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Foliar Application of Salicylic Acid as Potent Inducer of Salt Tolerance in Radish (*Raphanus sativus* L.)

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Abstract: Major objective of the present study were to determine ameliorative effect of salicylic acid (SA) on chlorophyll pigments and gas exchange attributes of radish under saline conditions. Two varieties of radish (Desi White and Lal Pari) were grown under non-saline (control) or saline (100 mM NaCl) conditions. Varying levels of salicylic acid (0, 100 mg L^{-1}) were applied exogenously. Salt stress significantly reduced shoot fresh, dry weight and chlorophyll contents (Chl. a, chl. b and chl. a/b ratio) of both radish varieties. Exogenously applied SA caused a significant improvement in biomass production and chlorophyll contents under salt regimes. Salt stress also markedly reduced gas exchange attributes (*A, E, gs, Ci and WUE*) in both radish varieties. Higher reduction was found in Lal Pari while lower in Desi White under salt stress under saline conditions. Foliarly applied SA ameliorates the adverse of salt stress and improve gas exchange attribute. It is inferred that SA is economical and environment friendly alternative and can be implicated to improve the plant growth of radish in current scenario of salt stress.

Key words: Foliar Application · Salicylic Acid · Salt Tolerance · Radish

INTRODUCTION

Throughout civilized history, environmental stress due to high concentrations of salt in soils or water is one of the most somber factors limiting growth and productivity of agricultural crops, which are mainly sensitive to salinity. High salt content in the soil affect the soil porosity and also decreases the soil water potential that results in a physiological drought [1]. Higher conc. of sodium and chloride severely affects the physiology of plants, both at the cellular as well as whole plant levels [2]. Excessive accumulation of Na^+ and Cl^- ions create ionic imbalance which results in hindrance the uptake of mineral nutrients such as K^+, Ca^{2+} and Mn^{2+} etc. [3]. Accumulation of sodium and chloride ions in cells cause enzyme inhibition which adversely affect various cell metabolisms such as those of rubisco and PEP carboxylase [4] and degradation of photosynthetic pigments [5, 4]. Salt stress negatively affects photosynthetic process [6, 7] which is arbitrated through a decrease stomatal conductance, internal CO_2 partial pressure [8] and water use efficiency [9]. The reduction in net photosynthetic rate under saline regimes is considered as one of the most important factor responsible for reduced plant growth and productivity [10]. It is the need of time that some quick and effective measures to be worked out so that the crops deteriorating stress factors such as salinity can be countered successfully. The applications of plant growth regulators by foliar methods are found to play a vital role in plant responses to abiotic stress [11]. Salicylic acid is one of the important phenolic compounds very effective for stress ameliorators that have recently been recognized as a plant growth hormone [12]. Salicylic acid plays diverse physiological roles in crops, which include plant growth, flower induction, nutrient uptake, ethylene biosynthesis, stomatal movements, photosynthesis and enzyme activities [12]. The disease resistance and abiotic stress tolerance are the other roles assigned to SA [13]. Among abiotic stresses, SA has been reported to counter water stress [14, 15], low temperature [16], high temperature [17] and salinity stress [18-20].

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The present study was carried out to appraise the effect of salicylic acid on biomass production, photosynthetic pigments and gas exchange attributes in winter vegetable radish treated to NaCl stress.

MATERIALS AND METHODS

An experiment was performed in the research area, University of Gujrat. Fresh river sand was taken, washed two times with tap water. Plastic pots of 25 cm diameter were filled with sand. Seeds of radish varieties were obtained from Ayub Agricultural Research Institute (AARI), Faisalabad, Pakistan. Ten seeds of each radish variety were sown in each pot. After germination Hoagland's nutrient solution was used for irrigation and then plants were treated with 100 m*M* NaCl with control (0 m*M* NaCl). The desired salinity level was obtained. To alleviate the adverse effect of sodium chloride two levels of salicylic acid (SA) (0 and 100 mg L^{-1}) were applied by foliar method.

After 35 days of germination, when plants were at vegetative stage, followings data was recorded.

Gas Exchange Characteristics: Photosynthetic attributes were measured with an imported LCA-4 ADC open system infrared gas analyzer (IRGA) (Analytical Development Company, Hoddesdon, England) at 11am when light intensity was full. For this purpose a third one youngest, expanded leaf was used. Net CO_2 assimilation rate (*A*), transpiration rate (*E*), sub stomatal CO_2 concentration and stomatal conductance were noted. The given conditions for IRGA were used; leaf surface area11.35 cm^2, ambient CO_2 conc. 352 µmol mol^{-1}, leaf chamber flow rate (V) 25, temperature of leaf chamber varied from 31.5 to 37.8 °C, ambient pressure 99.2 kpa, molar flow of air per unit leaf area (Us) 22.06 mol m^{-2} s^{-1}, chamber vapor pressure varied from 4.4 to 6.6 mbar and PAR (Qleaf) at was maximum up to 1048 µmol m^{-2} s^{-1}.

Water Use Efficiency: Ratio of water use efficiency was measures dividing A over E

Chlorophyll Contents: Concentrations of chlorophyll contents were determined with the method as described by Arnon (1949). Fresh leaves sample (0.5g) were cut into very small segments and extracted overnight with 80% acetone at -10°C. The extract was filtered and made volume 10ml with 80% acetone and then readings was collected at 645, 652 and 663 nm using a spectrophotometer (Hitachi, Model U2001, Tokyo, Japan).

These Calculations Were Calculated Using the Following Formulae:

- Chl. a (mg g^{-1}f. wt.) = [12.7 (OD 663) - 2.69 (OD 645)] x V/1000 x W
- Chl. b (mg g^{-1}f. wt.) = [22.9 (OD 645) - 4.68 (OD 663)] x V/1000 x W

V = Volume of the leaf extract (ml)
W = Weight of the fresh leaf (g)

After recording all these data, plants were removed from pots and separated into shoots and roots. Then washed with distilled water and dried with filter paper. Fresh samples of shoots and roots were over dried at 70°C for 5 days and their dry weight recorded.

Experimental Design and Statistical Analysis: The experiment was set in a completely randomized design (CRD) with three replicates. Analysis of variance of all parameters was computed using the MSTAT computer package (MSTAT Development Team, 1989). The least significance difference (LSD) values for different attributes were calculated following Snedecor & Cochran (1980) for assessing the significant differences among mean values.

RESULTS

Analysis of variance of the data showed that salt stress significantly reduced shoot fresh weight of both radish varieties. Varieties showed different response in this attribute. Value of shoot fresh weight was improved by foliar application of salicylic acid (SA) under saline conditions. Variety Desi White showed higher rate of shoot fresh weight while, Lal Pari lower under saline regimes with foliar application of SA.

Imposition of salt to the rooting medium significantly decreased the shoot dry weight of both radish varieties. A variable response was noted in this parameter in both varieties. Shoot dry weight was improved by foliar application of salicylic acid in both varieties under saline conditions. Higher value of shoot dry weight was found in Desi White as compared to Lal Pari under saline condition with foliar application of SA.

Root zone salinity caused a significant reduction in chlorophyll a contents of both radish varieties. Exogenous application of salicylic acid (SA) caused a considerable increase in chlorophyll a of both varieties under saline conditions. A substantial response was observed in this attribute. Both radish varieties showed variable results at 100 m*M* NaCl and 100 m*M* SA. Chlorophyll a had highest value in Desi White as compared to Lal Pari under saline condition with foliar application of SA.

Saline growth medium caused a significant reduction in chlorophyll b contents of both radish varieties. Exogenous application of salicylic acid (SA) caused a significant improvement in chlorophyll b of both radish varieties under saline conditions. Both radish varieties showed variable results in this parameter. Higher value of chlorophyll b was observed in Desi White, while lower in Lal Pari under saline condition with foliar application of SA.

Salt stress caused a marked reduction in chlorophyll a/b ratio of both radish varieties. Value chlorophyll a/b ratio was improved under saline conditions by foliar application of salicylic acid (SA). Both radish varieties showed variable response in Chlorophyll a/b ratio. Chlorophyll a/b ratio was higher in Desi White while, lower in Lal Pari under saline condition with foliar application of SA.

Net photosynthetic rate (A) of both radish varieties was significantly reduced in both radish varieties. Foliar application of salicylic acid (SA) had improved CO_2 assimilation rate (A) under saline conditions. Both radish varieties showed changeable response in net photosynthetic rate. Higher rate of CO_2 assimilation rate (A) was observed in Desi White than those in Lal Pari under saline conditions with foliar application of SA.

Transpiration rate (E) of both radish varieties was markedly reduced due to addition of root zone salinity. Salicylic acid (SA) was applied as foliar method caused improvement in transpiration rate (E) of both radish varieties under saline conditions. Both radish varieties showed variable results in this parameter. Higher rate of transpiration rate (E) was observed in Desi White than those in Lal Pari under salt regimes with foliar applications of salicylic acid.

Salt stress significantly reduced the stomatal conductance of both radish varieties. By foliar application of salicylic acid (SA), improvement was found in stomatal conductance (g_s) under saline conditions. Both radish varieties showed variable in this parameter. Stomatal conductance (g_s) had higher value in Lal Pari as compared to Desi White under saline conditions with foliar application of SA.

Water use efficiency of both radish varieties was markedly reduced under saline condition. Both radish varieties illustrated changeable response at in this attribute. Water use efficiency had highest value in Desi White as compared to Lal Pari under saline conditions with foliar application of SA.

DISCUSSION

Salinity stress significantly reduced plant growth and development of agriculture crops [21, 7]. Salt stress severely reduced biomass production. Such reduction in plant growth due to less water uptake which influenced by soil osmotic potential [22, 23]. Signaling compounds that are able to reduce the effect of salt stress on crops and thus increase productivity could be of great importance to restoration of natural ecosystems as well as agricultural, horticultural and forestry production systems at world level. Foliar application of salicylic acid reduced adverse effects of salt stress and improved biomass of salinity treated plants. Results of present study are in agreements with the earlier findings where exogenously applied salicylic acid alleviates adverse effects of salt in various crops [24-27, 12].

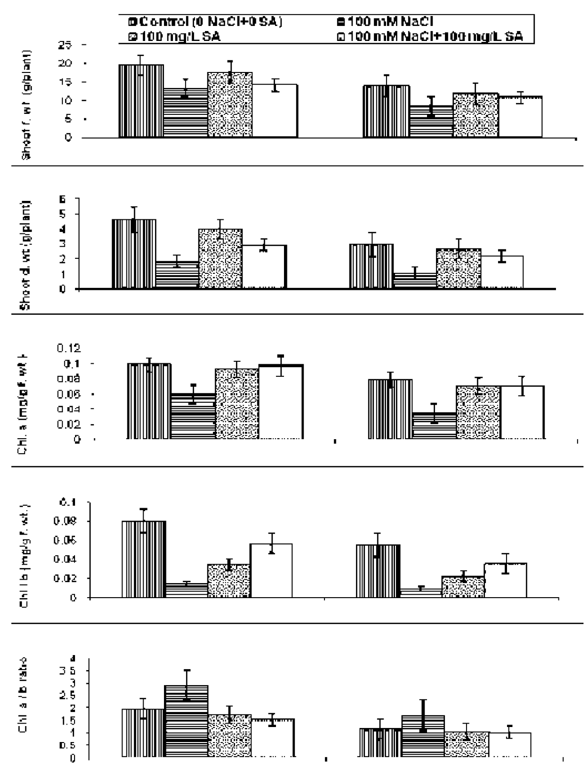

Fig. 1: Foliar application of SA on shoot fresh, dry weight and chlorophyll contents (Chl. a, chl. b, chl. a/b ratio), when 35 days old plant of two varieties were subjected to salt stress. (Mean ± S.E; n= 3).

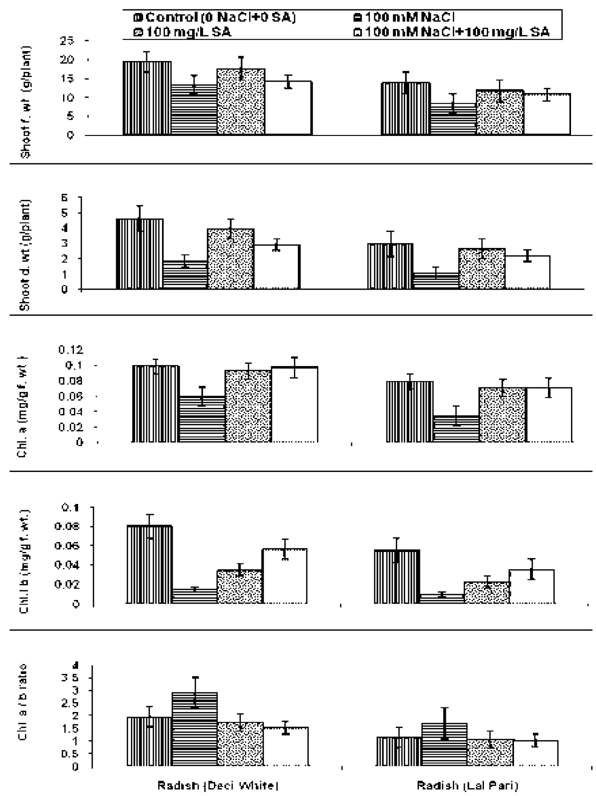

Fig. 2: Foliar application of SA on gas exchange attribute (A, E, gs, Ci, WUE), when 35 days old plant of two varieties were subjected to salt stress. (Mean ± S.E; n= 3).

Boughalleb *et al.* [28] reported that salt stress destroyed thylakoid membranes results in leakage of chloroplast contents. It has been documented that increases salt stress increases activity of the chlorophyll degrading enzyme cholorophyllase [29], resulting in reduction of chlorophyll contents [30, 7]. Salt stress significantly decreased chlorophyll contents of both radish varieties but improved by the foliar application of salicylic acid. Results of the present study are in agreements with findings of Rafique *et al.* [31] while working with pumpkin where salicylic acid alleviates the harmful effect of salt stress. Photosynthetic pigments play a key role in photosynthetic process. Salinity stress adversely effects net CO_2 assimilation rate in two radish varieties but exogenously applied salicylic acid ameliorate negative effects of salt stress. Results of the present study are in accordance with findings of Rafique *et al.* [31] while working with pumpkin and Fariduddin *et al.* [27], with *Brassica juncea* L. where exogenously applied salicylic acid mitigates negative effects of salinity stress. Transpiration rate markedly reduced under salt stress in both radish varieties, exogenously applied salicylic acid improved transpiration rate. Results of the present study are in agreements with the earlier findings of Stevens *et al.* [32] where salicylates reduced the negative effect of NaCl stress on transpiration rate in tomato. Value of stomata conductance reduced in both radish varieties under saline conditions while foliar applied salicylic acid mitigates this harmful effect and improved stomatal conductance.

From all these findings it is suggested that SA-induced improvement in growth of radish varieties might have been due to SA-induced increase in chlorophyll contents and net CO_2 assimilation rate. It is inferred that SA is economical and environment friendly alternative and can be implicated to improve the plant growth of radish in current scenario of salt stress.

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