Acta Physiologiae Plantarum (2024) 46:41
https://doi.org/10.1007/s11738-023-03639-z

ORIGINAL ARTICLE

Exogenous application of salicylic acid via seed soaking improved growth and photosynthetic efficiency by maintaining stomatal organisation, redox homeostasis, and antioxidant defense system in tomato (*Solanum lycopersicum* L.)

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Received: 15 October 2022 / Revised: 3 February 2023 / Accepted: 21 November 2023 / Published online: 10 March 2024

Abstract
Salicylic acid (SA) is a versatile phenolic plant growth regulator (PGR) which is involved in regulation of several processes of plant growth and development. It confers tolerance against both biotic and abiotic stresses in plants by modulating different morphological and physio-biochemical aspects of plants. Therefore, the present experiment was intended to reveal the impact of SA by seed soaking in *Solanum lycopersicum* L. (varieties, S-22 and PKM-1). Seeds of both varieties were soaked in 0, 10^{-4}, 10^{-5} or 10^{-6} M of SA for 3, 6 or 9 h, before sowing. The respective treated seeds were sown in nursery beds to create nursery and then seedlings were transplanted at 20 days after sowing (DAS) and at 40 days after transplantation (DAT), various growth, photosynthetic, microscopic, histochemical and biochemical attributes were assessed. It was observed that irrespective of the concentration and duration, treatment with SA through seed soaking had enhanced growth, photosynthesis, improved stomatal width, activity of antioxidant enzymes (peroxidase (POX), superoxide dismutase (SOD) and catalase (CAT)), nitrate reductase (NR), carbonic anhydrase (CA) and greater accumulation of proline than the non-treated plants. Remarkably, SA supplementation reduced the accrual of reactive oxygen species (ROS; H_2O_2 and $O_2^{\bullet-}$ content) and also decreased the electrolyte leakage (EL). Soaking of seeds with SA improved growth and photosynthesis by regulating stomatal organisation, ROS levels and antioxidant enzymes. Among two dissimilar varieties of tomato and three different concentrations of SA, seed soaking of S-22 variety with 10^{-5} M for 6 h showed significant increase in growth and photosynthesis than PKM-1 variety.

Keywords Antioxidants · Photosynthesis · Reactive oxygen species · Salicylic acid · Stomata · Tomato

Introduction

Tomato (*Solanum lycopersicum* L.) is the second chief cultivated fruit–vegetable crop in the world. However, due to fluctuating environmental conditions (global warming, population explosion, pollution), the growth and production of tomatoes are drastically affected. Therefore, one of the interesting issues to agriculture globally is to provide 70% more food by the year 2050 (Food Summit FAO 2009). Various strategies have been proposed to improve the productivity, performance and tolerance of plants under challenging environments, however, one of the approach using plant growth regulators (PGRs) proved very potential. Out of several PGRs, exogenous application of salicylic acid (SA) through seed soaking improves growth, seed germination, photosynthetic efficiency, antioxidant machinery and various other biochemical attributes (Farooq et al. 2019; Boukari et al. 2019; Mir et al. 2020a,b; Gul et al. 2020; Jelali et al. 2021; Ahmad et al. 2020). SA, chemically known as ortho-hydroxybenzoic acid, an omnipresent phenolic hormone in the plant kingdom, plays vital role in the regulation of various morphological, cytological and physio-biochemical attributes in normal and unfavourable environmental conditions (Chen et al. 2009; Wani et al.

Communicated by A. Gniazdowska-Piekarska.

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2017; Fariduddin et al. 2018; Khan et al. 2020; Saleem et al. 2021a).

SA is regarded as the best seed primer to improve the tolerance in various crops like wheat, maize and rice by stimulating the photosynthesis, metabolism of carbon and protein, accumulation compatible solutes like proline and the antioxidant machinery in many abiotic stresses (Farooq et al. 2019; Saruhan et al. 2012; Sharma et al. 2017). Exogenous application of SA in lower doses showed beneficial for improving morpho-physiological and biochemical attributes of plant (Hayat et al. 2010). Beside this, supplementation of SA increased growth, water use efficiency (WUE), decreases reactive oxygen species (ROS) levels and yield in peanuts and maize plants (Kong et al. 2014). SA modulates several metabolic and physiological processes thereby improving the growth and development in plants (Khodary 2004; Arfan et al. 2007; Manaa et al. 2014; Saleem et al. 2021b). Furthermore, SA upregulate the activities of antioxidant enzyme and increase the tolerance of plants to various abiotic stresses (Yusuf et al. 2008; Parashar et al. 2014; Fariduddin et al. 2018; Tayyab et al. 2020).

Therefore, bearing in mind, current work was aimed to investigate the role of SA on morpho-physiological, biochemical, and the antioxidant defence system in *S. lycopersicum* plant through seed soaking. This study explores the SA-induced microscopic (stomatal), histochemical (ROS levels), photosynthetic, biochemical and antioxidant enzymatic changes in two varieties (S-22 and PKM-1) of tomato treated with different concentrations of SA through seed soaking. Moreover, how SA impacts the growth, photosynthetic potential and ROS-homeostasis is vital to give mechanistic-insights of SA-induced changes in plants.

Materials and methods

Plant materials

Tomato (*S. lycopersicum* L. varieties, PKM-1 and S-22) seeds were taken from Indian Agricultural Research Institute (IARI), New Delhi. Uniform and healthy seeds of both varieties were selected followed by sterilization with sodium hypochlorite (1% NaOCl) for about 10 min, and then repeated washing with double distilled water (DDW) before soaking. These disinfected seeds were soaked in 0, 10^{-4}, 10^{-5} or 10^{-6} M of SA for 3, 6 or 9 h and then sown in earthen pots to create nursery. At 20 DAT, seedlings were transplanted from nursery beds to earthen pots. Two different varieties of tomato (PKM-1 and S-22) were chosen for the study based on our preliminary study depicted in Supplementary File 1. Various growth, physiological and biochemical attributes were assessed at 40 days after transplantation (DAT).

Preparation of SA

SA was bought from Sigma–Aldrich Chemicals Pvt. Ltd. India. 1 M stock of SA solution was made in 200 mL flask by solvating 27.6242 g of SA in 10 mL of ethyl alcohol, and the absolute measurements was adjusted by adding DDW. The concentrations required of SA (10^{-4}, 10^{-5} or 10^{-6} M) were set by diluting the solution of SA stock with DDW. Tween-20 (0.5 mL) a surfactant, was also added before seed soaking. Concentrations were opted based on early findings of Fariduddin et al. (2003) and Hayat et al. (2012).

Growth conditions and experimental setup

The surface-sterilized and respective soaked seeds were propagated in nursery beds to produce the nursery. Twenty DAS, the seedlings were transplanted into pots (23 cm diameter), filled with loamy sand soil and farmyard compost in a ratio of 6:1, v/v. each pot was added with the basal dose of NP and K. An equivalent amount of tap water was provided to maintain the moisture content in the soil. The experiment was carried out in simple sequential block layout in the net house of the Department of Botany, Aligarh Muslim University (27° 34′ N and 78° 31′ E), India. Seeds were treated with three diverse concentrations 10^{-4}, 10^{-5} or 10^{-6} M of SA for 3, 6 or 9 h. Consequently, three sets were obtained and set up for different durations, and each set was added with three different concentrations of SA. Five replicates were applied in each treatment. To reveal the effect of different concentrations and durations of seed soaking with SA, plants were assessed at 40 days after transplantation (DAT).

Analysis of growth parameters

Changes in the growth biomarkers were determined by uprooting plants cautiously from pots and these uprooted plants were gently washed to make sure that no soil particle remains with the plant. Root and shoot length, root and shoot fresh weight and leaf area were measured with the help of meter scale, electronic balance and leaf area meter (LA 211, Systronics, New Delhi, India), respectively. For root and shoot dry weight, the samples of root and shoot were retained in a hot air oven, at 80 ℃ for 72 h, then dry samples were again weighted by electronic balance.

Photosynthesis and related attributes

Determination of SPAD value of chlorophyll and maximum quantum yield of PSII (Fv/Fm)

SPAD chlorophyll meter (SPAD-502; Konica, Minolta sensing, Inc., Japan) was used to determine the chlorophyll value on extended leaves of a plant. Before determining Fv/Fm, leaf samples were adjusted in dark for 30 min. After dark adaptation, Fv/Fm was measured using a weak measuring light photosynthetic photon flux density (PPFD) of less than 1 µmol m^{-2} s^{-1} for Fo followed by a saturating pulse (PPFD = 4200 µmol m^{-2} s^{-1}) for Fm.

Leaf gas exchange attributes

The gas exchange attributes were analyzed with the aid of a portable photosynthetic system Infrared gas analyser (IRGA) (LI-COR 6400, LI-COR, and Lincoln, NE, USA) during the day time (11:00 to 12:30 h) on the third fully extended leaves of a plant. Net photosynthetic rate (PN), and its related attributes like transpiration rate (*E*), internal CO_2 concentration (Ci) and stomatal conductance (gs) were measured at ~25 ℃ of air temperature, 80% of relative humidity, 600 µmol mol^{-1} of CO_2 concentration and 800 µmol mol^{-2} s^{-2} of PPFD.

Determination of electrolyte leakage (EL) and leaf water potential (LWP)

According to method of Sullivan and Ross (1979), EL was determined. Twenty leaf segments were placed in a boiling tube holding 10 mL of DDW. The samples were heated at 45 ℃ (ECa) and 55 ℃ (ECb) for 30 min each in a water container and a conductivity meter (Eutech Instruments; Part of Thermo Fisher Scientific-Singapore) was used to measure the consequent EC. Finally, the samples were boiled in a test tube at 100 ℃ for 10 min and the EC (ECc) was estimated, and EL was determined by the following formula;

$$\text{Electrolyteleakage}(\%) = \frac{(\text{ECb} - \text{ECa})}{\text{ECc}} \times 100,$$

where the ECa stands for original conductance, ECb for higher temperature conductance, and ECc for concluding conductance.

With the help of the Psypro water potential system (Wescor, Inc. USA), LWP was determined in third fully expanded leaves of plant during day time at 13:00 h which is based on the principle that loss of water via evaporation from exterior cool down the surface.

Biochemical attributes

Activity of NR and CA

The activity of nitrate reductase (NR) was estimated by the protocol proposed by Jaworski (1971), in which freshly cut leaves were placed in test tubes filled with 2.5 mL of 5% isopropanol, 2.5 mL of 0.1 M phosphate buffer at pH of 7.5 and 0.5 mL of 0.2 M potassium nitrate (KNO_3) and this reaction mixture was incubated for 2 h at 30 ℃. Besides this, another set of test tubes were retained with 0.02% of *N*-1-napthyl-ethylendiaminhydrochloride (NED-HCl) and 0.3 mL of 1% sulfanilamide solution. About 1 mL of the incubated reaction mixture was added from each treatment to this set of test tubes. The pink colour generated was observed at absorbance of 540 nm by a spectrophotometer (Spectronic-20D, Milton Roy, USA) and expressed as fresh weight (FW) basis.

The carbonic anhydrase (CA) activity was estimated by method proposed by Dwivedi and Randhawa (1974). Leaf samples were equally weighted and cut into small pieces and placed in test tubes filled with 0.2 M of cysteine-HCl solution and incubated at 4 ℃ for 20 min, then the filtrate of this extract was filled in other test tubes having a mixture of 0.2 M sodium bicarbonate ($NaHCO_3$) solution (2 mL), Na-phosphate buffer (2 mL) and 0.002% of bromothymol blue (0.1 mL). Finally, the test tubes were left for about 20 min and the liberation of CO_2 by the action of CA was evaluated by titration of the reaction mixture versus hydrochloric acid (0.05 N HCl). The methyl red ($C_{15}H_{15}N_3O_2$) was used as an indicator and activity of CA activity was stated as per gram fresh weight basis by adding the following values in the equation:

$$\text{Carbonicanhydraseactivity} = \frac{V \times 22 \times N}{W},$$

where V = Volume difference (mL of HCl used, in control and test sample titrations), 22 = Equivalent weight of CO_2, N = Normality of HCl, and W = Fresh weight of leaf used.

Estimation of ROS

H_2O_2 and $O_2^{\bullet-}$ content

Method described by Patterson et al. (1984) was used to determine H_2O_2 content. Fresh leaves of about 0.5 g were taken in order and ground in ice-cold mortar and pestle mixed with acetone. Homogenate formed was then centrifuged for about 15 min at 5000 g. Later on 20% of titanium chloride ($TiCl_4$) (prepared in concentrated HCl)

and 17 M of ammonia solution was added in the test tubes containing supernatant (1 mL). Once these solutions were added, the precipitate was taken and dissolved in 2 N H_2SO_4 (10 mL) and again centrifuged to take out undissolved substances. Finally, absorbance of the supernatant was detected at 410 nm and H_2O_2 content was calculated with the help of a standard curve plotted with a familiar H_2O_2 concentration and stated in terms of µmol g^{-1} FM.

Method of Wu et al. (2010) was used to determine the superoxide anion content. Fresh samples of leaves were ground in sodium phosphate buffer of about 65 mM and 1% of Poly vinyl pyrrolidone (PVP). The homogenate was set to centrifugation for 15 min at 5000 g. Later on, the supernatant was mixed with 10 mM hydroxylamine hydrochloride and 65 mM phosphate buffer in test tubes and incubated at 25 ℃ for about 30 min. Later on, 58 m of metanilic acid and 7 mM of 1-naphthyl amine was added and then again retained for incubation (20 min) at 25 ℃. At 530 nm, absorbance was recorded and content of superoxide anion was assessed with respect to the calibration curve and expressed in terms of the µmol g^{-1} FM.

Histochemical detection of H_2O_2 and $O_2^{\bullet-}$ content

The method of Kaur et al. (2016) was used to determine the histochemical staining of leaves to detect the ROS (H_2O_2 and $O_2^{\bullet-}$ content) as explained by Saleem and Fariduddin (2022). The H_2O_2 and $O_2^{\bullet-}$ content was detected by dipping leaf samples in 3, 3-diaminobenzidine (DAB) (maintained at pH ~ 3.8) and 6 mM of nitro blue tetrazolium (NBT) dyes, respectively, for about 6 h at normal room temperature. These leaves were further immersed in 100% ethanol and boiled at 100 ℃ to remove pigments and then samples were cooled, sequentially cleaned with glycerol solution (20%). Finally, images were taken with the help of the camera (NIKON-D53).

Assessment of antioxidant enzymes and protein content

Activities of the antioxidant enzymes and the content of protein was determined following the homogenization of fresh leaves with the extraction buffer having 1 mM of EDTA (ethylenediaminetetraacetic acid), 0.5% of Triton X-100, 1 mM of PMSF (phenyl methane sulfonyl flouride) and 2% of PVP (polyvinyl pyrollidone). After that, centrifugation of homogenate was done for 20 min at 12,000 g at 4 ℃ and supernatant generated was used further to measure the total protein and activities of antioxidant enzyme.

Protein content

Protocol of Bradford (1976), was followed to determine protein content. In this method supernatant (200 mL of enzyme extract) and Bradford reagent (4 mL) were mixed intensely in test tubes. After mixing, samples were incubated at 25 ℃ for 10 min and at absorbance of 595 nm, protein content was measured with the aid of a spectrophotometer and the content of protein was stated as mg g^{-1} FW. Finally, the linear standard equation was attained by laying a graph of well-known concentration of BSA (bovine serum albumin) v/s absorbance.

Antioxidant enzyme activity

The activity of superoxide dismutase (SOD) was measured by following protocol of Beauchamp and Fridovich (1971) which is based on the principle of photochemical reduction inhibition of NBT (nitro blue tetrazolium). The reaction mixture having 50 mM of phosphate buffer prepared at pH of 7.8, EDTA (2 mM), L-methionine (9.9 mM), 0.02% of Triton X-100, NBT (55 mM) and lastly 1 mM riboflavin were mixed with extract of the enzyme. Blank and control were also measured in a similar manner and SOD activity were observed at regular intervals for 2 min, at 560 nm absorbance. The activity of SOD (one-unit) was calculated as per the quantity of requisite enzyme to cause the 50% decrease of the NBT at 25 ℃.

The catalase (CAT) activity was measured according to protocol of Aebi (1984) based on loss of H_2O_2. In this method, 100 µL enzyme extract was added to the reaction mixture having 50 mM phosphate buffer prepared at pH 7.0 and 15 mM of H_2O_2. Finally, the optical density was observed for 2 min at 25 ℃ with the short-term of 30 s at absorbance of 240 nm.

The activity of peroxidase (POX) was measured by following protocol proposed by Sanchez et al. (1995). In this method, 100 µL enzyme extract was mixed to reaction mixture having phosphate buffer (50 mM) prepared at the pH of 7.0, 20 mM guaiacol and 1.5 mM of H_2O_2. Finally, at 436 nm of absorbance, the activity of POX was measured with the help of spectrophotometer (Spectronic-20D, Milton Roy, USA).

Proline content

According to the protocol of Bates et al. (1973), content of proline was determined (with slight variation). Fresh leaves were ground with 3% sulphosalicylic acid (extract reagent) and then centrifugation at 10,000 g was carried for 10 min. A mixture of 2 mL each of glacial acetic acid (CH_3COOH), sulphosalicylic acid ($C_7H_6O_6S$) and freshly prepared acid-ninhydrin solution filled in the test tube was added with 2 mL of supernatant. Then, test tubes were kept in a hot water bath for 1 h at 100 ℃ and then transferred in an ice bath to terminate the reaction. Finally, the absorbance of the

reaction mixture was observed at 528 nm and the quantity of proline was calculated on the basis of fresh weight.

Stomatal physiology

Compound microscopy

Fresh leaves were taken in which epidermal peels were removed with the aid of forceps from the abaxial surface of the leaf. These peels were then mounted on a glass slide. Then, these mounted peels were observed in a camera fixed compound microscope (OLYMPUS BX 51) at magnification of 40×. The aperture of stomata was revealed by micrometer scale.

Scanning electron microscopy (SEM)

The outer organization of stomata was observed via visualizing the freeze and dehydrated sections of leaf with the aid of a scanning electron microscope (JEOL JSM-JSM 6510 SEM) as explained by Khan et al. (2022). Leaf samples were cut (2×2 mm segments) and fixed for 2 h in smallest polyoxymethylene paraformaldehyde (2%), glutaraldehyde (2.5%) and buffer 0.1 M of sodium cacodylate (pH of 7.3). After fixation, the segments of leaf samples were compressed by aid of 1% osmium oxide. Finally, the samples were dried out sequentially by graded series of 50%, 70%, 80%, 90%, and 100% of ethanol. With the help of gold–palladium, the samples were glazed in a coater splatter instrument (JEOL JFC-1600). At a magnification about 4000× and 15 kV voltage images of stomata were taken.

Statistical analysis

With the aid of the SPSS version 17 for windows (IBM Corporation, New York, USA) statistical analysis was done. Analysis of variance (ANOVA) and standard error was calculated on the data taking five replicates ($n=5$). For comparison among means for significant differences Duncan Multiple Range test (DMRT) was performed at $P \leq 0.05$. Means have been separated by the Duncan's multiple range test (DMRT). Beside this the Pearson's correlation between the various attributes and non-metric multidimensional scaling (NMDS) was done with the aid of software Past.

Results

Growth parameters

Plants upraised from seed soaking with SA under three different durations (3, 6 or 9 h) and concentrations (10^{-4}, 10^{-5} or 10^{-6} M) improved growth traits like shoot and root length, fresh and dry weight of shoot and root and leaf area with respect to control plants. Soaking of seeds with SA for 6 h generated significant impact on growth as compared to other sets of seed soaking and the response was more prominent in S-22 variety than PKM-1. The maximum length of shoot and root (53 and 48%), the fresh weight of shoot and root (51 and 36%), dry weight of shoot and root (52 and 45%) and leaf area (50%) of S-22 variety was observed by soaking of seeds (10^{-5} M of SA for 6 h) as compared to control (Figs. 1, 2A). Soaking of seeds with 10^{-5} M of SA for 6 h considerably improved all parameters of growth.

Photosynthesis and stomatal traits

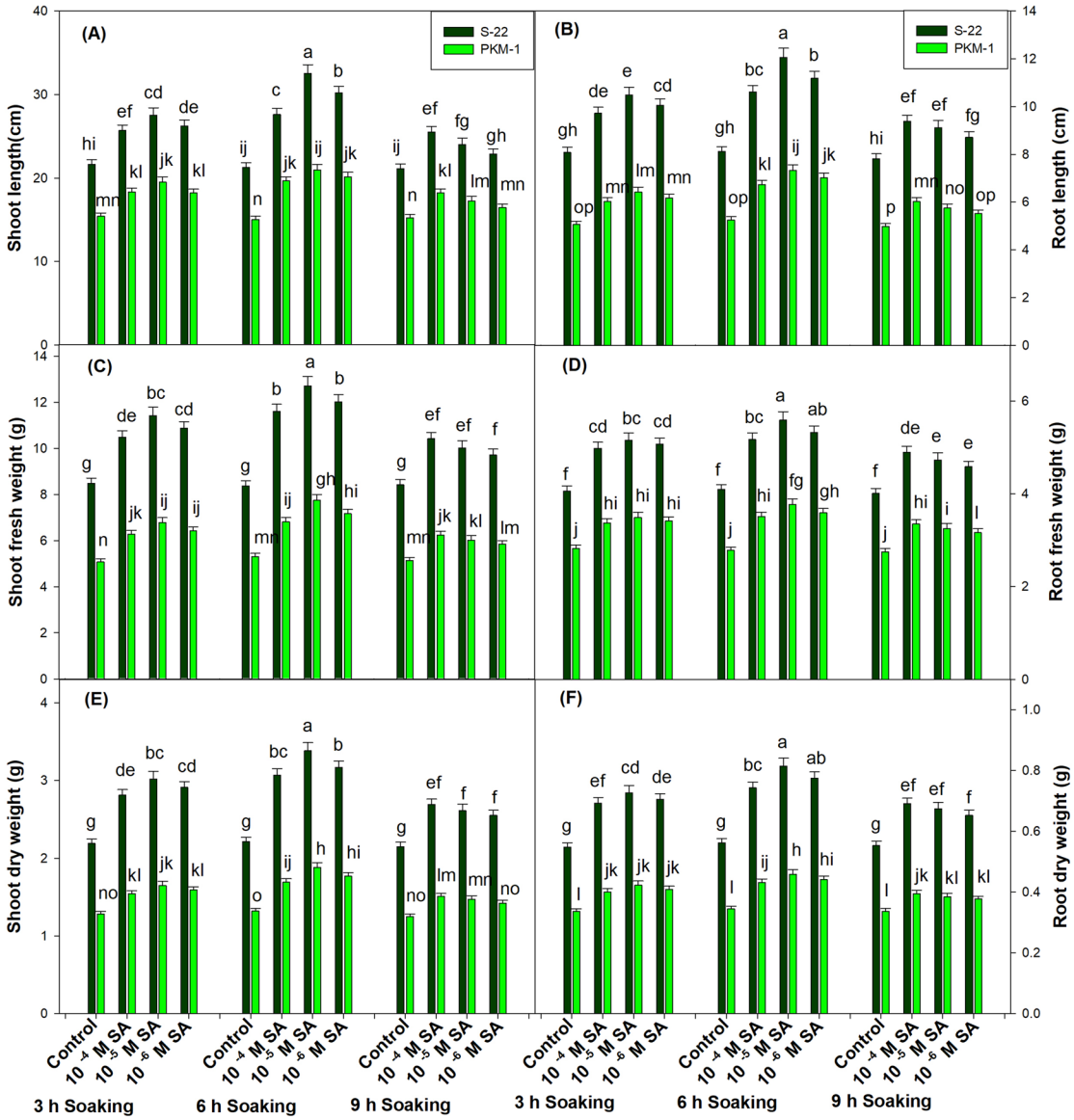
Seed soaking with SA increased the chlorophyll content (SPAD value) and Fv/Fm as compared to their respective control. However, the plants raised from seed soaking with 10^{-5} M of SA for 6 h had significantly increased SPAD level and Fv/Fm than control, and the impact was more pronounced in S-22 than the PKM-1 variety. The values of SPAD and Fv/Fm were increased by (53 and 52%) and (45 and 43%) in S-22 and PKM-1 variety by seed soaking with 10^{-5} M of SA for 6 h (Fig. 2B, C).

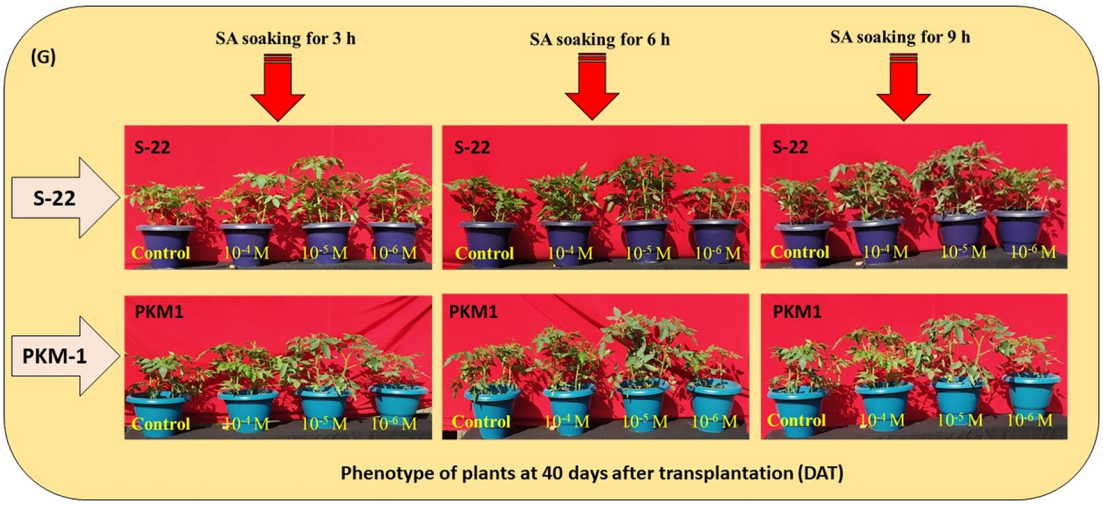
Furthermore, treatment of SA by soaking of seeds triggered a substantial increase in gas exchange parameters (net photosynthetic rate (PN), internal CO_2 concentration (Ci), stomatal conductance (gs) and the transpiration rate (E)). But soaking of seeds with 10^{-5} M of SA for 6 h triggered a significant increase in comparison to their respective control, and the outcome was seen more pronounced in S-22 than the PKM-1 variety. Pre-sowing treatment of seed with 10^{-5} M of SA for 6 h increased about PN by 52 and 39%, gs by 43 and 28%, Ci by 51 and 38% and E by 45 and 36% in S-22 and PKM-1 variety, respectively (Figs. 2D–F, 3A).

Soaking of seeds for different durations and concentrations of SA significantly decreased the EL and increased the LWP with respect to control. But, the plants raised from treatment of seeds for 6 h with 10^{-5} M of SA decreased the EL by 21 and 16% as compared to control in S-22 and PKM-1 variety, respectively, (Fig. 3C). Besides this, the pre-sowing seed treatment with 10^{-5} M of SA for 6 h significantly increased the LWP by 50 and 32% in S-22 and PKM-1 variety, respectively, with respect to control plant (Fig. 3B).

Stomatal physiology

Treatment of seeds with SA and the developed plant had improved width of stomata with respect to control. However,


(A)
(B)
(C)
(D)
(E)
(F)
S-22
PKM-1
Shoot length(cm)
Root length (cm)
Shoot fresh weight (g)
Root fresh weight (g)
Shoot dry weight (g)
Root dry weight (g)
Control
10-4 M SA
10-5 M SA
10-6 M SA
3 h Soaking
6 h Soaking
9 h Soaking


(G)
SA soaking for 3 h
SA soaking for 6 h
SA soaking for 9 h
S-22
PKM-1
Control
10-4 M
10-5 M
10-6 M
Phenotype of plants at 40 days after transplantation (DAT)

◂**Fig. 1** Effect of salicylic acid (SA) through seed soaking on **A** shoot and **B** root length; **C** shoot and **D** root fresh weight; **E** shoot and **F** root dry weight; and **G** phenotype of two varieties of tomato seedlings at 40 DAT. All the data are the means of five replicates ($n=5$); vertical bars show standard errors (±SE). Means with different letters above the bars are significantly different at $P \leq 0.05$

the outstanding improvement in the stomatal width was observed in the plants raised from soaking for 6 h in SA (10^{-5} M) of as evident in the (Fig. 4A). The compound microscopy examinations were corroborated from SEM observations (Fig. 4B).

Biochemical traits

Activity of carbonic anhydrase (CA) and nitrate reductase (NR)

Plants raised with the soaking of seeds with SA had substantial increase in the activities of CA and NR as compared to their respective control. However, out of various concentrations and duration of soaking treatment, 10^{-5} M of SA for 6 h maximally improved the CA and NR activity by 45 and 49% in the S-22 variety, as compared to their respective control. Although similar impact was observed in PKM-1 variety, but S-22 was more responsive than PKM-1 where activity of CA and NR increased by 34 and 33%, respectively, as compared to their respective control (Fig. 3D, E).

ROS (Reactive oxygen species) indicators

H_2O_2 and $O_2^{\bullet-}$ content

Plants raised from the soaking of seeds with SA showed lesser content of both H_2O_2 and superoxide anion as compared to the control. But soaking with 10^{-5} M of SA for 6 h decreased the H_2O_2 and superoxide anion by 26 and 20% in S-22 variety; 21 and 16% in PKM-1 variety, respectively, as compared to the respective control (Figs. 5A, 6A).

Histochemical staining for H_2O_2 and $O_2^{\bullet-}$

Histochemical staining of plants depicted the level of H_2O_2 and superoxide anion, where H_2O_2 was observed by brownish spots, whereas superoxide anion by blue spots on leaf discs. It was observed that plants raised from seed soaking with SA had lesser stained spots than the control. However, less number of spots (accretion of H_2O_2 and $O_2^{\bullet-}$) were observed in the leaf discs produced by treatment of SA (10^{-5} M) for 6 h as compared to the respective controls (Figs. 5A–D, 6A–D) and S-22 was more responsive than PKM-1 variety.

Total protein content

Pre-sowing seed treatment with SA enhanced the protein content with respect to control. However, soaking of seeds with 10^{-5} M of SA for 6 h triggered maximum increase in the protein content by 25 and 18% in S-22 and PKM-1 variety, respectively, as compared to the control (Fig. 7A).

Activities of antioxidant enzymes

Pre-sowing seed treatment of SA via seed soaking increased the antioxidant enzyme activities like CAT, POX and SOD as compared to control. However, the maximum activities of POX (63%), CAT (57%) and SOD (61%) were noticed in the S-22 variety subjected to soaking with 10^{-5} M of SA for 6 h, whereas, 45%, 54% and 49% rise in the activity of CAT, POX and SOD was observed in PKM-1 variety by soaking for 6 h with the 10^{-5} M of SA as compared to the control (Fig. 7B–D).

Proline content

The content of proline amino acid observed was improved by all the treatments of seed soaking with SA with respect to control. But, plants raised from soaking by 10^{-5} M of SA for 6 h showed maximum accretion in proline by 48 and 36% in S-22 and PKM-1 varieties, respectively, then the respective control (Fig. 3F).

A strong correlation among the several growth biomarkers, photosynthetic and biochemical attributes was observed in our experiment on tomato plants treated with SA via seed soaking. The correlation-matrix of both varieties (S-22 and PKM-1) are depicted in Fig. 8, respectively, which portrays a significant positive correlation (red colour) and negative correlation (blue colour). We observed a significant positive relationship between the various growth, photosynthetic and biochemical attributes except EL, H_2O_2 and superoxide anion content which exhibited a negative correlation with all these attributes. Moreover, we found that photosynthetic gas exchange attributes were positively correlated with the growth attributes, SPAD chlorophyll value and antioxidant enzymes but, negatively correlated with EL, H_2O_2 and $O_2^{\bullet-}$ content in both S-22 and PKM-1 (Fig. 8) variety. The impact of SA on two different varieties of tomato has been statistically seen by NMDS analysis (Fig. 9). It shows the effect of SA within and between two different varieties of tomato was significant

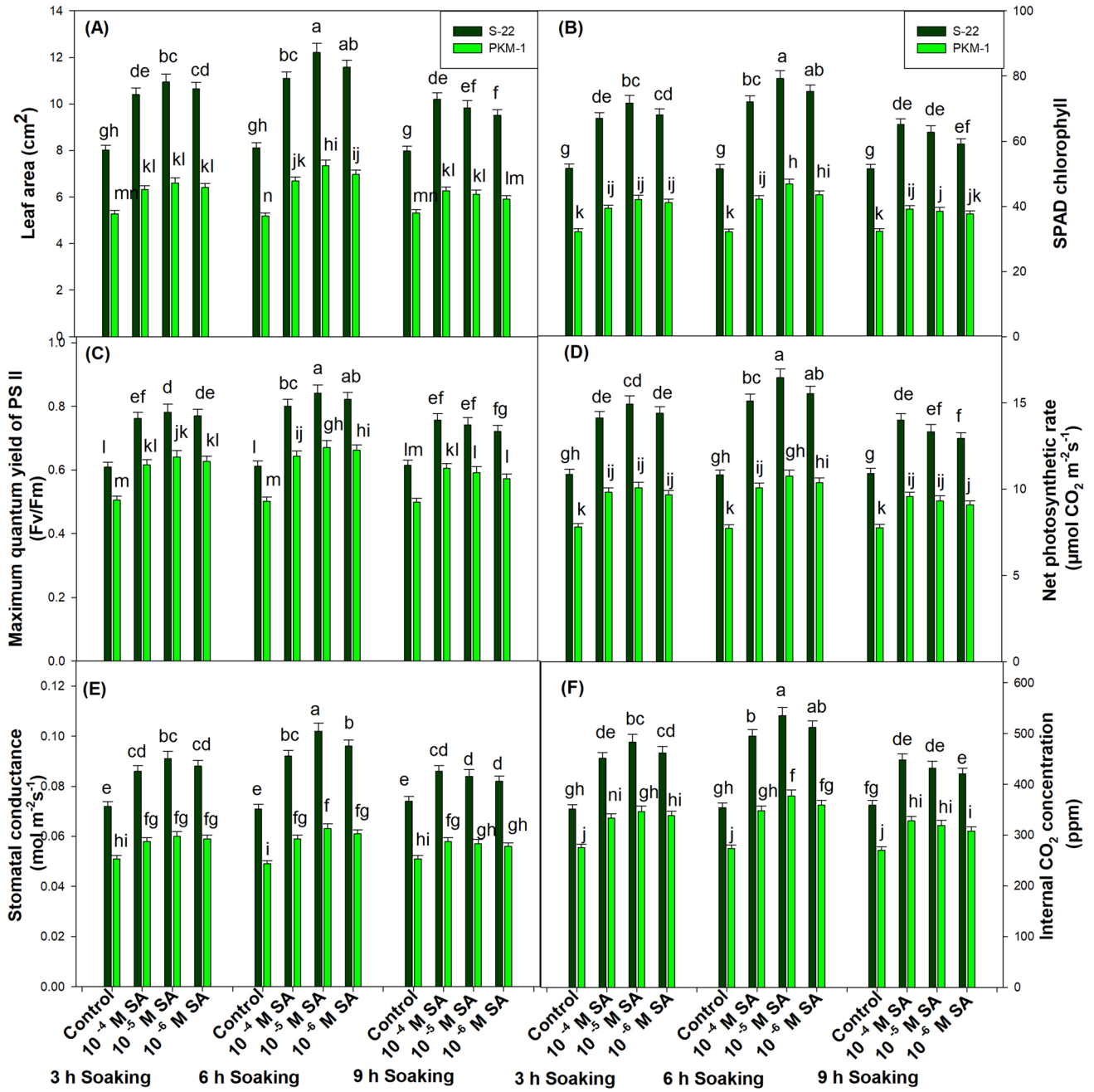


Fig. 2 Effect of SA through seed soaking on **A** leaf area, **B** SPAD Chlorophyll content, **C** maximum quantum yield of PSII (Fv/Fm), **D** net photosynthetic rate (PN), **E** stomatal conductance (gs) and **F** internal CO_2 concentration (Ci) in two different varieties of *Solanum lycopersicum* L. plants at 40 DAT. All the data are the means of five replicates ($n=5$); vertical bars show standard errors (±SE). Means with different letters above the bars are significantly different at $P \leq 0.05$

in all growth, physiological and biochemical attributes. SA enhanced the growth and photosynthesis in both varieties but the response was different in both varieties, as depicted in NMDS diagram that mean value of various parameters of both varieties clustered separately as shown in Fig. 9 cluster a (S-22 variety; black colour) and cluster b (PKM-1 variety; blue colour). Variety S-22 was more responsive to exogenous treatment of SA than PKM-1 variety.

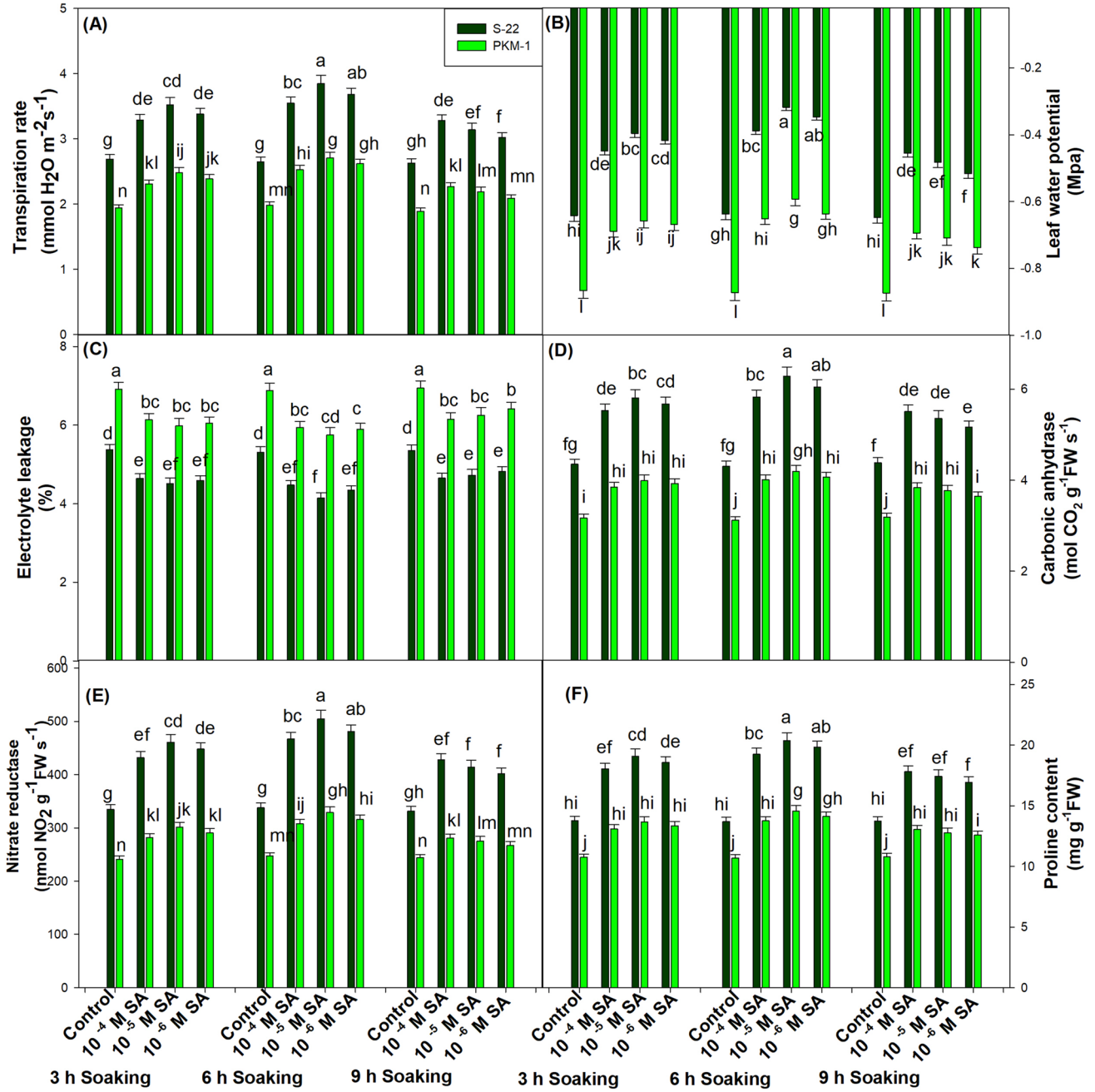


Fig. 3 Effect of SA through seed soaking on **A** transpiration rate, **B** leaf water potential, **C** electrolyte leakage, **D** carbonic anhydrase, **E** nitrate reductase and **F** proline content in two different varieties of *S. lycopersicum* L. plants at 40 DAT. All the data are the means of five replicates ($n=5$); vertical bars show standard errors ($\pm$SE). Means with different letters above the bars are significantly different at $P \leq 0.05$

Discussion

In the current study, the potentiality of PGR, SA through the exogenous application by seed soaking was assessed, and it was hypothesized to enhance the morphological and physio-biochemical traits of tomato plants in normal condition. Growth of tomato plants, i.e., the length of shoot and root, fresh and dry weight of shoot and root and leaf area were increased by SA treatment as depicted in Figs. 1 and 2A. Tayyab et al. (2020) and Mohammed (2020) revealed that SA applied exogenously by seed soaking improved the growth biomarkers like dry weight of root and shoot, plant height and leaf area in maize and oat plants. Beside this, SA also promoted the morphological attributes in various plants like

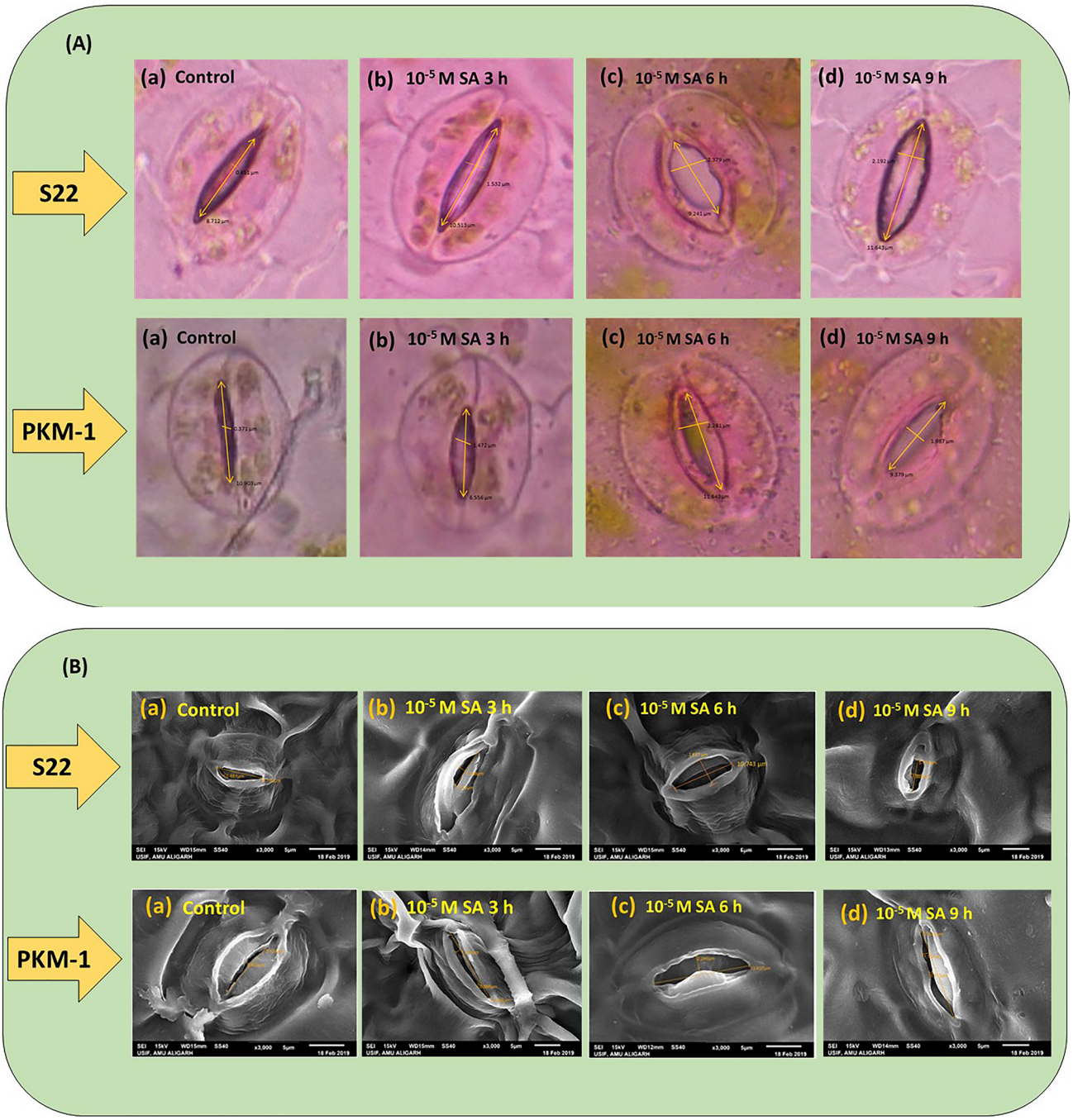


Fig. 4 Stomatal response of two different varieties of *S. lycopersicum* L. plants at 40 DAT under control (a), 10^{-5} M of SA soaking for 3 h (b), 10^{-5} M of SA soaking for 6 h (c) 10^{-5} M of SA soaking for 9 h (d), at 40×using compound microscope (**A**) and at 3000×using scanning electron microscope (**B**)

mustard, tomato, soybean, wheat, alfalfa and violet plants in a concentration-dependent manner (Gutiérrez-Coronado et al. 1998; Fariduddin et al. 2003, 2018; Shakirova et al. 2003; Hayat et al. 2005; Yusuf et al. 2012; Parashar et al. 2014; Boukari et al. 2019; El-Mergawi and Abd El-Wahed 2020). Our results were analogous with the findings of Rady and Mohamed (2015) and Kaydan et al. (2007) in which it was exposed that soaking of seeds with SA enhanced growth traits like shoot length, number and leaf area per plant, and plant dry mass in common bean and wheat. The possible reason for this increase in the morphological characters could be a due increase in cell division, photosynthetic attributes and nutrient uptake efficiency by exogenous application of SA (Zhu 2001; El-Tayeb 2005; Pacheco et al.

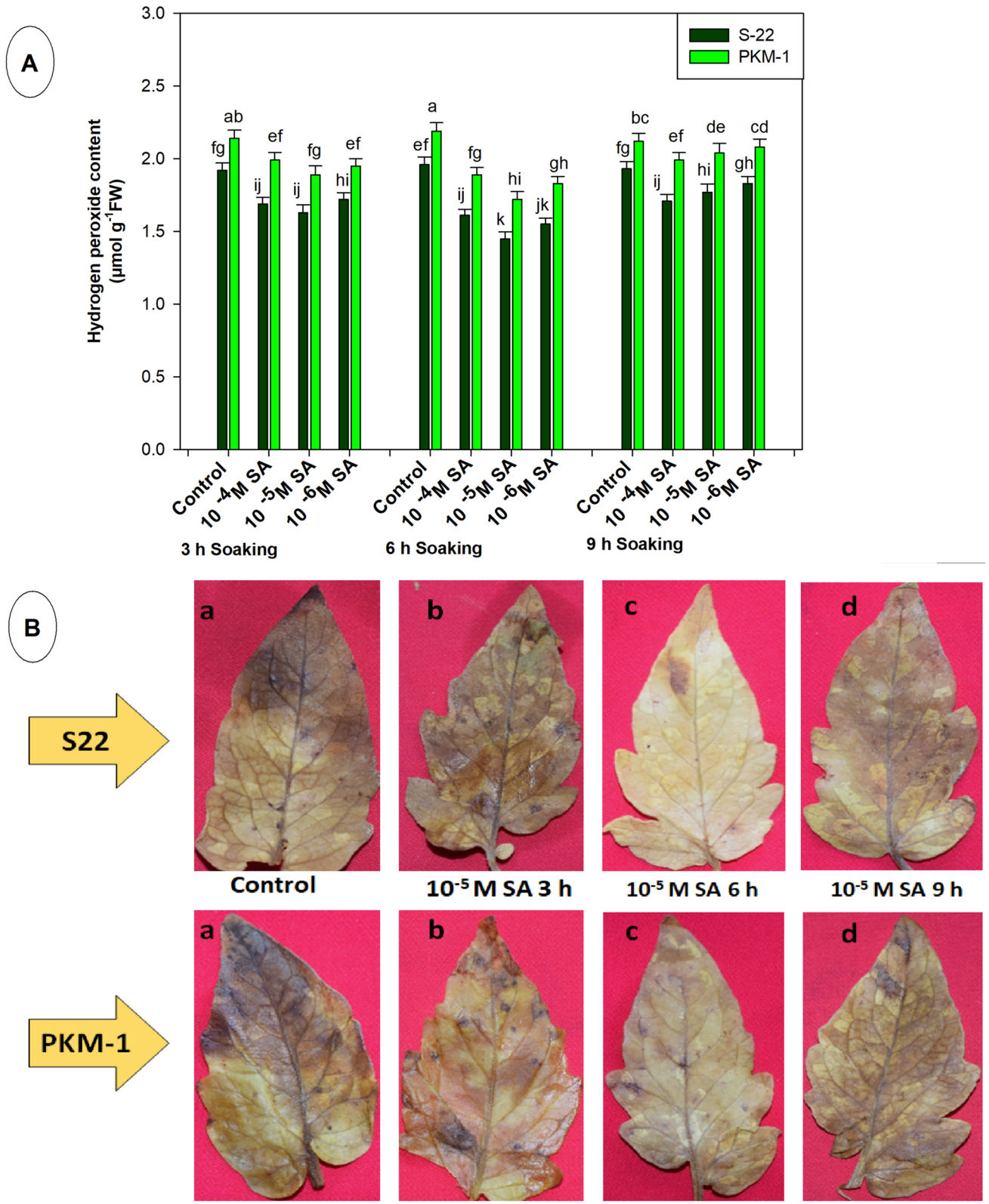


Fig. 5 Effect of SA through seed soaking on **A** hydrogen peroxide content and **B** histochemical detection of hydrogen peroxide in leaves by DAB staining at 40 DAT in different varieties of *S. lycopersicum* L. plants under control (a), 10^{-5} M of SA soaking for 3 h (b), 10^{-5} M of SA soaking for 6 h (c) 10^{-5} M of SA soaking for 9 h (d). All the data are the means of five replicates ($n=5$); vertical bars show standard errors (±SE). Means with different letters above the bars are significantly different at $P \leq 0.05$

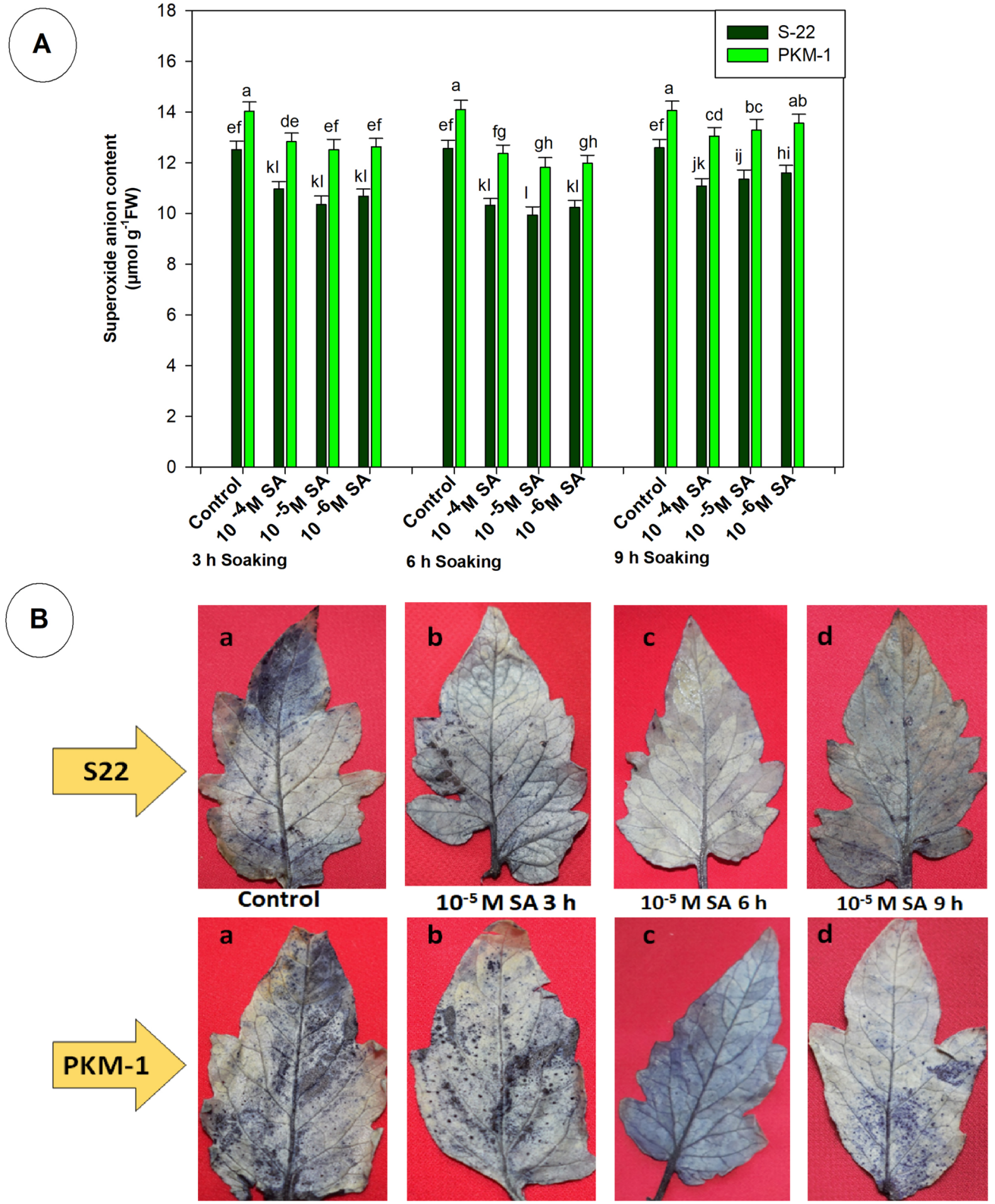


Fig. 6 Effect of SA through seed soaking on **A** superoxide anion content and **B** histochemical detection of superoxide anion content in leaves by NBT staining at 40 DAT in different varieties of *S. lycopersicum* L. plants under control (a), 10^{-5} M of SA soaking for 3 h (b), 10^{-5} M of SA soaking for 6 h (c) 10^{-5} M of SA soaking for 9 h (d). All the data are the means of five replicates ($n=5$); vertical bars show standard errors (±SE). Means with different letters above the bars are significantly different at $P \leq 0.05$

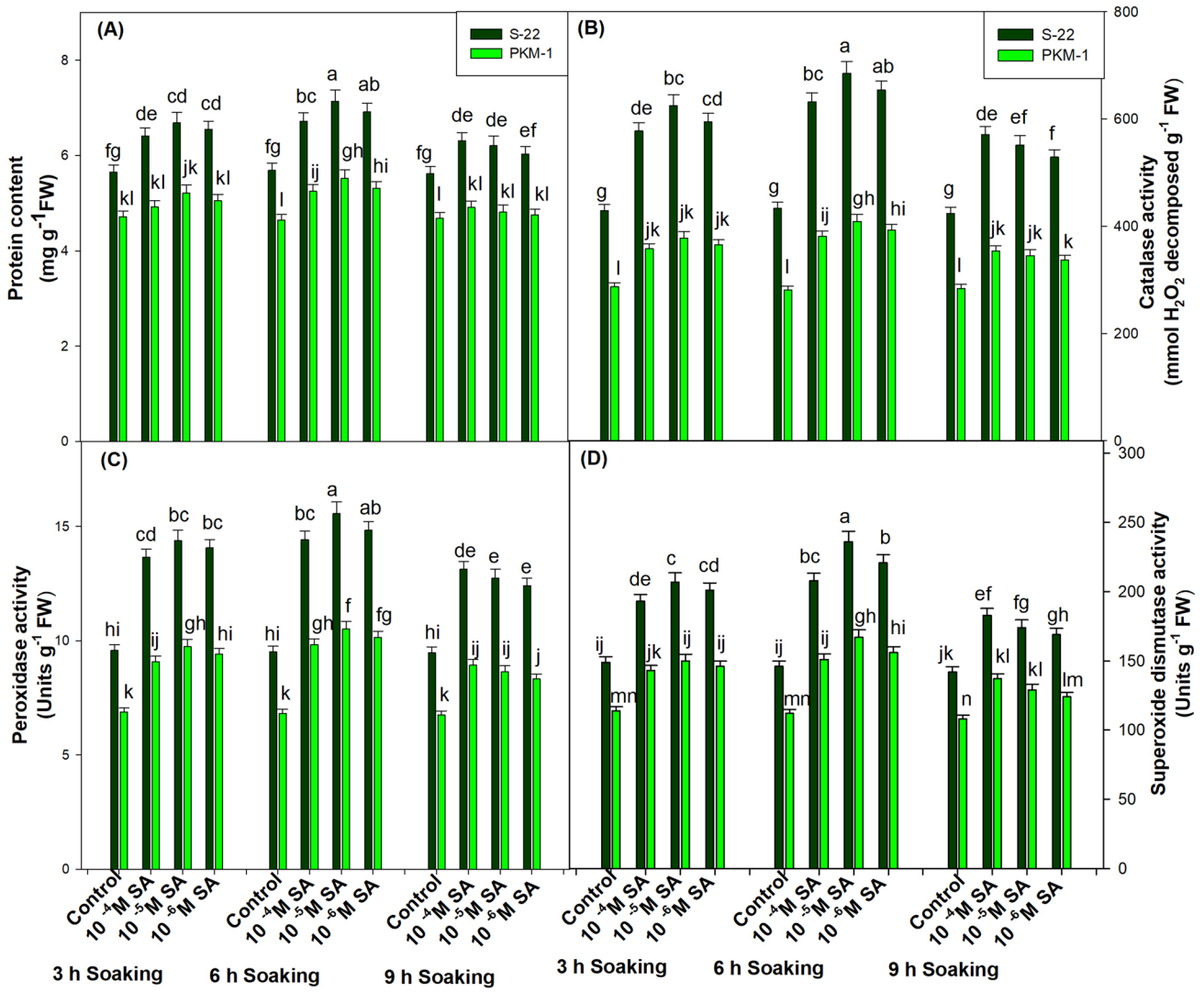


Fig. 7 Effect of SA through seed soaking on **A** protein content, **B** catalase, **C** peroxidase and **D** superoxide dismutase in two different varieties of *S. lycopersicum* L. plants at 40 DAT. All the data are the means of five replicates ($n=5$); vertical bars show standard errors (±SE). Means with different letters above the bars are significantly different at $P \leq 0.05$

2013; Rady and Mohamed 2015; Pradhan et al. 2016; Jelali et al. 2021).

The photosynthetic attributes like SPAD value, Fv/Fm (quantum yield of PSII), gas exchange parameters, stomatal behaviour were increased along with the decrease in the EL and increase in LWP by SA supplementation through seed soaking as depicted in Figs. 2, 3A–C and 4. Our results are in agreement with the findings of Kohli et al. (2018) in *Brassica juncea*, Boukari et al. (2019) in alfalfa and Jelali et al. (2021) in *Sulla carnosa* plants. It is revealed that SA increased the rate of photosynthesis by accretion of α-ALA (α-aminolevulinic acid) a close precursor molecule of biosynthetic pathway of chlorophyll (Kumar et al. 2010). Potentiality of SA in enhancing the content of chlorophyll pigments might be due to its positive impact on leaf and chloroplast organization, stomatal behaviour, activation of ATPase pump that facilitates the iron uptake, and ability to inhibit the chlorophyll degrading enzyme chlorophyllase by decreasing the expression of *CHLASE* gene (Uzunova and Popova 2000; Kong et al. 2014; Kohli et al. 2018). The increased morpho-physiological traits including gas exchange attributes and ionic uptake were enhanced by SA seed priming in *Medicago sativa* which are in accordance with our findings (Boukari et al. 2019). SA possibly maintains the stability and functionality of PSII reaction centers by increasing the expression of D1, D2 and LHC protein, and hence oxygen evolving complex (oec) (Luo et al. 2009; Wang et al. 2010). SA is recognized as a vital regulator of photosynthesis as it increases photosynthesis, water relations and other related attributes (Uzunova and

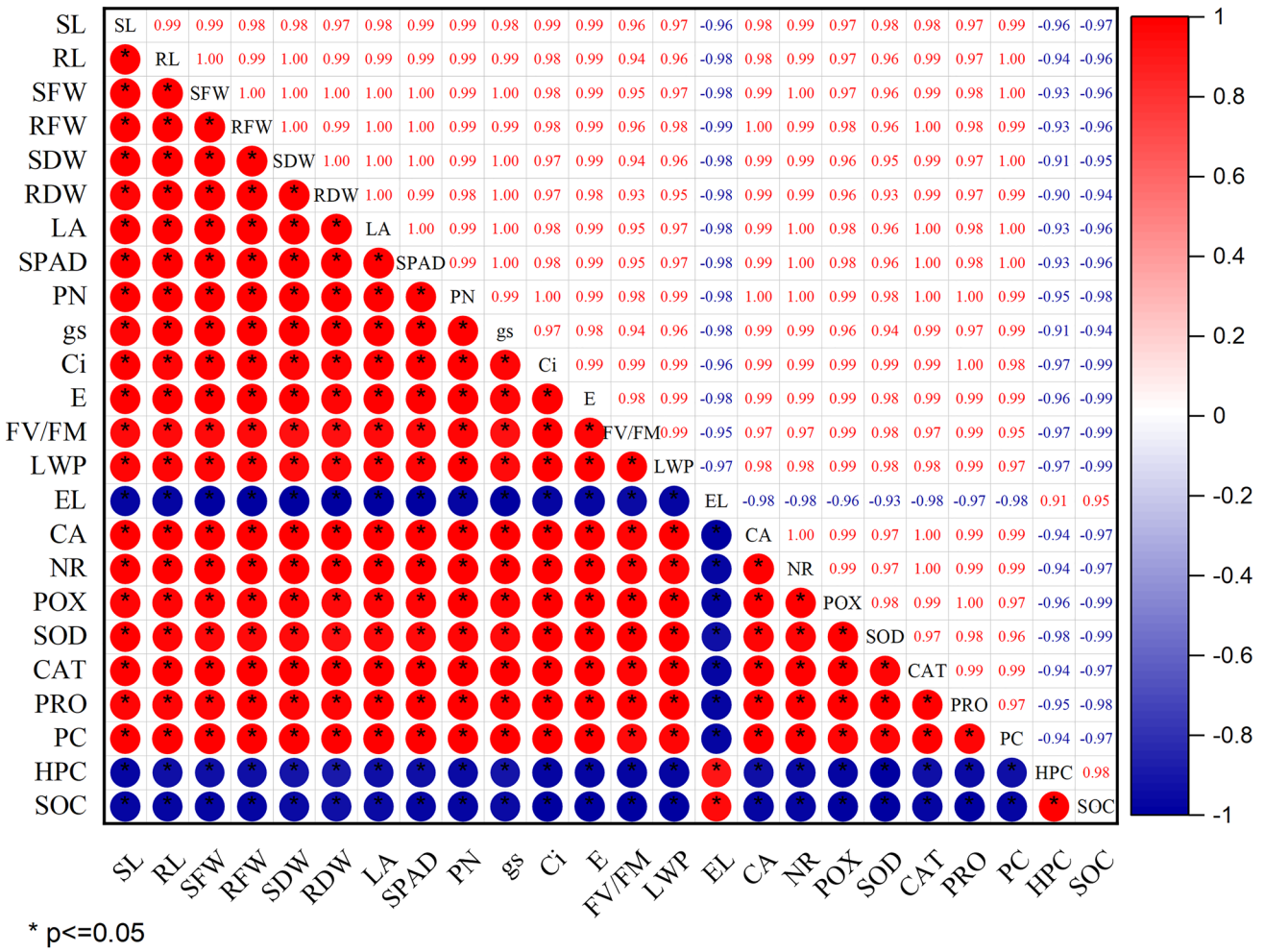


Fig. 8 Pearson's correlation matrix of two different varieties of tomato at 40 DAT S-22 and PKM-1 are depicted. It portrays a significant positive correlation (red colour) and negative correlation (blue colour). All parameters except electrolyte leakage, hydrogen peroxide content and superoxide anion content shows positive correlation. Various abbreviations used are SL (shoot length), RL (root length), SFW (shoot fresh weight), RFW (root fresh weight), SDW (shoot dry weight), RDW (root dry weight), LA (leaf area), SPAD (chlorophyll content), PN (net photosynthetic rate), Ci (internal CO_2 concentration), gs (stomatal conductance), E (transpiration rate), FV/FM (represents the maximum potential quantum efficiency of Photosystem II), LWP (leaf water potential), EL (electrolyte leakage), CA (carbonic anhydrase), NR (nitrate reductase), POX (peroxidase), SOD (superoxide dismutase), CAT (catalase), PRO (proline content), HPC (hydrogen peroxide content), SOC (superoxide anion content) and PC (protein content)

Popova 2000; Ashraf et al. 2010; Parashar et al. 2014; Janda et al. 2014; Fariduddin et al. 2018; Singh et al. 2021). An increase in the photosynthesis by SA could be probably due to its ability to control the Rubisco enzyme and PSII activity (Pacheco et al. 2013; Alam et al. 2022). Study of Krantev et al. (2008), Shao et al. (2018) and Sharma et al. (2018) revealed that SA pre-treatment increases the efficiency of photosynthesis by enhancing the activities of regulatory enzymes of CO_2 assimilation like Rubisco, Rubisco activase, CA, phosphoenol pyruvate carboxylase (PEPC) and various transcription factors (*Rbc L, ZmRCAβ mRNA* and *ZmRCAα*) involved in photosynthesis.

NR and CA activity was increased by supplementation of SA exogenously as depicted in Fig. 3D–E. Our results were analogous with the findings of Khalil et al. (2021) in *Phaselous vulgaris*, Aftab et al. (2011) in *Artemesia*; Zanganeh et al. (2019) in *Zea mays*. The main reason for elevation in NR activity by SA could be due to enhancement in the activity of H^+-ATPase pump associated with plasma membrane, membrane stability index thereby facilitating the nutrient uptake particularly NO_3, which act as the stimulator for NR activity (Campbell 1999; Agarwal et al.2005; Dong et al. 2016; Khalil et al. 2021). Our present outcomes are concurrent with the study of Hayat et al. (2005), Yusuf et al. (2008), Idrees et al. (2012) and Fariduddin et al. (2018). In addition soaking of seeds with SA improved the growth of *Z. mays* L. and *P. vulgaris* plants by increasing CO_2 fixation, activity of Rubisco, PEPC, photosynthetic pigments, content

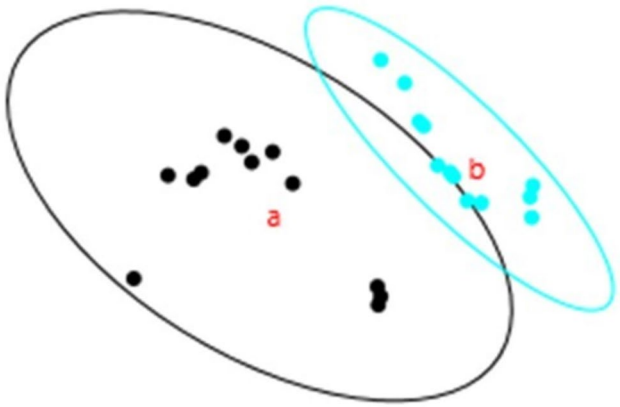


Fig. 9 Non-metric multidimensional scaling ordination diagram (NMDS with Bray–Curtis similarity measure) of two different varieties of tomato which assembled separately in two clusters (a) S-22 and (b) PKM-1 and it signifies the differential response of both varieties of tomato to SA at 40 DAT

of nitrogen and carbohydrate, along with increased activities of CA and NR (Gunes et al. 2005; Krantev et al. 2008; Khalil et al. 2021).

In the current study, SA has been revealed to enhance the activities of antioxidant enzymes like CAT, SOD and POX and proline content while decreasing the H_2O_2 and superoxide anion content in the plants (Fig. 7). SA enhanced the activities of SOD, glutathione reductase (GR), CAT and ascorbate peroxidase (APX) as reported in soybean plants by Chen et al. (2016) and Sharma et al (2018). In present study, SA treatment decreased the hydrogen peroxide and superoxide anion content (Figs. 5, 6) which improved the growth and photosynthetic potential of plant by regulating balance between ROS and antioxidant enzymes (Mutlu et al. 2016; Tayyab et al. 2020). Enhancement in the activities of antioxidant enzymes by pre-treatment of seeds with SA might be due to regulation of amino acid content and hence the proteins of ascorbate and glutathione cycle (Zanganeh et al. 2019). Besides this, elevation in antioxidative enzymes could be due to accrual of osmolytes (proline, sugar alcohol, etc.) and enhanced activity of aldose reductase. SA is known to reduce the oxidative burst by increasing the antioxidant enzymatic activities and accumulation of osmolytes (Kazemi et al. 2010; Agami 2013; Hussain et al. 2016; Faghih et al. 2017). Application of SA by exogenous mode decreases the accrual of ROS and maintains the homeostasis of plant, and analogous results were observed in several plants like *Artemisia anua* L., mustard and wheat (Singh and Usha 2003; Fariduddin et al. 2003; Aftab et al. 2010; Yadu et al. 2017; Dutra et al. 2017). Moreover, SA increase the tolerance against stress by enhancing the antioxidant machinery (Mutlu et al. 2009; Li et al. 2014; Anaya et al. 2017; El-Esawi et al. 2017; Fariduddin et al. 2018; Saleem et al. 2021a). In addition, it was reported by Briache et al. (2020) in faba bean that out of three different modes (foliar spray; FS, seed soaking; SS and combined; FS + SS) of SA application, seed soaking in SA induced the maximum resistance against *Orobanche crenata* a parasitic weed as compared to other modes of SA application or untreated control plants.

Apart from inducing antioxidant machinery, we also observed that the content of proline and protein was increased by exogenous treatment of SA. The interaction of SA with other hormones could contribute to the enhancement of protein content and other osmolytes (Song et al. 2011; Anosheh et al. 2014). Proline, stress amino acid maintains the osmoticum of the plant cell and function as molecular chaperons which protect the protein integrity and activate various vital enzymes essential for sustenance of plant life (Hong et al. 2000; Anosheh et al. 2014; Naliwajski and Skłodowska 2014). As proline acts as an osmolyte which acts together with other proteins and enzymes of the cell and helps in upregulation, activation and stabilization of other proteins (Efimova et al. 2014; Fariduddin et al. 2018). Accumulation of proline was observed in various plants by SA application, and this increased level of proline could be due to the regulation of amino acid content or inhibition of hydrolyzing enzymes thereby causing accrual of osmolytes (Agami 2013; Anosheh et al. 2014; Fariduddin et al. 2018). The soaking of common bean seeds in SA increased the overall growth by enhancing membrane stability index, relative water content (RWC), osmolytes concentrations, and activity of antioxidant enzymes, along with decreased EL and peroxidation of lipids (Sadeghipour and Aghaei 2012; Semida and Rady 2014).

Conclusion

Finally, our results signify that the exogenous application of SA increased the growth, photosynthesis, stomatal physiology, water balance and chlorophyll content. Further the performance of plant is enhanced by SA via increasing activity of antioxidant enzymes, CA, NR, proline content, total protein content and decreasing the ROS level. Therefore, the pre-treatment of SA via seed soaking improves the morphological and physio-biochemical attributes with respect to control in two varieties of tomato plants. However, out of two different varieties of tomato and different concentrations and durations of seed soaking the supplementation of S-22 variety with 10^{-5} M of SA for 6 h generated promising impact as compared to all the concentrations tested. Response was more prominent in S-22 than PKM-1 variety. Therefore, in conclusion, SA improved the growth of tomato plants by controlling

various morphological and physio-biochemical attributes. SA application through seed soaking could be a promising technique for crop improvement and these outcomes would be vital for understanding the molecular and physiological mechanisms associated with performance of plants in changing environment.

Author contribution statement Study conception, design and data collection, analysis and interpretation of manuscript, draft manuscript preparation by MS; overall supervision by QF. Both authors reviewed and approved the final version of the manuscript.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11738-023-03639-z.

Acknowledgements M. Saleem and co gratefully acknowledges the financial support in the form of Senior Research Fellowship rendered by CSIR UGC, New Delhi Ref. NO. 677/ (CSIR-UGC NET JUNE 2018) at Aligarh Muslim University, Aligarh, India. We are also grateful to University Sophisticated Instrumentation Facility (USIF) A.M.U., Aligarh for carrying out SEM analysis.

Data availability The authors declare that the data supporting the findings of this study are made available on request in supplementary file 2.

Declarations

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

References

Aebi H (1984) Catalase in vitro. Methods Enzymol 105:121–126. https://doi.org/10.1016/S0076-6879(84)05016-3

Aftab T, Masroor M, Khan A, Idrees M, Naeem M (2010) Salicylic acid acts as potent enhancer of growth, photosynthesis and artemisinin production in *Artemisia annua* L. J Crop Sci Biotechnol 13:183–188. https://doi.org/10.1007/s12892-010-0040-3

Aftab T, Khan MM, da Silva JA, Idrees M, Naeem M (2011) Role of salicylic acid in promoting salt stress tolerance and enhanced artemisinin production in *Artemisia annua* L. J Plant Growth Regul 30:425–435. https://doi.org/10.1007/s00344-011-9205-0

Agami RA (2013) Alleviating the adverse effects of NaCl stress in maize seedlings by pretreating seeds with salicylic acid and 24-epibrassinolide. S Afr J Bot 88:171–177. https://doi.org/10.1016/j.sajb.2013.07.019

Agarwal S, Sairam RK, Srivastava GC, Tyagi A, Meena RC (2005) Role of ABA, salicylic acid, calcium and hydrogen peroxide on antioxidant enzymes induction in wheat seedlings. Plant Sci 169:559–570. https://doi.org/10.1016/j.plantsci.2005.05.004

Ahmad F, Kamal A, Singh A, Ashfaque F, Alamri S, Siddiqui MH (2020) Salicylic acid modulates antioxidant system, defense metabolites, and expression of salt transporter genes in *Pisum sativum* under salinity stress. J Plant Growth Regul 24:1–4. https://doi.org/10.1007/s00344-020-10271-5

Alam P, Balawi TA, Faizan M (2022) Salicylic acid's impact on growth, photosynthesis, and antioxidant enzyme activity of *Triticum aestivum* when exposed to salt. Molecules 28:100

Anaya F, Fghire R, Wahbi S, Loutfi K (2017) Antioxidant enzymes and physiological traits of *Vicia faba* L. as affected by salicylic acid under salt stress. J Mater Environ Sci 8:2549–2563

Anosheh HP, Ranjbar G, Emam Y, Ashraf M (2014) Salicylic-acid-induced recovery ability in salt-stressed *Hordeum vulgare* plants. Turkish J Bot 38(112):121

Arfan M, Athar HR, Ashraf M (2007) Does exogenous application of salicylic acid through the rooting medium modulate growth and photosynthetic capacity in two differently adapted spring wheat cultivars under salt stress? J Plant Physiol 164:685–694. https://doi.org/10.1016/j.jplph.2006.05.010

Ashraf M, Akram NA, Arteca RN, Foolad MR (2010) The physiological, biochemical and molecular roles of brassinosteroids and salicylic acid in plant processes and salt tolerance. Crit Rev Plant Sci 29:162–190. https://doi.org/10.1080/07352689.2010.483580

Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water-stress studies. Plant Soil 39:205–207. https://doi.org/10.1007/BF00018060

Beauchamp C, Fridovich I (1971) Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal Biochem 44:276–287. https://doi.org/10.1016/0003-2697(71)90370-8

Boukari N, Jelali N, Renaud JB, Youssef RB, Abdelly C, Hannoufa A (2019) Salicylic acid seed priming improves tolerance to salinity, iron deficiency and their combined effect in two ecotypes of Alfalfa. Environ Exp Bot 167:103820. https://doi.org/10.1016/j.envexpbot.2019.103820

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254. https://doi.org/10.1016/0003-2697(76)90527-3

Briache FZ, Ennami M, Mbasani-Mansi J, Lozzi A, Abousalim A, El Rodeny W, Mentag R (2020) Effects of salicylic acid and indole acetic acid exogenous applications on induction of faba bean resistance against *Orobanche crenata*. Plant Pathol J 36:476

Campbell WH (1999) Nitrate reductase structure, function and regulation: bridging the gap between biochemistry and physiology. Ann Rev Plant Biol 50:277–303. https://doi.org/10.1146/annurev.arplant.50.1.277

Chen Z, Zheng Z, Huang J, Lai Z, Fan B (2009) Biosynthesis of salicylic acid in plants. Plant Signal Behav 4:493–496. https://doi.org/10.4161/psb.4.6.8392

Chen YE, Cui JM, Li GX, Yuan M, Zhang ZW, Yuan S, Zhang HY (2016) Effect of salicylic acid on the antioxidant system and photosystem II in wheat seedlings. Biol Plant 60:139–147. https://doi.org/10.1007/s10535-015-0564-4

Dong Y, Chen W, Liu F, Wan Y (2016) Effects of exogenous salicylic acid and nitric oxide on peanut seedlings growth under iron deficiency. Commun Soil Sci Plant Anal 47:2490–2505. https://doi.org/10.1080/00103624.2016.1254790

Dutra WF, Soares de Melo A, Suassuna JF, Dutra AF, Chagas da Silva D, Maia JM (2017) Antioxidative responses of cowpea cultivars to water deficit and salicylic acid treatment. Agron J 109:895–905. https://doi.org/10.2134/agronj2015.0519

Dwivedi RS, Randhawa NS (1974) Evaluation of a rapid test for the hidden hunger of zinc in plants. Plant Soil 40:445–451. https://doi.org/10.1007/BF00011531

Efimova MV, Savchuk AL, Hasan JA, Litvinovskaya RP, Khripach VA, Kholodova VP, Kuznetsov VV (2014) Physiological mechanisms of enhancing salt tolerance of oilseed rape plants

with brassinosteroids. Russ J Plant Physiol 61:733–743. https://doi.org/10.1134/S1021443714060053
El-Esawi MA, Elansary HO, El-Shanhorey NA, Abdel-Hamid AM, Ali HM, Elshikh MS (2017) Salicylic acid-regulated antioxidant mechanisms and gene expression enhance rosemary performance under saline conditions. Front Physiol 8:716. https://doi.org/10.3389/fphys.2017.00716
El-Mergawi RA, Abd El-Wahed MS (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. https://doi.org/10.1186/s42269-020-00416-7
El-Tayeb MA (2005) Response of barley grains to the interactive e. ect of salinity and salicylic acid. Plant Growth Regul 45:215–224. https://doi.org/10.1007/s10725-005-4928-1
Faghih S, Ghobadi C, Zarei A (2017) Response of strawberry plant cv. '*Camarosa*' to salicylic acid and methyl jasmonate application under salt stress condition. J Plant Growth Regul 36:651–659. https://doi.org/10.1007/s00344-017-9666-x
Fariduddin Q, Hayat S, Ahmad A (2003) Salicylic acid influences net photosynthetic rate, carboxylation efficiency, nitrate reductase activity, and seed yield in *Brassica juncea*. Photosynthetica 41:281–284. https://doi.org/10.1023/B:PHOT.0000011962.05991.6c
Fariduddin Q, Khan TA, Yusuf M, Aafaqee ST, Khalil RR (2018) Ameliorative role of salicylic acid and spermidine in the presence of excess salt in *Lycopersicon esculentum*. Photosynthetica 56:750–762. https://doi.org/10.1007/s11099-017-0727-y
Farooq M, Usman M, Nadeem F, ur Rehman H, Wahid A, Basra SM, Siddique KH (2019) Seed priming in field crops: potential benefits, adoption and challenges. Crop Pasture Sci 70:731–771. https://doi.org/10.1071/CP18604
Food Summit FAO (2009) Declaration of the world summit on food security. World Food Summit 16–18
Gul F, Arfan M, Shahbaz M, Basra S (2020) Salicylic acid seed priming modulates morphology, nutrient relations and photosynthetic attributes of wheat grown under cadmium stress. Int J Agric Biol 23:197–204. https://doi.org/10.17957/IJAB/15.1277
Gunes A, Inal A, Alpaslan M, Cicek N, Guneri E, Eraslan F, Guzelordu T (2005) Effects of exogenously applied salicylic acid on the induction of multiple stress tolerance and mineral nutrition in maize (*Zea mays* L.) (Einfluss einer Salicylsäure-Applikation auf die Induktion von Stresstoleranz sowie Nährstoffaufnahme von Mais [*Zea mays* L.]). Arch Agron Soil Sci 51:687–695
Gutiérrez-Coronado MA, Trejo-López C, Larqué-Saavedra A (1998) Effects of salicylic acid on the growth of roots and shoots in soybean. Plant Physiol Biochem 36:563–565. https://doi.org/10.1016/S0981-9428(98)80003-X
Hayat S, Fariduddin Q, Ali B, Ahmad A (2005) Effect of salicylic acid on growth and enzyme activities of wheat seedlings. Acta Agron Hung 53:433–437. https://doi.org/10.1556/AAgr.53.2005.4.9
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. https://doi.org/10.1016/j.envexpbot.2009.08.005
Hayat Q, Hayat S, Alyemeni MN, Ahmad A (2012) Salicylic acid mediated changes in growth, photosynthesis, nitrogen metabolism and antioxidant defense system in *Cicer arietinum* L. Plant Soil Environ 58:417–423. https://doi.org/10.17221/232/2012-PSE
Hong Z, Lakkineni K, Zhang Z, Verma DP (2000) Removal of feedback inhibition of Δ1-pyrroline-5-carboxylate synthetase results in increased proline accumulation and protection of plants from osmotic stress. Plant Physiol 122:1129–1136. https://doi.org/10.1104/pp.122.4.1129
Hussain S, Khan F, Hussain HA, Nie L (2016) Physiological and biochemical mechanisms of seed priming-induced chilling tolerance in rice cultivars. Front Plant Sci 7:116. https://doi.org/10.3389/fpls.2016.00116
Idrees M, Naeem M, Khan MN, Aftab T, Khan MM (2012) Alleviation of salt stress in lemongrass by salicylic acid. Protoplasma 249:709–720. https://doi.org/10.1007/s00709-011-0314-1
Janda T, Gondor OK, Yordanova R, Szalai G, Pál M (2014) Salicylic acid and photosynthesis: signalling and effects. Acta Physiol Plant 36:2537–2546. https://doi.org/10.1007/s11738-014-1620-y
Jaworski EG (1971) Nitrate reductase assay in intact plant tissues. Biochem Biophys Res Commun 43:1274–1279. https://doi.org/10.1016/s0006-291x(71)80010-4
Jelali N, Youssef RB, Boukari N, Zorrig W, Dhifi W, Abdelly C (2021) Salicylic acid and H_2O_2 seed priming alleviates Fe deficiency through the modulation of growth, root acidification capacity and photosynthetic performance in *Sulla carnosa*. Plant Physiol Biochem 159:392–399. https://doi.org/10.1016/j.plaphy.2020.11.039
Kaur N, Sharma I, Kirat K, Pati PK (2016) Detection of reactive oxygen species in *Oryza sativa* L. (Rice). Bio Protoc 6:2061. https://doi.org/10.21769/Bio-Protoc.2061
Kaydan D, Yagmur M, Okut N (2007) Effects of salicylic acid on the growth and some physiological characters in salt stressed wheat (*Triticum aestivum* L.). Tarim Bilimleri Dergisi 13:114–119
Kazemi N, Khavari-Nejad RA, Fahimi H, Saadatmand S, Nejad-Sattari T (2010) Effects of exogenous salicylic acid and nitric oxide on lipid peroxidation and antioxidant enzyme activities in leaves of *Brassica napus* L. under nickel stress. Sci Hort 126:402–407. https://doi.org/10.1016/j.scienta.2010.07.037
Khalil R, Haroun S, Bassyoini F, Nagah A, Yusuf M (2021) Salicylic acid in combination with kinetin or calcium ameliorates heavy metal stress in *Phaseolus vulgaris* plant. J Agri Food Res 5:100182
Khan TA, Fariduddin Q, Nazir F, Saleem M (2020) Melatonin in business with abiotic stresses in plants. Physiol Mol Biol Plants 14:1–4. https://doi.org/10.1007/s12298-020-00878-z
Khan TA, Saleem M, Fariduddin Q (2022) Melatonin influences stomatal behavior, root morphology, cell viability, photosynthetic responses, fruit yield, and fruit quality of tomato plants exposed to salt stress. J Plant Growth Regul 25:1–25. https://doi.org/10.1007/s00344-022-10713-2
Khodary SEA (2004) Effect of salicylic acid on the growth, photosynthesis and carbohydrate metabolism in salt stressed maize plants. Int J Agric Biol 6:5–8. http://www.ijab.org
Kohli SK, Handa N, Sharma A, Gautam V, Arora S, Bhardwaj R, Wijaya L, Alyemeni MN, Ahmad P (2018) Interaction of 24-epibrassinolide and salicylic acid regulates pigment contents, antioxidative defense responses, and gene expression in *Brassica juncea* L. seedlings under Pb stress. Environ Sci Pollut Res 25:15159–15173. https://doi.org/10.1007/s11356-018-1742-7
Kong J, Dong Y, Xu L, Liu S, Bai X (2014) Effects of foliar application of salicylic acid and nitric oxide in alleviating iron deficiency induced chlorosis of *Arachis hypogaea* L. Bot Stud 55:1–2. https://doi.org/10.1186/1999-3110-55-9
Krantev A, Yordanova R, Janda T, Szalai G, Popova L (2008) Treatment with salicylic acid decreases the effect of cadmium on photosynthesis in maize plants. J Plant Physiol 165:920–931. https://doi.org/10.1016/j.jplph.2006.11.014
Kumar P, Tewari RK, Sharma PN (2010) Sodium nitroprusside-mediated alleviation of iron deficiency and modulation of antioxidant responses in maize plants. AoB Plants 2010:plq002. https://doi.org/10.1093/aobpla/plq002
Li T, Hu Y, Du X, Tang H, Shen C, Wu 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. https://doi.org/10.1371/journal.pone.0109492
Luo MH, Yuan S, Chen YE, Liu WJ, Du JB, Lei T, Wang MB, Lin HH (2009) Effects of salicylic acid on the photosystem 2 of barley

seedlings under osmotic stress. Biol Plant 53:663. https://doi.org/10.1007/s10535-009-0120-1
Manaa A, Gharbi E, Mimouni H, Wasti S, Aschi-Smiti S, Lutts S, Ahmed HB (2014) Simultaneous application of salicylic acid and calcium improves salt tolerance in two contrasting tomato (*Solanum lycopersicum*) cultivars. South African J Bot 95:32–39. https://doi.org/10.1016/j.sajb.2014.07.015
Mir AR, Siddiqui H, Alam P, Hayat S (2020a) Foliar spray of Auxin/IAA modulates photosynthesis, elemental composition, ROS localization and antioxidant machinery to promote growth of *Brassica juncea*. Physiol Mol Biol Plants 26:2503–2520. https://doi.org/10.1007/s12298-020-00914-y
Mir AR, Siddiqui H, Alam P, Hayat S (2020b) Melatonin modulates photosynthesis, redox status, and elemental composition to promote growth of *Brassica juncea*—a dose-dependent effect. Protoplasma 257:1685–1700. https://doi.org/10.1007/s00709-020-01537-6
Mohammed LS (2020) Effect of seed stimulation and spraying of salicylic acid in germination, growth and yield of oats (*Avena sativa* l.) in gypsum soils. Plant Arch 20:777–781
Mutlu SA, Atici Ö, Nalbantoglu B (2009) Effects of salicylic acid and salinity on apoplastic antioxidant enzymes in two wheat cultivars differing in salt tolerance. Biol Plant 53:334–338. https://doi.org/10.1007/s10535-009-0061-8
Mutlu S, Atıcı Ö, Nalbantoğlu B, Mete E (2016) Exogenous salicylic acid alleviates cold damage by regulating antioxidative system in two barley (*Hordeum vulgare* L.) cultivars. Front Life Sci 9:99–109. https://doi.org/10.1080/21553769.2015.1115430
Naliwajski MR, Skłodowska M (2014) The oxidative stress and antioxidant systems in cucumber cells during acclimation to salinity. Biol Plant 58:47–54. https://doi.org/10.1007/s10535-013-0378-1
Pacheco AC, da Silva CC, da Silva Fermino ES, Aleman CC (2013) Salicylic acid-induced changes to growth, flowering and flavonoids production in marigold plants. J Med Plants Res 7:3158–3163. https://doi.org/10.5897/JMPR2013.5208
Parashar A, Yusuf M, Fariduddin Q, Ahmad A (2014) Salicylic acid enhances antioxidant system in *Brassica juncea* grown under different levels of manganese. Int J Biol Macromol 70:551–558. https://doi.org/10.1016/j.ijbiomac.2014.07.014
Patterson BD, MacRae EA, Ferguson IB (1984) Estimation of hydrogen peroxide in plant extracts using titanium (IV). Anal Biochem 139:487–492. https://doi.org/10.1016/0003-2697(84)90039-3
Pradhan M, Tripathy P, Mandal P, Sahoo BB, Pradhan R, Mishra SP, Mishra HN (2016) Effect of salicylic acid on growth and bulb yield of onion (*Allium cepa* L.). Int J Bio-Resource Environ Agric Sci 7:960–963
Rady MM, Mohamed GF (2015) Modulation of salt stress effects on the growth, physio-chemical attributes and yields of Phaseolus vulgaris L. plants by the combined application of salicylic acid and *Moringa oleifera* leaf extract. Sci Hort 193:105–113
Sadeghipour O, Aghaei P (2012) Response of common bean (*Phaseolus vulgaris* L.) to exogenous application of salicylic acid (SA) under water stress conditions. Advan Environ Biol 6:1160–1168
Saleem M, Fariduddin Q (2022) Novel mechanistic insights of selenium induced microscopic, histochemical and physio-biochemical changes in tomato (*Solanum lycopersicum* L.) plant. An account of beneficiality or toxicity. J Hazard Mat 434:128830. https://doi.org/10.1016/j.jhazmat.2022.128830
Saleem M, Fariduddin Q, Castroverde CD (2021a) Salicylic acid: a key regulator of redox signalling and plant immunity. Plant Physiol Biochem 168:381–397. https://doi.org/10.1016/j.plaphy.2021.10.011
Saleem M, Fariduddin Q, Janda T (2021b) Multifaceted role of salicylic acid in combating cold stress in plants: a review. J Plant Growth Regul 40:464–485. https://doi.org/10.1007/s00344-020-10152-x
Sanchez M, Revilla G, Zawrra I (1995) Changes in peroxidase activity associated with cell walls during pine hypocotyle growth. Ann Bot 75:415–419. https://www.jstor.org/stable/42761733
Saruhan N, Saglam A, Kadioglu A (2012) Salicylic acid pretreatment induces drought tolerance and delays leaf rolling by inducing antioxidant systems in maize genotypes. Acta Physiol Plant 34:97–106. https://doi.org/10.1007/s11738-011-0808-7
Semida WM, Rady MM (2014) Pre-soaking in 24-epibrassinolide or salicylic acid improves seed germination, seedling growth, and antioxidant capacity in *Phaseolus vulgaris* L. grown under NaCl stress. J Hortic Sci Biotechnol 89(3):338–344
Shakirova FM, Sakhabutdinova AR, Bezrukova MV, Fatkhutdinova RA, Fatkhutdinova DR (2003) Changes in the hormonal status of wheat seedlings induced by salicylic acid and salinity. Plant Sci 164:317–322. https://doi.org/10.1016/S0168-9452(02)00415-6
Shao RX, Xin LF, Guo JM, Zheng HF, Mao J, Han XP, Jia L, Jia SJ, Du CG, Song R, Yang QH (2018) Salicylic acid-induced photosynthetic adaptability of *Zea mays* L. to polyethylene glycol-simulated water deficit is associated with nitric oxide signaling. Photosynthetica 56:1370–1377. https://doi.org/10.1007/s11099-018-0850-4
Sharma M, Gupta SK, Majumder B, Maurya VK, Deeba F, Alam A, Pandey V (2017) Salicylic acid mediated growth, physiological and proteomic responses in two wheat varieties under drought stress. J Proteom 163:28–51. https://doi.org/10.1016/j.jprot.2017.05.011
Sharma M, Gupta SK, Majumder B, Maurya VK, Deeba F, Alam A, Pandey V (2018) Proteomics unravel the regulating role of salicylic acid in soybean under yield limiting drought stress. Plant Physiol Biochem 130:529–541. https://doi.org/10.1016/j.plaphy.2018.08.001
Singh B, Usha K (2003) Salicylic acid induced physiological and biochemical changes in wheat seedlings under water stress. Plant Growth Regul 39:137–141. https://doi.org/10.1023/A:1022556103536
Singh P, Yadav VK, Yadav PC, Pandey GC (2021) Effect of salicylic acid on growth, bio-chemical changes and yield of wheat (*Triticum aestivum* L.) under different date of sowing condition. Pharma Innov J 10:126–131
Song H, Xu X, Wang H, Tao Y (2011) Protein carbonylation in barley seedling roots caused by aluminum and proton toxicity is suppressed by salicylic acid. Russ J Plant Physiol 58:653–659. https://doi.org/10.1134/S1021443711040169
Sullivan CY, Ross WM (1979) Selection for drought and heat tolerance in grain sorghum. In: Mussell H, Staples RC (eds) Stress physiology in crop plants. Wiley, New York
Tayyab N, Naz R, Yasmin H, Nosheen A, Keyani R, Sajjad M, Hassan MN, Roberts TH (2020) Combined seed and foliar pre-treatments with exogenous methyl jasmonate and salicylic acid mitigate drought-induced stress in maize. PLoS One 15:e0232269. https://doi.org/10.1371/journal.pone.0232269
Uzunova AN, Popova LP (2000) Effect of salicylic acid on leaf anatomy and chloroplast ultrastructure of barley plants. Photosynthetica 38:243–250. https://doi.org/10.1023/A:1007226116925
Wang LJ, Fan L, Loescher W, Duan W, Liu GJ, Cheng JS, Luo HB, Li SH (2010) Salicylic acid alleviates decreases in photosynthesis under heat stress and accelerates recovery in grapevine leaves. BMC Plant Boil 10:10. https://doi.org/10.1186/1471-2229-10-34
Wani AB, Chadar H, Wani AH, Singh S, Upadhyay N (2017) Salicylic acid to decrease plant stress. Environ Chem Lett 15:101–123. https://doi.org/10.1007/s10311-016-0584-0
Wu GL, Cui J, Tao L, Yang H (2010) Fluroxypyr triggers oxidative damage by producing superoxide and hydrogen peroxide in rice (*Oryza sativa*). Ecotoxicol 19:124–132. https://doi.org/10.1007/s10646-009-0396-0
Yadu S, Dewangan TL, Chandrakar V, Keshavkant S (2017) Imperative roles of salicylic acid and nitric oxide in improving salinity tolerance

in *Pisum sativum* L. Physiol Mol Biol Plant 23:43–58. https://doi.org/10.1007/s12298-016-0394-7

Yusuf M, Hasan SA, Ali B, Hayat S, Fariduddin Q, Ahmad A (2008) Effect of salicylic acid on salinity-induced changes in *Brassica juncea*. J Int Plant Biol 50:1096–1102. https://doi.org/10.1111/j.1744-7909.2008.00697.x

Yusuf M, Fariduddin Q, Varshney P, Ahmad A (2012) Salicylic acid minimizes nickel and/or salinity-induced toxicity in Indian mustard (*Brassica juncea*) through an improved antioxidant system. Environ Sci Pollut Res 19:8–18. https://doi.org/10.1007/s11356-011-0531-3

Zanganeh R, Jamei R, Rahmani F (2019) Role of salicylic acid and hydrogen sulfide in promoting lead stress tolerance and regulating free amino acid composition in *Zea mays* L. Acta Physiol Plant 41:1–9. https://doi.org/10.1007/s11738-019-2892-z

Zhu JK (2001) Cell signaling under salt, water and cold stresses. Curr Opin Plant Boil 4:401–406. https://doi.org/10.1016/S1369-5266(00)00192-8