NaCl-treated plants. Application of SA decreased $Na^+$ concentration and increased $K^+$ concentration, thus increasing the $K^+/Na^+$ ratio. The application of SA mitigated the effect of NaCl by improving plant water status, ion homeostasis and decreased oxidative damage. Foliar application of 0.5 mM SA was more effective in mitigating the salt stress while 2 mM SA was inhibitory, and the second spray of SA showed no significantly enhanced ameliorating effect over the first spray.

**Key words:** membrane lipids, Oryza sativa, photosynthesis, reactive oxygen species, salicylic acid, salinity.
**Abbreviations:** APX, ascorbate peroxidase; CAT, catalase, DW, dry weight; EL, electrolyte leakage; FW, fresh weight; MDA, malondialdehyde; ROS, reactive oxygen species; RWC, relative water content; SA, salicylic acid; SOD, superoxide dismutase; TW, turgid weight.

## Introduction

*Oryza sativa* L. is the primary sustenance for nearly one-half of the world's inhabitants; with an ever growing population, only high productivity of food crops will ensure food security. Abiotic stresses such as salt, drought, extreme temperatures, and heavy metals impact the yield of all crops, including rice (Waqas et al. 2019). Salinity stress is increasing in intensity and area due to the effects of global warming, which causes vaporization and sea level rise, as well as agricultural practices such as improper irrigation, which causes salts to accumulate in the soil (Tahjib et al. 2018). Of the 25 million hectares of irrigated lands in India, 7 million hectares are adversely affected by salinity (Parihar et al. 2015). Rice, a glycophyte, is sensitive to salinity at all life stages.

Salinity influences a sequence of morphological, physiological, biochemical, and molecular changes leading to reduced growth and yield, initially due to decreased water potential as a result of dissolved salts, leading to osmotic stress (Zhang et al. 2018), followed by salt accumulation resulting in ion toxicity, ultimately leading to oxidative stress by generating reactive oxygen species (ROS) and affecting cell functions (Botella et al. 2007). Salt adaptation strategies include osmotic stress tolerance by synthesis of organic osmolytes (Misra, Saxena 2009), maintenance of ion homeostasis (Azooz 2009), associated with ion exclusion (Chen et al. 2018), accumulation and compartmentation (Munns, Tester 2008), as well as ROS detoxification (Yancey 2005).

Plants also respond to abiotic stresses through the synthesis of jasmonates and salicylic acid (SA) (Gunes et al. 2007). SA, a seven-carbon phenolic plant growth hormone, is essential in regulation of various physiological processes and protection against biotic and abiotic stresses (Gunes et al. 2007; Azooz 2009; Tahjib et al. 2018). Extensive studies against biotic stress have been undertaken to elucidate the molecular mechanism of SA-induced systemically acquired resistance; however, its physiological and biochemical mechanism of signal regulation in plants against abiotic stress is still being studied (Shakirova et al. 2003). Currently, considerable interest has been stimulated by the effect of SA in protection against different abiotic stresses (Sakhabutdinova et al. 2003). Several studies have reported SA-induced increased resistance against salinity (Gunes et al. 2007), temperature (Senaratna et al. 2000), and heavy metals (Mishra, Choudhuri 1999) in various crop plants and cultivars. Studies indicate that the protective role of SA may

be due to the regulation of ROS and antioxidants (Shi, Zhu 2008; Jini, Joseph 2017; Mahmud et al. 2017), enhancing concentration of photosynthetic pigments (Alamri et al. 2018) and induction of biosynthesis of organic solutes such as proline and glycine betain (Khan et al. 2015). The mitigating effect of SA on abiotic stresses has been investigated through different modes of application such as seed priming (Azooz 2009), through rooting medium (Poor et al. 2011), or foliar spray (Yildirim, Dursun 2008). The effect of SA on cellular and molecular metabolism varies depending on the concentration of SA, plant species and environmental conditions, promoting some processes while inhibiting others (El-Mergawi, Abd El-Wahed 2004). The aim of this study was to perform foliar application of SA in different concentrations in rice seedlings grown under normal and salinity stressed conditions to identify the effective concentration of SA in alleviating the salinity effect by examining growth and photosynthesis, plant water status, membrane integrity, ion homeostasis, ROS and enzymatic antioxidants, and level of SA within the plants.

## Materials and methods

### *Plant material and growth conditions*

Seeds of Oryza sativa L. cv. Jaya (salt-sensitive variety) were procured from the Department of Agriculture, Pernem, Goa, India. Seeds were surface sterilized with 3% sodium hypochlorite (Merck, A.R. grade), washed with distilled water repeatedly and soaked for five days. Seeds were sown in plastic pots containing vermiculite in a plant growth chamber with controlled conditions (temperature 25 ± 2 °C, photon flux density of photosynthetically active radiation ≈ 500 μmol $m^{-2}$ $s^{-1}$, 16 h photoperiod, relative humidity 70 to 75%) and watered on alternate days with NaCl solution (0, 40, 120 mM NaCl; Merck, A.R. Grade) prepared in Hoagland solution (pH 6.5) (Hoagland, Arnon 1950) for a total duration of 28 days. SA (Merck, tissue culture grade) was dissolved in water (0.5, 1 and 2 mM; aqueous solubility of SA being 2.2 mg $mL^{-1}$; Trissel et al. 2018). One set of plants was treated through foliar application on the 14$^{th}$ day (first spray), and the second set of plants was given the first spray on the 14$^{th}$ day, followed by a second foliar spray on the 21$^{st}$ day (second spray). Both sets of plants were harvested on the 28th day for analysis using the second leaf. Based on our preliminary studies on phenotypic observations with a wider range of SA between 0.1 to 2 mM, the experimental concentration of exogenous SA was selected. Exogenous SA ≤ 0.4 mM showed no significant change compared to the control (data not shown). A constant level of salicylic acid spray was provided to plants with an atomizer (60 sprays per pot; ~12.5 to 13.0 mL per pot). Saline and non-saline control plants were sprayed with distilled water. The experimental design was done using the randomized block method. Measurements were performed 3 to 5 times with five replicates using the middle portion of the 2$^{nd}$ leaf.

### *Morphological, physiological and biochemical measurements*

Shoot and root length of ten randomly selected plants of each treatment were measured. Dry biomass of shoot and root was measured using ten plants dried at 60 °C for 72 h.

Relative water content (RWC) in rice leaves was determined using the second leaf of randomly selected five plants per treatment. Fresh weight (FW) was recorded immediately after detachment and the leaves were soaked and rubbed gently in distilled water containing a few drops of Tween 20 for 6 h at room temperature under constant light conditions to measure turgid weight (TW). After dehydrating for 72 h at 60 °C, dry weight (DW) was measured. RWC was calculated according to Barrs and Weatherley (1962):

$$RWC = (FW - DW) / (TW - DW) \times 100.$$

For determination of sodium and potassium concentration, fresh tissue (0.5 g) was oven-dried at 60 °C for 72 h. Dry tissue samples were placed in a muffle furnace in a crucible at 500 °C for 4 to 5 h to convert to ash, which was dissolved in 0.1N $HNO_3$ and filtered using Whatman Paper No. 1. Sodium and potassium concentration in the sample was determined with a Digital Flame Photometer (Esico Model 381) and calculated using standard solutions (Chapman, Pratt 1962).

Chlorophyll *a*, chlorophyll *b*, and carotenoid concentration was estimated using 0.2 g of fresh tissue homogenized in acetone and centrifuged at 8000 × $g_n$ for 10 min. The absorbance of the supernatant at 663, 645, and 470 nm was measured using a Shimadzu UV-2450 spectrophotometer and calculated as follows (Lichtenthaler, Wellburn 1983):

$$\text{Chlorophyll } a = 12.21\ A_{663} - 2.81\ A_{646};$$
$$\text{Chlorophyll } b = 20.13\ A_{646} - 5.03\ A_{663};$$
$$\text{Carotenoids} = (1000\ A_{470} - 3.27\ \text{Chl } a - 104\ \text{Chl } b) / 229.$$

Chlorophyll *a* fluorescence was measured according to Sharma et al. (1997) using a fluorescence monitoring system (PAM 101, Walz, Germany) on fully expanded leaves. A weak modulated beam of 3 μmol $m^{-2}$ $s^{-1}$ to the dark-adapted (30 min) leaf was established to measure initial fluorescence ($F_0$), after which it was exposed to saturating pulse of the white light of 3000 μmol $m^{-2}$ $s^{-1}$ to obtain maximum fluorescence ($F_m$). Steady-state fluorescence ($F_s$) was measured when leaves were exposed to actinic light of 330 μmol $m^{-2}$ $s^{-1}$. $F'_m$ was obtained by exposing leaves to another pulse of saturated light, followed by infrared radiation to obtain $F'_0$. Variable fluorescence ($F_v$) was calculated as $F_m - F_0$ to obtain $F_v/F_m$ ratio. Photochemical quenching ($q_p$) was calculated as $(F'_m - F_s) / (F'_m - F'_0)$ and PSII efficiency ($e'_{PSII}$) was measured as $(F'_m - F_s) / F'_m$ according to Schreiber et al. (1986).

Concentration of $H_2O_2$ was measured spectrophotometrically by homogenizing 0.2 g of tissue with 5% trichloroacetic acid. A reaction mixture of potassium thiocyanate and iron ammonium sulfate was made to react

with the supernatant and absorbance was measured at 480 nm using $H_2O_2$ as standard (Sagisaka 1976).

The amount of lipid peroxidation was estimated by measuring 2-thiobarbituric acid-reactive malondialdehyde formation spectrophotometrically (Sankhalkar, Sharma 2002). Absorbance was determined at 532 nm, subtracting from the absorbance at 600 nm for non-specific turbidity. The extinction coefficient of 155 $mM^{-1}$ $cm^{-1}$ was used to calculate malondialdehyde concentration.

For measurement of electrolyte leakage (EL), leaf disks (total weight 0.2 g) were cut and suspended in 25 mL of MilliQ water, incubated at 30 °C in a water bath for 2 h to measure the initial electrical conductivity ($EC_1$). After incubation at 100 °C for 15 min to release all the electrolytes and cooling, the final electrical conductivity ($EC_2$) was measured using a conductivity meter. The EL (%) was calculated according to Gong et al. (1998):

$$EL\ (\%) = (EC_1 - EC_2) \times 100.$$

The proline concentration was estimated spectrophotometrically by homogenizing the tissue with 3% sulfosalicylic acid; the supernatant was reacted with ninhydrin and glacial acetic acid. The absorbance was measured at 520 nm after incubating in a dry bath at 95 °C for 1 h and adding toluene. L-Proline standard was used for calculation, and results were expressed as nmol proline $g^{-1}$ FW (Bates et al. 1973).

Superoxide dismutase (SOD) activity was measured spectrophotometrically by homogenizing tissue with 0.05 M phosphate buffer. The supernatant was reacted with 13 mM methionine, 75 µM nitro blue tetrazolium, 2 µM riboflavin and 0.1 mM EDTA. One unit of SOD activity is defined as the amount of enzyme required to cause 50% inhibition of nitro blue tetrazolium reduction, monitored at 560 nm (Giannopolitis, Ries 1977).

For determination of salicylic acid concentration in plants, plant tissue (5 g) was homogenized with 20 mL distilled water and centrifuged at 3000 × $g_n$ for 10 min. Freshly prepared 0.2% $FeCl_2$ was reacted with the supernatant to form a violet-colored complex and SA was determined spectrophotometrically at 540 nm using $FeCl_2$ as blank. SA concentration within plants was calculated using standard SA (Merck, Tissue culture grade) and was calculated as nmol $g^{-1}$ FW (Warrier et al. 2013).

### *Statistical analysis*

RWC, shoot-root length, biomass, EL and chlorophyll fluorescence parameters were repeated five times with five replicates to reduce variability and other experiments were repeated three times with five replicates and the data was expressed as mean ± SE. Two-way ANOVA was performed to confirm the variability of the results and Duncan's multiple range test to determine significant ($p \leq 0.05$) difference between treatment groups by using Microsoft Excel XL-STAT (version 2020.4.1). Correlation between physiological parameters was evaluated by using Karl Pearson correlation coefficients significant at ($p \leq 0.05$).

## Results

### *Morphological parameters*

Shoot and root length decreased with an increase in salt stress (Appendix 1). Shoot length decreased by 34.7 and 55.8% ($p < 0.05$) and root decreased by 26.4 and 41.7% ($p < 0.05$) in 40 and 120 mM NaCl plants, respectively (Table 1). Spraying control plants with SA showed no significant change ($p > 0.05$) in the length of the shoot and root. The first and second spray of 0.5 mM SA improved the shoot and root length up to 21 and 17% ($p < 0.05$), respectively, in plants treated with 40 mM NaCl and up to 17.5 and 21.4%, respectively, in plants at 120 mM NaCl. Spray with 1 mM SA also resulted in significant increase in shoot and root length ($p < 0.05$) but to the lesser degree as compared to 0.5 mM SA spray. Spray with 2 mM SA showed reduced ($p < 0.05$) shoot length but no significant change ($p > 0.05$) in root length as compared to salt-treated rice seedlings.

Shoot and root biomass of rice seedlings decreased with an increase in salt concentration (Table 1). Shoot-root biomass were both improved by the spraying of 0.5 mM SA. Both sprays of 0.5 mM SA improved the shoot fresh weight up to 19.5% and dry weight up to 17.8% ($p < 0.05$) in 40 mM NaCl seedlings and up to 14.3 and 16.7% in 120 mM NaCl seedlings, however, a spray of 2 mM SA showed a significant ($p < 0.05$) decrease in the shoot biomass. On application of first and second sprays of 0.5 mM SA, FW and DW of root improved up to 59.1 and 12.2% respectively in 40 mM NaCl seedlings and up to 88.8 and 42.3% ($p < 0.05$) respectively in 120 mM NaCl-treated seedlings compared to the salt-stressed control. A spray of 2 mM SA showed no significant ($p > 0.05$) change in root FW.

### *Relative water content*

RWC decreased with an increase in the salinity stress of 40 and 120 mM NaCl by 7.1 and 19.3%, respectively ($p < 0.05$), compared to the non-saline control (Fig. 1A). In plants grown with 40 and 120 mM NaCl, spraying with 0.5 mM SA improved the RWC up to 1.28 fold ($p < 0.05$) compared to unsprayed plants. However, at a higher concentration of SA (1 and 2 mM), RWC of salt-stressed plants decreased significantly ($p < 0.05$) compared to salt-treated controls. Application of SA to non-saline control showed no significant change ($p > 0.05$) in RWC nor in other parameters studied.

### *Sodium and potassium concentration*

In response to salt stress, sodium concentration increased in both the shoot (10 fold) and root (7.5 fold) ($p < 0.05$) (Fig. 1B). The first spray of 0.5 mM SA to salt-treated plants decreased the sodium concentration by 18.0% in the shoot ($p < 0.05$) compared to their salt-treated control (Fig. 1B). However, no such lowering effect of SA on sodium concentration was observed in the root ($p > 0.05$) (Fig. 1C). In general, $K^+$ concentration (Appendix 2) in shoots (~1150 µg $g^{-1}$ DW) was ~2.8 folds higher than in roots (~400 µg $g^{-1}$

**Table 1.** Effect of exogenous salicylic acid on shoot, root length and biomass in 28 days old salt grown *Oryza sativa* cv. 'Jaya' seedlings. SA, salicylic acid; FS, first spray on the 14th day; SS, second spray on the 21st day. Data represent the means of five independent experiments with five replicates and ± represents standard error. According to Duncan's multiple range test, different lettters indicate significant differences at $p < 0.05$

| Treatment | Shoot length(cm) | Root length (cm) | Shoot fresh weight (g) | Shoot dry weight (g) | Root fresh weight (g) | Root dry weight (g) |
|---|---|---|---|---|---|---|
| Control | 28.5 ± 0.2 a | 7.2 ± 0.1 a | 1.26 ± 0.03 a | 0.167 ± 0.004 a | 0.47 ± 0.001 a | 0.053 ± 0.001 a |
| Control, SA 0.5 mM FS | 28.9 ± 0.1 a | 7.3 ± 0.1 a | 1.28 ± 0.05 a | 0.170 ± 0.003 a | 0.48 ± 0.002 a | 0.055 ± 0.002 a |
| Control, SA 1 mM FS | 28.7 ± 0.1 a | 7.2 ± 0.1 a | 1.27 ± 0.05 a | 0.169 ± 0.003 a | 0.47 ± 0.001 a | 0.053 ± 0.002 a |
| Control, SA 2 mM FS | 28.2 ± 0.2 a | 7.1 ± 0.1 a | 1.27 ± 0.04 a | 0.166 ± 0.004 a | 0.47 ± 0.001 a | 0.053 ± 0.003 a |
| Control, SA 0.5 mM SS | 28.6 ± 0.2a | 7.2 ± 0.1 a | 1.27 ± 0.02 a | 0.170 ± 0.002 a | 0.48 ± 0.003 a | 0.054 ± 0.001 a |
| Control, SA 1 mM SS | 28.5 ± 0.1a | 7.2 ± 0.1 a | 1.26 ± 0.03 a | 0.170 ± 0.003 a | 0.47 ± 0.002 a | 0.054 ± 0.002 a |
| Control, SA 2 mM SS | 28.1 ± 0.2a | 7.1 ± 0.1 a | 1.27 ± 0.03 a | 0.166 ± 0.003 a | 0.46 ± 0.002 a | 0.053 ± 0.002 a |
| 40 mM NaCl | 18.6 ± 0.2 c | 5.3 ± 0.2 d | 0.82 ± 0.02 c | 0.073 ± 0.006 c | 0.22 ± 0.002 c | 0.047 ± 0.002 b |
| 40 mM NaCl, SA 0.5 mM FS | 22.5 ± 0.3 b | 6.2 ± 0.1 b | 0.98 ± 0.05 b | 0.086 ± 0.002 b | 0.35 ± 0.003 b | 0.052 ± 0.001 a |
| 40 mM NaCl, SA 1 mM FS | 21.6 ± 0.2 b | 5.8 ± 0.3 bc | 0.96 ± 0.06 b | 0.081 ± 0.004 b | 0.33 ± 0.002 b | 0.048 ± 0.001 b |
| 40 mM NaCl, SA 2 mM FS | 17.4 ± 0.4 d | 5.1 ± 0.2 d | 0.74 ± 0.03 d | 0.068 ± 0.004 d | 0.20 ± 0.001 c | 0.031 ± 0.002 c |
| 40 mM NaCl, SA 0.5 mM SS | 21.5 ± 0.5 b | 6.0 ± 0.3 b | 0.95 ± 0.02 b | 0.083 ± 0.005 b | 0.32 ± 0.003 b | 0.052 ± 0.002 a |
| 40 mM NaCl, SA 1 mM SS | 21.3 ± 0.4 b | 5.7 ± 0.1 c | 0.93 ± 0.03 b | 0.080 ± 0.004 b | 0.31 ± 0.002 b | 0.051 ± 0.001 a |
| 40 mM NaCl, SA 2 mM SS | 17.2 ± 0.5 d | 5.0 ± 0.1 d | 0.73 ± 0.02 d | 0.067 ± 0.003 d | 0.21 ± 0.002 c | 0.035 ± 0.000 c |
| 120 mM NaCl | 12.6 ± 0.5 g | 4.2 ± 0.2e | 0.56 ± 0.04 f | 0.054 ± 0.003 e | 0.09 ± 0.001 e | 0.016 ± 0.001 e |
| 120 mM NaCl, SA 0.5 mM FS | 14.8 ± 0.3 e | 5.1 ± 0.0 d | 0.64 ± 0.02 e | 0.063 ± 0.002 d | 0.17 ± 0.002 d | 0.023 ± 0.002 d |
| 120 mM NaCl, SA 1 mM FS | 14.1 ± 0.3 e | 4.8 ± 0.1 d | 0.63 ± 0.04 e | 0.061 ± 0.004 d | 0.16 ± 0.002 d | 0.022 ± 0.002 d |
| 120 mM NaCl, SA 2 mM FS | 10.3 ± 0.4 h | 4.1 ± 0.1 e | 0.54 ± 0.03 f | 0.045 ± 0.005 f | 0.09 ± 0.001 e | 0.014 ± 0.000 e |
| 120 mM NaCl, SA 0.5 mM SS | 14.2 ± 0.5 e | 5.0 ± 0.1d | 0.61 ± 0.01 e | 0.061 ± 0.004 d | 0.16 ± 0.002 d | 0.023 ± 0.001 d |
| 120 mM NaCl, SA 1 mM SS | 13.5 ± 0.4 f | 4.8 ± 0.1 d | 0.61 ± 0.01 e | 0.055 ± 0.005 e | 0.15 ± 0.003 d | 0.022 ± 0.001 d |
| 120 mM NaCl, SA 2 mM SS | 10.0 ± 0.6 h | 4.0 ± 0.2 e | 0.53 ± 0.03 f | 0.043 ± 0.005 f | 0.08 ± 0.003 e | 0.013 ± 0.001 e |

DW) ($p < 0.05$). $K^+$ concentration decreased in the shoot as a result of salt stress, more so at 120 mM NaCl; however, the application of SA increased the $K^+/Na^+$ ratio significantly (19.3%) by increasing the $K^+$ concentration of the shoot to 13.7% ($p < 0.05$) at 40 mM NaCl. However, such an increase was not observed in plants treated with a higher level of salt and sprayed with SA. Potassium concentration in the root remained more or less the same due to salt stress or application of SA ($p > 0.05$) (Fig. 1B, C).

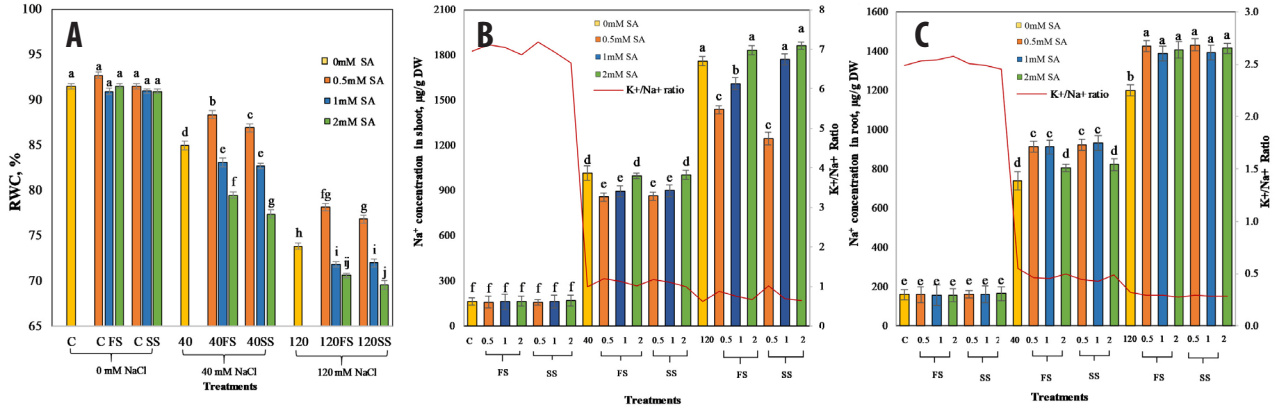


**Fig. 1.** Effect of NaCl treatment and salicylic acid (SA) foliar application on relative water content (RWC) (A), $Na^+$ concentration and $K^+/Na^+$ concentration ratio in shoots (B), and $Na^+$ concentration and $K^+/Na^+$ concentration ratio in roots (C) of *Oryza sativa* cv. 'Jaya' seedlings grown for 28 days. C, non-saline control; FS, first spray on the 14th day with SA; SS, second spray with SA on 21st day. Data represent the means of five independent experiments with five replicates in (A) and three independent experiments with five replicates in (B) and (C). Vertical bars represent standard error. Different letters indicate significant differences at $p < 0.05$ according to the Duncan's multiple range test.

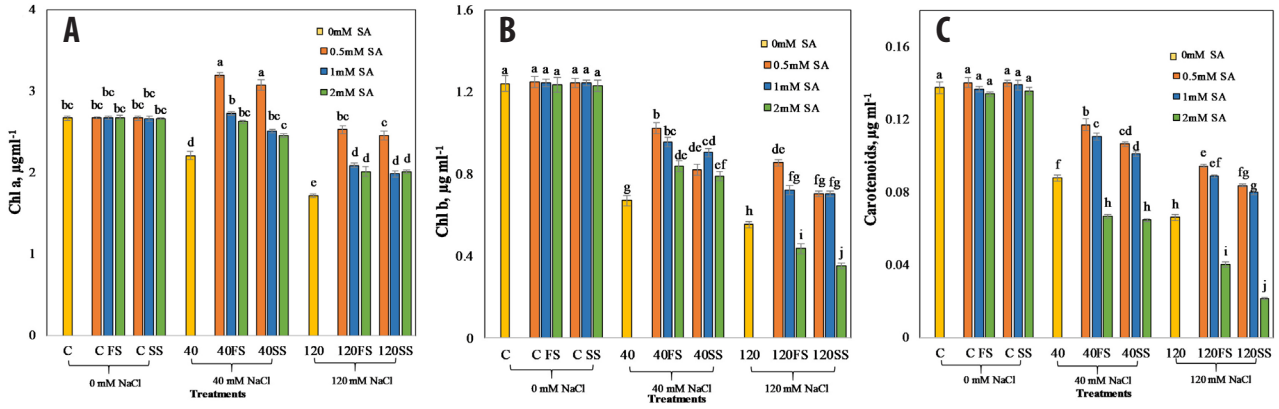


**Fig. 2.** Effect of NaCl treatment and salicylic acid (SA) foliar application on concentration of chlorophyll *a* (A), chlorophyll *b* (B) and carotenoids (C) in *Oryza sativa* cv. 'Jaya' seedlings grown for 28 days. C, non-saline control; FS, first spray on the 14th day with SA; SS, second spray with SA on 21st day. Data represent the means of three independent experiments with five replicates. Vertical bars represent standard error. Different letters indicate significant differences at $p < 0.05$ according to the Duncan's multiple range test.

### *Photosynthetic pigments*

Chlorophyll *a* concentration decreased in plants stressed with 40 and 120 mM NaCl by 17.2 and 35.6%, respectively ($p < 0.05$), compared to the non-saline control (Fig. 2A). Application of 0.5 mM SA reduced the salt-induced decline in chlorophyll a in plants treated with 40 and 120 mM NaCl up to 44.7 and 47.3%, respectively ($p < 0.05$), compared to salt-treated controls (Fig. 2A). A similar trend of reversal in the salt-induced decrease in chlorophyll *b* ($p < 0.05$; Fig. 2B) and carotenoid concentration ($p < 0.05$; Fig. 2C) was also observed on the application of 0.5 mM SA. The second SA spray showed a lesser recovery in chlorophyll *a* and *b* concentration than the first spray to salt-treated plants. Application of 1 and 2 mM SA, however, showed lower recovery of pigments than 0.5 mM.

### *Chlorophyll a fluorescence*

Photosynthetic efficiency ($F_v/F_m$), quantum efficiency of PS II ($e'_{PSII}$) and photochemical quenching ($q_p$) decreased with an increase in salt treatment. Foliar spray with 0.5 mM SA increased $F_v/F_m$ up to 23.5 and 26.6% ($p < 0.05$) in plants treated with 40 and 120 mM NaCl, respectively, compared to their salt-treated controls (Fig. 3A). Similarly, salinity-induced inhibition of $e'_{PSII}$ was reduced by 19.8 and 18.7% ($p < 0.05$) by 0.5 mM SA spray in plants treated with 40 and 120 mM NaCl, respectively, compared with salt-treated controls (Fig. 3B). $q_p$ also showed a similar trend with an increase of up to 21.5% ($p < 0.05$) after spraying with 0.5 mM SA at both NaCl concentrations compared to their salt- treated control plants (Fig. 3C). However, 2 mM SA had an inhibitory effect on $F_v/F_m$, $e'_{PSII}$ and $q_p$ in salt-treated plants. There was a strong positive correlation ($p < 0.05$) between $F_v/F_m$ and RWC ($R^2 = 0.8852$, Fig. 3D), and $F_v/F_m$ and total chlorophyll ($R^2 = 0.9063$, Fig. 3E).

### *Malondialdehyde concentration*

MDA, indicative of lipid peroxidation, amplified with an increase in salinity by 89.9 and 189.0% ($p < 0.05$) in plants treated with 40 and 120 mM NaCl, respectively, compared to the non-saline control (Fig. 4A). Application of 0.5 mM SA to 40 and 120 mM salt-treated plants decreased the MDA concentration by 35.9 and 18.3%, respectively ($p < 0.05$), compared to their salt-treated control. The first spray of 1 mM SA in salt-treated plants decreased the MDA concentration less than 0.5 mM SA. However, a spray of 2 mM SA increased the MDA level compared to their respective salt-treated control plants ($p < 0.05$) (Fig. 4A). There was a negative relationship ($p < 0.05$) between increased lipid peroxidation and RWC ($R^2 = 0.9214$; Fig. 4B).

### *Electrolyte leakage*

Electrolyte leakage (EL), indication of membrane damage, increased with salinity by 6.5 and 12.5% in 40 and 120 mM NaCl-treated plants, respectively, compared to non-saline control (Fig. 4C). EL reduced up to 3.8 and 5.7% ($p < 0.05$) on the application of 2nd spray of 0.5 mM SA in 40 and 120 mM NaCl-treated plants, respectively. A spray with 1 mM SA decreased EL by 6.5% ($p < 0.05$) in 120 mM NaCl-treated plants; however, a spray with 2 mM SA increased the EL by 10.8 and 5.3% ($p < 0.05$) in plants treated with 40 and 120 mM NaCl, respectively, as compared to their saline controls.

### *Hydrogen peroxide concentration*

The concentration of $H_2O_2$, a relatively stable form of ROS, increased up to 66.0% ($p < 0.05$) in 120 mM NaCl-treated plants compared to the non-saline control (Fig. 5A). The salt-induced rise in $H_2O_2$ concentration declined by 9.4% ($p < 0.05$) in 40 mM salt-treated plants with the 1st spray of 0.5 mM SA, and decreased by 30.6% ($p < 0.05$) on the subsequent 2nd spray compared to its salt-stressed control. Likewise, application of 0.5 mM SA on plants treated with 120 mM NaCl reduced $H_2O_2$ concentration by 36.4%

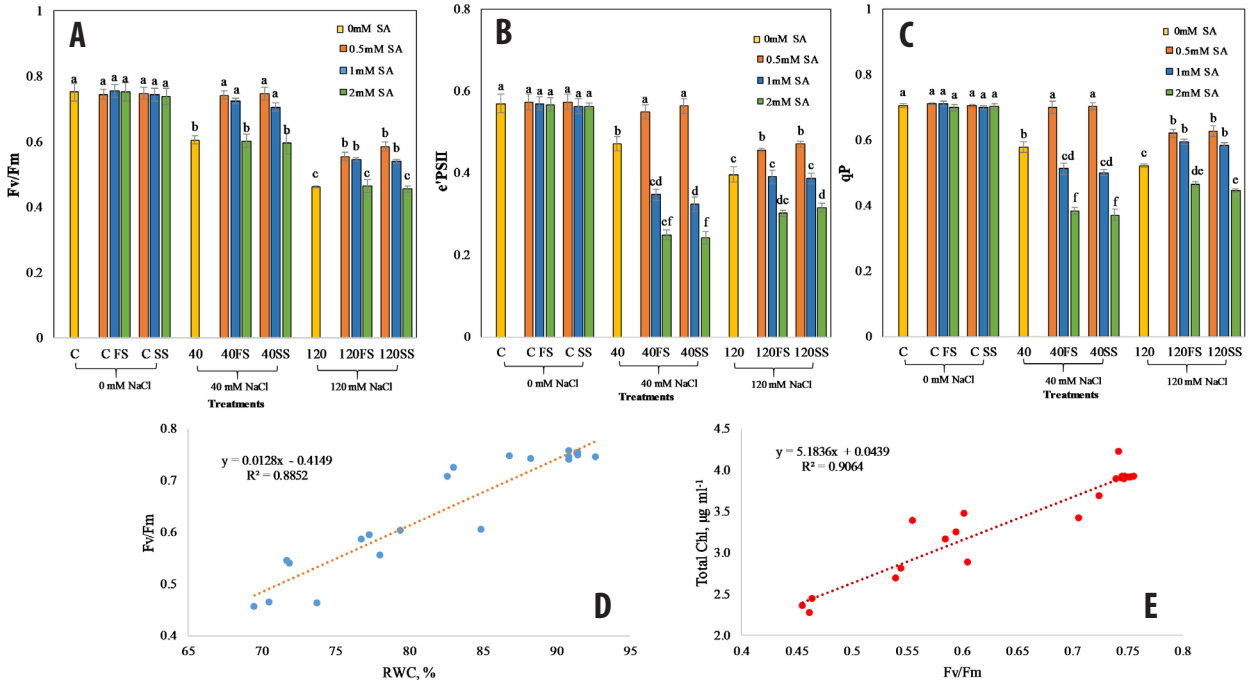


**Fig. 3.** Effect of NaCl treatment and salicylic acid (SA) foliar application on photosynthetic efficiency of photosystem II ($F_v/F_m$) (A), quantum efficiency of photosystem II (e'PSII) (B); photochemical quenching (qP) (C). Relationship between relative water content (RWC) and photosynthetic efficiency of photosystem II ($F_v/F_m$) (D), relationship between total chlorophyll concentration and photosynthetic efficiency of photosystem II ($F_v/F_m$) (E) in *Oryza sativa* cv. 'Jaya' grown for 28 days. C, non-saline control; FS, first spray on the 14th day with SA; SS, second spray with SA on 21st day. Data represent the means of five independent experiments with five replicates. Vertical bars represent standard error. According to the Duncan's multiple range tests, different letters indicate significant differences at $p < 0.05$.

($p < 0.05$) in comparison with its salt-treated control. Application of 1 mM SA resulted in a decrease ($p < 0.05$) in $H_2O_2$ concentration, but to a lesser extent than that for 0.5 mM SA. In contrast, a spray of 2 mM SA to salt-treated plants ($p < 0.05$) increased $H_2O_2$ concentration compared to salt-treated control.

### *Superoxide dismutase activity*

The activity of superoxide dismutase increased by 39.9 and 49.0% ($p < 0.05$) in 40 and 120 mM NaCl-treated plants compared to non-saline control (Fig. 5B). It was evident that the 1st and 2nd spray with 0.5 mM SA on 40 mM NaCl-treated plants further increased the activity of SOD by 28.0 and 14.0%, respectively ($p < 0.05$), and a similar trend was

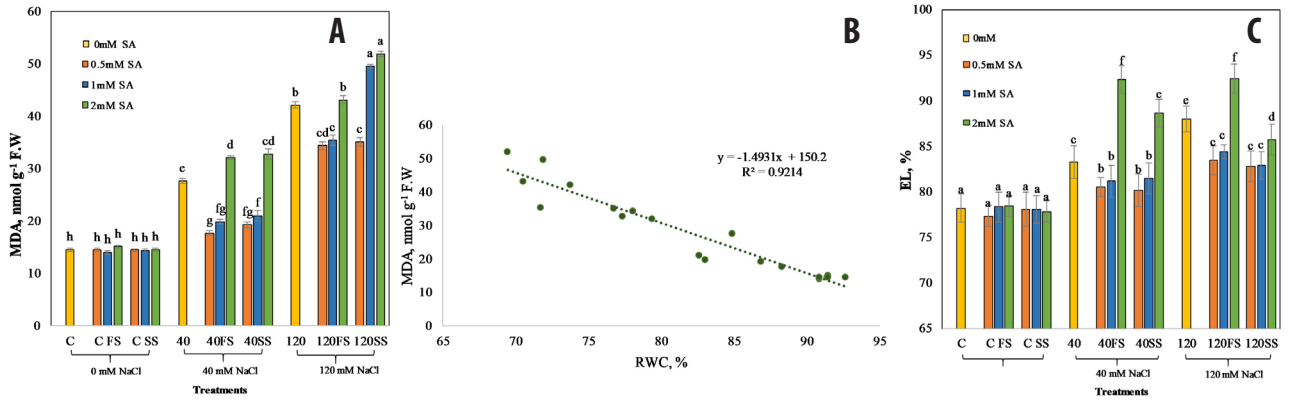


**Fig. 4.** Effect of NaCl treatment and salicylic acid (SA) foliar application on malondialdehyde (MDA) concentration (A), relationship between relative water content (RWC) and MDA concentration (B), electrolyte leakage (EL) (C) in *Oryza sativa* cv. 'Jaya' grown for 28 days. C, non-saline control; FS, first spray on the 14th day with SA; SS, second spray with SA on 21st day. Data represent the means of five independent experiments with five replicates. Vertical bars represent standard error. According to Duncan's multiple range test, different letters indicate significant differences at $p < 0.05$. $R^2$ is the coefficient of determination.

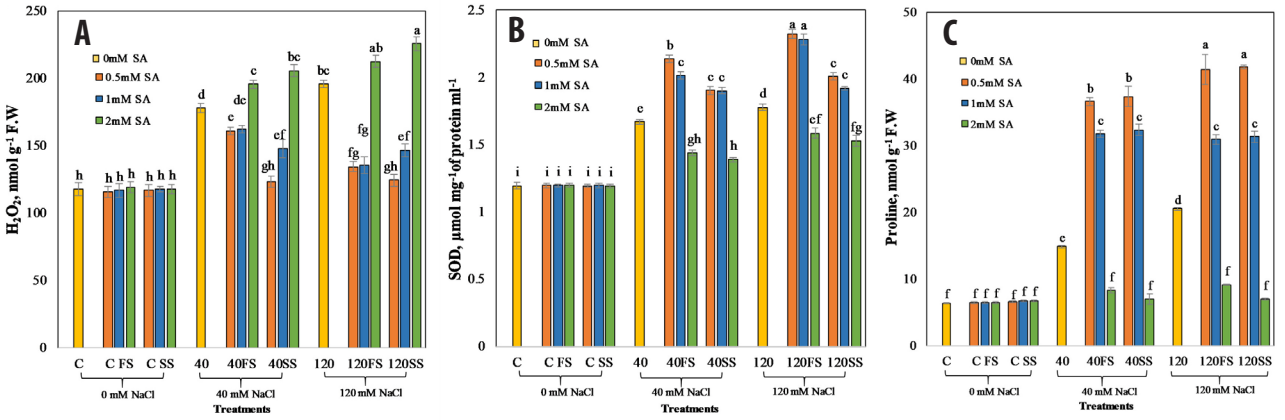


**Fig. 5.** Effect of NaCl treatment and salicylic acid (SA) foliar application on $H_2O_2$ concentration (A), activity of superoxide dismutase (SOD) (B), and proline concentration (C) in *Oryza sativa* cv. 'Jaya' grown for 28 days. C, non-saline control; FS, first spray on the 14th day with SA; SS, second spray with SA on 21st day. Data represent the means of three independent experiments with five replicates. Vertical bars represent standard error. According to Duncan's multiple range test, different letters indicate significant differences at $p < 0.05$.

seen for plants treated with 120 mM NaCl. In contrast, SOD activity decreased ($p < 0.05$) when sprayed with a higher concentration of SA (2 mM) in comparison with the salt-stressed control.

### *Proline concentration*

Proline, an organic solute, increased by 132.7 and 221.6% ($p < 0.05$) in plants treated with 40 and 120 mM NaCl, respectively, compared to the non-saline control (Fig. 5C). In the first application, 0.5 mM SA increased proline concentration up to 151.6% in 40 and 120 mM salt-treated plants; however, on the second spray, the increase in proline was only up to 103.7% compared to the salt-stressed control. We observed a similar increasing trend in proline concentration on the spray of 1 mM SA to 40 and 120 mM NaCl-treated plants ($p < 0.05$). However, applying a higher concentration of 2 mM SA to salt-treated plants decreased ($p < 0.05$) the proline level compared to salt-stressed controls.

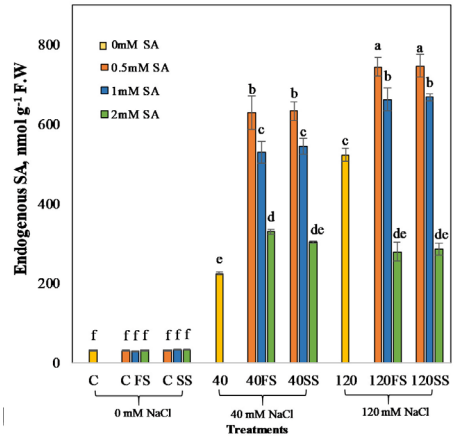


**Fig. 6.** Effect of NaCl treatment and salicylic acid (SA) foliar application on SA levels within *Oryza sativa* cv. 'Jaya' grown for 28 days. C, non-saline control; FS, first spray on the 14th day with SA; SS, second spray with SA on the 21st day. Data represent the means of three independent experiments with five replicates. Vertical bars represent standard error. According to Duncan's multiple range test, different letters indicate significant differences at $p < 0.05$

### *Salicylic acid concentration in plants*

SA concentration within plants was measured to determine its level and relate it to the parameters studied in salt-stressed control and SA sprayed plants. An increase in SA concentration by 592.9 and 1512.2% ($p < 0.05$) in 40 and 120 mM NaCl-treated plants, respectively, was observed, compared to the non-saline control (Fig. 6). The salinity-induced rise in SA within the plants further increased by 181.8 and 42.9% ($p < 0.05$) after spray with 0.5 mM SA in 40 and 120 mM NaCl-treated plants, respectively, compared to salt-treated controls. A similar increasing trend in SA was observed in plants sprayed with 1 mM SA ($p < 0.05$) in both salt concentrations compared to their salt-treated controls. However, the application of 2 mM SA to 120 mM NaCl plants decreased SA concentration compared to the salt-treated control.

## Discussion

This study demonstrated improved growth, biomass, RWC, and ion homeostasis, as well as increased photosynthetic pigment concentration, $F_v/F_m$, $e'_{PSII}$, $q_P$, proline concentration, SOD activity, and lowered ROS and lipid peroxidation as a result of foliar spray of SA in NaCl-treated rice plants, suggesting ameliorating salt stress-induced damaging effects. The mitigating effect of SA was more effective at lower concentrations (0.5 mM), improving the analyzed parameters, but higher concentration of SA (2 mM) was inhibitory. However, non-saline plants sprayed

with SA showed no significant change in these parameters.

RWC, determining the water status of a shoot relative to its fully hydrated state, was negatively related to salt stress but improved with the foliar spray of 0.5 mM SA (Fig. 1A), suggesting the role of SA in better plant water status by maintaining cell turgor and improved shoot-root growth and biomass (Khoshbakkht, Asgharei 2015). Leaf water potential has often been used as an indicator of plant physiological status under stressed conditions, as there is a positive relationship between RWC and leaf water potential (Katerji et al. 2008).

Similar results have been obtained also in previous studies. Enhanced seedling growth in wheat (Shakirova 2007) and enhanced leaf area and dry mass production in corn and soybean (Khan et al. 2003) as a result of treatment with SA under salt-stressed conditions has been observed. An observed relationship between increased RWC (Fig. 1A) and proline concentration (Fig. 5C) as a result of SA application in the present study may suggest its role in increasing solute potential, thereby facilitating the intake of water to maintain higher RWC. It was also reported that improved leaf water potential and RWC coincided with increased proline concentration in tomato plants (Hayat et al. 2008). It was demonstrated that higher osmolyte concentration in the metal tolerant variety of green gram resulted in the maintenance of comparatively higher RWC on spraying with SA (Misra, Dwivedi 2004). Improved RWC with the application of exogenous SA has been demonstrated in citrus (Khoshbakht, Asgharei 2015) and *Vigna angularis* (Ahanger et al. 2020) plants. Application of SA also helped in increasing the $K^+/Na^+$ ratio in the shoot (Fig. 1B), further indicating better plant-water relations. A relationship between RWC and higher $K^+/Na^+$ ratio on the application of SA in salt-treated rosemary plants has been also shown (Hassan et al. 2017).

The present results not only indicated a higher $K^+/Na^+$ ratio due to the application of SA, but facilitated competitive intake of $Na^+$ and $K^+$ in the shoot, probably through a non-specific cation channel, as the level of $Na^+$ decreased (Fig. 1B) and potassium concentration increased (Appendix 2), further improving the water status with SA treatment to salt-treated plants (Fig. 1C). The mitigation of salt stress depends on ion homeostasis and the neutralization of ion toxicity, which are critical components of salinity tolerance (Wu, Li 2019). Again, the ameliorating effect was more effective at a low concentration of SA. It was reported that decreased sodium and increased potassium concentration on the application of SA in rice might be due to the influence of $Na^+$ on the transport of $K^+$ within plant cells owing to chemical similarity (Jini, Joseph 2017). Similar changes in $Na^+$ and $K^+$ on the application of SA in *Arabidopsis thaliana* have been shown (Jayakannan et al. 2013).

The observed increase in concentration of photosynthetic pigments, as well as chlorophyll *a* fluorescence parameters $F_v/F_m$, $e'_{PSII}$, and $q_P$ resulting from the application of low concentration of exogenous SA in our study again suggests higher photosynthetic productivity leading to increase in shoot and root growth and biomass accumulation (Appendix 1, Table 1). The same was further substantiated with a positive correlation between maximum quantum efficiency of photosystem II ($F_v/F_m$), RWC and total chlorophyll concentration (Fig. 3D, E). Variable effects of SA regarding chlorophyll *a* fluorescence parameters and photosynthetic pigments have been reported previously (Janda et al. 2014). Improved $F_v/F_m$ value as a result of SA treatment has been reported for citrus (Khoshbakht, Asgharei 2015) and mustard plants (Nazar et al. 2015), while there were no changes no in $F_v/F_m$ value in alfalfa plants with exogenous SA (Palma et al. 2013). There are variable results, as an increase of photosynthesis-related parameters on the application of 1 mM SA in rice genotypes was observed (Jini, Joseph 2017), but a decrease on the application of SA (50 to 250 mg $kg^{-1}$) in wheat (Moharekar et al. 2003). Changes in chlorophyll concentration due to SA treatment are suggested to be concentration-dependent and species-specific (Miura, Tada 2014).

Decreased damage to the cell membrane was suggested, as the level of lipid peroxidation measured as MDA declined in response to the application of SA, due to lower production of $H_2O_2$ (Fig. 5A) and a higher level of antioxidants such as SOD activity (Fig. 5B) and proline concentration (Fig. 5C). ROS is known to cause oxidation of polyunsaturated fatty acids and membrane proteins, thereby affecting their structure, resulting in altered fluidity, permeability and catalytic functions of the membrane (Sharma et al. 2012). An increase in the activity of the primary antioxidant enzyme SOD in the present study also suggested tolerance against salt-induced oxidative stress by modulating the cell redox balance (Fig. 5B). Osmotic stress, resulting in lowered RWC, leads to oxidative stress (Borsani et al. 2001). The application of SA to salt-stressed plants improved the water status of the cell by increasing RWC (Fig. 1A), thereby lowering the osmotic stress. The same is also suggested by an increase in the proline concentration (Fig. 5C) and consequential reduction in ROS and oxidative stress (Fig. 5A). A negative relationship between increased peroxidation of membrane lipids (MDA concentration) and decreased plant water status (RWC) is shown (Fig. 4B). A similar ameliorative effect of SA on inhibiting MDA production has been shown for other plant species (Shaki et al. 2017). Stimulation of the activity of many antioxidant enzymes, SOD, APX and CAT by SA has been also shown (Jini, Joseph 2017). Salinity-induced increased damage to the membrane and decreased antioxidant enzyme activity was shown in SA-deficient NahG transgenic *Arabidopsis* lines (Cao et al. 2009). A reduction in MDA concentration with an increase in RWC after the application of SA in salt-stressed *Torreya grandis* has been reported (Li et al. 2014).

Increased proline concentration observed with salt stress was further increased with the application of lower

SA concentration (Fig. 5C), indicating a role of SA in synthesizing proline an organic osmoticum to maintain a favorable solute potential. Higher osmoticum due to proline and higher $K^+/Na^+$ indicate better water potential (Khaleghi et al. 2019), implying lesser osmotic stress, also seen as better RWC in the present study (Fig. 1A). Proline is known to act as an excellent osmolyte to balance the cell turgor and stabilize the cell membrane by preventing membrane leakage (Matysik et al. 2002; Hayat et al. 2012). In addition, it is also known to aid in preventing oxidative bursts and increasing the threshold level of antioxidant enzymes in plants (Khaleghi et al. 2019). The function of proline as a sink for energy, regulating the redox potential and mitigating salt stress, and acting as an antioxidant to quench singlet oxygen species by decreasing oxygenase and carboxylase activities of Rubisco and protecting plants have been reported (Misra, Saxena 2009). The present results on changes in proline concentration are in accordance with studies showing that the application of SA increases the proline concentration in water-stressed tomato plants to reverse the inhibitory effect of salinity more efficiently (Hayat et al. 2008).

A positive correlation has been established between improved physiological parameters such as RWC (Fig. 1A), ion homeostasis (Fig. 1B, C), antioxidant activity (Fig. 5B) and negative correlation with ROS production (Fig. 5A), oxidative damage to the membranes (Fig. 4A) and membrane leakage (Fig. 4C) with a higher level of SA within the plants (endogenous, Fig. 6) after the application of SA at 0.5 mM concentration, which was not observed on the application of SA at 2 mM concentration. Plant hormones develop various signaling pathways to balance plant growth and defense response, and this crosstalk between plant hormones is the core of plant stress response (Yang et al. 2019). Plant hormones are known to act within a specific concentration range depending on tissue specificity (Borsani et al. 2001). Also, hormones work in interrelation with other hormones, synergistically or antagonistically. Shakirova et al. (2003) reported that SA enhanced indole-3-acetic acid, cytokinin and abscisic acid concentration under salt stress resulting in improved cell division in the root apical meristem and increased growth in wheat plants. SA was also reported to act as an activator of protein kinases through MPK4, positively regulating SA signaling while negatively regulating MYC2 in the JA signaling pathway (Wasternack, Hause 2013). In *Arabidopsis thaliana* seedlings, increased tolerance to salt stress was associated with higher endogenous SA levels (Alonso-Ramírez et al. 2009). The present results also suggest the same, as increased level of endogenous SA observed in plants sprayed with 0.5 mM SA was related to a higher level of photosynthesis, RWC, proline concentration and SOD activity, and lower level of ion toxicity, ROS production and oxidative damage to the cell membranes, while application of 2 mM SA resulted in a low level of endogenous SA and showed no such positive mitigating effect in the parameters studied.

The decrease in the endogenous SA observed on the application of 2 mM SA in our study may not be due to conjugation. Conversion of free SA to conjugated SA due to salt stress has been reported in tobacco plants, but not in rice seedlings (Savada et al. 2006). Tobacco cell suspension culture supplemented with a higher concentration of SA showed in de novo induction of SA excretion, mediated by generation of ROS and cascade of $Ca^{2+}$ signaling and protein phosphorylation (Hayat et al. 2010). Concentration dependence and species-specific effects of SA on physiological parameters has been also shown (Miura, Tada 2014). SA was reported to cause variations in physiological response in salt-sensitive and resistant cultivars of rice (C3 plant) (Jini, Joseph 2017) and maize (C4 plant) (El-Mergawi, El Wahed 2020). In addition, the method of application of SA might also affect the plant responses (Horvath et al. 2007).

Our study indicates that applying exogenous SA to a non-saline control plant showed no stimulation in the endogenous level of SA, which may suggest that induction of SA is stimulated only under stress conditions, even on the application of exogenous SA. No improvement in plant growth and leaf RWC on the application of SA to non-saline plants compared to control plants has been shown (Tahjib et al. 2018) suggesting no further improvement of SA under non-stress conditions; however, endogenous level of SA was not analyzed in this study. In contrast, a higher endogenous SA level in control plants was reported (Jini, Joseph 2017). Rice plants given two sprays of SA showed no enhanced ameliorating effect over one spray except

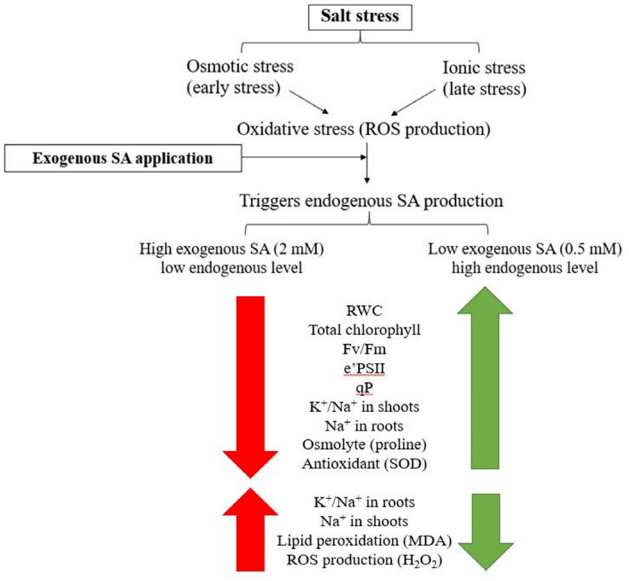


**Fig. 7.** Schematic presentation of variable changes occurring in various studied parameters in response to the endogenous level of salicylic acid (SA) in salt-stressed plants treated with foliar application of exogenous SA (0.5 to 2 mM).

for ROS production, probably because no difference was observed in the endogenous level of SA (Fig. 6). In general, our results provide insight into the possible mechanisms of salt tolerance of rice with a correlation between the parameters mediated by a range of SA concentrations, which may provide the basis to improve its productivity in saline areas (Fig. 7).

## Conclusions

The present study demonstrated that increased salt stress reduced the photosynthetic efficiency by inducing oxidative damage, which reversed to an extent with the foliar application of SA. Data also demonstrated a threshold value of 0.5 mM SA for mitigating salt stress by increased levels of RWC, ion homeostasis, photosynthetic pigments, antioxidant activity, and proline concentration while lowering the membrane damage and ROS production, preventing oxidative damage. Application of 1 mM SA showed no further improvement in physiological parameters over 0.5 mM SA; however, a higher concentration of 2 mM SA had a negative effect on the growth of salt-stressed plants.

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### References

Ahanger M.A., Aziz U., Alsahli A.A., Alyemeni M.N., 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: 42.

Alamri S.A., Siddiqui M.H., Al-Khaishani M.Y., Ali H.M. 2018. Response of salicylic acid on seed germination and physio-biochemical changes of wheat under salt stress. *Acta Sci. Agric.* 2: 36–42.

Alonso-Ramírez A., Rodríguez D., Reyes D., Jiménez J.A., Nicolás G., López-Climent M., Gomez-Cadenas A., Nicolás C. 2009. Crosstalk between gibberellins and salicylic acid in early stress responses in *Arabidopsis thaliana* seeds. *Plant Signal. Behav.* 4: 750–751.

Azooz M.M. 2009. Salt stress mitigation by seed priming with salicylic acid in two faba bean genotypes differing in salt tolerance. *Int. J. Agric. Biol.* 11: 343–350.

Barrs H.D., Weatherley P.E. 1962. A re-examination of the relative turgidity technique for estimating water deficits in leaves. *Austr. J. Biol. Sci.* 15: 413–428.

Bates L.S., Waldren R.P., Teare I.D. 1973. Rapid determination of free proline for water-stress studies. *Plant Soil* 39: 205–208.

Borsani O., Valpuesta V., Botella M.A. 2001. Evidence for a role of salicylic acid in the oxidative damage generated by NaCl and osmotic stress in *Arabidopsis* seedlings. *Plant Physiol.* 126: 1024–1030.

Botella M.A., Rosado A., Bressan R., Hasegawa P.M. 2007. Plant adaptive responses to salinity stress. In: Jenks M.A., Hasegawa P.M. (Eds.) *Plant Abiotic Stress.* Blackwell Publishing, Iowa, pp. 37–70.

Cao Y., Zhang Z.W., Xue L.W., Du J.B., Shang J., Xu F., Yuan S., Lin H.H. 2009. Lack of salicylic acid in *Arabidopsis* protects plants against moderate salt stress. *Z. Naturforsch. C J. Biosci.* 64: 231–238.

Chapman H.D., Pratt P.F. 1962. Methods of analysis for soils, plants and waters. *Soil Sci.* 93: 68.

Chen M., Yang Z., Liu J., Zhu T., Wei X., Fan H., Wang B. 2018. Adaptation mechanism of salt excluders under saline conditions and its applications. *Int. J. Mol. Sci.* 19: 3668.

El-Mergawi R.A., Abd El-Wahed M.S. 2020. Effect of exogenous salicylic acid or indole acetic acid on their endogenous levels, germination, and growth in maize. *Bull. Natl. Res. Cent.* 44: 1–8.

Giannopolitis C.N., Ries S.K. 1977. Superoxide dismutases: I. Occurrence in higher plants. *Plant Physiol.* 59: 309–314.

Gong M., Li Y.J., Chen S.Z. 1998. Abscisic acid-induced thermotolerance in maize seedlings is mediated by calcium and associated with antioxidant systems. *J. Plant Physiol.* 153: 488–496.

Gunes A., Inal A., Alpaslam M., Erslan F., Bagsi E.G., Cicek N. 2007. Salicylic acid induced changes on some physiological parameters symptomatic for oxidative stress and mineral nutrition in maize (*Zea mays* L.) grown under salinity. *J. Plant Physiol.* 164: 728–736.

Hassan F.A.S., Morsi M.M., Aljoudi N.G.S. 2017. Alleviating the adverse effects of salt stress in rosemary by salicylic acid treatment. *Res. J. Pharm. Biol. Chem. Sci.* 8: 1980–1995.

Hayat Q., Hayat S., Irfan M., Ahmad A. 2010. Effect of exogenous salicylic acid under changing environment: A review. *Environ. Exp. Bot.* 68: 14–25.

Hayat S., Hasan S.A., Fariduddin Q., Ahmad A. 2008. Growth of tomato (*Lycopersicon esculentum*) in response to salicylic acid under water stress. *J. Plant Interact.* 3: 297–304.

Hayat S., Hayat Q., Alyemeni M.N., Wani A.S., Pichtel J., Ahmad A. 2012. Role of proline under changing environments: a review. *Plant Signal. Behav.* 7: 1456–1466.

Hoagland, D. R., Arnon, D. I. 1950. The water-culture method for growing plants without soil. *California Agricultural Experiment Station Circular* 347: 1–32.

Horvath E., Szalai G., Janda T. 2007. Induction of abiotic stress tolerance by salicylic acid signaling. *J. Plant Growth Regul.* 26: 290–300.

Janda T., Gondor O.K., Yordanova R., Szalai G., Pal M. 2014. Salicylic acid and photosynthesis: signalling and effects. *Acta Physiol Plant*. 36: 2537–2546.

Jayakannan M., Bose J., Babourina O., Rengel Z., Shabala S. 2013. Salicylic acid improves salinity tolerance in *Arabidopsis* by restoring membrane potential and preventing salt-induced $K^+$ loss via a GORK channel. *J. Exp. Bot.* 64: 2255–2268.

Jini D., Joseph B. 2017. Physiological mechanism of salicylic acid for alleviation of salt stress in rice. *Rice Sci.* 24: 97–108.

Katerji N., Mastrorilli M., Rana G. 2008. Water use efficiency of crops cultivated in the Mediterranean region: Review and analysis. *Eur. J. Agron.* 28: 493–507.

Khaleghi A., Naderi R., Brunetti C., Maserti B.E., Salami S.A., Babalar M. 2019. Morphological, physiochemical and

antioxidant responses of *Maclura pomifera* to drought stress. *Sci. Rep.* 9: 19250.

Khan M.I.R., Fatma M., Per T.S., Anjum N.A., Khan N.A. 2015. Salicylic acid-induced abiotic stress tolerance and underlying mechanisms in plants. *Front. Plant Sci.* 6: 462.

Khan W., Balakrishnan P., Smith D.L. 2003. Photosynthetic responses of corn and soybean to foliar application of salicylates. *J. Plant Physiol.* 160: 485–492.

Khoshbakht D., Asgharei M.R. 2015. Influence of foliar-applied salicylic acid on growth, gas-exchange characteristics, and chlorophyll fluorescence in citrus under saline conditions. *Photosynthetica* 53: 410–418.

Li T., Hu Y., Du X., Tang H., Shen C., Wu J., Zhang J. 2014. Salicylic acid alleviates the adverse effects of salt stress in *Torreya grandis* cv. Merrillii seedlings by activating photosynthesis and enhancing antioxidant systems. *PLoS ONE* 9: e109492

Lichtenthaler H.K., Wellburn A.R. 1983. Determinations of total carotenoids and chlorophylls *a* and *b* of leaf extracts in different solvents. *Biochem. Soc. Trans.* 11: 591–592.

Mahmud S., Sharmin S., Chowdhury B.L., Hossain M.A. 2017. Effect of salinity and alleviating role of methyl jasmonate in some rice varieties. *Asian J. Plant Sci.* 16: 87–93.

Matysik J., Alia A., Bhalu B., Mohanty P. 2002. Molecular mechanisms of quenching of reactive oxygen species by proline under stress in plants. *Curr Sci.* 82: 525–532.

Mishra A., Choudhuri M. 1999. Effects of salicylic acid on heavy metal-induced membrane deterioration mediated by lipoxygenase in rice. *Biol. Plant.* 42: 409–415

Misra N., Dwivedi U.N. 2004. Genotypic difference in salinity tolerance of green gram cultivars. *Plant Sci.* 166 :1135–1142.

Misra N., Saxena P. 2009. Effect of salicylic acid on proline metabolism in lentil grown under salinity stress. *Plant Sci.* 177: 181–189.

Miura K., Tada Y. 2014. Regulation of water, salinity, and cold stress responses by salicylic acid. *Front. Plant Sci.* 5: 4.

Moharekar S.T., Lokhande S.D., Hara T., Tanaka R., Tanaka A., Chavan P.D. 2003. Effect of salicylic acid on chlorophyll and carotenoid contents of wheat and moong seedlings. *Photosynthetica* 41: 315–317.

Munns R., Tester M. 2008. Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.* 59: 651–681.

Nazar R., Umar S., Khan N.A., Sareer O. 2015. Salicylic acid supplementation improves photosynthesis and growth in mustard through changes in proline accumulation and ethylene formation under drought stress. *South African J. Bot.* 98: 84–94.

Palma F., López-Gómez M., Tejera N.A., Lluch C. 2013. Salicylic acid improves the salinity tolerance of *Medicago sativa* in symbiosis with *Sinorhizobium meliloti* by preventing nitrogen fixation inhibition. *Plant Sci.* 208: 75–82.

Parihar P., Singh S., Singh R., Singh V.P., Prasad S.M. 2015. Effect of salinity stress on plants and its tolerance strategies: A review. *Environ. Sci. Pollut. Res.* 22: 4056–4075.

Poór P., Gémes K., Horváth F., Szepesi Á., Simon M. L., Tari I. 2011. Salicylic acid treatment via the rooting medium interferes with stomatal response, $CO_2$ fixation rate and carbohydrate metabolism in tomato, and decreases harmful effects of subsequent salt stress. *Plant Biol.* 13: 105–114.

Sagisaka S. 1976. The occurrence of peroxide in a perennial plant, *Populus gelrica. Plant Physiol.* 57: 308–309.

Sakhabutdinova A.R., Fatkhutdinova D.R., Bezrukova M.V., Shakirova F.M. 2003. Salicylic acid prevents the damaging action of stress factors on wheat plants. *Bulg. J. Plant Physiol.* SI: 314–319.

Sankhalkar S., Sharma P.K. 2002. Protection against photooxidative damage provided by enzymatic and non-enzymatic antioxidant system in sorghum seedlings. *Indian J. Exp. Biol.* 40: 1260–1268.

Schreiber U., Schliwa U., and Bilger W. 1986. Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. *Photosynth. Res.* 10: 51–62.

Senaratna T., Touchell D., Bunn E., Dixon K. 2000. Acetyl salicylic acid (aspirin) and salicylic acid induce multiple stress tolerance in bean and tomato plants. *Plant Growth Regul.* 30: 157–161.

Shaki F., Ebrahimzadeh M.H., Niknam V. 2017. Central role of salicylic acid in resistance of safflower (*Carthamus tinctorius* L.) against salinity. *J. Plant Interact.* 12: 414–420.

Shakirova F.M. 2007. Role of hormonal system in the manifestation of growth promoting and anti-stress action of salicylic acid. In: Hayat S., Ahmad A. (Eds.) *Salicylic Acid, A Plant Hormone.* Springer, Dordrecht, Netherlands. P. 69–89.

Shakirova F.M., Sakhabutdinova A.R., Bezrukova M.V., Fatkhutdinova R.A., Fatkhutdinova D.R. 2003. Changes in the hormonal status of wheat seedlings induced by salicylic acid and salinity. *Plant Sci.* 164: 317–322.

Sharma P., Jha A.B., Dubey R.S., Pessarakli M. 2012. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *J. Bot.* 2012: 217037.

Sharma P.K., Shetye R., Bhonsle S. 1997. Effect of supplementary ultraviolet-B radiation on young wheat seedlings. *Curr. Sci.* 72: 400–405.

Shi Q., Zhu Z. 2008. Effects of exogenous salicylic acid on manganese toxicity, element contents and antioxidative system in cucumber. *Environ. Exp. Bot.* 63: 317–326.

Tahjib-Ul-Arif M., Siddiqui M.N., Sohag A.A.M., Sakil M.A., Rahman M.D., Polash M.A.S., Mostofa M.G., Tran L.P. 2018. Salicylic acid-mediated enhancement of photosynthesis attributes and antioxidant capacity contributes to yield improvement of maize plants under salt stress. *J. Plant Growth Regul.* 37: 1318–1330.

Trissel L. A., Ashworth L. D., Ashworth, J. 2018. Ubidecarenone–Ursodiol. *Trissel's Stability of Compounded Formulations.* 6th Ed.; American Pharmacists Association: Washington, DC, USA.

Waqas M.A., Kaya C., Riaz A., Farooq M., Nawaz I., Wilkes A., Li Y. 2019. Potential mechanisms of abiotic stress tolerance in crop plants induced by thiourea. *Front. Plant Sci.* 10: 1336.

Warrier R.R., Paul M., Vineetha M.V. 2013. Stimation of salicylic acid in *Eucalyptus* leaves using spectrophotometric methods. *Genet. Plant Physiol.* 3: 90–97.

Wasternack C., Hause B. 2013. Jasmonates: biosynthesis, perception, signal transduction and action in plant stress response, growth and development. *Ann. Bot.* 111: 1021–1058.

Wu H., Li Z. 2019. The importance of $Cl^-$ exclusion and vacuolar $Cl^-$ sequestration: Revisiting the role of $Cl^-$ transport in plant salt tolerance. *Front. Plant Sci.* 10, 1418.

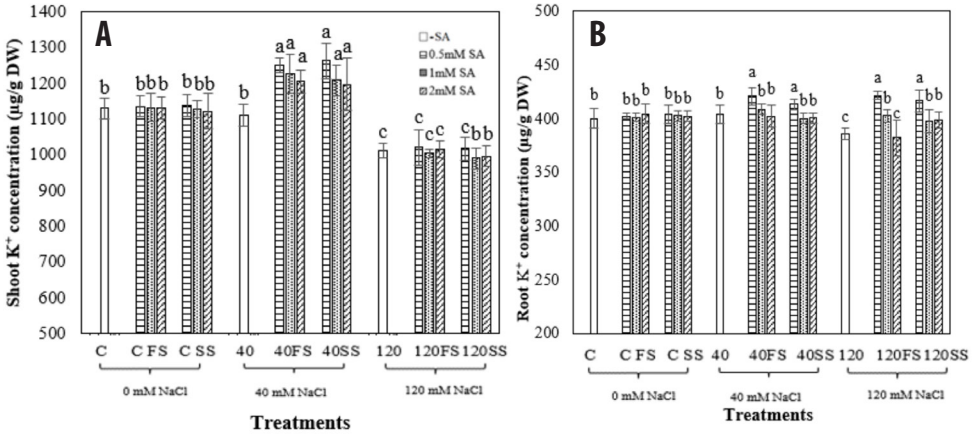
Yancey P.H. 2005. Organic osmolytes as compatible, metabolic and counteracting cytoprotectants in high osmolarity and other stresses. *J. Exp. Biol.* 208: 2819–2830.

Yang J., Duan G., Li C., Liu L., Han G., Zhang Y., Wang C. 2019. The crosstalks between jasmonic acid and other plant hormone

signaling highlight the involvement of jasmonic acid as a core component in plant response to biotic and abiotic stresses. *Front Plant Sci.* 10: 1349.

Yildirim E., Dursun A. 2008. Effect of foliar salicylic acid applications on plant growth and yield of tomato under greenhouse conditions. *Acta Hortic.* 807: 395–400.

Zhang Yu., Yang L., Jahan, N., Chen, G., Deyong R., Guo L. 2018. Sensing of abiotic stress and ionic stress responses in plants. *Int. J. Mol. Sci.* 19: 3298.




**Appendix 1.** Effect of NaCl treatment and salicylic acid (SA) foliar application on $K^+$ concentration in shoots (A, and $K^+$ concentration in roots (B) of *Oryza sativa* cv. 'Jaya' seedlings grown for 28 days. C, non-saline control; FS, first spray on the 14th day with SA; SS, second spray with SA on 21st day. Data represent the means of three independent experiments with five replicates. Vertical bars represent standard error. Different letters indicate significant differences at $p < 0.05$ according to the Duncan's multiple range test.


**Appendix 2.** *Oryza sativa* cv. 'Jaya' plants grown for 28 days. Non-saline control (a), plants treated with 120 mM NaCl (b), plants treated with 120 mM NaCl and sprayed only once with 0.5 mM SA (c), 1 mM SA (d) or 2 mM SA (e).

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