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ORIGINAL ARTICLE

Effect of Salicylic Acid and Salinity in Rosemary (*Rosmarinus officinalis* L.): Investigation on Changes in Gas Exchange, Water Relations, and Membrane Stabilization

Sharareh Najafian, Morteza Khoshkhui, Vahid Tavallali

Department of Horticultural Science, College of Agriculture, Shiraz University, Shiraz-Iran.

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ABSTRACT

The present study investigates the role of salicylic acid (SA) in inducing plant tolerance to salinity. The application of 150, 300 and 450 ppm SA to rosemary [*Rosmarinus officinalis* L.] plants via foliar spraying provided protection against 50, 100 and 150 mM NaCl stress. SA treated plants had greater shoot and root dry weights compared to untreated plants when exposed to salt stress. At each salinity level, SA application at 300 ppm significantly increased shoots dry weight. Application of SA increased photosynthetic rates, stomatal conductance, mesophyll efficiency and water use efficiency in salt stressed plants. Transpiration rates were significantly lower in SA treated plants under saline stress conditions. SA application decreased electrolyte leakage compared to untreated plants. Beneficial effects of SA in saline conditions include sustaining the photosynthetic/transpiration activity and consequently growth, and may have contributed to the reduction or total avoidance of necrosis. SA, when used in appropriate concentrations, alleviates salinity stress without compromising the plants ability for growth under a favorable environment.

Key words: Photosynthesis, Salicylic acid, Salinity, Stress tolerance, Transpiration

Introduction

Crops are exposed to many environmental stresses limiting their yield potential. These stresses may be of a biotic (infection caused by fungi, bacteria and viruses, and/or damage by herbivores including insects) or abiotic (water, temperature, or ionic stresses) nature. Plants initially perceive environmental stresses and activate a range of defensive mechanisms [29]. These mechanisms may also be induced or enhanced by the application of some chemicals to the plants [23,13,21]. The application of salicylic acid (SA) has reported to induce tolerance in plants to many biotic and abiotic stresses including fungi, bacteria, and viruses [7]. chilling [26,13,25,26], drought [25,26], and heat [5,26,25]. Since SA is effective in inducing stress tolerance when applied as a soil drench [26], foliar or seed treatment [1] it appears that SA has a regulatory effect on activating biochemical pathways associated with tolerance mechanisms in plants [29,16].

As a part of the strategy in the development of stress resilient plants it is important that we understand particular mechanisms that plants use to tolerate stresses and how such mechanisms are induced. The application of chemical signals to alleviate stresses will facilitate both the maintenance

Corresponding Author

Vahid Tavallali, Department of Horticultural Science, College of Agriculture, Shiraz University, Shiraz-Iran
Email: tavallali@shirazu.ac.ir
phone number: +989177171937

of arable lands and allow for the expansion of plants into marginal areas that are currently unavailable. However, detailed understanding of the effects of these chemicals on key physiological processes determining plant productivity in relation to stress tolerance is warranted prior to practical application. Furthermore such studies may provide insight into molecular mechanisms governing stress tolerance in plants and may also facilitate genetic engineering of plants to tolerate stresses.

SA alters plants physiological functions including; nutrient uptake [8,9,10], membrane functioning [11], water relations [3], stomatal functioning [18,20,1], inhibition of ethylene biosynthesis [19,28], and increased growth [12,21]. These functions may have a key role in plants tolerance to salinity stress. To our knowledge, SA interactions with basic plant physiological functions have not been investigated under salinity stress in medicinal plants yet. The objective of this study was to determine the physiological responses (photosynthesis, transpiration and membrane functioning) associated with enhanced tolerance resulting from the application of SA to plants grown under saline condition.

Materials and methods

Plant material

Prepared cuttings were established under mist system with a temperature of 25/18 °C day/night, relative humidity of 75% and soil moisture at saturation level. One month after cuttings planting, callus tissue was formed at the bottom of cutting. In order to root facility, cuttings were sprayed 3 times a day at 10, 12 am and 2 pm with mist system until soil moisture reached field capacity.

Uniform rooted cuttings (*Rosmarinus officinalis* L.) were grown in a controlled environment greenhouse at 25/18 °C day/night temperature, 12 h photoperiod (PAR 300 µmol m^{-2} s^{-1}) and 70% relative humidity. Potting mix was a sandy-loam texture soil with pH 7.6, EC 1.8 dS m^{-1}, 1.77% organic matter, 0.06% N, 14 mg P kg^{-1}, 275 mg K kg^{-1}, 3.75 mg Fe kg^{-1}, 0.9 mg Zn kg^{-1}, 0.07 mg Cu kg^{-1}. Plants were irrigated with distilled water daily.

Salt treatments

Four months after transplanting, Rosemary plants were subjected to 0 mM (control), 50 mM, 100 mM and 150 mM NaCl concentrations at 7-day intervals using 0.5 L irrigation water per pot. Water content of the pots was maintained at 80% field capacity with distilled water till the end of the experiment.

SA application

SA was dissolved in absolute ethanol and then added drop wise to water (ethanol:water, 1:1000, v/v) [30]. SA application (0, 150, 300, 450 ppm) consisted of foliar spray, which occurred after covering the soil surface in order to omission of SA interfering via soil. Initial SA treatments occurred one week after salt treatments with 7-day intervals. This allowed for a known amount of SA for plant uptake. Untreated plants received ethanol:water 1:1000, v/v over the two application times.

Measurements

One hundred fifty days after SA treatment, Rosemary plants were harvested and following parameters were measured.

Shoots and roots dry weight

Plants divided into shoot (stem + leaves), and root components. Roots were separated from the soil by washing them onto sieves and then separating roots from any remaining soil and organic debris. After the harvest, plant materials were washed first with tap water and then twice with deionized water, before being oven-dried at 70°C to attain a constant weight for biomass estimation (dry weight).

Gas exchange and water relations

Measurements were conducted one month before harvest. The youngest fully expanded leaves (4th leaf counting from stem top) of individual plants were used for gas exchange measurements (n = 4). Gas exchange characteristics were measured 4–6 h during the 12 h photoperiod using a LC*i* (ADC, Bioscientific, England) portable photosynthesis system (block temperature 25 °C, CO_2 reference 360 µmol CO_2 mol^{-1}, PAR 500 µmol m^{-2} s^{-1}, flow rate 300 µmol s^{-1}). Water use efficiency and mesophyll efficiency were calculated according to the following equations (Ashraf et al., 2002):

Water use efficiency (WUE) = P_N/E, Mesophyll efficiency (ME) = P_N/Ci.

Electrolyte leakage percentage

Electrolyte leakage percentage was used to assess membrane permeability based on the method of Sairam *et al.* [24]. Leaves washed three times with distilled water to remove surface contamination and then they were cut and placed in individual stopper vials containing 10 ml of distilled water. These samples were incubated at 40°C for 30 min. Electrical conductivity of solution (EC1) was

measured after incubation using a conductivity meter (Model Ohm-419). Samples were then placed in boiling water for 10 min and the second measurements (EC2) were done after cooling the bathing solutions to room temperature. ELP was calculated as [1– (EC1/EC2)] × 100.

Statistical analysis

Treatments were arranged in a completely randomized design with 16 treatments. The measurements were made on 4 pots and 4 plants in each. The layout was a 4×4 factorial arrangement with four replications. Analysis of variance was performed using the SPSS software package and means were separated using Duncan's test ($p \leq 0.05$).

Results and discussion

Dry weight of shoots and roots. Shoot and root dry weights were reduced as the salinity level increased in irrigation water, whereas, the main effects of salinity on shoot and root dry weights were not statistically significant as compared with control (Fig. 1). SA application enhanced Shoot and roots dry weights under stress conditions compared with only salt treatment. There were no significant differences between SA concentrations.

Gas exchange and water relations

As the NaCl levels increased in irrigation water, P_N, g_s and E were decreased compared to the control, Whereas, *ME* and *WUE* was showed increased. SA application caused a noticeable enhancement of P_N, g_s, E and ME in contrast with salt stress alone, and 150 ppm SA was more effective.In addition, g_s was showed significant increased at 150 ppm SA and 50 mM NaCl (Fig. 2).Plant treated with SA had a significant effect on transpiration rate (E) in compared to the control.

Electrolyte leakage percentage

Electrolyte leakage percentage (ELP) represents cell membrane injury. With increasing salinity, the ELP was increased significantly at 150 mM NaCl in leaves of seedlings compared to the control. The conductivity in NaCl-treated plants with sprayed SA showed a significant decrease (at 150 mM NaCl) which SA application at 150, 300, and 450 ppm significantly reduced ELP. Treatments with only SA showed lesser differences in conductivity (Fig. 3).

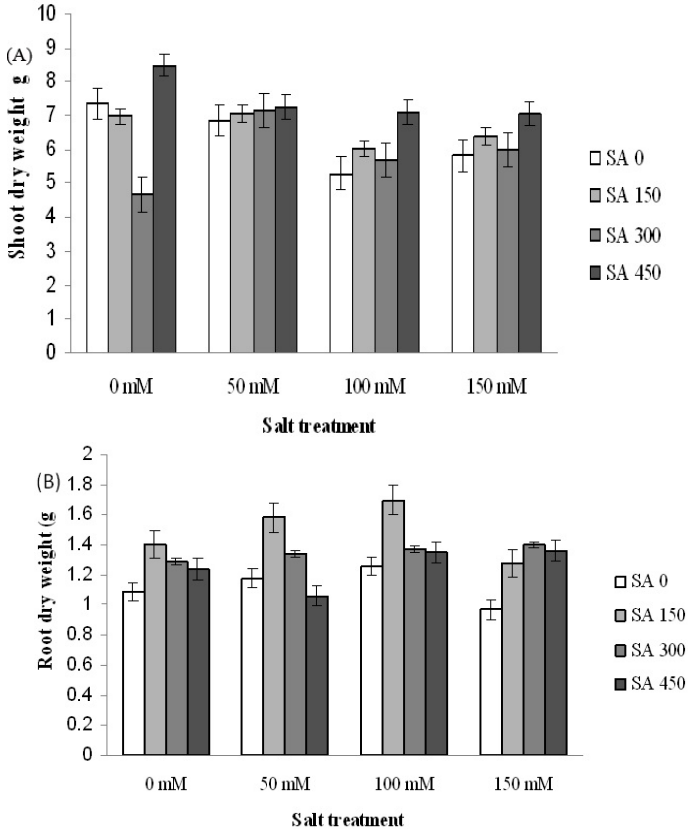

Fig. 1: Effect of exogenous SA application on mean (A) shoot dry weight and (B) root dry weight of Rosemary seedlings subjected to salinity stress. Vertical bars represent SE of the means (n = 4).

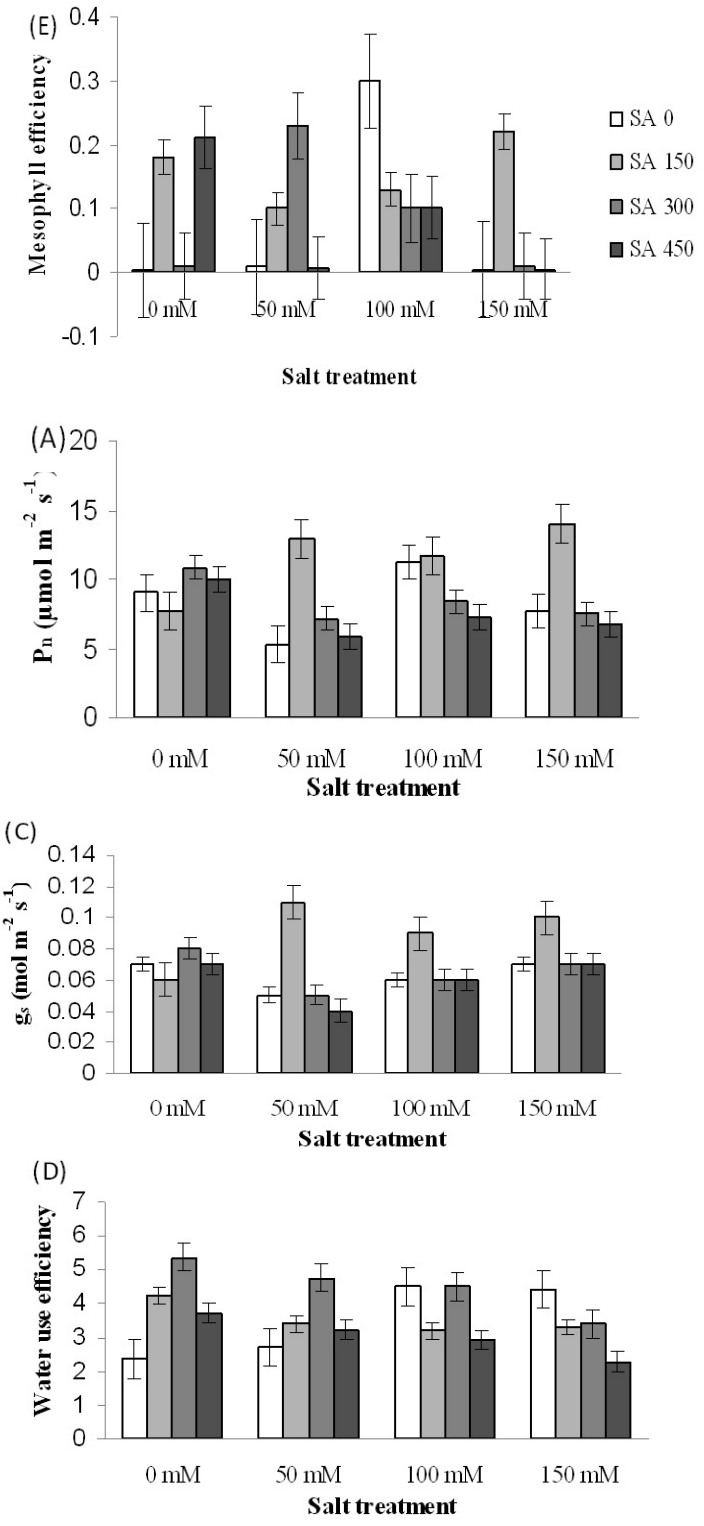

Fig. 2: Effects of exogenous SA application on mean (A) photosynthetic rate (B) transpiration rate (C) stomatal conductance (D) water use efficiency and (E) mesophyll efficiency of Rosemary seedlings subjected to salinity stress. Vertical bars represent SE of the means (n = 4).

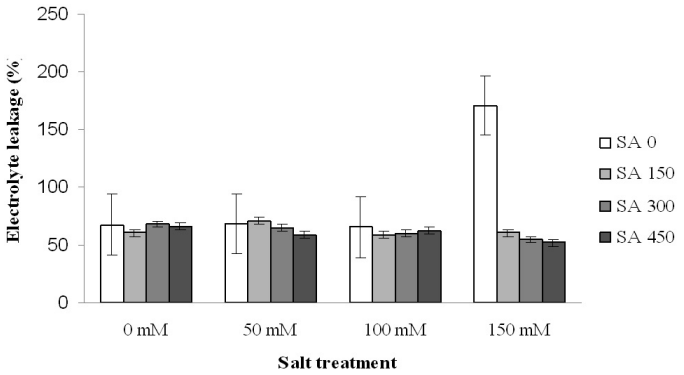

Fig. 3: Effect of exogenous SA application on mean of electrolyte (%) of Rosemary seedlings subjected to salinity stress. Vertical bars represent SE of the means (n = 4).

Discussion

Signalling compounds that are able to reduce the effect of stresses on plants and thus increase productivity could be of great importance to restoration of natural ecosystems as well as agricultural, horticultural and forestry production systems around the world. This is the first study linking increased growth of saline stressed Rosemary plants with protection of the photosynthetic system. This study demonstrates that the spray of SA after to the exposure to salinity stresses increases survival and decreases the severity of the stress injury in Rosemary seedlings. This agrees with the findings of others that SA induces tolerance to many biotic [7] and abiotic stresses [7,5,26,13].

As shown in Fig. 1 and 2, SA could completely eliminate the deleterious effects of the severe abiotic stresses as shown by an increase in shoot and root dry weights, photosynthesis, stomatal conductance and mesophyll efficiency. It does improve plant tolerance to salinity in comparison to untreated plants.

SA reduced the negative affects of salinity stress on shoot and root dry weights in Rosemary seedlings, which has previously been unreported. The present study demonstrated that concentration of 150 ppm SA has positive effect on plant photosynthesis and stomatal conductance when plants were under stress conditions. The protection of the photosynthetic apparatus has also been shown in SA treated drought stressed Jack pine [21] and wheat [27]. Enhanced activity of certain antioxidant enzymes with SA treatment has been reported [13,28,14]. These enzymes may have an important role in protecting the photosynthetic apparatus by scavenging active oxygen species arising during stress.

Abscisic acid (ABA) is an important plant signaling molecule induced by stress conditions and controls stomatal functioning leading to the decrease in leaf transpiration under water stress conditions. SA has been shown to reverse ABA controlled stomatal closure [20]. Transpiration rates of SA treated plants under unstress conditions was showed a significantly decreased in our study may be linked to the influence of SA on ABA regulated stomatal functioning (especially at 150 ppm).

Transpiration rates may be decreased by the application of salicylates and are likely to be concentration and plant species dependent. Stomatal closure has been observed within 13 min when *Commelina communis* leaves were treated with a 10 mM acetyl-salicylic acid (ASA) solution [18]. Other studies have shown SA application to decrease stomatal apertures at much lower concentrations [20,3] In the current study, SA applied via spraying at 150, 300 and 450 ppm had noticeable effects on transpiration rates of Rosemary plants under unstress conditions. In Arabidopsis, SA has been proposed to have a dual role, which may explain differences in plant responses to SA [4]. SA is necessary for the induction of antioxidant defenses and is essential for plant protection against oxidative stress [22]. Excessive SA accumulation can induce a programmed cell death pathway [22], with adverse effects of SA above 1 mM being observed in tomato and bean plants [26]. However, in our finding, it appears that the true beneficial effect of SA on Rosemary plant transpiration apparent at 150 ppm concentrations, however this critical concentration may vary between species. It is therefore evident that SA regulates stomatal behavior although the exact mechanism is yet to be elucidated. Maintaining integrity of cellular membranes under stress conditions is considered an integral part of salinity tolerance mechanisms. This study showed that SA reduced the amount of ion leakage (measured as electrolytes) in salt stressed Rosemary seedlings indicating that SA treatment has facilitated the maintenance of membrane functions (i.e., semi-permeability) under stress conditions (at 150Mm NaCl). Supporting evidence was shown when SA reduced electrolyte leakage in corn leaf, rice leaf and cucumber hypocotyls under chilling stress [15].

SA allows maintenance of photosynthesis, transpiration, stomatal conductance and growth at higher rates in saline stress conditions compared to untreated plants. To our knowledge such information has not been previously reported. Reduction in such physiological parameters previously reported may be a result of higher dosages of SA used where toxic effects are more pronounced than beneficial effects of SA [3]. It appears that SA may be used to alleviate adverse effects of salinity stress with compromising the plants ability to grow under unstressed conditions in rosemary (especially at 150 ppm).

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