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Effect of Salicylic Acid and Salinity in Thyme (*Thymus Vulgaris* L.): Investigation on Changes in Gas Exchange, Water Relations, and Membrane Stabilization and Biomass Accumulation

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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 thyme [*Thymus vulgaris* 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. SA application at 150 ppm significantly increased roots dry weight. Application of SA increased photosynthetic rates, mesophyll efficiency and water use efficiency in salt stressed plants. Transpiration rates and stomatal conductance were also significantly iesser in SA treated plants under saline stress conditions. SA application increased 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 (Sticher *et al.* 1997). These mechanisms may also be induced or enhanced by the application of some chemicals to the plants (Raskin 1995; Janda *et al.* 1999; Rajasekaran and Blake 1999). The application of salicylic acid (SA) has reported to induce tolerance in plants to many biotic and abiotic stresses including fungi, bacteria, and viruses (Delany *et al.* 1994), chilling (Senaratna *et al.* 1998; Janda *et al.* 1999; Senaratna *et al.* 2000; 2003), drought (Senaratna *et al.* 1998; 2000; 2003), and heat (Dat *et al.* 1998; Senaratna *et al.* 2000; Senaratna *et al.* 2003). Since SA is effective in inducing stress tolerance when applied as a soil drench (Senaratna *et al.* 2000), foliar or seed treatment (Aldesuquy *et al.* 1998) it appears that SA has a regulatory effect on activating biochemical pathways associated with tolerance mechanisms in plants (Sticher *et al.* 1997; Klessig *et al.* 1998).

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 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 (Glass 1973, 1974, 1975), membrane functioning (Glass and Dunlop 1974), water relations (Barkosky and Einhellig 1993), stomatal functioning (Larque-Saavedra 1979; Rai *et al.* 1986; Aldesuquy *et al.* 1998), inhibition of ethylene biosynthesis (Leslie and Romani 1988; Srivastava and Dwivedi 2000), and increased growth (Gutierrez-Coronado *et al.* 1998; Rajasekaran and Blake 1999). These functions may have a key role in plants tolerance to salinity stress. To

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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:

One year old thyme seedlings (*Thymus vulgaris* L.) were grown in a controlled environment greenhouse at 25/18 $^{\circ}$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}, 0.7 mg Cu kg^{-1}. Plants were irrigated with distilled water daily.

Salt Treatments:

Four months after transplanting, thyme seedlings were subjected to 0 mM (control), 50 mM, 100 mM and 150 mM NaCl concentrations at 5-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) (Williams *et al.* 2003). 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, thyme seedlings 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 $^{\circ}$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.* (1997). 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 iriigation 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 at 150, ppm significantly enhanced roots dry weights. There were no significant differences between SA concentrations at 300 and 450 ppm.

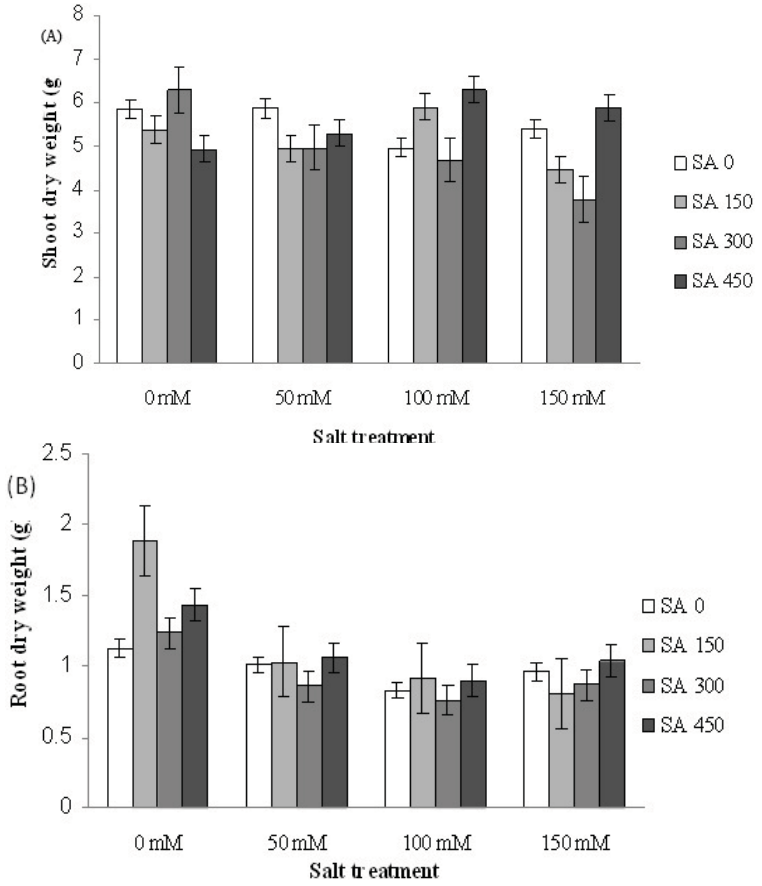

Fig. 1: Effect of exogenous SA application on mean (A) shoot dry weight and (B) root dry weight of thyme seedlings subjected to salinity stress. Vertical bars represent SE of the means (n = 4).

Gas Exchange and Water Relations:

As the NaCl levels increased in irrigation water, P_N, g_s, E, ME, and WUE were decreased compared to the control, Whereas, P_N was significantly decreased at 100 and 150 mM NaCl concentrations. SA application caused a noticeable enhancement of P_N, g_s, E, WUE and ME in contrast with salt stress alone, and 450 ppm SA was more effective (Fig. 2). SA at 150 ppm had no a significant effect on stomatal conductance (g_s) in compared to the control and ME was showed increased *at* 150 ppm.

Electrolyte Leakage Percentage:

With increasing salinity level, the ELP was increased in leaves of seedlings compared to the control. SA application increased the ELP significantly at 450 ppm,Whereas,*it was showed no* significantly at 150 and 300 ppm, Regardless of salt concentration, growing plants with SA application, decreased ELP of plants leaves, especially at 150 ppm SA with 50 mM and 150 mM NaCl. Treatments with only SA also showed lesser differences in conductivity (Fig. 3).

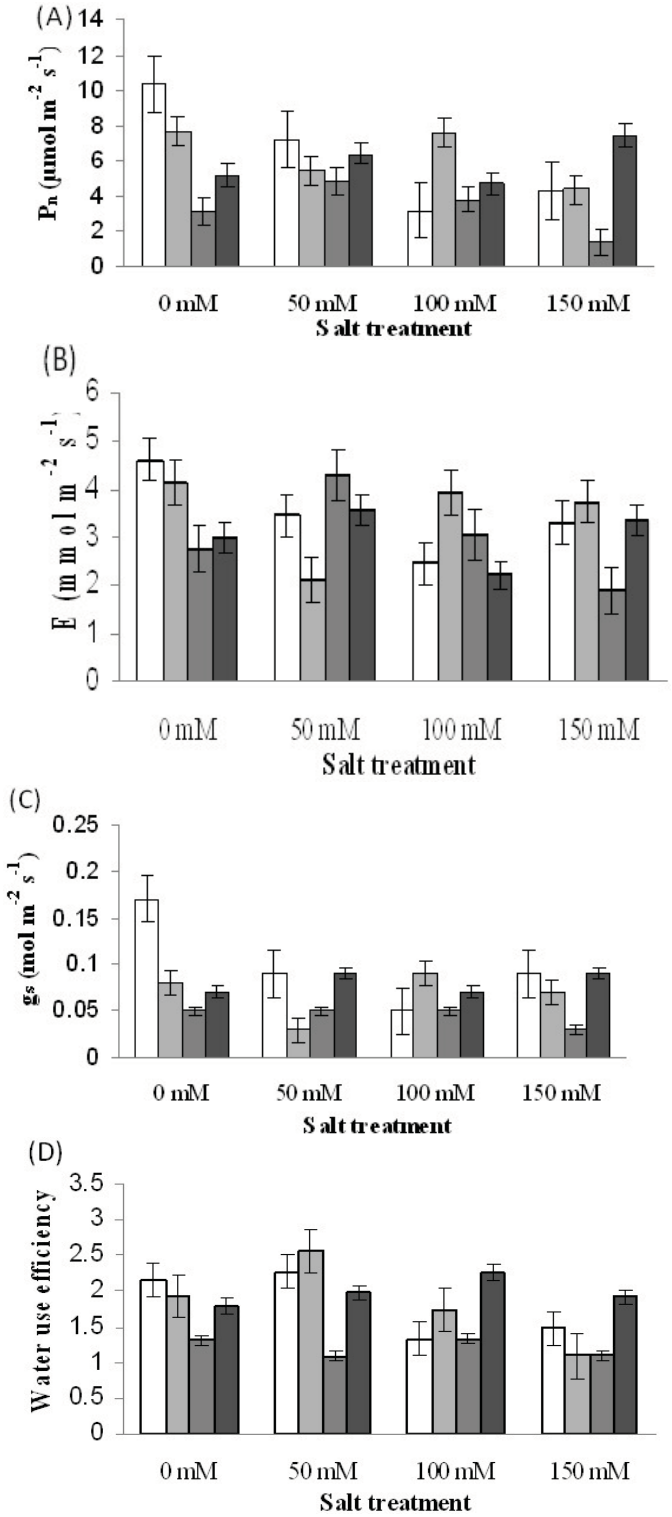

Fig. 2: Effects of exogenous SA application on mean (A) photosynthetic rate (B) transpiration rate (C) stomatal conductance (D) water use efficiency and (E) mesophyll efficiency of thyme seedlings subjected to salinity stress. Vertical bars represent SE of the means (n = 4).

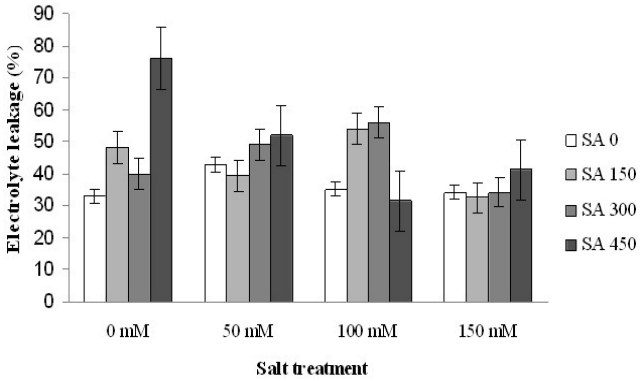

Fig. 3: Effect of exogenous SA application on mean of electrolyte (%) of thyme seedlings subjected to salinity stress. Vertical bars represent SE of the means (n = 4).

Discussion:

Signaling compounds that are able to reduce the adverse effects of stresses on plants could be of great importance to restoration of natural ecosystems as well as agricultural, horticultural and forestry production systems. This is the first study linking increased growth of saline stressed thyme with protection of the photosynthetic system.

This study demonstrates that SA application after exposure to salinity stress, increases survival and decreases the severity of the stress injuries in thyme seedlings. This agrees with the findings of others that reported SA induces tolerance to many biotic (Delany *et al.* 1994) and abiotic stresses (Delany *et al.* 1994; Dat *et al.* 1998; Senaratna *et al.* 1998; Janda *et al.* 1999; Senaratna *et al.* 2000).

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 root dry weights, mesophyll efficiency at 150 ppm SA. It does improve plant tolerance to salinity in comparison to untreated plants.

Reduction in the negative affects of salinity on biomass accumulation of thyme seedlings by SA application has not previously been reported. SA did not have any deleterious effects on biomass of unstressed plants at levels used in the present study. Our results demonstrated that concentrations of 150 ppm SA have positive effects on plants biomass in root when plant were under stress or non-stressed conditions. The protection of the photosynthetic apparatus has also been reported in SA treated drought stressed Jack pine (Rajasekaran and Blake 1999) and wheat (Singh and Usha 2003). Enhanced activity of certain antioxidant enzymes with SA treatment has been reported (Janda *et al.* 1999; Srivastava and Dwivedi 2000; Kang *et al.* 2003). 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 closure leading to the decrease in leaf transpiration under water stress conditions (Leung and Giraudat 1998). SA has been shown to reverse ABA controlled stomatal closure (Rai *et al.* 1986). Relatively lower transpiration rates of SA treated plants under salt stress conditions observed in this study may be linked to the influence of SA on plant species.

Transpiration rates may be decreased by the application of salicylates and are likely to be dependent on concentration and plant species. Stomatal closure has been observed within 13 min when *Commelina communis* leaves were treated with a 10 mM acetyl-salicylic acid solution (Larque-Saavedra 1979). Other studies have shown SA application to decrease stomatal apertures at much lower concentrations (Rai *et al.* 1986; Barkosky and Einhellig 1993). However, in the current study, SA application at 150, 300 and 450 ppm had noticeable effects on transpiration rates of thyme plants under stress and non-saline conditions. In Arabidopsis, SA has been proposed to have a dual role, which may explain differences in plants responses to SA (Borsani *et al.* 2001). SA is necessary for the induction of antioxidant defenses and is essential for plant protection against oxidative stress (Rao and Davis 1999). Excessive SA accumulation can induce senescence of cells (Rao and Davis 1999), with adverse effects of SA above 1 mM being observed in tomato and bean plants (Senaratna *et al.* 1998). However, based on our finding, it appears that the true beneficial effect of SA on thyme plant transpiration apparented, so the critical concentration may vary in different 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. Electrolyte leakage percentage (ELP) represents cell membrane injury. This study showed that SA application increased the ion leakage in salt stressed thyme seedlings at higher concentrations, indicating that SA concentrations was very important under saline conditions. Supporting evidence was shown when SA reduced electrolyte leakage in corn leaf, rice leaf and cucumber hypocotyls under chilling stress (Kang and Saltveit 2001).

To our knowledge such information has not been previously reported. Reduction in such physiological processes previously reported may be a result of higher dosages of SA used which toxic effects of SA are more pronounced than its beneficial effects (Barkosky and Einhellig 1993). It appears that SA may be used to alleviate adverse effects of salinity stress at 150 ppm with compromising the plants ability to grow under unstressed conditions.

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