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Complementary Effects of Salicylic Acid and Pseudomonas yamanorum Seed Priming on Growth, Physiology, and Biochemical Responses of Salt Stressed Tomato (Solanum lycopersicum L.)

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Abstract

Salt stress is a problem that significantly harms vegetable crops such as tomato. A greenhouse experiment was carried out at the Regional Centre for Agricultural Research (CRRA), Sidi Bouzid, Tunisia, to determine the effect of seed priming with two biostimulants, salicylic acid (SA) (1 mM) and/or PGPR (Pseudomonas yamanorum) (PY), on tomato plants subjected to four salinity levels (C0 = 0 mM, C1 = 50 mM, C2 = 100 mM, and C3 = 150 mM NaCl). To our knowledge, this is the first study to evaluate *Pseudomonas yamanorum* as a seed priming agent, alone or in combination with SA, under salt stress. Increased salinity, especially at C3, reduced stem diameter (SD), root length (RL), leaf area (LA), and plant dry weight (PDW) by 49%, 62%, 56%, and 50%, respectively. Gas exchange and pigment concentrations were also impaired. Salinity significantly increased H_2O_2 (+ 330%) and MDA (+ 109%), reducing the salt stress tolerance index (SSTI) (-62%) and membrane stability index (MSI) (-32%). However, seed priming with SA and/or PY alleviated these negative effects, with the best performance attributed to combined SA and PY priming, recording the highest increases in PDW, net photosynthesis (Pn), evapotranspiration rate (Et), and chlorophyll a (Chla). Combined priming also increased secondary metabolites at C3, notably flavonoids (Flv) (+ 83%) and proline (Pro) (+ 60%), accompanied by reductions in H_2O_2 (-42%) and MDA (-20%). These results indicate that combining biostimulants is a promising technique for substantially alleviating salt stress, even at high concentrations, though full recovery under extreme stress remains partial.

1. Introduction

Tomato is one of the most widely produced crops worldwide; in 2021, tomato production surpassed 189 million tons across approximately 5 million hectares (Romdhane et al. 2023), with Tunisia's production reaching 1.416 million tons that year (FAO 2024). Among the environmental stressors intensified by climate change (Yuan et al. 2024), salinity is a major threat to crop production, affecting approximately 7% of the earth's land surface (Chele et al. 2021), with around 50% of arable land projected to become salt-affected (Kumar and Sharma ,2020). Salinity has been shown to impair the physiological and biochemical processes of plants (Atta et al., 2023). In many crops, salt stress results in a decrease in the biomass of the shoots and roots as well as in the length, volume, and diameter of the stem and leaf area in maize Vennam et al. 2024). Salt stress reduces seed germination rates, inhibits photosynthesis, causes ionic toxicity, limits water absorption, causes chlorosis, and causes senescence, all of which lead to oxidative stress, decreased growth, and yield losses (Zhou et al. 2024). According to Nahar et al. (2023), the breakdown of chloroplast ultrastructure is triggered by Na^+ toxicity or salt-induced oxidative damage. Photosynthetic activity is limited by either a decrease in the synthesis of chlorophyll molecules or an increase in the breakdown of these molecules under salt stress.

Several studies have reported that increased formation of reactive oxygen species (ROS) such as singlet oxygen, superoxide, hydrogen peroxide, and hydroxyl radicals is frequently observed in plants grown under salt stress (Kesawat et al. 2023), accelerating lipid peroxidation and disrupting membrane integrity, which leads to electrolyte leakage, pigment, protein, and enzyme degradation, inhibited nutrient

uptake, and eventually cell death (Khan et al. 2022), as reflected by elevated oxidative stress indicators (MDA and H_2O_2) that decrease membrane stability under salt stress ((Ellouzi et al. 2023; Sachdev et al. 2021). One such adaptive strategy is producing osmolytes such as proline, which help regulate osmotic potential, maintain cell turgidity, support cellular homeostasis, and protect protein function (Khademi Astaneh et al. 2022). They also serve as sources of nitrogen and carbon for post-stress recovery (Spormann et al. 2023) and scavenge ROS and free radicals to protect the photosynthetic apparatus and prevent protein denaturation (Hayat et al. 2012).

Phenolic compounds are well known for their potential antioxidant properties, which help to scavenge ROS under salt stress (Kumar et al. 2023). It has been discovered that the phenylpropanoid biosynthesis pathway is induced by salt stress, and this raises the production of many phenolic compounds (Sharma et al. 2019). Plants have a wide family of secondary metabolites called flavonoids, which are capable of efficiently removing too many reactive oxygen species (ROS) from plant cells. (Mohammed et al. 2023). In their study, Shomali et al. (2022) stated that Plants under stress build up flavonoids by overexpressing the genes that code for important enzymes involved in their manufacture.

Seed priming, a pre-sowing treatment that initiates germination before planting, increases seed performance and subsequently boosts overall plant development (Habibi et al. 2024). It also affects several photosynthesis-related factors critical to plant growth, including chlorophyll production, activation of carbon-fixing enzymes, carbon absorption, and stomatal conductance, thereby improving the overall photosynthetic rate (Hasanović et al. 2025), while also enhancing water-use efficiency and reducing the negative effects of abiotic stress on photosynthesis (Sneha et al. 2024). Seed-primed plants may better withstand stressful environments through altered biochemical and molecular pathways (Biswas et al., 2023).

One of the major players in reducing heat, salt, osmotic, and oxidative stressors is salicylic acid (SA), a phenolic plant hormone that controls growth, development, and several physiological responses (Song et al. 2023). Numerous studies have shown that physiological process adjustments are shown through seed priming with SA when plants are subjected to different environmental conditions. (Apon et al. 2023 ; Ben Youssef et al. 2025). Gunes et al. (2007) proved that through its ability to decrease the accumulation of Na^+ and Cl^- in Zea mays grown under salt stress conditions, SA can have antagonistic or cooperative interactions with the nutrition, growth, and development of plants in ideal and stressful environmental conditions. According to many studies, SA increased the plants resistance to abiotic stressors, particularly salt, via regulating antioxidant metabolism (Yang et al. 2023). In a recent study, Carrot seeds primed with SA showed increased levels of antioxidants and total phenolic contents (Mahmood-Ur-Rehman et al. 2020). In salt-stressed chickpea, seed priming with SA stopped nodule senescence, enhanced photosynthetic efficiency, and upregulated the machinery that quenches free radicals, restoring biomass and yield (Kaur et al. 2022). SA was found benefiting yield-related attributes of plants via modulating physiological and biochemical responses. According to reports, plants including Arabidopsis, rice, wheat, tomato, and cucumber may tolerate salt better when exogenous SA is applied as a foliar spray or as seed priming (El-Hawary et al. 2023; Miao et al. 2020; Shan et al. 2024).

Pseudomonas yamanorum is a recently described gram-negative rhizobacterial species isolated from tomato rhizosphere soils at the Regional Centre for Agricultural Research (CRRA) in Sidi Bouzid, Tunisia (GenBank accession number PQ555427). The strain has been characterized for multiple plant growth-promoting (PGP) traits, including the biosynthesis of indole-3-acetic acid (IAA) and the solubilization of inorganic phosphate, while atmospheric nitrogen fixation activity was not detected (Hajji-Hedfi et al. 2025a; Hajji-Hedfi et al. 2025b). These two traits are of direct mechanistic relevance to salt stress alleviation: bacterially produced IAA helps stimulate root cell elongation and lateral root initiation, partially compensating for the suppression of endogenous auxin signaling that occurs under salinity (Del Carmen Orozco-Mosqueda et al. 2019), while phosphate solubilization by *Pseudomonas* strains improves plant nutrition through the release of inorganic phosphorus from insoluble soil complexes, supporting macromolecular biosynthesis, photosynthesis, respiration, and energy transfer under stressed conditions (Pandey and Gupta. 2020). Indeed, comparable *Pseudomonas* strains sharing these traits, including IAA production and phosphate solubilization, have demonstrated significant improvements in root and shoot length, chlorophyll content, osmolyte accumulation, and antioxidant enzyme activities in tomato plants subjected to 100 mM NaCl (Pandey and Gupta. 2020), and *Pseudomonas sp. UW4* maintained root length, shoot biomass, and chlorophyll content at levels comparable to non-stressed control plants under 0.2 M NaCl through IAA supplementation and complementary metabolite production (Del Carmen Orozco-Mosqueda et al. 2019). To date, no study has specifically evaluated the salt stress-alleviating capacity of *P. yamanorum* as a priming agent in any crop system, making the present study the first to address this gap directly.

While seed priming with SA or with PGPR strains has been studied individually under salt stress, few studies have examined their combined application, and whether such combinations produce additive or synergistic effects remains largely unexplored. The present study aimed to investigate the effect of seed priming with SA (1 mM) and/or *Pseudomonas yamanorum* (PY), applied separately and in combination, on the growth, gas exchange, physiological, and biochemical responses of tomato (*Solanum lycopersicum* L., cv. Firenze) subjected to four levels of NaCl salinity (0, 50, 100, and 150 mM NaCl). We hypothesized that the combined SA×PY seed priming would produce a significantly greater alleviation of salt stress than either agent applied alone, through complementary mechanisms: SA acting via hormonal regulation and antioxidant defense priming, and PY acting through IAA-mediated root stimulation and phosphate-solubilization-dependent nutritional improvement. Multivariate analyses, including principal component analysis (PCA) and hierarchical cluster analysis (HCA), were additionally employed to characterize overall physiological response patterns and the differential efficacy of treatments across salinity levels.

2. Materials and methods

2.1. Experimental design and growth conditions

A greenhouse experiment was conducted at the Regional Center of Agricultural Research (CRRA), Sidi Bouzid, Tunisia (9°430 E, 35° 010 N) on the tomato Firenze variety seeds. The experiment was conducted

using a factorial design with two factors: The first factor, saline stress, includes four different levels of irrigation with varying concentrations of NaCl: C0, C1, C2, and C3 corresponding to 0, 50, 100, and 150 mM NaCl, respectively. The second factor concerns the treatment of seeds soaking with biostimulants. NB: seed not treated, SA: Seeds were soaked with salicylic acid for 24h, PY: seeds were soaked with bacterial solution containing *Pseudomonas yamanorum*, and SA×PY: seeds were soaked with SA for 24 followed by subsequent soaking with PY for another 24h.

The *Pseudomonas yamanorum* strain was cultured in sterile Luria-Bertani (LB) broth after incubation in a rotary shaker incubator (Optic Ivymen System, Spain) at 150 rpm and 28°C, and the bacterial concentration was adjusted to 10^8 CFUs/mL using a spectrophotometer at 600 nm. It was obtained from the Plant Protection and Biological Sciences Laboratory at the Regional Center for Agricultural Research (CRRA), Tunisia, under GenBank accession number (PQ555427) (Hajji-Hedfi et al., 2025a).

Pseudomonas yamanorum was assessed in vitro for several traits associated with plant growth-promoting potential, including indole-3-acetic acid (IAA) production, atmospheric nitrogen fixation, and phosphate solubilization (Hajji-Hedfi et al. 2025b)

After the seed priming as described above, they were transferred to Pots (upper diameter: 45 cm; depth: 66 cm) equipped with a drainage system consisting of gravel and an effluent collection tray. The growing medium consisted of a ternary mixture (1/3 each) of soil, quartz sand, and peat of the “DOMOFLOR MIX”, under natural light, temperature of 25°C-30°C ± 2°C, day /night, and relative humidity of about 70%.

The nutrient solution as mentioned by Hewitt, (1966): $Ca(NO_3)_2$ (1.25 mM), KNO_3 (1.25 mM), KH_2PO_4 (1.60 mM), $MgSO_4$ (0.50 mM), K_2SO_4 (1.50 mM), $CaSO_4$ (3.50 mM), H_3BO_3 (4 µM), $MnSO_4$ (4 µM), $ZnSO_4$ (1 µM), $CuSO_4$ (1 µM), $CoCl_2$ (0.12 µM), $(Na)_6(Mo)_7O_{24}$ (0.12 µM) was used for irrigation, until the plants reached the 4- to 5-leaf stage, 30 days after sowing, at which point salt stress was applied by adding the necessary amount of NaCl to reach the required concentration.

At the end of the experiment (plants at the age of 75 days), the plants were collected to perform necessary measurments

2.2. Growth parameters

Stem diameter (SD), leaf area (LA), and root length (RL) were measured manually using a Digital Vernier Caliper (T304B.W-1215, France), a Laser area meter (CI-203, USA), and a regular measuring ruler, respectively.

The harvested plants were washed with distilled water and dried in an oven for 3 days at 70°C to determine the plant dry weight (PDW) (Hajlaoui et al. 2023).

2.3. Net photosynthesis, evapotranspiration, and leaf water potential

A portable photosynthesis system (CI-340-USA) was employed to measure gas exchange parameters. This device measures net photosynthetic activity (Pn) and evapotranspiration (Et). Measurements were taken on the same plants used for photosynthetic pigment analysis, 24 hours before the final harvest. Photosynthesis was induced using saturating light (1000 µmol m^{-2} s^{-1}), which was applied using the standard 6-cm^2 clamp on the leaf chamber. The sample pCO_2, flow rate, and temperature were maintained at 362 mbar, 500 µmol s^{-1}, and 25°C, respectively.

Leaf water potential (Ψw) was measured following the method described by Hajlaoui et al. (2023). This measurement was taken on the upper, fully expanded leaves, two hours after sunrise, using a Model 600 Pressure Chamber Instrument manufactured by PMS Instrument Company (Germany).

2.4. Biochemical parameters

Chlorophyll a and b were analyzed using the method described by Dere et al. (1998). One gram of fresh leaves was homogenized for one minute with 50 ml of pure acetone and then centrifuged at 2500 rpm for 10 minutes. The supernatant was collected to measure absorbance (A) at two wavelengths (646 and 662 nm) with acetone as the blank. The pigment concentrations were calculated and expressed as µg g^{-1} fresh weight.

$Chla = 11.75A_{662} - 2.35A_{645}$

$Chlb = 18.61A_{645} - 3.96A_{662}$

For the quantification of phenols (Phl), Flavonoids (Flv), and proline (Pro), 200 mg of fresh leaf material was ground in liquid nitrogen and suspended in 5 mL of 95% ethanol. The samples were then incubated overnight in the dark at 4°C and centrifuged at 8000× g for 15 minutes. The supernatants were collected and stored at − 20°C until analysis.

Total polyphenols were quantified using the method described by Santangeli et al. (2019). In this process, 50 µL of each sample and standard solution was mixed with 0.475 mL of 0.25 N Folin-Ciocalteu reagent and incubated in the dark for 2 minutes. Next, 0.475 mL of 1 M sodium carbonate was added. After incubating in the dark at room temperature for one hour, absorbance was measured at 724 nm using a spectrophotometer. Phenolic compound levels were calculated from a chlorogenic acid standard curve with concentrations of 10, 20, 40, and 50 µg/mL. Results were expressed in micrograms of chlorogenic acid equivalents per gram of fresh weight (µg CAE/g FW).

Flavonoids were estimated following the method mentioned by Borromeo et al. (2023) with minor modifications. Each standard solution and sample (0.5 mL) was separately mixed with 1.5 mL of a reaction solution consisting of 95% ethanol, 0.1 mL of 10% $AlCl_3$, 0.1 mL of 1 M $C_2H_3KO_2$, and 2.8 mL of distilled water. After incubating at room temperature for 30 minutes, the absorbance was measured at 415 nm using a spectrophotometer. To determine flavonoid concentrations, a calibration curve was

constructed using quercetin at concentrations of 10 µg, 20, 40, and 80 µg mL^{-1}. Flavonoid content was expressed as µg quercetin equivalent per gram of fresh weight (µg quercetin equivalent · g $f.w.^{-1}$).

The determination of proline content was carried out using the method outlined by Stassinos et al. (2021). 500 µL of the extract was added to 1 mL of a reaction mixture containing 1% (w/v) ninhydrin dissolved in a blend of 60% (v/v) acetic acid and 20% (v/v) ethanol. The samples were shielded from light and heated at 95°C for 20 minutes. Proline concentration was assessed by measuring the absorbance at 520 nm with a spectrophotometer. A calibration curve was created using L-Proline standard solutions at concentrations of 5, 10, 15, and 20 µg mL^{-1}. The results were expressed as µg proline per gram of fresh weight (µg proline · g $f.w.^{-1}$).

2.5. Parameters of oxidative stress induced by salinity

The concentration of H_2O_2 was measured following the method of Hajlaoui et al. (2009). Fresh leaf samples (0.5 g) were homogenized in 5 ml of cold acetone and then centrifuged for 10 minutes at 1500 × g. The resulting supernatant was used for the H_2O_2 assay. After allowing the reaction to proceed for 10 minutes at 25°C, 0.1 mL of 20% TiCl4 and 0.2 mL of concentrated ammonia were added to 1 mL of the supernatant. The mixture was then centrifuged again for 10 minutes at 1500 × g. The absorbance was measured at 410 nm, and the H_2O_2 concentration was determined using a standard curve.

The lipid peroxidation rate was determined as equivalents of malondialdehyde using the method described by Hajlaoui et al. (2009), as fresh leaf samples (0.5 g) were ground in 5 ml of 0.1% (w/v) trichloroacetic acid (TCA) at 4°C. After centrifuging at 12,000 × g for 5 minutes, 1 mL of the supernatant was mixed with 4 ml of 0.5% (w/v) thiobarbituric acid (TBA) in 20% (w/v) TCA. The mixture was heated at 90°C for 30 minutes, then the reaction was stopped by placing the samples in an ice bath. Following centrifugation at 10,000 × g for 5 minutes, the absorbance of the supernatant was measured at 532 nm using a spectrophotometer (Model Jenway-6300-UK) and corrected for non-specific turbidity by subtracting the absorbance at 600 nm. The malondialdehyde (MDA) content was calculated using its absorption coefficient (ε) and expressed as nmol MDA per gram of fresh mass using the formula:

$$MDA\ (nmol\ g^{-1}\ FM) = ((A532 - A600) \times V \times 1000)/ \varepsilon \times W$$

where ε is the specific extinction coefficient (= 155 mM cm^{-1}), V is the volume of crushing medium, W is the fresh weight of the leaf, A600 is the absorbance at 600 nm wavelength, and A532 is the absorbance at 532 nm wavelength

The membrane stability index (MSI) of the cells was evaluated using the method described by Rehman et al. (2016). Samples of leaves and roots, each weighing 1g, were collected and cut into small pieces. The pieces were washed with distilled water to remove any electrolytes attached to their surface, then each sample was soaked in 18 mL of distilled water for 24 hours at 25°C. This soaking allows the cellular electrolytes to diffuse into the water. After this soaking period, the electrical conductivity of the water was measured using a conductive meter, denoted as EC_1. The samples were then autoclaved for

20 minutes at 120°C using an autoclave. After cooling to 25°C, the second measurement, representing the total conductivity, was taken as EC_2. The membrane stability index is determined using the following formula:

$MSI\% = (EC_1/EC_2) \times 100$

The salt stress tolerance index (SSTI) is determined based on the root length according to the formula used by Borromeo et al. (2023)

SSTI (%) = (Mean root length in stress/Mean root length in control) × 100

2.6. Statistical analysis

Statistical analyses were performed on the mean values of the replicates. The data were subjected to analysis of variance (ANOVA) using R software (version 4.4.2). Differences among treatments were assessed using Duncan's multiple range test at a 5% significance level ($P \leq 0.05$). Multivariate analyses, including principal component analysis (PCA), hierarchical cluster analysis (HCA), and Z-score–based heat-map visualization, were conducted using R (version 4.4.2) to explore relationships among parameters and treatments

3. Results

3.1. *Pseudomonas yamanorum* plant growth-promoting traits

Pseudomonas yamanorum demonstrated positive phosphate-solubilizing capacity and indole-3-acetic acid (IAA) biosynthesis in vitro, whereas atmospheric nitrogen-fixation activity was not observed. (Hajji-Hedfi et al. 2025b)

3.2. Growth parameters and leaf water potential

Salinity progressively constrained all four growth traits in a consistent, dose-dependent manner (Fig. 1.a–d). Relative to non-stressed plants (C0), stem diameter (SD), leaf area (LA), and plant dry weight (PDW) declined by 24%, 56%, and 50%, respectively, for C3 plants, while root length (RL) fell steadily from 33 cm at C0 to 13 cm at C3.

Seed priming with SA and/or PY consistently attenuated this decline across all four traits and at every salinity level, with the combined SA×PY seed priming treatment outperforming either agent alone in nearly every case. This advantage was very noticeable under C3, where SA×PY priming increased SD, LA, and PDW by 38%, 57%, and 48%, respectively, relative to non-primed (NB) plants, while RL reached 20 cm. SA×PY seed Priming also enhanced growth in the absence of salt stress, increasing SD by 33%, RL by 27%, LA by 53%, and PDW by 40% relative to NB plants.

Even applied individually, SA and PY each produced substantial improvements, with their strongest single-agent effects concentrated under C2 and C3 salinities. PY alone achieved its largest increase in RL at C2 (+ 45%) and in LA at C3 (+ 46%). SA alone, meanwhile, reached its peak efficacy for PDW at C3 (+ 35%).

3.3. Net photosynthesis, evapotranspiration, and leaf water potential

Salt stress impaired gas exchange, pigment content, and leaf water potential (Fig. 2.a–e). for C3 plants that showed the highest decrease rates, Net photosynthesis (Pn), evapotranspiration (Et), chlorophyll a (Chla), and chlorophyll b (Chlb) declined by 60%, 63%, 57%, and 50%, compared to C3 plants. Over the same gradient, leaf water potential (Ψw) dropped by134%.

Seed priming with SA and/or PY almost improved all parameters, with SA×PY again the most effective treatment, particularly under extreme salinity. At C3, SA×PY priming increased Pn by 208%, Et by 136%, Chla by 140% and Ψw by 31% relative to non-primed (NB) plants. Seed Priming also improved these parameters in the absence of salt stress: at C0, SA×PY increased both Pn and Et by 67%, Ψw by 28%, Chla by 101%, and Chlb by 143% relative to NB plants.

Applied individually, SA and PY each produced substantial increases, for Chlb in particular. The single agents matched or exceeded the combined treatment, a pattern distinct from the growth traits. Under C2 conditions, SA and PY alone increased Chlb by 301% and 147% compared to non-treated plants. Similarly, under C3 salinity, the increase rates attributed to SA and PY alone seed priming treatments were 83% and 115%, respectively.

3.4. Biochemical parameters

Salt stress increased the accumulation of the three secondary metabolites presented in Table 1, consistent with their role as antioxidants and osmoprotective compounds mobilized under abiotic stress. Phenolic content (Phl) rose approximately by 2, 5, and 11 fold at C1, C2, and C3, respectively, relative to non-stressed plants. Flavonoid content (Flv) followed a similar upward trend with salinity, though less steeply; it increased approximately two-fold at C3, while proline (Pro) remained largely unchanged between C0 and C1 before increasing by 21% and 41% at C2 and C3, respectively.

Table 1

Effect of seeds priming by salicylic acid (SA) and/or PGPR (*Pseudomonas yamanorum*) (PY), on Phenols, Flavonoids, and Proline content variations of tomato subjected to varying salt stress levels (C0:0 mM NaCl, C1:50 mM NaCl, C2:100 mM NaCl, C3:150 mM NaCl). Values are presented as mean ± SE (n = 5). For each parameter, lowercase letters compare seed priming treatments (NB, SA, PY, SA×PY) within the same salinity level, and uppercase letters compare salinity levels (C0, C1, C2, C3) within the same seed pretreatment; means sharing the same letter (lowercase or uppercase, respectively) are not significantly different according to Duncan's multiple range test at $P < 0.05$.

Treatments	Polyphenols, µg.gmf^{-1}	Flavonoids, µg.gmf^{-1}	Proline, µg.gmf^{-1}
C0 NB	119.4 ± 12.2 Db	97 ± 6 Dc	207.8 ± 19.1 Ca
C0 SA	186.2 ± 18.7 Cb	169.1 ± 1.9 Ca	140.1 ± 19.8 Bb
C0 PY	133.7 ± 21.9 Db	88.7 ± 7.4 Cd	103.8 ± 7.6 Dc
C0 SA×PY	429.8 ± 14.3 Da	116.7 ± 1.9 Db	125 ± 8 Db
C1 NB	234 ± 21.9 Cc	159.3 ± 6 Bc	209.3 ± 5 Cb
C1 SA	854.8 ± 93.2 Ba	256.6 ± 5 Aa	149.1 ± 10 Bc
C1 PY	682.9 ± 103.6 Cb	252.1 ± 5.8 Aa	239.2 ± 5 Ca
C1 SA×PY	864.4 ± 87.5 Ca	211.6 ± 1.1 Bb	256.3 ± 7 Ca
C2 NB	611.3 ± 21.3 Bc	140.7 ± 4.6 Cd	252.4 ± 1.1 Bc
C2 SA	883.5 ± 64.6 Bb	215.8 ± 2.2 Bb	299.7 ± 2.8 Ab
C2 PY	816.6 ± 28.7 Bb	250.1 ± 2.2 Aa	279.4 ± 6.3 Bb
C2 SA×PY	1103.2 ± 114.6 Ba	183.6 ± 0.5 Cc	322.8 ± 5.3 Ba
C3 NB	1389.7 ± 28.7 Ac	180.4 ± 1.2 Ad	292.3 ± 5.5 Ac
C3 SA	1580.7 ± 8.3 Ab	219.2 ± 0.1 Bb	298 ± 1.5 Ac
C3 PY	1647.6 ± 37.9 Aab	200 ± 0.7 Bc	322.1 ± 4.4 Ab
C3 SA×PY	1728.7 ± 29.8 Aa	330.5 ± 4.2 Aa	468.8 ± 37.2 Aa

Seed priming further amplified this stress response for Phl and Flv in most conditions, with SA×PY treatment generally producing the largest increases. At C1, SA×PY increased Phl by 269%, only narrowly exceeding SA alone (265%), under C2 salinity, PY alone produced the largest increase in Flv (78%), exceeding SA×PY (31%). And for plant cultivated under C3 condition, SA×PY regained its lead for both Phl (+ 24%) and Flv (+ 83%).

Proline responded less predictably to priming than either Phl or Flv under C0 and C1 conditions. SA priming reduced Pro content relative to NB plants (by 33% and 29%, respectively), while PY and SA×PY instead increased it modestly at C1 (+ 14% and + 22%). The change of the growth medium to C2 and C3

conditions reversed this pattern: SA, PY, and SA×PY all increased Pro relative to NB plants at C2 (19%, 11%, and 28%), and PY and SA×PY continued to boost Pro at C3 (+ 10% and + 60%), while SA had no significant effect.

3.5. Parameters of oxidative stress induced by salinity

Salinity induced progressive oxidative damage and altered membrane stability and tolerance index (Table 2). Hydrogen peroxide (H_2O_2) rose by 103%, 220%, and 331% at C1, C2, and C3, respectively, relative to C0, accompanied by a parallel rise in malondialdehyde (MDA) of 14%, 64%, and 109%, indicating escalating lipid peroxidation alongside reactive oxygen species accumulation. This oxidative burden was mirrored by a steady decline in membrane stability (MSI), which fell from 80.2% at C0 to 47.7% at C3, and in the salt stress tolerance index (SSTI), which dropped from 100% to 38% at C3.

Table 2
Effect of seeds priming by salicylic acid (SA) and/or PGPR (*Pseudomonas yamanorum*) (PY), on H_2O_2, MDA, SSTI, and MSI of tomato subjected to varying salt stress levels (C0:0 mM NaCl, C1:50 mM NaCl, C2:100 mM NaCl, C3:150 mM NaCl).

Traitements	H_2O_2, µg.gmf^{-1}	MDA,mmol.gmf^{-1}	SSTI (%)	MSI (%)
C0 NB	29.4 ± 1.1 Da	4.7 ± 0.7 Ca	100 ± 0.00 Ab	80.22 ± 3.9 Ab
C0 SA	25.8 ± 0.7 Dab	2.8 ± 0.4 Cb	118.3 ± 8.6 Aa	89.1 ± 4.3 Aa
C0 PY	25.2 ± 0.6 Dab	3.2 ± 0.2 Cb	115.2 ± 5.7 Aa	87.5 ± 2.7 Aa
C0 SA×PY	24 ± 1 Db	2.4 ± 0.2 Cb	126.2 ± 7.4 Aa	90 ± 4.6 Aa
C1 NB	59.6 ± 4.2 Ca	5.4 ± 0.4 Ca	75.3 ± 8.4 Bc	73.3 ± 0.8 Bb
C1 SA	47.1 ± 1.4 Cb	4.1 ± 0.7 Bb	91.7 ± 8 Bb	77.4 ± 3.4 Bb
C1 PY	47.2 ± 1.8 Cb	3.7 ± 0.4 Cb	93.2 ± 7.2 Bb	83.1 ± 2.6 Aa
C1 SA×PY	30.1 ± 1.5 Cc	3.2 ± 0.6 Cb	108 ± 4.8 Ba	77.8 ± 0.8 Bb
C2 NB	94.1 ± 3.3 Ba	7.7 ± 0.6 Ba	52.9 ± 2.6 Cc	60.6 ± 2.5 Cb
C2 SA	80.8 ± 1.3 Bb	5.6 ± 0.7 Ab	75.2 ± 8.4 Cb	67.3 ± 1.2 Ca
C2 PY	79.2 ± 3.4 Bb	5.4 ± 0.4 Bb	77.3 ± 11.1 Cb	67.6 ± 2.8 Ba
C2 SA×PY	59.2 ± 2.8 Bc	4.5 ± 0.6 Bb	92.2 ± 5.8 Ca	69.8 ± 3.5 Ca
C3 NB	126.5 ± 3.6 Aa	9.9 ± 1 Aa	38 ± 4.1 Dc	47.7 ± 0.2 Dc
C3 SA	98.9 ± 2.5 Ab	6.2 ± 1 Ac	48.1 ± 5.9 Dab	60.9 ± 0.9 Db
C3 PY	102 ± 3.8 Ab	7.1 ± 0.2 ABc	52 ± 2.3 Db	59.8 ± 1 Cb
C3 SA×PY	73.8 ± 4.3 Ac	8 ± 0.4 Ab	60.2 ± 5.7 Da	65.8 ± 0.7 Ca

Values are presented as mean ± SE (n = 5). For each parameter, lowercase letters compare seed priming treatments (NB, SA, PY, SA×PY) within the same salinity level, and uppercase letters compare salinity levels (C0, C1, C2, C3) within the same seed pretreatment; means sharing the same letter (lowercase or uppercase, respectively) are not significantly different according to Duncan's multiple range test at $P < 0.05$.

Seed priming alleviated this oxidative damage and improved stability across nearly all conditions, with SA×PY being approximately the most consistently effective treatment. At C3, SA×PY reduced H_2O_2 by 42% and MDA by 20%, while raising MSI to 65.8% and SSTI by 22% relative to non-primed (NB) plants. This pattern held broadly across C1 and C2 as well, where SA×PY produced the largest reductions in

H_2O_2 (49% and 37%) and the largest gains in SSTI (33% and 40%). The pattern of SA×PY seed Priming superiority was also seen under non-saline conditions. At C0, SA×PY reduced H_2O_2 by 18% and MDA by 50%, and raised MSI to 90% and SSTI by 26.2%, compared to NB plants.

There were two exceptions to this pattern. For MDA, seed priming by SA alone induced a reduction for this oxidative stress marker by 37% in the case of C3 plants. For MSI, PY alone was best at C1, raising membrane stability to 83.1%.

3.6. Interrelations between parameters and treatments.

Principal component analysis (PCA) and Pearson correlation were conducted to determine relationships among the 16 parameters and 16 treatments done in this study. The PCA biplot (Fig. 3) captured 88.7% of the total variance, with PC1(77.6%) and PC2 (11.1%). Based on PC1, stress markers, and. Eleven parameters, Pn, Chla, Chlb, Et, LA, PDW, Ψw, MSI, SD, RL, and SSTI, exhibited high positive loadings (0.76–0.99) on PC1 (right side of the biplot), indicating strong positive intercorrelations, which is supported by the Pearson correlation heat map, r ranging from 0.67 (Pn-Chlb) to 0.99 (RL-SSTI), as corroborated by the Pearson correlation heatmap. On the other hand, the remaining parameters (H_2O_2, MDA, Phl, Flv, and Pro) exhibited negative loadings (-0.40 to -0.96) on PC1 (left side of the plot), indicating their positive associations. According to the correlation heat map, the Pearson coefficient (Fig. 4) of these parameters ranged from 0.27 (Flv-MDA) to 0.89 (MDA-H_2O_2). The PC1 highlighted also that the right-sided and the left-sided parameters have inverse behavior to each other, r between the two groups varied from − 0.20 (Flv-Chlb) to -0.95 (RL-H_2O_2). This dominant axis, PC1, delineated a clear trade-off between two plant patterns. The first pattern displayed by the parameters located at the right side collectively represented good growth indicators via photosynthetic efficiency, water relations, and biomass accumulation, and the second pattern is presented by the parameters located at the left side that defined via oxidative stress (ROS accumulation and lipid peroxidation) and adaptive responses (antioxidant and osmoprotectant synthesis).

PC2, which captured only 11.1% of the total variance, revealed another pattern with positive loadings for Flv, Phl, Pro, Pn, Chla, and Chlb (0.29–0.76). This suggested a positive influence of antioxidants on photosynthetic efficiency under certain conditions, despite overall negative correlations between these parameters, r ranged from − 0.05 (Pn-Flv) to -0.52 (Chla-Pro),

In addition to partitioning the parameters, PC1 captured most of the variation among treatments, reflecting a clear gradient of salt stress severity modulated by biostimulant application. The treatments, C0:NB, C0:PY, C0:SA, and C0:SA×PY had high positive scores to PC1 (1.8, 4.3, 4.4, 6.5), associated with the right-sided parameters of the PCA plot and revealing optimal growth conditions in the absence of salt stress, and the use of SA, PY, and SA×PY further supported this optimality. At Moderate salinity, the treatment C1:NB shifted leftward (-0.6), but biostimulants restored positive scores (C1:PY, 1.6; C1:SA, 1.1; C1:SA×PY, 3), indicating substantial mitigation of salinity by the biostimulants, with the most pronounced effect attributed to SA×PY. In Severe salinity conditions, the C2:NB score was negative, referring to PC1 (-3.4). This score became less negative under biostimulants (C2:PY, -1.2; C2:SA, -1.3;

C2:SA×PY, 0.4), indicating a partial mitigation of salinity, despite the predominance of stress symptoms. The treatments with Extreme salinity showed the most exacerbated negative scores to PC1 (C3:NB, -6.7; C3:PY, -3.5; C3:SA, -3.5; C3:SA×PY, -3). This indicated the worst growth conditions marked with a high reduction of the right-sided parameters at the expense of the stress marks and antioxidants located at the left sideas a response to the extreme salinity. Despite the further accumulation of antioxidants by biostimulants (Phl, Flv, and Pro), it was not enough to fully counteract the extreme salinity. Overall, PC1 scores declined with salinity intensity but increased with biostimulant effect, emphasizing SA×PY treatment superiority in shifting the profiles of the plants good development.

Based on the inverse relationship between salt stress indicators and growth parameters identified by PCA and Pearson correlation, a hierarchical cluster analysis (HCA), coupled with a Z-score heatmap (Fig. 5), provided a Complementary detailed view of treatment-specific physiological responses and parameters co-regulation. The Z-score hierarchical clustering heat map revealed three principal clusters Consistent with the outputs of the PCA analysis.

Cluster 1 (the red Rectangle): This cluster included the following treatments (C3:SA×PY, C3:NB, C2:NB, C3:PY, C3:SA), forming a sub-dendrogram with short intrabranch distances, indicative of similar physiological impact. For this first cluster, the Z-score patterns revealed pronounced suppression of growth and photosynthetic parameters (ΨW, SD, LA, Et, MSI, PDW, RL, SSTI, Chlb, Pn, Chla), corresponding to G1 (they are the same parameters located at the right side of the PCA plot discussed above), Appearing as blue blocks in the left columns with Z varied from − 1 to -2, particularly for C3:NB and C2:NB. Concurrently the cluster 1 revealed that oxidative stress markers and antioxidant metabolites corresponding to G2 parameters (H_2O_2, MDA, Phl, Flv, and Pro) (the same parameters located at the left side of the PCA plot) expressed high Z-scores, varying from + 1 to + 2, as red boxes. This reflected ROS accumulation, lipid peroxidation, and osmoprotectant synthesis as an adaptation to ionic toxicity and osmotic imbalance under salinity. The treatments (C3:NB, C2:NB), where extreme and severe salt stress was applied without biotimulants, exhibited the most extreme divergences, consistent with unmitigated salt stress damage. For the other treatments of cluster 1 (C3:SA×PY, C3:PY, C3:SA), corresponding to extreme salt stress and biostimulation application, the corresponding Z-scores were slightly higher in G1 parameters (Pn and Chla shifts toward 0 for C3:SA×PY) and amplified in G2 parameters (Pro and Flv boxes shifted toward dark red), indicating partial activation of antioxidant pathways. However, this was insufficient for full mitigation at extreme salinity, since the Z scores of the majority of G1 parameters were still negative.

Cluster 2 (the Blue Rectangle): This cluster included the following treatments (C0:SA×PY, C0:PY, C0:SA), corresponding to no stress condition with biostimulants treatment. This cluster showed the inverse pattern of cluster 1. The G1 parameters Z -scores were the highest (from 1 to 2), especially in C0:SA×PY. Conversely, the G2 parameters Z-scores were very low (from − 1 to 2). This cluster is representative of the optimal growth condition; this performance is attributable to biostimulant use under no salt stress conditions. This cluster corresponds to the extreme right side of the PCA biplot, where these treatments align closely with the G1 parameters loadings. In fact, under these circumstances, the beneficial effect of

biostimulants was fully directed towards the boost of the G1 parameters since no oxidative burden appeared under these conditions.

Cluster 3 (the Green Rectangle): This cluster covered the remaining treatments (C0:NB, C1:NB, C1:SA, C1:PY, C2:SA, C2:PY, C1:SA×PY, C2:SA×PY), characterized by intermediate dendrogram branching with Z-score transitions from non-stress to moderate stress conditions with varying ameliorations by the biostimulants. Passing from C0:NB to C1:NB, a minor blue shift appeared in G1 parameters (Z-scroes from 0.5 to -0.5 approximately) due to moderate salt stress. Biostimulants demonstrated differential efficacy depending on their combined or separate use. The treatments C1:SA×PY and C2:SA×PY, when biostimulants were used in combination for both moderate and severe salinity, showed the most pronounced alleviation effects, with redder color in G1 parameters (Z-scores from 0.5 to 1.5) and less bluer in G2 parameters (Z-score from − 1 to 0), indicating the success of the antioxidant system in alleviating salinity to certain extent via ROS scavenging and osmotic adjustment. In treatments where biostimulants were applied separately (C1:SA, C1:PY, C2:SA, and C2:PY), a moderate improvement in G1 parameters was observed compared to C1:NB and C2:NB (especially Pn and MSI, Z-score from 0 to + 1). This amelioration was less significant than the treatments with the combined application of biostimulants. This cluster is aligned with the central part of the PCA plot, where treatments shift rightward with biostimulant efficacy and tend to increase G1 parameters.

In summary, the Z-score hierarchical clustering heat map elucidated a salinity stress gradient modulated by biostimulants, with clusters outlining severe impairment with the worst growth conditions (Cluster 1), optimal performance (Cluster 2), and progressive mitigation depending on biostimulant use (Cluster 3). These findings were validated by the PCA outputs, suggesting SA×PY as the most efficacious intervention for salt stress alleviation.

4. Discussion

Tomatoes are one of the most important pillars of agriculture in Tunisia, both in terms of production and export, and are cultivated in regions characterized by arid and semi-arid conditions with limited precipitation and high evaporation rates, which increase the salinity of water and soil. This condition will be further aggravated by climate change. Under those circumstances, finding a solution to cope with salt stress is a necessity. This study demonstrated the effect of the exogenous application of SA and PGPR (*Pseudomonas yamanorum*) either separately or combined on plant growth parameters, leaf gas exchange, and biochemical attributes.

Our results demonstrated that salt stress significantly reduced plant dry weight, stem diameter, root length, and leaf area, especially in plants grown under C3 conditions. Similar results were found by Ors et al. (2021) for tomato, who reported that Plants grown under saline conditions experienced water loss in the cell, degradation of the plasma membrane, and release of hydrolytic enzymes, which in turn led to the degradation of cytoplasm and an overall slowdown in growth and reduction in turgor, our findings were also consistent Ahmed et al. (2013) for barley. Ali et al. (2021) further attributed the decline in

morphological attributes and biomass accumulation in tomato subjected to 150 mM NaCl to the cumulative effect on the key metabolic and assimilatory attributes like uptake, transport, and assimilation of mineral elements. The high electrical conductivity (EC) of the soil or the irrigation water may restrict water availability to the plants as it increases soil osmotic potential, resulting in reduced water uptake and partial dehydration of the cell cytoplasm, ending up with a slowdown or even cessation of growth (Munns and Tester2008). The root is the most sensitive portion of the plant to salinity stress (Arif et al. 2019). Salt stress-mediated growth reduction may be due to the high energy cost of NaCl detoxification mechanisms (Faizan et al. 2021).

Regarding stem diameter specifically, Parkash and Singh(2020), Nassar et al. (2020), and Castillo-Campohermoso et al. (2020) attributed this reduction to osmotic stress-induced inhibition of cell expansion rates. Regarding leaf area, Tanveer et al. (2020), Ullah et al. (2020), and Miceli et al. (2020) noted a significant decrease in leaf area of tomato and sweet peppers subjected to varying NaCl concentrations, reaching 150 mM NaCl, which is in parallel to our results. They attributed the reduction of leaf area under saline conditions to retarded cell division and expansion and an impairment of nitrogen metabolism, reducing photosynthetic capacity as shown by Kashem et al. 2000.

Increasing salinity also reduced leaf water potential, consistent with Xue et al. (2021) and Metoui Ben Mahmoud et al. (2020) for barley and tomato, respectively, subjected to 100 mM and 150 mM NaCl. Salinity, primarily through the accumulation of Na^+, disrupts both soil water potential and leaf water potential (Zörb et al. 2019). The presence of Na^+ in the soil solution alters osmotic potential and reduces water availability for uptake (Arif et al. 2020), hampering hydration (Naorem et al. 2023) and further lowering leaf water potential as Na^+ accumulates in plant tissues, ultimately reducing turgor pressure and impairing water uptake and the physiological processes essential for growth (Methenni et al. 2018)

The reduction in overall growth parameters is preceded by a reduction in net photosynthesis. Our results showed that salt stress greatly reduced net photosynthesis (Pn) and evapotranspiration (Et), consistent with Souana et al. (2020) for *Vicia faba* and Win et al. (2018) for tomato. Reductions in transpiration rates reflect decreases in stomatal conductivity occurring due to stomatal closure for maintenance of cell turgor pressure under osmotic stress (Arif et al. 2020), which is associated with decreases in photosynthetic rates and CO_2 assimilation (Dourado et al. 2022). The reduced supply of CO_2 into leaves primarily governs Pn under salinity stress conditions (Zhao and Chen.2025), and marked reductions in total leaf area in stressed plants further limit whole-plant photosynthesis, contributing to low biomass production. Diminution in photosynthesis may also be attributable to non-stomatal causes, including perturbations in pigment content, this finding was consistent with the results of Ali et al. (2022) in maize and Jha and Subramanian (2012) in paddy. The decrease in chlorophyll content for plants subjected to salinity has been a typical symptom of oxidative stress and may be the result of pigment photo-oxidation, chlorophyll degradation, or lack of chlorophyll synthesis (Lucas et al., 2023). Also, Jumpa et al. (2022) found that under salinity stress, chlorophyllase breaks down chlorophyll and other enzymes, which lowers photosynthetic pigment synthesis.

The determination of H_2O_2 and MDA provides a useful indicator of oxidative damage induced by salinity. Our results demonstrated that increasing salinity significantly elevated both molecules. Various authors reported similar increases in H_2O_2 and MDA contents due to salinity, reflecting the extent of membrane damage, including Farhadi and Ghassemi-Golezani. (2020) in *Mentha pulegium*, Hajlaoui et al. (2026) in tomato, and Ma et al. (2017) in *Dianthus superbus*. Salt stress induces osmotic disturbance that reduces vacuole and cytoplasm volume, leading to cellular dehydration, while higher salt concentrations induce oxidative stress via the generation of ROS, including superoxide anions, hydrogen peroxide, and hydroxyl radicals (Chen et al., 2019). Reactive oxygen species attack linolenic and linoleic polyunsaturated fatty acids, producing lipid hydroperoxides that reduce membrane permeability (Ahmad et al., 2018). The increase in H_2O_2 is consequently the main cause of decreased membrane stability index (MSI), as reported by Souana et al. (2020) for *Vicia faba* and Jha and Subramanian. (2012) for paddy, and by Guo et al. (2019), who attributed the increase in electrolyte leakage to decreases in phospholipids and sterols in response to salinity.

One of the strategies used by the plants to maintain their water status is the production of proline. Our findings demonstrated that the increase in salinity is accompanied by an increase in this osmoprotectant content. Similar findings were demonstrated by (Kumar et al. 2022) for *Capsicum annuum* subjected to salinities varying from 25 to 150 mM NaCl, which maintains water retention in the cell, protects the plant under salinity stress, and plays a role in stress tolerance. Proline accumulation is a crucial part of the physiological adaptation in many plant species to stress (Hosseinifard et al. 2022). Besides its osmoprotectant role, proline acts as a scavenger of OH, $^1O_2$. It also inhibits lipid peroxidation, chelates metals, stabilizes proteins, supports protein synthesis, and protects against redox cycling, which is most important in antioxidant defense in plants under biotic and abiotic stress (Sachdev et al. 2021; Zulfiqar and Ashraf, 2023).

To counter the adverse effects of salinity, a differential accumulation of specific secondary metabolites occurs in plants. Phenolic compounds are well known for their potential antioxidant properties, which help to scavenge ROS under salt stress. Our result mentioned a significant increase in polyphenols under salinity. This is in line with the results of Hossain et al. (2022) and Kiani et al. (2021) in the case of *Amaranthus lividus* and wheat subjected to salt stress. Various reports mentioned that Salt stress induces the phenylpropanoid biosynthetic pathway, which consequently elevates various kinds of phenolic compound biosynthesis (Al-Ghamdi and Elansary. 2018; Guo et al. 2017). Also, in tobacco, an upregulation of the flavone synthase gene, responsible for the production of flavonoids, was seen in tobacco grown under salinity, which indicates that the flavonoids may play a crucial role in coping with salinity stress (M. Wang et al., 2021).

Seed soaking with SA, *P. yamanorum*, or their combination increased all parameters related to plant growth, leaf gas exchange, and biochemical attributes.

Similarly to our results, Miao et al. (2020) found that relative growth rate and whole-plant dry weight increased significantly in 0.5 mM SA-treated cucumber plants subjected to 50 mM NaCl compared with

non-treated plants, attributing this to SA-enhanced water and nutrient absorption through accelerated root growth and increased carbohydrate accumulation via promoted leaf photosynthesis. Various reports found that SA enhanced almost all growth parameters, including plant height, leaf area, and number of leaves. These effects may be connected to SA's alleviation of salinity's harmful effects by inducing growth hormones such as IAA and cytokinins, which in turn reduce the uptake of toxic ions and stimulate cell division, while also maintaining cell membrane integrity, thereby improving cell division and overall plant growth (Mady et al.2023; Sousa et al.2021; Da Silva et al. 2020). Additionally, Tang et al. (2012) demonstrated that SA significantly regulates transcriptional levels of root system architecture (RSA) genes, with NAC transcription factor family members involved in root development and abiotic stress responses, leading to better nutrient availability.

Our results demonstrated that *P. yamanorum* improves plant growth parameters under both stress and non-stress conditions, consistent with Omer et al. (2022), who showed that inoculation with A. *brasilense*, P. *geniculata*, and their co-inoculation significantly promoted flax growth characteristics (shoot and root lengths, fresh and dry weights, and leaf number) as a means of mitigating salinity's negative effects. Abdel Latef et al. (2020) reported that the growth-parameter increases observed in PGPR-treated plants reflect the bacteria's ability to produce IAA, which stimulates cell elongation. Ali et al. (2023) further demonstrated that PGPR promotes plant growth through enhanced nutrient assimilation, biological nitrogen fixation, phosphorus solubilization, and iron acquisition, while Ali et al. (2022) reported that bacterially produced IAA directly promotes root growth by inducing cell elongation and/or regulating cell division, adapting root structure to stress conditions.

The plant growth-promoting effects of *P. yamanorum* observed here are mechanistically grounded in two confirmed in vitro traits of this strain: IAA biosynthesis and inorganic phosphate solubilization (Hajji-Hedfi et al. 2025b). IAA is the principal native auxin regulating root architecture, and its bacterial supply via seeds priming may partially compensate for the disruption of endogenous auxin signaling under salt stress, in which ROS-mediated degradation of tryptophan-derived auxin precursors and suppression of auxin-responsive gene expression collectively impair root system development (Spaepen and Vanderleyden, 2011). Del Carmen Orozco-Mosqueda et al. (2019) demonstrated that *Pseudomonas* sp. UW4, with IAA-producing capacity, stimulates root growth and partially reverses salt stress-induced growth inhibition in shoot and root while improving chlorophyll content, establishing a mechanistic precedent for the root length and biomass improvements observed here under *P. yamanorum* biopriming. Similarly, Pandey and Gupta (2020) reported that a multi-trait *Pseudomonas* sp. strain S3, producing IAA and solubilizing phosphate, significantly improved root length, shoot length, shoot biomass, chlorophyll content, proline accumulation, and antioxidant enzyme activities in tomato under 100 mM NaCl, closely paralleling our own findings and supporting IAA-mediated root stimulation as a primary mechanism of *P. yamanorum* driven salt stress alleviation in tomato.

As phosphorus is an obligate constituent of ATP, NADPH, membrane phospholipids, and nucleic acids, its enhanced availability under *P. yamanorum* biopriming may support the macromolecular biosynthesis, photosynthesis, and energy transfer processes needed to sustain growth under salt stress, a mechanism

explicitly attributed by Pandey and Gupta (2020) to the phosphate- and nitrogen-solubilization capacity of Pseudomonas strains. Taken together, the mechanistic evidence from these analogous Pseudomonas studies provides a strong biological rationale for the growth-promoting effects of *P. yamanorum* observed here, operating through IAA-mediated restoration of root architecture and phosphate-solubilization-dependent nutritional improvement as two complementary, partially independent pathways.

Regarding gas exchange and photosynthetic pigments, our findings are consistent with Lotfı et al. (2020) and Souana et al. (2020), who demonstrated the positive effect of SA on leaf gas exchange in mung bean and *Vicia faba* under salt stress. They reported that SA restricted ethylene formation to its optimal range by inhibiting ACC synthase activity, reducing ethylene-induced inhibition of K^+ absorption and thereby regulating stomatal behavior, improving photosynthesis and transpiration rate, which may explain our results. In parallel with our findings, Lotfı et al. (2020) also found that Chla and Chlb increased with SA application in mung bean subjected to salinity up to 9 dS m^{-1}, attributable to SA's suppression of ROS production, protection of pigment–protein complexes, and regulation of enzymes in the chlorophyll biosynthesis pathway. Previous studies by Ahmad et al. (2018) and Talaat (2021) further reported that SA increases pigment content, improves transpiration and photosynthetic electron transport rates, sustains higher Rubisco and antioxidant enzymatic activities, and boosts PSII efficiency under salt stress.

Furthermore, Omer et al. (2022) found that PGPR such as *Azospirillum brasilense* and *Pseudomonas geniculata* enhanced chlorophyll content and photosynthetic activity in flax. Cassán et al. (2014) attributed chlorophyll increases in treated plants to the development of microbial metabolites such as auxin, gibberellin, and cytokinin, which facilitate photosynthetic material supply in plant cells. Bhat et al. (2020) noted that *Pseudomonas* strains under salt stress enhanced production of auxiliary photoprotective pigments such as zeaxanthin and β-carotene, which may protect Chla and Chlb from oxidation. These findings are corroborated by Pandey and Gupta (2020), who demonstrated that *Pseudomonas* sp. S3 improved total chlorophyll content in tomato under normal conditions and substantially restored chlorophyll levels reduced under 100 mM NaCl, confirming that *Pseudomonas* PGP traits directly support maintenance of the photosynthetic apparatus under ionic and osmotic stress. Costa-Gutierrez et al. (2020) similarly found that Pseudomonas strains such as P. putida improved the photosynthetic activity of soybean and corn under salt stress, attributing this to the bacteria's capacity to solubilize nitrogen and phosphorus required for macromolecular biosynthesis, photosynthesis, respiration, and energy transfer.

Plant secondary metabolite accumulation serves as an adaptive response to salinity stress. Phenolic compounds and ascorbic acid scavenge ROS-generated free radicals, supporting the antioxidant defense system (Osman et al., 2021), and can stabilize cell membranes by reducing membrane fluidity, limiting free-radical transport across membranes and reducing membrane peroxidation (Hussein et al. 2022). Our results demonstrated an increase in phenolic compound content following SA application, attributable to SA's stimulation of phenylalanine ammonia-lyase (PAL), the key enzyme of the

phenylpropanoid pathway responsible for biosynthesis of flavonoids, lignins, coumarins, and other phenolic compounds, as confirmed by Misra et al. (2014) for *Catharanthus roseus*, where a sharp increase in PAL activity was followed by increased soluble phenolics in SA-treated plants under salinity.

Our results also showed a significant increase in proline content, particularly for plants under C3 conditions treated with SA, consistent with Farhadi and Ghassemi-Golezani (2020) in *Mentha pulegium* and Misra and Saxena (2009) in lentil. This advantageous effect may be credited to SA's capacity to decrease proline oxidase activity, the enzyme responsible for proline degradation, while enhancing P5CR (pyrroline-5-carboxylate reductase) activity, which converts pyrroline-5-carboxylate to proline.

Our results are also in agreement with Omer et al. (2022) and Khan et al. (2023), who documented the role of PGPR in abolishing the deleterious effects of salinity stress through increases in phenolic compound and ascorbic acid content. Similarly, Pandey and Gupta (2020) reported that *Pseudomonas* sp. S3 inoculation of salt-stressed tomato plants (100 mM NaCl) increased proline accumulation compared with uninoculated stressed plants, confirming the role of Pseudomonas-based PGP inoculation in enhancing osmolyte production as an adaptive response to salinity.

Our results suggested that the increase in proline and phenolic compounds under the influence of SA and PGPR is associated with the reduction in H_2O_2 and MDA content relative to untreated stressed plants. SA preserves membrane integrity by improving osmotic adjustment and increasing ROS-scavenging activity (Nazar et al. 2015), inducing a pre-adaptive response to salt stress that promotes protective reactions and maintains membrane integrity, accordingly, improving plant growth, reducing MDA, and increasing MSI (Samea-Andabjadid et al. 2018). Our findings are also in line with reports documenting PGPR's, particularly Pseudomonas's, role in diminishing oxidative damage from salinity through decreased MDA and H_2O_2 content, which in turn increases MSI (Omer et al. 2022; Samaddar et al. 2019). Jha and Subramanian (2014) likewise found that PGPR treatment increased MSI under both normal and saline conditions. Since ROS causes oxidation of cellular lipids, nucleic acids, and proteins and can trigger caspase-like activity (Wang et al., 2010), the improvement in MSI observed with P. yamanorum treatment may be attributable, at least in part, to inhibition of this caspase-like activity.

The capacity of SA and PGPR to enhance the plant's antioxidant defense, via increased phenolic compounds and proline content, translates into an increase in the plant's salt tolerance index and MSI. This is consistent with Roshdy et al. (2021) and Khalil et al. (2022), who reported that SA application increased the salt tolerance index in strawberry and pomegranate subjected to 40 and 70 mM NaCl, respectively. Similar effects of PGPR on salinity tolerance have been reported by Safari et al. (2018) and Ali et al. (2022) attributed the increased salt tolerance of PGPR-inoculated plants to elevated antioxidant gene expression under PGPR influence.

5. Conclusion

The overall results demonstrated that increased salinity has a drastic effect on plant growth, gas exchange parameters, leaf water potential, and photosynthetic pigments. Higher salinity levels led to

increased H_2O_2 and MDA, resulting in a significant decrease in the membrane stability index (MSI) and the salt stress tolerance index (SSTI). However, seed priming with SA and/or PY proved effective in alleviating salt stress effects by boosting phenols and flavonoids, known for their high antioxidant activity, along with proline, which plays a role as an antioxidant and osmotic adjustment agent. This led to an increase in both MSI and SSTI.

The use of either SA or PGPR alone had a positive effect on overall plant behavior against salinity, though their relative efficacy compared to the combined treatment varied by parameter and salinity level. The best results were observed in plants whose seeds were primed with both SA and PY under most salt stress conditions and for most parameters measured, although single-agent priming occasionally matched or exceeded the combined treatment for specific traits. Based on our findings, we suggest employing both SA and PY as priming agents to ensure the best overall efficacy and performance of plants, particularly when salt stress becomes severe. These findings were obtained under controlled greenhouse conditions and at the vegetative growth stage, and they support the combination of SA and *P. yamanorum* seed priming as a promising approach for alleviating salinity stress in tomato; however, further experiments under open-field conditions, including assessment of fruit yield and quality, are needed to confirm the agronomic relevance of this approach for sustainable tomato production in semi-arid regions.

Declarations

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Conflict of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Author Contributions

All authors: Conceptualization; **Fedi Hajlaoui, Hichem Hajlaoui, and Lobna Hajji-Hedfi**: Methodology and material preparation**; Hichem Hajlaoui**: Project administration and Funding acquisition; **Fedi Hajlaoui:** Writing original draft, Investigation, Formal Analysis, Software, Data Curation, Visualization; **Hichem Hajlaoui and Abdelmajid Krouma**: Validation and Supervision; **Hichem Hajlaoui and Lobna Hajji-Hedfi:** Resources; **Hichem Hajlaoui, Abdelmajid Krouma, and Lobna Hajji-Hedfi**: Writing Review & Editing; **all authors** approved the final version.

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Figures

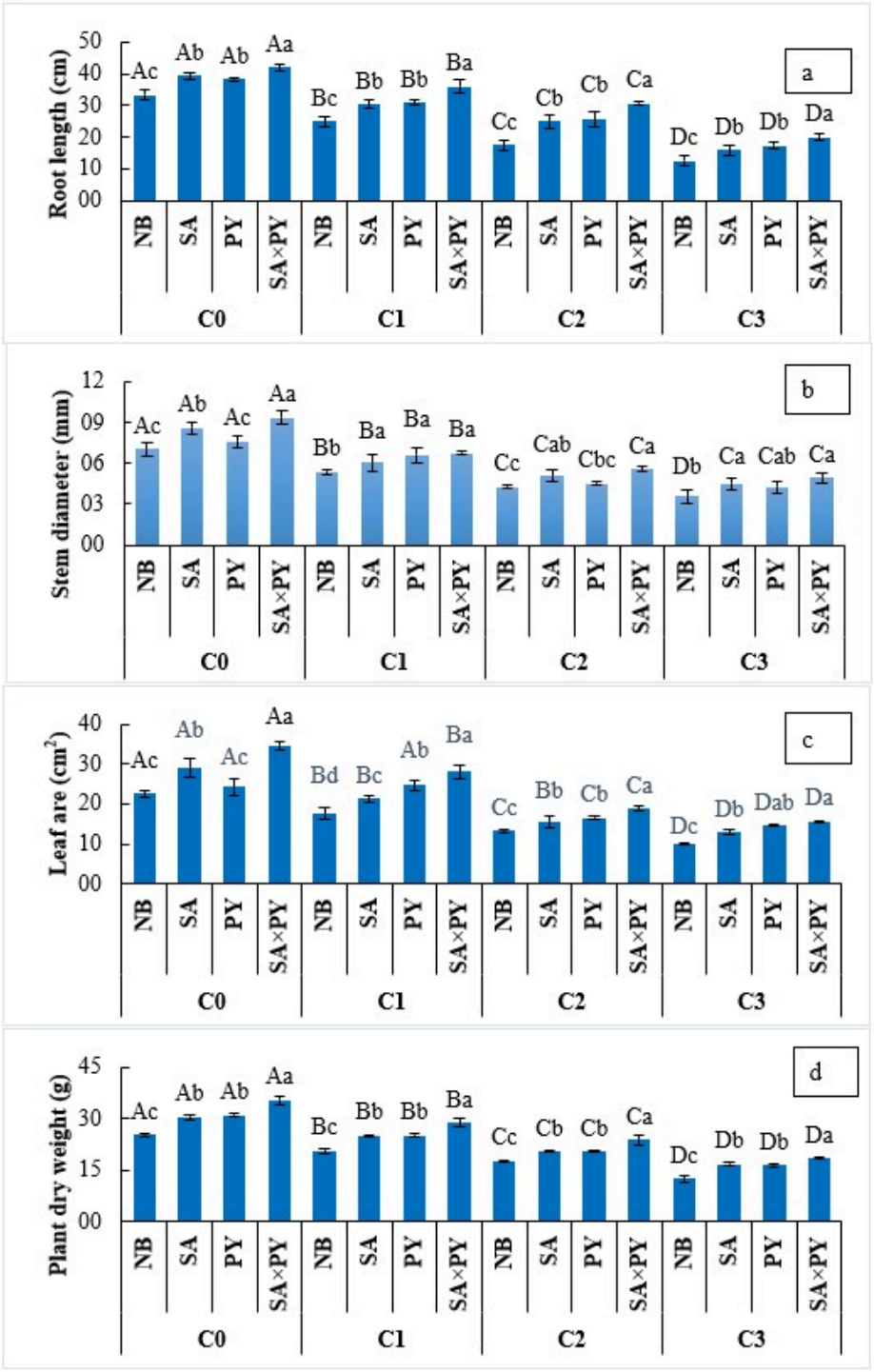


Fig. 1

Figure 1

Effect of seeds priming by salicylic acid (SA) and/or PGPR (*Pseudomonas yamanorum*) (PY), on Root length, stem diameter, Leaf area, and Plant dry weight variation of tomato subjected to varying salt stress levels (C0:0 mM NaCl, C1:50 mM NaCl, C2:100 mM NaCl, C3:150 mM NaCl). Values represent the

mean ± SE (n = 5). Bars marked with different lowercase letters indicate significant differences among seeds priming treatments within the same salinity level; bars marked with different uppercase letters indicate significant differences among salinity levels within the same seed pretreatment according to Duncan's multiple range test $P < 0.05$.

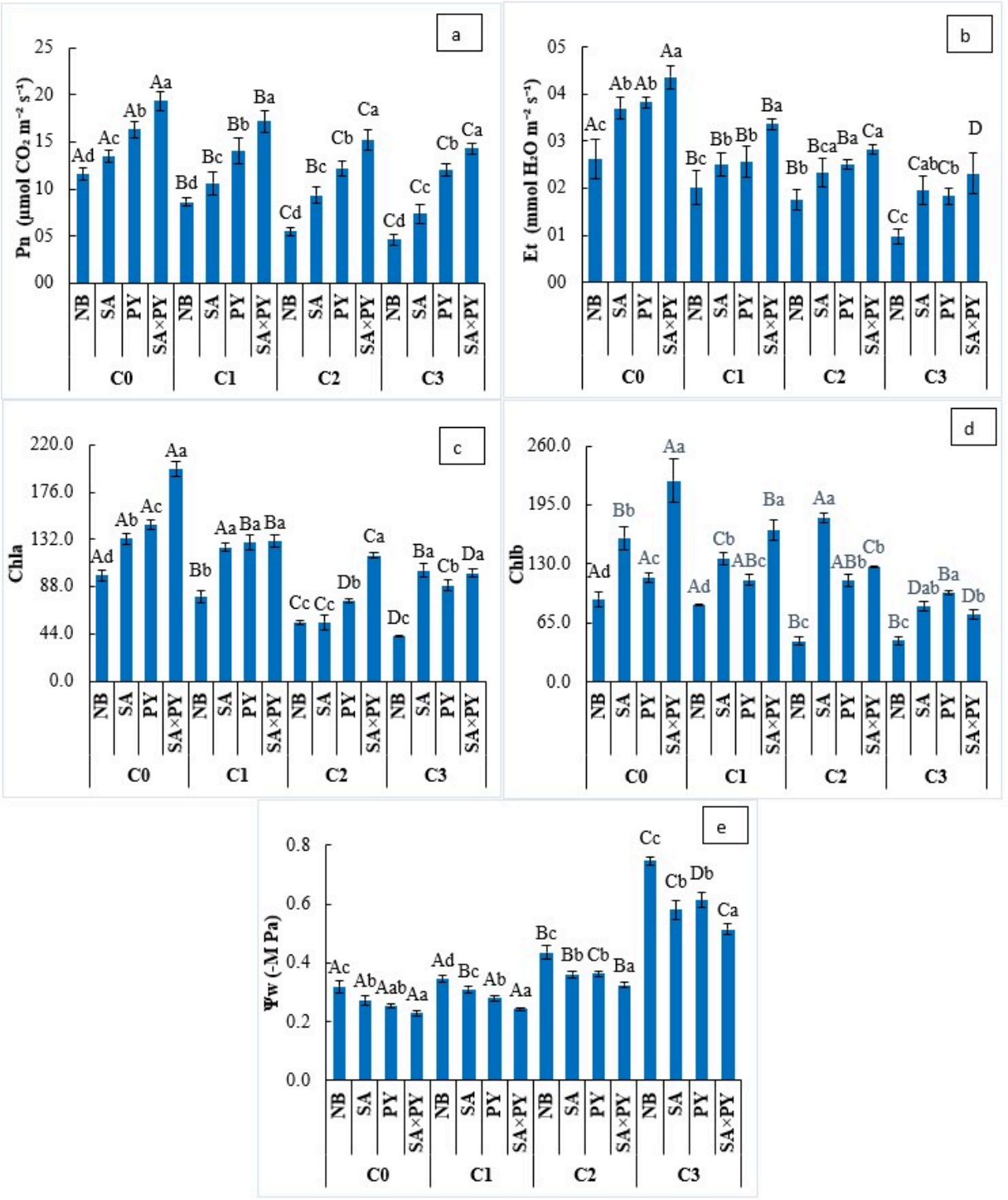


Fig. 2

Figure 2

Effect of seeds priming by salicylic acid (SA) and/or PGPR (*Pseudomonas yamanorum*) (PY), on Net photosynthesis, Evapotranspiration rate, Chlorophyll a, Chlorophyll b and Leaf water potential variation of tomato subjected to varying salt stress levels (C0:0 mM NaCl, C1:50 mM NaCl, C2:100 mM NaCl, C3:150 mM NaCl). Values represent the mean ± SE (n = 5). Bars marked with different lowercase letters indicate significant differences among seeds priming treatments within the same salinity level; bars marked with different uppercase letters indicate significant differences among salinity levels within the same seed pretreatment according to Duncan's multiple range test $P < 0.05$.

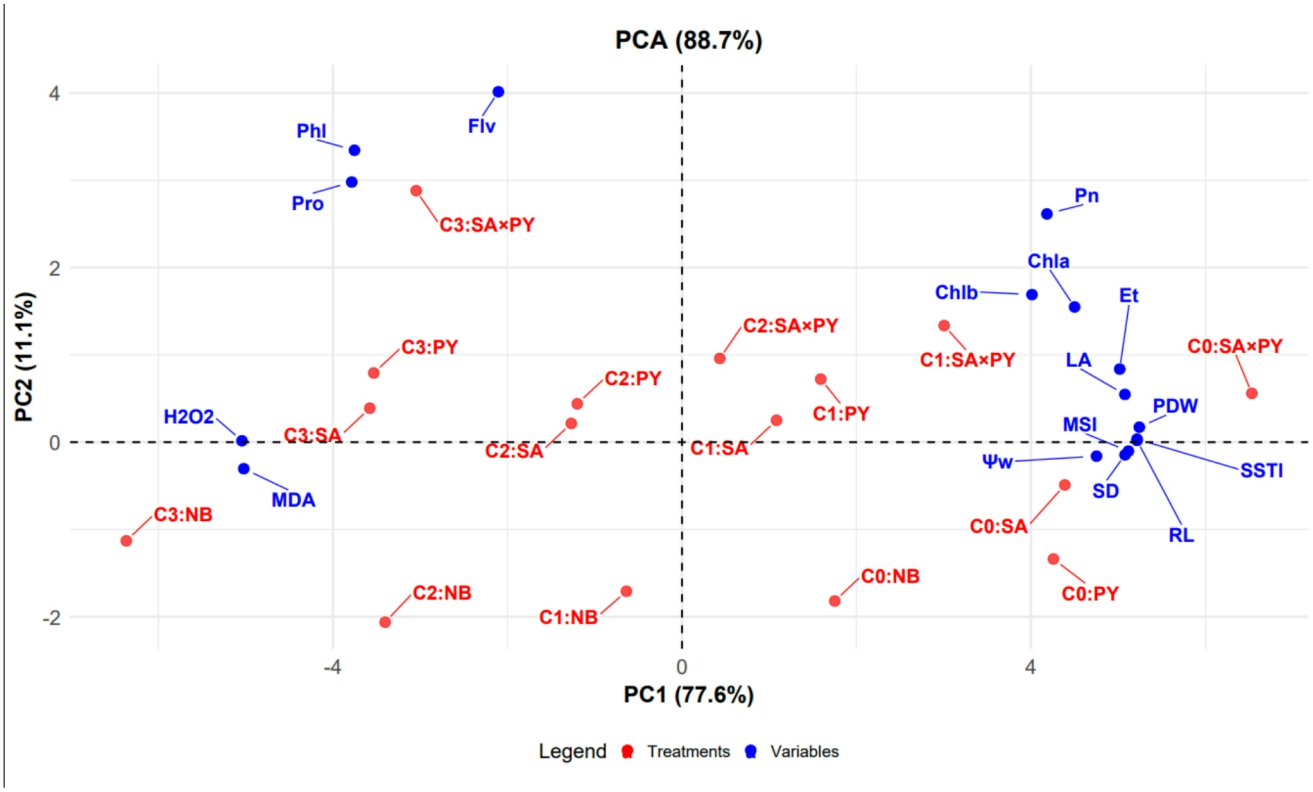


Figure 3

Principal Component analysis of sixteen studied parameters (blue dots) and treatments (red dots) applied on tomato firenze variety, Stem Diameter (SD), Root length (RL), Leaf Area (LA), plant dry weight (DW), Net photosynthesis (Pn), Evapotranspiration (ET), Leaf Water Potential (LWP), Chlorophyll a (Chla), Chlorophyll b (Chlb), Proline (Pro), Phenols (Phl), Flavonoids (Flv), Hydrogen peroxide (H_2O_2) Malondialdehyde (MDA), Membrane stability index (MSI), salt stress tolerance index (SSTI), C0:NB (plants cultivated at 0 mM NaCl without biostimulants application), C0:SA (plants cultivated at 0 mM NaCl with seeds priming by 1mM SA), C0:PY (plants cultivated at 0 mM NaCl with seeds priming by *Pseudomonas yamanorum*), C0:SA×PY (plants cultivated at 0 mM NaCl with seeds priming by 1mM SA and *Pseudomonas yamanorum*), C1:NB (plants cultivated at 50 mM NaCl without biostimulants application), C1:SA (plants cultivated at 50 mM NaCl with seeds priming by 1mM SA), C0:PY (plants

cultivated at 50 mM NaCl with seeds pretreated with *Pseudomonas yamanorum*), C0:SA×PY (plants cultivated at 50 mM NaCl with seeds seeds priming by 1Mm SA and *Pseudomonas yamanorum*), C2:NB (plants cultivated at 100 mM NaCl without biostimulants application), C0:SA (plants cultivated at 100 mM NaCl with seeds priming by 1mM SA), C0:PY (plants cultivated at 100 mM NaCl with seeds priming by *Pseudomonas yamanorum*), C0:SA×PY (plants cultivated at 100 mM NaCl with seeds priming by 1mM SA and *Pseudomonas yamanorum*), C3:NB (plants cultivated at 150 mM NaCl without biostimulants application), C3:SA (plants cultivated at 150 mM NaCl with seeds priming by 1mM SA), C3:PY (plants cultivated at 150 mM NaCl with seeds priming by *Pseudomonas yamanorum*) and C3:SA×PY (plants cultivated at 150 mM NaCl with seeds priming by 1mM SA and *Pseudomonas yamanorum*),

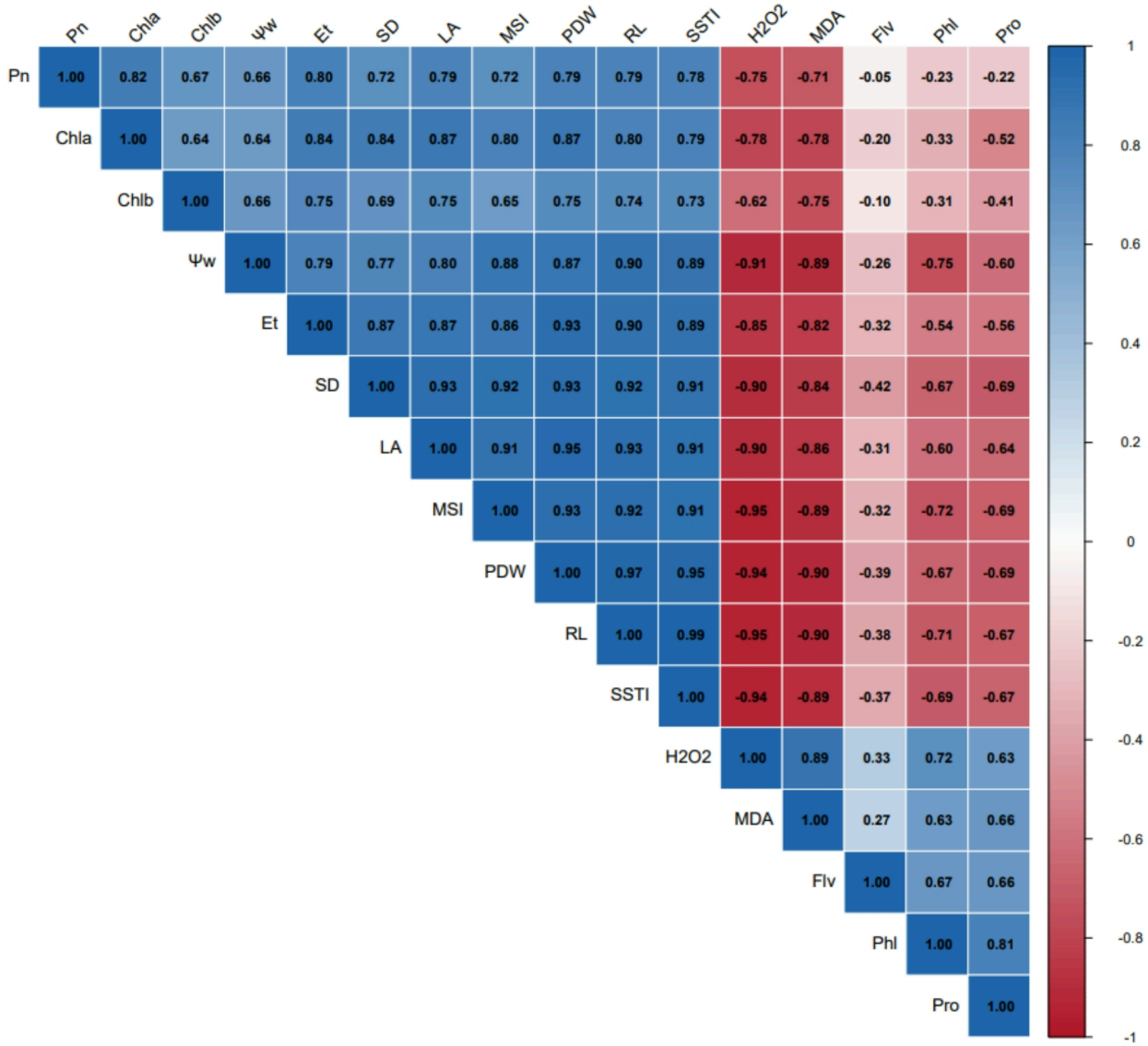


Figure 4

Pearson correlation heat map of the sixteen evaluated parameters of the Firenze tomato plant with seeds priming by salicylic acid (SA) and/or *Pseudomonas yamanorum* (PY) and subjected to C0 (0 mM NaCl), C1 (50 mM NaCl), C2 (100 mM NaCl), and C3 (150 mM NaCl) salt stress, Stem Diameter (SD), Root length (RL), Leaf Area (LA), plant dry weight (DW), Net photosynthesis (Pn), Evapotranspiration (ET), Leaf Water Potential (LWP), Chlorophyll a (Chla), Chlorophyll b (Chlb), Proline (Pro), Phenols (Phl), Flavonoids (Flv), Hydrogen peroxide (H_2O_2) Malondialdehyde (MDA), Membrane stability index (MSI), and salt stress tolerance index (SSTI).

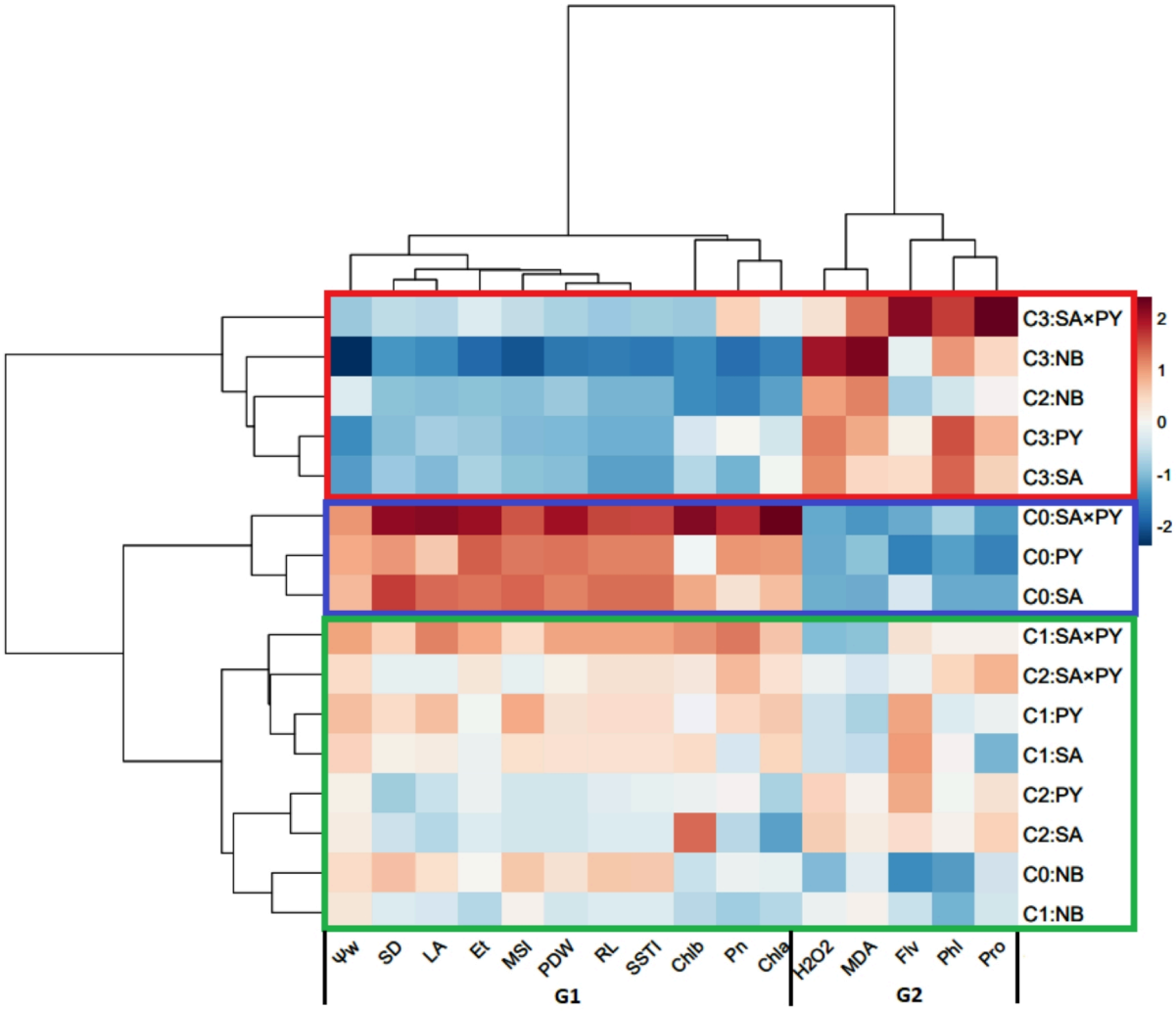


Figure 5

Hierarchical clustering heat map sixteen studied parameters (blue dots) and treatments (red dots) applied on tomato firenze variety, Stem Diameter (SD), Root length (RL), Leaf Area (LA), plant dry weight (DW), Net photosynthesis (Pn), Evapotranspiration (ET), Leaf Water Potential (LWP), Chlorophyll a (Chla),

Chlorophyll b (Chlb), Proline (Pro), Phenols (Phl), Flavonoids (Flv), Hydrogen peroxide (H_2O_2) Malondialdehyde (MDA), Membrane stability index (MSI), salt stress tolerance index (SSTI), C0:NB (plants cultivated at 0 mM NaCl without biostimulants application), C0:SA (plants cultivated at 0 mM NaCl with seeds priming by 1mM SA), C0:PY (plants cultivated at 0 mM NaCl with seeds priming by *Pseudomonas yamanorum*), C0:SA×PY (plants cultivated at 0 mM NaCl with seeds priming by 1mM SA and *Pseudomonas yamanorum*), C1:NB (plants cultivated at 50 mM NaCl without biostimulants application), C1:SA (plants cultivated at 50 mM NaCl with seeds priming by 1mM SA), C0:PY (plants cultivated at 50 mM NaCl with seeds priming by *Pseudomonas yamanorum*), C0:SA×PY (plants cultivated at 50 mM NaCl with seeds priming by 1mM SA and *Pseudomonas yamanorum*), C2:NB (plants cultivated at 100 mM NaCl without biostimulants application), C0:SA (plants cultivated at 100 mM NaCl with seeds priming by 1mM SA), C0:PY (plants cultivated at 100 mM NaCl with seeds priming by *Pseudomonas yamanorum*), C0:SA×PY (plants cultivated at 100 mM NaCl with seeds priming by 1mM SA and *Pseudomonas yamanorum*), C3:NB (plants cultivated at 150 mM NaCl without biostimulants application), C3:SA (plants cultivated at 150 mM NaCl with seeds priming by 1mM SA), C3:PY (plants cultivated at 150 mM NaCl with seeds priming by *Pseudomonas yamanorum*) and C3:SA×PY (plants cultivated at 150 mM NaCl with seeds priming by 1mM SA and *Pseudomonas yamanorum*),