

Enhancing the Salt Tolerance Potential of Watermelon (*Citrullus lanatus*) by Exogenous Application of Salicylic Acid

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Abstract

Salicylic acid (SA) is a vital plant growth regulator providing promising role in plant development and adopts defense mechanism to abiotic stresses. Salinity is the most limiting abiotic factor for plant development and growth changes in watermelon by producing reactive oxygen species and ultimately oxidative stress. The present study was aimed to investigate the mechanism involved in salt stress alleviation in watermelon (*Citrullus lanatus* Thanb. Mavs.) through the foliar application of salicylic acid. Watermelon cv. Charleston Gray was grown under moderate saline regime of 3 ds·m⁻¹ NaCl and sprayed with salicylic acid with four level (0.5, 1.0, 2.5 and 5.0 mmol/L) compared along with control. SA @ 5.0 mmol/L was found to be very effective in mitigation of salt stress. SA was found to be very effective in alleviation of salinity stress by produced antioxidants and acted as osmo-regulator.

Keywords

Citrullus lanatus, Salicylic Acid, Salinity Stress, NaCl, Abiotic Stress

1. Introduction

Different parts of the world are facing two of the most serious soil problems *i.e.* salinity and water logging [1].

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There are different types of salt-affected soils that cover about 10% of the total land surface. At present, there are nearly 954 million hectares of saline soils on the earth's surface. Pakistan occupies 6.3 million hectares salt affected soils [2] while about 40% are saline and 60% are saline sodic while 80% of salt affected area in Punjab is saline sodic [3]. Salt-affected soils exist mostly under arid, semi-arid climate and cover about 955 million hectares of world [4]. Despite the various control measures, about 20 million hectares of land go out of production annually [5]. Ahmad and Riffat (2005) [6] evaluated the effect of salinity on some physio-biochemical parameters in pea. They concluded that all these changes were associated with decrease in relative water contents and K^+ uptake. There was significantly increase in proline and sugar contents while nitrate reductase activity and chlorophyll contents were decreased. Salt stress also reduces the photosynthetic activity by reducing chlorophyll contents, number of stomata and stomatal conductance [7].

Salicylic acid is very important plant hormone that plays very effective role in plant developmental process and defense responses to biotic and abiotic stresses. It has ability to act as osmotic adjustors and metabolic signals in the establishment of plant defense responses. It alleviated the drastic effect of salt stress in cucumber [8]. To reveal the effects of SA on sugar metabolism, salicylic acid can inhibit the catalase activity. Catalase activity reduction leading to increased hydrogen peroxide that it can enhance some germination of seeds. It is possible that 2 - 6 dihydroxy benzoic acids and salicylic acid stimulate the germination of seed via GA bio-synthesis and act as thermogene inducers [9]. Tomato salicylic acid can maintain membrane stability and photosynthesis. It can also regulate metabolism properly in salinity stress [10]. Plants treated with SA (0.1 and 0.5 mM). Salinity strongly reduced the nitrogenase activity by 60% - 75% in all the parameters determined apparent nitrogenase activity (ANA), total nitrogenase activity (TNA) and nitrogen fixation rate (NFR). Interestingly, SA pretreatment fully abolished the negative effect of salinity in the above mentioned parameters. In the absence of salt stress, nitrogenase activity displayed different responses depending on the SA dose: 0.1 mM SA induced a significant increase of the ANA, TNA and NFR whereas 0.5 mM slightly reduced the three determined nitrogenase parameters by 20% [11].

Watermelon is moderately sensitive to salinity. Its economic losses start above $2.0 \text{ ds}\cdot\text{m}^{-1}$ and yield decrease about 10% at $EC 2.5 \text{ ds}\cdot\text{m}^{-1}$, 10% at 3.3, 25% at 3.5 and 50% at $4.5 \text{ ds}\cdot\text{m}^{-1}$ [12]. Time of watermelon seeds germination decreased by salicylic acid in stress conditions. So, seeds come out of the soil earlier [13]. The objective was to investigate the effect of foliar application of salicylic acid under salinity stress and its alleviation in watermelon and also to find the optimum dose of salicylic acid for salt tolerance.

2. Materials and Methods

Study was carried out at mushroom glass house in Institute of Horticultural Sciences, University of Agriculture, Faisalabad. Charleston Gray, a variety of watermelon, was grown under moderate saline regime of ($3 \text{ ds}\cdot\text{m}^{-1}$ NaCl). Exogenous application of salicylic acid with four doses (0.5, 1.0, 2.5 and 5.0 mmol/L) was applied along with control. Seeds were sown in plastic pots containing sand rinsed with distilled water. The number of seedlings per pot was adjusted to seven and the seeds were watered according to the need of plant by observing the moisture of sand. There were three replications for each treatment. Seeds were watered according to the need of plant by observing the moisture of sand. After sowing, half strength (0.5) Hoagland's nutrient solution was applied to plants for nourishment. Salinity of sand in pots was maintained through occasional checking by EC meter. Experiment was continued till four to five plant leaves stage.

At three to four leaf stages plants were harvested. Following parameters of both phases regarding shoot length (cm), root length (cm), total length (cm), total fresh weight (g), total dry weight (g), chlorophyll contents (SPAD unit), nitrogen (N) contents ($\text{mg}\cdot\text{g}^{-1}$ DW), phosphorus contents ($\text{mg}\cdot\text{g}^{-1}$ DW), potassium contents ($\text{mg}\cdot\text{g}^{-1}$ DW) and protein contents (mg) [14] were tested. Foliar application of different levels of salicylic acid was compared with control regarding alleviation of salinity stress.

The experiment was laid out in completely randomized design (CRD) single factor arrangement was applied to the experiment with 3 replications. Collected data were analyzed statistically by applying Fisher's analysis of variance technique and significance of treatments will be tested by using LSD test [15].

3. Results

3.1. Morphological Attributes

Maximum shoot length (3.52 cm) was observed at 2.5 mmol/L while 0.5 mmol/L minimum shoot length (2.60

cm) was observed at control. On overall basis all concentrations of salicylic acid showed increasing trend in shoot length in alleviation of salinity stress (**Table 1**).

The effect of different concentration of SA on the root length of watermelon cv. Charleston gray is graphically shown in **Table 1**. The SA treatment showed the significant result on root length of the seeds under salinity stress of $3 \text{ ds}\cdot\text{m}^{-1}$. Maximum root length (13.18 cm) was observed at 1.0 mmol/L and minimum root length of was observed at 0.5 mmol/L (**Table 1**).

Analysis of variance revealed that SA treatment had significant effect on fresh weight of the plant grown under salinity stress of $3 \text{ ds}\cdot\text{m}^{-1}$. Maximum fresh weight (1.97 g) was observed at 2.5 mmol/L followed by 1.0 mmol/L, 5 mmol/L, 0.5 mmol/L resulted and minimum (1.04 g) fresh weight of was observed at control (**Table 1**).

Analysis of variance revealed that the SA treatment had significant effect on plant dry weight under salinity stress of $3 \text{ ds}\cdot\text{m}^{-1}$. Maximum dry weight (0.59 g) was observed at 5 mmol/L which followed by 2.5 mmol/L and 1.0 mmol/L and 0.5 mmol/L and minimum value (0.32 g) was observed at control. Plant dry weight decreased significantly as the concentration of salicylic acid increased from control to 5 mM when applied on plants (**Table 1**).

SA treatment had significant effect on number of leaves under salinity stress of $3 \text{ ds}\cdot\text{m}^{-1}$. Maximum number of leaves (4.14) were observed at 5 mmol/L, followed by 2.5 mmol/L, 1.0 mmol/L, control and lowest (3.21) were observed at 0.5 mmol/L (**Table 1**).

3.2. Chlorophyll Contents

SA treatment had significant effect on chlorophyll contents under salinity stress of $3 \text{ ds}\cdot\text{m}^{-1}$. Maximum chlorophyll contents (23.93 SPAD units) were observed at 5 mmol/L, followed by 2.5 mmol/L showed, 1.0 mmol/L showed and 0.5 mmol/L were at par and minimum chlorophyll contents (9.07 SPAD units) was observed at control (**Table 1**).

3.3. N-P-K Contents

Under saline regime ($3 \text{ ds}\cdot\text{m}^{-1}$) remarkable effect of SA with maximum nitrogen contents ($0.48 \text{ mg}\cdot\text{g}^{-1} \text{ DW}$) were observed at 5.0 mmol/L followed by 2.5 mmol/L, 0.5 mmol/L, 1 mmol/L and least ($0.32 \text{ mg}\cdot\text{g}^{-1} \text{ DW}$) were recorded at control. Maximum phosphorous contents ($0.297 \text{ mg}\cdot\text{g}^{-1} \text{ DW}$) were observed at 1.0 mmol/L followed by 2.5 mmol/L, 5.0 mmol/L while 0.5 mmol/L and lowest phosphorous contents ($0.081 \text{ mg}\cdot\text{g}^{-1} \text{ DW}$) were ob-

Table 1. Response of different concentrations of salicylic acid on mortality (%), shoot length (cm), root length (cm), plant fresh weight (g), plant dry weight (g), chlorophyll contents (CCI), (h) nitrogen (N) concentration ($\text{mg}\cdot\text{g}^{-1} \text{ DW}$), phosphorus concentration ($\text{mg}\cdot\text{g}^{-1} \text{ DW}$), potassium concentration ($\text{mg}\cdot\text{g}^{-1} \text{ DW}$) and protein contents ($\text{mg}\cdot\text{g}^{-1} \text{ DW}$) of Charleston gray.

Attributes	Units	Levels of salicylic acid				
		Control	0.5 mmol/L	1.0 mmol/L	2.5 mmol/L	5.0 mmol/L
Shoot length	(cm)	2.60 (c)	2.75 (bc)	3.10 (ab)	3.37 (a)	3.52 (a)
Root length	(cm)	9.26 (b)	7.79 (b)	13.18 (a)	9.24 (a)	13.03 (b)
Plant fresh weight	(g)	1.04 (b)	1.39 (b)	1.75 (b)	1.97 (ab)	1.73 (a)
Plant dry weight	(g)	0.34(b)	0.32 (b)	0.40 (b)	0.46 (ab)	0.59 (a)
Number of leaves		3.22 (b)	3.21 (b)	3.44 (b)	3.55 (ab)	4.14 (ab)
Chlorophyll contents	(SPAD unit)	9.07 (d)	14.10 (c)	14.80 (bc)	19.03 (b)	23.93 (c)
Nitrogen	($\text{mg}\cdot\text{g}^{-1} \text{ DW}$)	0.32 (b)	0.35 (b)	0.42 (ab)	0.40 (ab)	0.48 (a)
Phosphorous	($\text{mg}\cdot\text{g}^{-1} \text{ DW}$)	0.081 (b)	0.092 (b)	0.297 (a)	0.101 (b)	0.098 (b)
Potassium	($\text{mg}\cdot\text{g}^{-1} \text{ DW}$)	11.6 (b)	14.2 (b)	19.6 (a)	20.5 (a)	20.8 (a)
Protein	($\text{mg}\cdot\text{g}^{-1} \text{ DW}$)	1.77 (b)	2.19 (b)	2.60 (ab)	2.50 (ab)	2.60 (a)

Figures sharing same letters do not differ significantly from each other at $P = 0.05$.

served at control. Maximum potassium contents ($20.8 \text{ mg}\cdot\text{g}^{-1} \text{ DW}$) were observed at 5 mmol/L, followed by 2.5 mmol/L, 1.0 mmol/L, 0.5 mmol/L showed and lowest potassium contents ($11.60 \text{ mg}\cdot\text{g}^{-1} \text{ DW}$) were observed at control. Maximum crude protein contents were observed at 5 mmol/L, followed by 2.5 mmol/L, 1.0 mmol/L, 0.5 mmol/L lowest were observed at control. Crude protein contents were in same trend as nitrogen contents (**Table 1**).

4. Discussion

If we want to face the challenges of the 21st century, we should have to pay attention to increasing the yield of crops by developing salt tolerance cultivar [16]. So, for mitigation of salinity salicylic acid was applied. The results in induction phase were justified by the finding of Khan *et al.* (2009) [17] that SA treatments showed significantly better results over control by improvement in root and shoot length. And it was reported remarkable reduction in shoot length as well as in the total plant biomass production. Results confirmed the finding of Mady (2009) [18] that different applied treatments of SA significantly increased all studied growth parameters as number of branches and leave per plant.

Guti Crrez-Coronado *et al.* (1998) [19] claimed that SA had intense effect on root development of *Glycine max*. The mechanism by which SA is causing such an effect is unknown, though the mechanism regulating cell enlargement and cell division must be altered.

These results were justified by the statement of Guti Crrez-Coronado *et al.* (1998) [19] that shoot growth increased with the application of three concentrations of salicylic acid used, on an average increase of 23% and 20% in plant length was detected under greenhouse conditions as well as in field experiments, respectively.

Ameliorative effect of SA was confirmed by Mady (2009) [18] results that different applied treatments significantly increased trend in dry weight as concentration of SA. And significantly increased all studied growth parameters, obviously increased photosynthetic pigment. This increase might be due to physiological role of SA in plant as phytohormone. These results were similar to the findings of (Palma *et al.*, 2013) [11]. Mady (2009) [18] studied the effect of SA on minerals contents, data discovered that all applied treatments were effectively improved N contents in tomato leaves compared with those of control plants in both seasons. Also, it could be noticed that SA at 50 ppm was superior to all concentrations. So, the results were matched with these findings. Mady (2009) [18] studied the effect of SA on minerals contents, data discovered that all applied treatments were effectively improved P contents in tomato leaves compared with those of control plants in both seasons. Also, it could be noticed that SA at 50 ppm was superior to all concentrations. So, the results were matched with these findings. The possible reason for decrease in K^+ concentration with increase in salinity could be that as Na^+ is similar to K^+ and many K^+ transporters do not discriminate sufficiently between these cations, excess external Na^+ can not only impair K^+ acquisition but also lead to accumulation of Na^+ in plant cells. More Na^+ influx inhibits K^+ permeability to the cells thus decreasing its concentration (Pardo and Quintero, 2002) [20]. Mady (2009) [18] studied the effect of SA on minerals contents, data discovered that all applied treatments were effectively improved K content in tomato leaves compared with those of control plants in both seasons. Also, it could be noticed that SA at 50 ppm was superior to all concentrations. So, the results were matched with these findings. This increased in trend was matched with the findings of (Radwan *et al.*, 2007) [21] which stated that no substantial increase was caused by (10 mM SA) treatment in soluble or total proteins. But higher concentrations, 50 mM and 100 mM of salicylic acid, demonstrate a conspicuous increase in all fractions. Mady (2009) [18] revealed that different applied treatments significantly increased all studied growth parameters especially crude protein concentrations in leaves of treated plants as compared with those of untreated ones. Data relating to protein contents represented that all salinity levels showed highly significant differences. On overall basis all concentrations of salicylic acid showed increasing trend in alleviation of salinity stress as was described by Volt *et al.* (2009) [22] that salicylic acid was involved in plant defense against stresses.

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