Mitigation of sodium chloride toxicity in *Solanum lycopersicum* L. by supplementation of jasmonic acid and nitric oxide

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**ABSTRACT**

We investigated the effects of exogenous application of jasmonic acid (JA) and nitric oxide (NO) on growth, antioxidant metabolism, physio-biochemical attributes and metabolite accumulation, in tomato (*Solanum lycopersicum* L) plants exposed to salt stress. Treating the plants with NaCl (200 mM) resulted in considerable growth inhibition in terms of biomass, relative water content, and chlorophyll content, all of which were significantly improved upon application of JA and NO under both normal and NaCl-stress treatments. Salt treatment particularly 200 mM NaCl caused an apparent increase in electrolyte leakage, lipid peroxidation, and hydrogen peroxide production, which were reduced by exogenous application of JA and NO. Salt treatment triggered the induction of antioxidant system by enhancing the activities of antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR). Application of JA and NO separately as well as in combination caused a significant improvement in activities of SOD, CAT, APX, and GR activities. JA and NO either applied individually or in combination boosted the flavonoid, proline and glycine betaine synthesis under NaCl treatments. In conclusion, the exogenous application of JA and NO protected tomato plants from NaCl-induced damage by up-regulating the antioxidant metabolism, osmolyte synthesis, and metabolite accumulation.

**1. Introduction**

As sessile organisms, plants frequently confront a variety of environmental stresses, which can result in significant alterations in their growth, metabolism, and development. Salinity stress is considered as one of the most severe abiotic stresses that hamper the normal growth and development of plants (Ahmad et al. 2015; Ahanger and Agarwal 2017). Salinity stress is increasing consistently at an alarming rate worldwide, leading to reduced fertility of land, especially in arid and semiarid regions. Agricultural malpractices, such as excessive use of saline water for irrigation, have further aggravated the problem, resulting in the conversion of arable land into salt-affected wasteland. Salinity induces primarily hyper-osmotic and ionic effects, thus causing impairment in growth by affecting important physio-biochemical processes such as photosynthesis, nitrogen and antioxidant metabolisms, and ion homeostasis (Khan et al. 2009; Ahmad et al. 2015). Negative effects on the growth of plants exposed to salinity have been reported in several species in a number of studies (Khan et al. 2009; Ahmad et al. 2010; Ahmad 2012; Ahmad et al. 2015; Iqbal et al. 2015). It is well documented that growing crops in saline soils triggers the over-production and accumulation of reactive oxygen species (ROS) such as superoxides, hydrogen peroxide, and hydroxyl radical (Ahmad et al. 2015; Ahmad, Abdel Latef, et al. 2016; Ahmad, Rasool, et al. 2016). Excess accumulation of ROS leads to the oxidation of important cellular molecules such as lipids, proteins, and chlorophyll, thereby hampering the cellular functioning and metabolism (Ahmad et al. 2010). Excess ROS affects plant growth by inducing peroxidation of important structural components such as membrane lipids, proteins, and nucleic acids (Ahmad et al. 2010; Iqbal et al. 2015). To counteract the salinity-induced oxidative stress, plants employ various mechanisms such as ion sequestration, osmoregulation, and up-regulation of the antioxidant defense system, so as to minimize the impact on their metabolism (Ahanger et al. 2014; Ahanger et al. 2015; Ahmad et al. 2015; Ahmad, Abdel Latef, et al. 2016). Osmoregulation is achieved by accumulating compatible osmolytes such as proline, glycine betaine (GB), and sugars (Ahanger et al. 2014; Ahanger et al. 2015; Ahanger and Agarwal 2017), while up-regulation of the antioxidant system keeps the ROS production and accumulation under control (Ahanger et al. 2015; Ahmad, Abdel Latef, et al. 2016). Enzymatic components such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and glutathione reductase (GR), and non-enzymatic components such as ascorbic acid (AsA) and glutathione, in addition to secondary metabolites, mediate the scavenging of ROS, thus, preventing oxidative damage to important cellular structures (Ahmad et al. 2010; Ahmad, Abdel Latef, et al. 2016). Furthermore, enhancement in synthesis and accumulation of osmolytes also contributes...
significantly to growth performance under stressful conditions (Ahanger et al. 2014; Ahanger et al. 2017; Ahanger and Agarwal 2017).

In order to mitigate salinity-induced adverse effects, a variety of growth regulators are being used. Of them, jasmonic acid (JA) and nitric oxide (NO) have been shown to be involved in the regulation of several metabolic pathways, under both normal and stressful conditions (Ahmad, Abdel Latef, et al. 2016; Ahmad, Rasool, et al. 2016; Sirhindi et al. 2016, 2017; Ahmad et al. 2017; Bali et al. 2017). In addition to their individual effects, it has been proposed that there is a crosstalk among them, for eliciting important growth responses via mediation of signaling events (Zhou et al. 2015; Ahmad, Rasool, et al. 2016). JA is an important cellular regulator playing an active role in maintaining developmental events, including germination, root growth, leaf movement, embryo development, sex determination, fruit ripening, and senescence (Dar et al. 2015; Ahmad, Rasool, et al. 2016). For example, in soybean, JA has been reported to regulate leaf and root morphogenesis (Xue and Zhang 2007). JA shares crosstalk networks with several important phytohormones such as auxins, gibberellic acid, and salicylic acid (SA), affecting several signaling events that regulate plant growth (Wasternack 2014). Similarly, NO abates ROS-induced oxidative stress by improving the antioxidant metabolism, nutrient assimilation, and regulation of stress-response genes (Fatma et al. 2014; Ahmad, Abdel Latef, et al. 2016). Ahmad, Abdel Latef, et al. (2016) have demonstrated that NO regulated the growth of chickpea (Cicer arietinum) under NaCl stress by up-regulating the antioxidant metabolism and maintaining the mineral assimilation. Based on such evidence highlighting the growth-promoting roles of JA and NO, the present study was undertaken to investigate the role of JA and NO in amelioration of NaCl-induced salt stress in tomato plants, because it has been widely reported that salt stress significantly reduces the production as well as quality of tomato crops. The present study forms the first report of its type, demonstrating the interactive role of JA and NO in ameliorating the NaCl-induced stress in tomato plants through the modifications of osmolytes, antioxidants, and secondary metabolites.

2. Material and methods

2.1. Plant materials and growth conditions

*Solanum lycopersicum* seeds were surface sterilized with sodium hypochlorite solution (1%) for 10 min and then rinsed briefly with distilled water. The seeds were sown in Petri-dishes in a growth chamber, and germinated seedlings (4-day-old) were transferred into pots filled with peat, perlite, and sand (1:1:1, v/v/v). Full strength nutrient solution (200 mL pot⁻¹) with pH 6.8 was provided to the germinated seedlings for 2 weeks, while they were being maintained under day/night temperature of 26/16°C, photoperiod 16 h and relative humidity of 70% in the growth chamber. After 18 days, on every alternate day, NaCl-supplemented (200 mM) nutrient solution was used to induce salinity stress, whereas the pots receiving just the nutrient solution served as a control. NO (50 µM) donor SNAP (S-nitroso-N-acetylpenicillamine) was applied along with the nutrient solution, while JA (1 mM) was sprayed onto the plants (10 mL per plant) every alternate day using a manual sprayer, from day 7 (25-days-old plants) to day 30 from the initiation of NaCl treatment (55-days-old plant), while the control plants were sprayed with an equal quantity of distilled water. The treatments of NaCl, JA, and SA were as follows: (1) 0 mM NaCl; (2) 0 mM + NO; (3) 0 mM + JA; (4) 0 mM + NO + JA; (5) 200 mM NaCl; (6) 200 mM + NO; (7) 200 mM + JA; (8) 200 mM + NO + JA. The pots were arranged in a completely randomized design in five replicates. After 30 days of treatment (55-days-old plants), plant samples were collected for analyses.

2.2. Growth and biomass

Morphological parameters such as shoot and root lengths were measured manually using a ruler, while the dry biomass was taken after drying the plant samples at 65°C for 72 h.

2.3. Estimation of pigment content

Photosynthetic pigments were estimated in fresh leaf tissues extracted in dimethyl sulfoxide (DMSO), and the absorbance of supernatant was recorded using a spectrophotometer at 480, 510, 645, and 663 nm (Beckman 640 D, USA), with DMSO as blank (Hiscox and Israelstam 1979).

2.4. Estimation of leaf relative water content (LRWC)

LRWC was obtained by punching leaf discs from each treatment plant to determine their fresh weight (FW). Thereafter, the leaf discs were floated on water for 4 h to determine their turgid weight (TW). Subsequently, they were oven-dried at 85°C to estimate their dry weight (DW) (Smart and Bingham 1974). RWC calculation was done using the following formula:

\[ \text{RWC} (\%) = \frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}} \times 100. \]

2.5. Determination of electrolyte leakage

The electrolyte leakage was measured by the method of D.nio-Seso and Tobita (1998), using the following formula:

\[ \text{Electrolyte leakage} (\%) = \left( \frac{\text{EC}_1 - \text{EC}_0}{\text{EC}_2 - \text{EC}_0} \right) \times 100, \]

EC₀ is the electrical conductivity at room temperature; EC₁ is the electrical conductivity at 60°C; and EC₂ is the electrical conductivity at 100°C.

2.6. Determination of proline and GB contents

Proline content was estimated following the method of Bates et al. (1973). After extraction with sulphosalicylic acid, a known volume of extract was reacted with ninhydrin reagent, and the absorbance was recorded at 520 nm using a spectrophotometer (Beckman 640 D, USA), with toluene as a blank.

The method of Grieve and Grattan (1983) was employed for the estimation of GB. Absorbance was recorded at 365 nm using a spectrophotometer and calculations were done using the reference standard of GB (50–200 mg mL⁻¹).

2.7. Estimation of hydrogen peroxide (H₂O₂) and lipid peroxidation (MDA)

For H₂O₂ estimation, fresh leaves (500 mg) were homogenized with 0.1% (w/v) trichloroacetic acid. The absorbance
was recorded at 390 nm, and calculation was done using a H$_2$O$_2$ standard (Velikova et al. 2000).

Lipid peroxidation was measured as MDA formation, following the method of Heath and Packer (1968). Optical density was recorded at 532 and 600 nm, and extinction coefficient of 155 mM$^{-1}$cm$^{-1}$ was used for MDA calculation.

2.8. Antioxidant enzymes assay

Extraction of enzymes was done by macerating fresh leaves (5 g) in Tris-HCl (100 mM, pH 7.5) containing 5.0 mM DTT, 10 mM MgCl$_2$, 1.0 mM ethylenediaminetetraacetic acid (EDTA), 5.0 mM magnesium acetate, 1.5% polyvinylpyrrolidone, and 1 μg mL$^{-1}$ aprotinin, followed by centrifugation at 10,000 rpm for 15 min at 4°C. The supernatant was used as the enzyme source. However, for APX extraction, the buffer was supplemented with 2.0 mM ascorbate. The soluble protein content was determined following Bradford (1976), and bovine serum albumin was used as the standard.

Superoxide dismutase (SOD, EC 1.15.1.1) activity was assayed by monitoring the photoreduction of nitroblue tetrazolium at 560 nm. One unit of SOD is the quantity of protein that causes 50% reduction of Nitro blue tetrazolium chloride and was expressed as Unit mg$^{-1}$ protein (van Rossum et al. 1997). Catalase (CAT, EC 1.11.1.6) was assayed using the method of Lück (1965). A decrease in absorbance was monitored at 240 nm for 3 min. For ascorbate peroxidase (APX, EC1.11.1.11), the method of Nakano and Asada (1981) was adopted, and change in absorbance was recorded at 290 nm using a spectrophotometer. Glutathione reductase (GR, EC 1.6.4.2) activity was assayed by following the oxidation of nicotinamide adenine dinucleotide phosphate (NADPH), and a decrease in absorbance was monitored at 340 nm for 2 min (Foyer and Halliwell 1976).

2.9. Non-enzymatic antioxidants

For the estimation of AsA and reduced glutathione (GSH), fresh leaves (0.5 g) were extracted in 3.0-mL metaphosphoric acid (5%) containing 1.0 mM EDTA. The mixture was subjected to centrifugation at 11,500 rpm for 15 min at 4°C, and the supernatant was used for AsA and GSH analyses. The method of Huang (2005) was employed for the estimation of AsA, while GSH was estimated using the method of Anderson (1985).

2.10. Estimation of total flavonoid content

The colorimetric method described by Zhishen et al. (1999) was adopted for the estimation of flavonoid content, and absorbance was measured at 510 nm. The flavonoid content was calculated from a standard curve of catechin and expressed as mg catechin equivalents g$^{-1}$ of the extract (mg g$^{-1}$).

2.11. Statistical analysis

Data were presented as the mean of five replicates ±SE. For statistical analysis, Duncan’s multiple range test was used, and $P \leq .05$ was considered as significant.

3. Results

3.1. Growth parameters

Effect of NaCl stress, JA, and NO on the growth parameters, including length and biomass, is depicted in Figure 1(A,B). Salinity reduced the shoot and root lengths by 69.95% and 63.23%, respectively. However, the application of JA and NO caused a substantial increase in these traits under normal as well as NaCl stress. Salt reduced the DW of shoots by 75.67% and of roots by 76.00%. However, an increase in shoot and root DW by 77.77% and 100% with JA and by 111.1% and 133.33%, respectively, with NO supplementation was observed, compared with the respective NaCl treatments. Moreover, plants supplemented with both JA and NO exhibited an increase of 200.0% in shoot DW and 250.0% in root DW over their respective NaCl-treated counterparts (Figure 1(B)).

3.2. Chlorophyll

Salinity (200 mM) treatment reduced the chl a, chl b, total chl, and car content by 71.91%, 58.13%, 62.41%, and 116.66%, respectively. Compared with control, chlorophyll (chl) a, chl b, total chl, and carotenoid (car) content increased by 10.11%, 51.16%, 22.69%, and 4.76%, respectively, with JA, and 22.47%, 79.06%, 39.00%, and 9.52%, respectively, with NO (Figure 2). However, plants treated with NaCl + JA + NO showed an increase of 208% in chl a, 100% in chl b, 162.79% in total chl, and 7.69% in carotenoid content, compared with the plants treated with NaCl alone (Figure 2).

3.3. LRWC and electrolyte leakage

The leaf RWC increased by 2.49%, 1.20%, and 5.86% with JA, NO, and JA + NO in the control plants. NaCl reduced the RWC in fresh leaves by 69.95% and 63.23%, respectively. However, an increase in shoot and root DW by 77.77% and 100% with JA and by 111.1% and 133.33%, respectively, with NO supplementation was observed, compared with the respective NaCl treatments. Moreover, plants supplemented with both JA and NO exhibited an increase of 200.0% in shoot DW and 250.0% in root DW over their respective NaCl-treated counterparts (Figure 1(B)).
LRWC by 47.77%, compared with control. Maximum mitigation was observed with JA + NO, where an enhancement of 32.41% was observed, compared with NaCl-stressed plants (Figure 3(A)).

NaCl stress increased the electrolyte leakage by 424.88%; however, the electrolyte leakage was reduced by 26.72% with JA, 31.90% with NO, and 45.17% with JA + NO supplementation, relative to the plants treated with NaCl only (Figure 3(A)).

### 3.4. Proline and GB

Relative to the control, accumulation of proline, and GB was enhanced 4.56- and 9.78-fold, respectively, in NaCl-treated plants (Figure 3(B)). JA and NO, individually as well as in combination, further enhanced the proline and GB content. However, the increase was more conspicuous in the case of NO + JA (4.80- and 13.98-fold for proline and GB, respectively) treated plants.

### 3.5. Hydrogen peroxide (H$_2$O$_2$) and malondialdehyde (MDA)

NaCl treatment caused a considerable increase in H$_2$O$_2$ (3-fold) and MDA content (2.18-fold) compared with the control plants. Reduced accumulation of H$_2$O$_2$ and MDA content was observed with the application of JA and NO. NaCl + JA + NO showed better results of in terms of very low accumulation of H$_2$O$_2$ (2.21-fold) and MDA content (1.57-fold), compared with that of the NaCl-treated plants (Figure 4(A,B)).

### 3.6. Antioxidant enzyme activity and content of non-enzymatic antioxidants

Salt stress significantly increased the activities of SOD, CAT, APX, and GR, and supplementation with JA and NO caused further up-regulation of their activities. Plants treated with both JA and NO maintained higher activity compared with the ones treated with JA or NO alone. Supplementation of JA + NO to control plants induced the SOD by 22.76%, CAT by 45.65%, APX by 52.35%, and GR by 65.96%. However, an increase of 77.78%, 132.36%, 125.14%, and 173.64% in SOD, CAT, APX, and GR activity was noted with NaCl treatment, compared with the control plants. A further increase of 66.85% in SOD, 51.25% in CAT, 54.20% in APX, and 71.68% in GR was observed with NaCl + JA + NO treatment, in comparison with NaCl-treated plants (Figure 5(A,B)).

Salt stress reduced the AsA content significantly and this salt stress triggered the accumulation of reduced glutathione (GSH). However, supplementation with JA and NO caused a
significant improvement in AsA and GSH accumulation under normal as well as salt-stressed conditions. NaCl treatment reduced the AsA content by 55.55% and increased GSH by 37.27%. AsA and GSH contents increased by 85.00% and 26.95%, respectively, with NaCl + JA + NO treatment, compared with that in NaCl-stressed counterparts (Figure 6(A,B)).

3.7. Flavonoids

Flavonoid content was reduced by 68.58% due to NaCl treatment; however, compared with that of NaCl-stressed plants, the flavonoid content increased by 63.06%, 100%, and 176.65% with NaCl + JA, NaCl + NO, and NaCl + JA + NO, respectively. When these treatments were applied to control plants, the flavonoids increased by 7.00%, 16.63%, and 27.36% due to JA, NO, and JA + NO, respectively (Figure 6(C)).

4. Discussion

Both JA and NO have been reported to mediate proper plant growth under normal as well as stressful conditions, in addition to playing a unique role in signaling under stress (Zhou et al. 2015; Ahmad, Abdel Latef, et al. 2016; Ahmad, Rasool, et al. 2016). In the present study, *S. lycopersicum* plants subjected to salinity showed a reduction in the growth parameters; such reduction in morphological parameters might have been due to the NaCl-induced inhibition of cell division and cell elongation (Yasseen et al. 1987). Supplementation with JA and NO enhanced the growth parameters and mitigated the NaCl-induced decline in growth to a considerable extent. Growth hormones enhance the resilience of plants to stress; they are key components for proper growth and maintenance under stressful conditions (Asgher et al. 2015). The positive influence of growth hormones such as JA (Sirhindi et al. 2016) and NO (Bai et al. 2015; Ahmad, Abdel Latef, et al. 2016) has been reported earlier as well. In the present study, application of JA and NO to NaCl-stressed *S. lycopersicum* enhanced growth and subsequent biomass accumulation, thereby depicting their active involvement in cell division and organ differentiation.

Reduced chlorophyll pigment synthesis under salinity stress substantiates the findings by Fatma and Khan (2014), Nazar et al. (2014), Rasool et al. (2013), and Ahmad, Abdel Latef, et al. (2016). Treatment with NaCl (200 mM) may have harmed the ultra-structure of pigment molecules by speeding up the chlorophyll degradation or by inducing chlorophyllase activity (Nazar et al. 2014). Reduced chlorophyll biosynthesis in NaCl-stressed plants has been reported to impede the net photosynthetic rate by affecting Rubisco synthesis and photosystem I functioning (Fatma et al. 2014). In the present study, the application of JA and NO protected NaCl-stressed *S. lycopersicum* plants by maintaining the contents of photosynthetic pigments, thereby contributing to the growth by maintaining production of photoassimilates. Hanaka et al. (2015) and Sirhindi et al. (2016), while working with JA on *Phaseolus coccineus*...
and *Glycine max* exposed to copper and nickel, respectively, found significant amelioration of negative effects on the pigment synthesis. The role of NO in protecting the photosynthetic pigments under salt stress has been reported in *Oryza sativa* (Habib and Ashraf 2014), *Brassica juncea* (Fatma and Khan 2014), and *C. arietinum* (Ahmad, Abdel Latef, et al. 2016). However, reports demonstrating the interactive role of JA and NO in protecting photosynthetic pigments are not available. In the present study, the combined treatment was much more effective in the promotion and protection of pigments, compared with individual JA and NO treatments. Apart from mediating free radical scavenging, carotenoids are believed to be effectively involved in controlling photo-protection against auto-oxidation in the photosynthetic reaction center (Gururani et al. 2015).

In the present study, the leaf RWC was negatively affected by NaCl stress, as the excess NaCl caused injuries in root system that resulted in a decreased uptake of water (Zeng et al. 2011). However, application of NO and JA had a positive impact on LRWC, suggesting the beneficial implications of NO and JA in preventing ionic stress; this might be due to the accumulation of osmolytes and the compartmentation of ions like Na through maintenance and optimal functioning of transport proteins (Shi et al. 2007). The increase in leaf RWC with NO application is supported by the findings of Habib and Ashraf (2014) and Ahmad, Abdel Latef, et al. (2016) in *O. sativa* and *C. arietinum*, respectively. JA application has also been reported to improve the LRWC in *Arabidopsis* (Brossa et al. 2011) and barley (Pazirandeh et al. 2015) under water stress. Although NO and JA were shown to improve the LRWC and mitigate the negative effects caused by NaCl, the exact mechanism is still unknown and needs to be studied. However, it is believed that NO could affect a decline in osmotic potential, with a parallel increase in water potential in osmotic-stressed plants (Ke et al. 2013).

Salt (NaCl) stress triggered the accumulation of compatible osmolytes like proline and GB in the present study, which corroborated the findings by Khan et al. (2012), Khan et al. (2009) and Ahmad et al. (2014) in different plant species. In NaCl-stressed *Morus alba* (Ahmad et al. 2010) and *Vigna radiata* (Khan et al. 2014), improved growth has been attributed to their potential to accumulate a significant amount of proline and GB, which help maintain the cell osmolarity and tissue water content. In the present study, application of NO and JA individually as well as in combination proved to be of immense significance, causing an increase in the LRWC. Enhancement in the accumulation of proline in JA- and NO-treated plants might have been due to an increased expression of its biosynthesizing genes. Proline accumulation under stressed conditions results from changes in the activities of proline synthesizing and degrading enzymes (Ahmad et al. 2010) and the up- and down-regulation of genes (*P5CS1*, *ProDH*) responsible for proline biosynthesis (Wang et al. 2013; Ahmad, Abdel Latef, et al. 2016). Both proline and GB are involved in the regulation of osmotic balance, ensuring that the damages caused by stress can be overcome (Ahmad, Abdel Latef, et al. 2016). Proline accumulation influences protein turnover and regulation of stress-protective proteins (Thakur and Sharma 2005). Under normal as well as salt stress treatments, exogenous application of JA and NO enhanced proline and GB accumulation compared with control, thereby providing extra protection to the photosynthetic machinery and energy for quick stress recovery once the stress is removed (Ahmad et al. 2014; Khan et al. 2014). In the present study, application of JA and NO to NaCl-stressed *S. lycopersicum* plants caused an apparent decline in the levels of ROS, which can be explained as a result of improved antioxidant metabolism and the accumulation of proline and GB. Enhanced accumulation of compatible osmolytes is contemplated as a potential indicator plant stress tolerance (Ahanger et al. 2015). Plants accumulating GB exhibit improved photosynthetic efficiency and nitrogen metabolism and are better able to protect important structures from oxidative stress (Khan et al. 2014). Kathuria et al. (2009) have reported a reduced ROS accumulation through the expression of stress-response genes, which could be due to over-expression of GB genes in rice seedlings. In the present study, the noticeable increase in the accumulation of proline and GB due to JA and NO application might contribute to salt tolerance of *S. lycopersicum* through osmotic regulation, consequently leading to regulation of tissue RWC, and improved growth and biomass yield. Application of NO to salt-stressed *B. juncea* has been reported to provoke calcium-induced antioxidant metabolism and the elemental sequestration for better amelioration of oxidative stress (Khan et al. 2012).

Salinity-stressed *S. lycopersicum* exhibited enhanced production of H$_2$O$_2$, which resulted in increased lipid peroxidation and membrane leakage. Our results corroborate with the findings of Ahmad (2012), who demonstrated increased membrane leakage and hence, reduced stability of membranes in *B. juncea* exposed to salt stress. Enhanced production of ROS in salinity-stressed plants triggers the loss of membrane integrity by causing peroxidation of lipids; in the present study, it is likely that both JA and NO application mitigated the NaCl-triggered oxidative damage to membranes by lowering the formation of toxic radicals. Ameliorative role of NO has also been reported by Fatma and Khan (2014) in mustard and Ahmad, Abdel Latef, et al. (2016) in chickpea. However, reports highlighting the interactive role of JA and NO in alleviating the ROS-induced membrane damage are inadequate and in the present investigation, combined treatment of JA and NO proved much more beneficial in protecting membranes compared with the individual treatments of JA and NO. NO interacts with superoxide and acts as a pro-oxidant for preventing lipid peroxidation (Violi et al. 1999). Sirhindhi et al. (2016) have confirmed reduced membrane peroxidation due to JA treatment in Ni-treated soybean by down-regulating the activity of plasma membrane NADPH oxidase. Our work supports the role of JA and NO in protecting the membrane lipids from ROS-induced peroxidation. A decrease in the production of ROS and NaCl toxicity occurred due to the application of JA and NO, which caused a substantial increase in antioxidant activities and endogenous levels of phytohormones (Ahmad, Abdel Latef, et al. 2016; Sirhindhi et al. 2016).

To counteract the deleterious effects of ROS on important molecules such as proteins, lipids, chlorophylls, and nucleic acids, plants increase the expression of antioxidants to avert ROS-induced damage. In the present study, application of JA and NO increased the activities of SOD, CAT, APX, and GR, and the synthesis of AsA, GSH, proline, and flavonoids, which helped the plants to counteract ROS-induced damage more precisely compared with that in the untreated counterparts. Improved antioxidant metabolism due to NO application has also been reported by Fan et al. (2013) in
Cucumis sativus, Fu et al. (2016) in Elymus mutans, and Ahmad, Abdel Latef, et al. (2016) in C. arietinum. Hasanuzzaman et al. (2011) also demonstrated the induction of antioxidant defense system upon NO application in salt-stressed wheat. However, reports on antioxidant regulation by the cumulative effects of JA and NO are not available. SOD is one of the key antioxidant enzymes involved in the scavenging of superoxide radicals, and in the present study, application of JA and NO mediated the improvement in SOD activity by modulating the substrate superoxide and H$_2$O$_2$, thereby reducing the chances of formation of hydroxyl (OH$^-$) radicals (Rasool et al. 2013; Ahanger et al. 2015). Similar to our results, an increased activity of CAT under salt stress has been reported in other studies as well (Rasool et al. 2013; Ahmad, Abdel Latef, et al. 2016). Mittal et al. (2012) reported increased CAT activity in B. juncea under high salinity. Both CAT and APX mediate the breakdown of H$_2$O$_2$ produced from SOD action, and further enhancement in CAT and APX by application of JA and NO may have strengthened the antioxidant defense system to reduce the oxidative damage. Exogenously sourced NO could promote the expression of antioxidant-coding genes (Groß et al. 2013). The relative expression of antioxidant enzymes by exogenous application of JA has also been studied in soybean (Sirhindi et al. 2016) and with NO in chickpea (Ahmad, Abdel Latef, et al. 2016). CAT has an indispensable role during stress, and APX and GR form important components of the ascorbate-glutathione pathway. In the present study, NO and JA application caused a significant increase in the contents of GSH and AsA, which might have imparted protection to photosynthesis by maintaining the redox state and the availability of NADP$, therefore, preventing the generation of superoxide radicals at the photosynthetic site (Fatma and Khan 2014; Ahmad, Abdel Latef, et al. 2016; Ahmad, Rasool, et al. 2016). However, a reduced AsA content was observed under NaCl treatment, and exogenous application of JA and NO enhanced its synthesis, thus, equilibrating the AsA levels for oxidative stress mitigation. Both AsA and NO play an important role in plant growth regulation and the mitigation of oxidative damage by maintaining the antioxidant metabolism (Fatma et al. 2014; Ahanger and Agarwal 2017).

The increased synthesis of flavonoids due to JA supplementation supports the findings by Horbowicz et al. (2011), Ishihara et al. (2002), and Wang et al. (2015). Xu et al. (2006) have reported that NO production induces the synthesis of JA, SA, and secondary metabolites, thereby mediating the regulated crosstalk between JA, SA, secondary metabolites, and NO signaling, for better stress amelioration. In the present study, NO application resulted in improved flavonoid synthesis, and this was maintained when applied in combination with JA, thus, showing the interactive role of NO and JA. Thiruvengadam et al. (2016) reported that the application of JA to Brassica rapa enhanced the synthesis of flavonoids by up-regulating the expression levels of genes controlling the biosynthesis of phenolic compounds and carotenoids. Studies illustrating the effect of exogenous NO $+$ JA on flavonoid biosynthesis under salt stress are hardly available. In UV-exposed Ginkgo biloba, Hao et al. (2009) demonstrated an improved accumulation of flavonoids due to NO application, via modulation of phenylalanine ammonia lyase (PAL) activity, and concluded that exogenously sourced NO induced an increase in NO synthase activity, causing a further enhancement in endogenous NO for better signaling in coordinating PAL activity, flavonoid synthesis, and stress tolerance. In the present study, the combined supplementation of NO and JA to S. lycopersicum plants might have ensured an optimal availability of JA for NO-mediated signaling cascades that regulate growth and development under NaCl stress; the enhancement in flavonoids and phytohormone synthesis might have further up-regulated these developmental events.

5. Conclusions
Salinity resulted in decreased growth of S. lycopersicum by altering its physiological and biochemical attributes. It increased the production of ROS, causing lipid peroxidation and leading to electrolyte leakage. Although salt tolerance is a complex process, the active roles of JA and NO in alleviating the salt stress are reported in the present study. From the present study, it can be conclusively explicated that exogenous application of JA and NO mitigates the NaCl-induced oxidative stress in S. lycopersicum plants to a significant extent, by improving the activity of antioxidants and accumulation of metabolites. Furthermore, optimization of JA and NO can bring about coordination between different signaling molecules like phytohormones, metabolites, and protein kinases, for efficient stress mitigation. Thus, more research in this direction can help unravel the exact mechanisms involved therein.

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