Amelioration of salt stress tolerance in *Solanum lycopersicum* L. using salicylic acid

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**ABSTRACT:** *Solanum lycopersicum* L. is a glycophytic species and vegetable crop grown worldwide for its edible fruits. Laboratory experiments were conducted to determine the ameliorating effects of salicylic acid on salt tolerance. Total ten germplasm seeds were germinated and grown under 50 mM and 150 mM salt stress and 0.5 mM salicylic acid (SA). Morphological and physio-biochemical variables such as seed germination, biomass, relative water content, Proline, protein content, total chlorophyll content and peroxidase activity were evaluated to assess the amelioration of salt stress tolerance. At 150 mM NaCl stress the seed germination of the germplasms were improved under the exogenous application of salicylic acid. Similarly, application of salicylic acid helps to maintain the Relative Water Content (RWC), chlorophyll and peroxidase activity in comparison to salt stress condition. After the application of SA the NB6 (EC-514013) and NB7 (EC-514101) germplasms were showed appreciably enhanced salt tolerance. The SA application could play an important role to induce plant resistance against salt stress.

**Key Words:** Sodium Chloride, Salicylic acid, Germination, Chlorophyll, Proline.

**INTRODUCTION:**

The physiological approaches from seed germination to plant development were greatly inclined by salt stress (Sivakumar et al., 2018; Rafique et al., 2011). Salinity is major environmental threat, which avert growth of crop, productiveness and noticed that among total world’s irrigated land ¾ th part is affected by salinity (Karlidag et al., 2009). At germination degree, reactive oxygen species (ROS) are generated basically in the course of depletion of food reserves and oxidative phosphorylation, while their quantitative stage is managed by means of seed’s protecting antioxidant method (Sivakumar et al., 2018; Basha et al., 2015). However, it has been mentioned that seeds include quite a lot of antioxidants in small quantities and compounds like ascorbic acid are not contribution. A synthetic expands within the cellular stage of an antioxidant such as ascorbic acid must be beneficial in bettering stress tolerance at germination degree (Hasanuzzaman et al., 2013).

The Salicylic acid (SA) is a phenolic phytohormone and is observed in crops with responsibility in plant development and development, photosynthesis, transpiration, ion uptake and transport (Hayat et al., 2009). The SA act as non permanent signaling molecule for adapting plant responses to environmental stress (Van Breusegem et al., 2001) and expanded stress attention was once familiar in remind salt tolerance in lots of plants (Aldesuquy et al., 1998; El-Tayeb, 2005). It plays a significant role in the plant response to adversarial environmental stipulations similar to salinity. It plays an essential position in plant development (Klessig and Malamy, 1994), ion uptake and transport (Harper and Balke, 1981), photosynthetic rate, stomatal conductance and transpiration (Khan et al., 2003) preventing oxidative harm in a plant by means of detoxifying superoxide radicals, produced as a consequence of salinity (Rajeshwari and Bhuvaneshwari, 2017). Exogenous software of SA enhances the pursuits of antioxidant enzymes and increase plant tolerance to the abiotic stress (He et al., 2002). The SA has received much awareness due to its position in plant responses to abiotic stresses akin to ozone (Koch et al., 2000), UV-B (Surplus et al., 1998), warmth stress (Clark et al., 2004; Dat et al., 1998), drought (Singh et al., 2003), salt and osmotic stress (El-Tayeb, 2005).

Hence, the present investigation was carried out in order to investigate the level of effectiveness of SA in ameliorating the stress tolerance by decreasing the deleterious effects of salinity on plants (Dolatabadian et al., 2008). Enhancing stress tolerance in plants has major implications in agriculture and horticulture (Senaratna et al., 2000). The present research work was planned with the aim to determine the morphological and physio-biochemical changes under salt stress in tomato and to determine the potential effect of SA for the reduction of salt stress in tomato.
MATERIALS AND METHODS:

Seed collection

To differentiate the effect of salinity stress on the growth of tomato seedlings and ameliorating responses of the SA. Ten variety of tomato (Solanum lycopersicum L) germplasm NB1 (EC-164259), NB2 (EC-164329), NB3 (EC-164334), NB4 (EC-164656), NB5 (EC-165751), NB6 (EC-514013), NB7 (EC-514101), NB8 (EC-514109), NB9 (EC-523851) AND NB10 (EC-550742) were collected. The germplasm was obtained from National Bureau of Plant Genetic Recourses (NBGR), Rajendra Nagar, Telangana, INDIA.

Seed germination percentage experiment design

Germination experiments were carried out in Petri dishes using sterilized cellulose germination papers. The first set was humidified with 10 ml of distilled water (Control), 50 mM NaCl and 150 mM NaCl regularly. In second set of tomato germplasms were pretreated with SA (0.5 M) for 24 h, then air dried before immersing in same above-mentioned NaCl concentrations. Both sets were kept in an incubator under continuous darkness for 3 days with 25 ± 2 °C temperature. After exposure of seed radicle as 2 mm length it may consider to calculate germination percentage. The triplicated results were used to calculate final germination percentage (FPG) according to Sivakumar et al., (2018) and Basha et al., (2015).

Plant growth conditions

The seeds were surface sterilized with 4% NaOCl solution for five minutes followed by ten repeated distilled water rinses. Seeds were inoculated in coconut peat and allowed to grow at 25 ± 2 °C. On 8th day from seed inoculation, two concentrations of NaCl i.e. 50 mM and 150 mM were supplemented to tomato seedlings. Another set were maintained with only distilled water for control. To the third set, the 0.5 M salicylic acid was exogenously applied in liquid form to the seedlings which were already under salt treatment. Daily 10 ml of SA was sprayed for each seedling till 30 days. Finally, fresh leaves were collected from each replicate for analysis.

Measurement of morphological variables

Plants were removed carefully from coconut peat and measured root length and shoot length by using a common measuring scale. All mean values of triplicate sample results were tabulated in Centimeter (cm) units. The seedlings were placed in aluminum foil and the samples were dried, then the dry matter was weighed and all obtained FW and DW results were tabulated.

Measurement of physio-biochemical variables

The physio-biochemical variables were estimated using fresh leaves of 30 days old tomato seedlings. The leaf relative water content (RWC) was estimated according to the method of Sairam et al., 2002. For estimating total chlorophyll content Arnon (1949) method was followed. Proline was estimated following the protocol described by Bates et al., 1973 with minor modification. The total protein concentration was determined through some modifications of Lowry et al., 1951 method. The peroxidase activity was determined based on the protocol observed via Putter (1974) with minor modifications.

Data analysis

The data analysis was made using the PRISM 5.0 software. Data were subjected to analysis of variance (ANOVA) to compare the effects of salt stress treatments and SA treatments. The differences between the means were compared using the least significant difference test (LSD, p < 0.05).

RESULTS:

Analysis of seed germination percentage

The seed germination percentage was presented in Figure 1. The studied ten tomato germplasms showed good germination strength under control (non salinity) condition (Figure 1). At 50 mM salt stress all the germplasms showed near or less than 50% reduction in germination except the NB6 (60.3%) and NB7 (60.8%) germplasms (Figure 1). At 150 mM salinity all germplasms lost their germination percentage to below 10% (Figure 1). But at both saline conditions Ameliorated germination percentage were found in all germplasms due to salicylic acid pre-immersion, among them NB5 (70.5%), NB6 (80.4%) and NB7 (77.1%) were showed substantial increase at 50 mM salinity. At 150 mM salt stress the NB5 (30.8%), NB6 (35.8%) and NB7 (36.1%) germplasms showed reasonably good germination (Figure 1).
Among the studied germplasms the NB5 (5.2 cm) and NB6 (5.2 cm) showed of highest shoot length and followed by NB3, NB4 and NB10 (Table 1). At 50 mM salt stress, the NB6 (3.6 cm) germplasm showed highest and NB1 (2.7 cm) germplasm showed lowest shoot length (Table 1). At 150 mM highest shoot length was observed in NB9 (2.1 cm) and lowest was found in NB1 (1.5 cm). However, application of the salicylic acid leads to increase in shoot length in all germplasm (Table 1). Among the studied ten germplasms the NB5 (5 cm), NB6 (5.1 cm) and NB9 (5.1 cm) were showed substantial increase in shoot length as close as to their controls at 50 mM salt stress when applied 0.5 M salicylic acid. Similarly at 150 mM salinity the treatment of salicylic acid leads the better elongated shoot length in NB10 (3.2 cm), NB9 (3.1 cm), NB8 (3.1 cm), NB7 (2.9 cm) and NB6 (2.9 cm) (Table 1). In overall, the germplasm NB9, NB6 and NB5 were maintained improved shoot length at 50 mM salinity and the NB10, NB9 and NB8 showed highest shoot length at 150 mM salinity after application of salicylic acid. At control condition, the root length of NB10, NB9 and NB8 germplasms were recorded as 6.5 cm, 6.4 cm and 6.4 cm respectively which is highest in comparison to other germplasms (Table 1). At 50 mM salt stress, the germplasms NB6 and NB7 were produced 3.1 cm of highest root length followed by NB10 (2.6 cm). Whereas, the germplasms NB10, NB9 and NB8 were maintained 1.6 cm, 1.5 cm and 1.3 cm of highest root length at 150 mM salt stress (Table 1). The 0.5 M salicylic acid treatment was improved the tomato germplasm root length at both studied salinity stress. At 50 mM salt stress the NB6 (3.8 cm) and NB7 (4.1 cm) germplasms were showed better root length among the studied germplasms and similar results were found even at 150 mM salt stress (Table 1).

Analysis of Shoot length and Root length (SL & RL)

Table 1. Shoot length, Root length, Fresh weight and Dry weight mean values of tomato germplasm.
Bio-mass

Fresh weight & Dry weight (FW & DW)

Among the studied ten germplasm the NB10 (0.338 mg) and NB9 (0.335 mg) were showed highest FW compared to other germplasm at control conditions (Table 1). At 50 mM salt stress the NB3 (0.19 mg) and NB2 (0.18 mg) germplasms were produced better fresh weight (Table 1). Whereas, at 150 mM salt stress the NB10 (0.085 mg) and NB9 (0.083 mg) germplasms were maintained highest fresh weight (Table 1). Due to application of the salicylic acid the NB6 and NB7 germplasms were showed significant enhanced fresh bio-mass under 50 mM and 150 mM salt stress (Table 1). Among the studied germplasms, the NB5 (1.65 µg) and NB9 (1.45 µg) were showed highest dry weight under non salinity condition (Table 1). At 50 mM salt stress, the NB5 (1.05 µg), NB10 (1.05 µg), NB3 (0.95 µg) and NB6 (0.9 µg) germplasms were maintained healthier dry weight (Table 1). At 150 mM salt stress, the germplasm NB6 had 0.88 µg of highest DW and followed by NB7, NB8, NB10 and NB5 germplasms. The application of salicylic acid was enhanced the dry weight of all germplasm at both saline conditions. At 50 mM salt stress the NB5 (1.32 µg) and NB6 (1.09 µg) germplasms showed substantial increased in dry weight (Table 1). At 150 mM salt stress the NB6 (0.92 µg) and NB8 (0.91 µg) were showed significant increase in dry weight in comparison to respective salt stress condition (Table 1).

Analysis of physio-biochemical parameters

Relative water content (RWC)

The RWC of the studied germplasms was showed in Figure 2. At 50 mM salt stress the NB10 (79.2%) germplasm had highest RWC compared to other germplasms (Figure 2). At 150 mM salt stress, the NB6 (49.8%) and NB7 (50.4 %) germplasm were able to maintained highest RWC (Figure 2). The application of salicylic acid was beneficial to all tomato germplasm in maintenance of the RWC (Figure 2). Among the studied tomato germplasm, the NB6 showed fine increase in RWC under 50 mM salt stress (88.6%) as well as 150 mM salt stress (58.9%) after application of salicylic acid.

![Figure 2. Effect of salicylic acid on Relative water content (RWC %) of tomato germplasm under control and salt stress. Each bar represents the mean (± SE) of six measurements.](image)

Total Chlorophyll content (CHL)

Among the studied tomato germplasms, the NB9 (36.86 mg/gm) and NB10 (37.5 mg/gm) were showed highest amount of total chlorophyll content under control condition (Figure 3). Under the 50 mM and 150 mM salt stresses the germplasm NB9 and NB10 showed the highest total chlorophyll content (Figure 3). Due to the application of salicylic acid, increased total chlorophyll content was observed in all germplasm. Among them NB6 (33.59 mg/gm and 21.63 mg/gm) and NB7 (34.08 mg/gm and 22.59 mg/gm) were placed in top position at 50 mM and 150 mM salt stresses (Figure 3).
Figure 3. Total chlorophyll content (mg/gm) of tomato germplasm under control, salt stress and salicylic acid treatments. Each bar represents the mean (± SE) of six measurements.

Total Protein content (TPC)

At control conditions, the NB8, NB3 and NB9 germplasms were showed highest total protein content (Figure 4). At 50 mM salt stress, the NB3 (0.21 µg/gm) and NB6 (0.22 µg/gm) maintained highest total protein content among the studied germplasms (Figure 4). At 150 mM salt stress, the NB9 and NB10 germplasms showed highest total protein content (Figure 4). After application of salicylic acid, the NB7, NB6 and NB10 germplasms were showed highest total protein content at 50 mM salt stress. The NB6 germplasm able to maintain high total protein content at 150 mM salt stress after application of salicylic acid (Figure 4).

Guaicol Peroxidase activity (GPX)

At control condition 20.82 ml/min average GPX activity was noted along the studied germplasms (Figure 5). At 50 mM salt stress, the NB10 germplasm showed highest GPX activity (Figure 5). At 150 mM salt stress, the NB7 and NB8 germplasms showed highest GPX activity (Figure 5). The NB10, NB7 and NB8 were showed increased GPX activity at 50 mM salt stress and NB10, NB3, NB4 and NB9 were showed enhanced GPX activity under 150 mM salinity after allocation of salicylic acid (Figure 5).

Figure 4. Total protein content (µg/gm) of tomato germplasms under control, salt and salicylic acid treatments. Each bar represents the mean (± SE) of six measurements.
Proline

Among the studied tomato germplasms, the NB2 showed the lowest proline content and NB10 showed the highest proline content at control condition (Figure 6). At 50 mM and 150 mM salt stress, increased proline content was observed in all germplasms. Among the studied germplasm, the NB1 (27.84 µg/gm), NB2 (28.53 µg/gm) and NB3 (29.78 µg/gm) showed low proline content at 50 mM and the same germplasm was produced lowest proline content even at 150 mM (Figure 6). After application of salicylic acid, the NB6 and NB7 germplasms showed low proline content at 50 and 150 mM salt stress (Figure 6).

DISCUSSION:

Salt stress diminishes by adversely affecting various physiological and biochemical parameters. Discount in plant growth expense is the outcome of the salt stress. The present results revealed that the salt stress prompted huge discount rates of all development variables akin to germination percent and growth parameters (shoot length, root length, recent weight and dry weight) below salt stress (figure 1; Table 1). These outcomes are in contract with these of Sivakumar et al., 2018; Vibhuti et al., 2015, Kapoor and Pande (2015) and Ahmed et al., (2009) who confirmed that salinity caused a marked reduction in germination and growth parameters of vegetable plants.

Salt stress tolerance in tomato may be extended now not best with the aid of genetic selection but in addition by way of using adapted physiological instruments. Researchers are trying to develop some powerful treatments comparable to exogenous salicylic acid software to curb the detrimental results of salinity (Karlidag et al., 2009). The salt tolerance of salicylic acid handled vegetation showed improved
photosynthesis and relative water content material and mirrored in phrases of improved progress rate within the gift record. Stevens et al., (2006) additionally mentioned a an identical increase within the shoots progress of tomato vegetation under 200 mM saline stress stipulations via addition of 0.1 mM salicylic acid by way of root medium. Similarly, salicylic acid therapies better the progress of maize (Khodary, 2004) and barley (El-Tayeb, 2005).

Salt stress reduced the relative water content material in all studied germplasms (Figure 2). Relative water content material, water capabilities and osmotic expertise of crops come to be more terrible with an expand in salinity (Sivakumar et al., 2018, Basha et al., 2015, Parida & Das 2005). This be trained confirmed that salicylic acid treatment caused an increase in RWC of the salt confused vegetation (Figure 2). Increases in RWC was noted when treated with salicylic acid were additionally said in barley (El-Tayeb, 2005) and tomato (Tari, 2002; Szepsi, 2005). This phenomenon is also attributed to the fact that foliar salicylic acid software can develop the leaf diffusive resistance and minimize transpiration charges.

Photosynthetic capability is a most important parameter to verify the plant recreation. Chlorophyll performs a main function in gentle absorption and energy transduction and is a primary aspect for photosynthesis. On this be taught, salt stress lowered the whole chlorophyll content in leaves in comparison with control crops (figure 3). Reduction in photosynthetic pigment (total chlorophyll) been reported in some prior experiences involving salt therapy in one of a kind plants i.e. Sunflower (Noreen et al., 2011) and wheat (Steven et al., 2006). Utility of salicylic acid accelerated the total Chl content of plants grown below NaCl 50 mM and 150 mM (figure 3). Mimouni et al., (2016) additionally observed a giant broaden in pigment content of tomato plant sprayed with salicylic acid. El-Tayeb (2005) located that salicylic acid software to barley precipitated a pre-adaptive response to salt stress that better the synthesis of Chl a, Chl b, and carotenoid, and maintained membrane integrity, main to development in plant progress.

Involvement of salicylic acid within the modulation of antioxidant metabolism has been largely stated in plant-tolerance to main abiotic stresses including ozone, UV-B, heat, steel, and osmotic stress (Khan MIR et al., 2015; Rajeshwari and Bhuvaneshwari, 2017). Salicylic acid pre-therapy was supported to alleviate the adversarial effects of salinity stress in green gram through enhancing the pursuits of antioxidant enzymes (Khan et al., 2014). In the gift study the 0.5 M salicylic acid decreases the salinity stress damage through regulation of GPX undertaking in all studied genotypes (Figure 5). Salicylic acid application multiplied activity of enzymes of AsA–GSH pathway resulted within the multiplied tolerance of B. juncea to salinity stress (Nazar et al., 2015). Redox lively compounds have been greatly reported to maintain a homeostatic balance of the mobile redo state, and are involved in protecting mechanisms towards both abiotic and biotic stresses (Anjum et al., 2014; Khan et al., 2015).

Proline accumulation is without doubt one of the most traditionally mentioned osmolyte that's brought on through salt stress in crops and is almost always measured to be worried in retaining the water content material. Additionally it's the foremost defense response to maintain the osmotic strain, osmotic adjustment, membrane stabilization, and detoxification of injurious ions in vegetation exposed to salt stress (Mimouni et al., 2016; Ashraf and Foolad, 2007). On this study the content of proline accelerated in leaves of tomato plant on salt cures (determine 6). Mimouni et al., 2016 and Wasti et al., (2012) also said that salinity brought about an increase of proline content in tomato. Other researchers also proved as identical in chamomile (Kovacik et al., 2009) and barley (El-Tayeb 2005), wheat seedlings collected big quantities of proline underneath salinity stress, which was once additional elevated when salicylic acid was utilized exogenously, thereby lowering the deleterious results of salinity (Arfan et al., 2007).

CONCLUSION:

Based on a copiousness of research conclusion, it is clear that salt stress has adverse effect on the germination percentage, growth and physio-biochemical development of plants. Among the studied germplasms NB6 (EC-514013) and NB7 (EC-514101) showed less adverse effect from salt injury because of salicylic acid application. The use of exogenous protectants under salt stress condition has been found to be very much effective to alleviate salt induced damages. Hence, usage of pre-seed treatments and exogenous application with salicylic acid at 0.5 M concentration can be preferable to alleviate the rigid effect at germination, plant growth and various physio-biochemical activities under salt treatment in tomato germplasm. In addition, further investigations considering molecular approaches are needed to reveal the underlying mechanisms of protection under stressful condition.

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Anjum NA, Aref IM, Duarte AC, Pereira E, Ahmad I, Iqbal M. Glutathione and proline can coordinately make plants withstand the joint attack of metal (loid) and salinity stresses. Frontiers in plant science. 2014 Nov 21;5:662.


Koch JR, Creelman RA, Eshita SM, Seskar M, Mullet JE, Davis KR. Ozone sensitivity in hybrid poplar correlates with insensitivity to both salicylic acid and jasmonic acid. The role of programmed cell death in lesion


- Parida AK, Das AB. Salt tolerance and salinity effects on plants: a review. Ecotoxicology and environmental safety. 2005 Mar 1;60(3):32-49.


- Stevens J, Senarathna T, Sivasthamparam K. Salicylic acid induces salinity tolerance in tomato (Lycopersicon esculentum cv. Roma): associated changes in gas exchange, water relations and membrane stabilisation. Plant Growth Regulation. 2006 May 1;49(1):77-83.

- Stevens J, Senarathna T, Sivasthamparam K. Salicylic acid induces salinity tolerance in tomato (Lycopersicon esculentum cv. Roma): associated changes in gas exchange, water relations and membrane stabilisation. Plant Growth Regulation. 2006 May 1;49(1):77-83.


