Ecotoxicology and Environmental Safety 166 (2018) 18–25

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

# Foliar sprays of salicylic acid and jasmonic acid stimulate $H^+$-ATPase activity of tonoplast, nutrient uptake and salt tolerance of soybean

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ARTICLE INFO

*Keywords:*
$H^+$-ATPase
Jasmonic acid
Nutrient uptake
Salicylic acid
Salinity
Soybean

ABSTRACT

This research was conducted as factorial on the basis of randomized complete block design with three replications to evaluate the effects of salicylic acid (1 mM SA), jasmonic acid (0.5 mM JA) and SA + JA on $H^+$-ATPase hydrolytic activity of tonoplast in soybean roots under 0, 4, 7 and 10 dS $m^{-1}$ NaCl levels. The $H^+$-ATPase hydrolytic activity of tonoplast was increased under 4 dS $m^{-1}$, but with rising salinity up to 7 and 10 dS $m^{-1}$, the activity of $H^+$-ATPase and ATP content were decreased in root cells. Root growth, potassium, calcium, magnesium and iron contents in plant tissues were decreased, while sodium, manganese, zinc and copper contents were increased by salinity, leading to a reduction in chlorophyll content index (CCI), relative water content (RWC), plant biomass and grain yield of soybean. Treatment of plants with SA, JA and SA + JA improved $H^+$-ATPase activity and ATP content in root cells. JA treatment also reduced root growth, thereby limited sodium uptake by roots and translocation to the shoots. Foliar spray of JA only increased magnesium and iron contents in plant tissues, with no significant effect on other cations. In contrast, SA and SA + JA improved root growth and enhanced most of the cations, CCI, RWC, plant biomass and consequently grain yield under different levels of salinity. The SA + JA was a superior treatment in diminishing the harmful effects of salinity on soybean plant performance, compared with individual application of these growth regulators.

## 1. Introduction

Soybean seed is a major source of high-quality oil and protein for human consumption (Puyang et al., 2015; Ghassemi-Golezani and Farhangi-Abriz, 2018). This plant is very sensitive to soil salinity and is known as a glycophyte. Soybean production limited by environmental stresses such as soil salinity (Ghassemi-Golezani and Farhangi-Abriz, 2018). Soil salinity is characterized by a high concentration of soluble salts that accounts for an electrical conductivity (EC) of 4 dS $m^{-1}$ or more (Munns and Gilliham, 2015). Above this EC, yields of most crops decline significantly (Parida and Das, 2005). Salt stress causes many adverse effects on plant growth and development, which are due to a low osmotic potential of soil solution (osmotic stress), specific ion effects (salt stress), nutritional imbalances or a combination of these factors (Munns and Gilliham, 2015; Farhangi-Abriz and Ghassemi-Golezan, 2018).

The major saline ions, $Na^+$ (Sodium) and $Cl^-$ (Chloride), can affect nutrient uptake through competitive interaction or by affecting membrane selectivity. For example, a high level of $Na^+$ frequently induces $Ca^{2+}$ (Calcium) and $K^+$ (Potassium) deficiencies (Munns and Gilliham, 2015). Mechanisms for salt resistance can be attributed to a number of strategies such as limited $Na^+$ uptake and reduction of $Na^+$ concentration in the cytoplasm in order to prevent poisoning levels in the plant cells (Tester and Davenport, 2003; Farhangi-Abriz and Torabian, 2017; Ghassemi-Golezani and Nikpour-Rashidabad, 2017). In tonoplast, $H^+$-ATPase pumps are pumping $H^+$ into the vacuoles, providing adequate protons for $Na^+/H^+$ antiporters. The Inclusion of $Na^+$ in root vacuoles is achieved by tonoplast $Na^+/H^+$ antiporters, which belong to the $Na^+/H^+$ exchanger (NHX) families (Yamaguchi et al., 2005). A positive correlation between salt resistance and $Na^+$ inclusion was already confirmed for sunflower and Arabidopsis in which relative activities and transcription of $Na^+/H^+$ antiporters in tonoplast was generally enhanced in salt-resistant genotypes (Queirós et al., 2009; Fan et al., 2018).

Salicylic acid (SA) is an endogenous growth regulator with phenolic nature, that plays an important role in the regulation of physiological and biochemical processes in plants. SA improves plant resistance to biotic and abiotic stresses (Shakirova et al., 2003; Hayat et al., 2010; Farhangi-Abriz and Ghassemi-Golezan, 2018). Exogenous application of SA enhances plant growth and photosynthetic capacity in saline conditions (Khan et al., 2014). It has been also reported that SA significantly reduces ion leakage and toxic ion accumulation (Hayat et al.,

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https://doi.org/10.1016/j.ecoenv.2018.09.059
Received 17 March 2018; Received in revised form 10 September 2018; Accepted 12 September 2018

2010), thereby mitigating some of the deleterious effects of environmental stresses (Shakirova et al., 2003). Shakirova et al. (2003) found that SA induces the resistance of wheat seedlings to salinity and improves plant growth under salt stress.

Jasmonates are a group of natural plant growth regulators, which are commonly distributed among higher plants, inducing a wide variety of physiological and developmental responses. Jasmonates also exploit plant defense mechanisms in response to different environmental stresses such as salinity (Qiu et al., 2014; Farhangi-Abriz and Ghassemi-Golezan, 2018). The role of jasmonic acid (JA) in the protection of plants from abiotic stress is the induction of expression of genes coding proteins such as jasmonate-induced protein 60 (JIP60, a 60 kDa protein). This protein by feature of its exclusive structure and processing is capable of reprogramming protein translation for increasing abiotic and biotic stress tolerance and controlling senescence (Rustgi et al., 2014). Qiu et al. (2014) reported that JA application significantly relieves the adverse effects of NaCl stress. Foliar application of jasmonic acid on soybean plants improves nitrogen and sulfur absorptions and also plant performance under salt stress (Farhangi-Abriz and Ghassemi-Golezani, 2016). Kang et al. (2005) found that the uptake of sodium decreases, but the uptake of the major ions partially increases as a result of foliar spray of JA on rice seedlings.

In the previous report (Farhangi-Abriz and Ghassemi-Golezan, 2018) we identified the mechanisms of salt mitigation in soybean plants by salicylic acid and jasmonic acid. These growth regulators showed different mechanisms of actions, so in this research, we tested individual and combined effects of these growth regulators on $H^+$-ATPase activity of tonoplast, cell energy, nutrient content and physiological performance of soybean plants under salt stress.

## 2. Materials and methods

### 2.1. Experimental design

The experiment was conducted in a greenhouse at the Faculty of Agriculture, University of Tabriz, with a factorial arrangement on the basis of randomized complete block design with three replications, to investigate the possible effects of salicylic acid (SA) and jasmonic acid (JA) on tonoplast $H^+$-ATPase hydrolytic activity in soybean roots. Treatments were different levels of NaCl salinity [0, 4, 7 and 10 dS $m^{-1}$ as non-saline and low (40 mM NaCl $L^{-1}$), moderate (70 mM NaCl $L^{-1}$) and high (100 mM NaCl $L^{-1}$) salinities, respectively] and foliar application of SA (0 and 1 mM) and JA (0 and 0.5 mM) and SA + JA. The dosages of foliar applications of these growth regulators were selected according to the previous works (Farhangi-Abriz and Ghassemi-Golezani, 2016; Farhangi-Abriz and Ghassemi-Golezan, 2018). In this experiment, 48 plastic pots (25 × 25 cm), each filled with 1 kg perlite, were used. Seeds were sown in each pot and all pots were then placed in a glass greenhouse under natural light with a day-night mean temperature of 28–26 °C and relative humidity of 35–40%. During the growth period, the pots were weighed and the losses were made up with Hoagland solution (EC = 1.3 dS $m^{-1}$, pH = 6.7–7.2) to achieve field capacity (FC). For avoiding excess salinity due to adding Hoagland solution, perlite substrate within the pots was washed every 30 days, and non-saline and salinity treatments were reapplied. The hormones were sprayed on plants at vegetative [V1 (First Trifoliate) and V3 (Third Trifoliate)] and full flowering (R2) stages (Pedersen et al., 2004) according to the treatments, with the foliar spray of distilled water for non-hormonal treatment. Measurements were started three days after the last hormonal treatment.

### 2.2. Isolation of tonoplast membrane

Tonoplast vesicles were isolated from roots of non-saline and salt-stressed plants of soybean according to Queirós et al. (2009). Three days after the last hormonal treatment, five plants from each pot were harvested and roots were cut and washed with double-distilled water. Then 20 g fresh roots were ground in 40 mL of extraction buffer with a pH of 8 [Glycerol 10% (v/v); 5 mM EDTA; bovine serum albumin 0.13% (w/v); 0.1 M Tris-HCl buffer;150 mM KCl; 3.3 mM dithiothreitol; 1 mM phenylmethylsulfonyl fluoride; polyvinylpyrrolidone 0.5% (w/v)]. Homogenized root materials were filtered, then the supernatant was collected and centrifuged at 10,000 g for 15 min and pellets were removed. The supernatant was centrifuged again at 10,000 g for 10 min and after removing the pellets, the supernatants were centrifuged at 100,000 g for 45 min at 4 °C. The microsomal pellet was suspended gently in an ice-cold buffer with a pH of 7.6 (10% (v/v) glycerol, 1 mM DTT, 1 mM EDTA and 10 mM TRIS-HCl). The resulting supernatant was then layered on top of discontinuous sucrose, consisting of 15 mL of 46%, 12 mL of 25% and 9 mL of 10% (w/v) sucrose solution centrifuged at 80,000 g for three and half hours. The gradient solutions consisted of 10, 25, 46% (w/w) sucrose, 10 mM Tris-HCl buffer, 1 mM DTT and 1 mM EDTA.

The tonoplast-enriched fraction was collected from the 10% and 25% sucrose interface (using a Pasteur pipette) and diluted three times in ice-cold water. It was then centrifuged at 100,000 g for 45 min at 4 °C. The resulting pellet was re-suspended in a medium containing 10 mM Tris-HCl, 10% (v/v) glycerol, 1 mM DTT, and 1 mM EDTA. Vacuolar membranes were stored in liquid $N_2$ for later use.

Protein content was determined by the method of Bradford (1976), using bovine serum albumin as a standard. The Bradford reagent was composed of 0.01% (w/v) Coomassie Brilliant Blue, 4.7% (w/v) ethanol and 8.5% (w/v) phosphoric acid. 20 µL of suspended membrane protein were mixed and augmented to 2.5 mL with Bradford reagent. After 45 min incubation at room temperature, protein was determined, using a spectrophotometer (Dynamica, Halo DB-20 - UV–Visible Spectrophotometer, United Kingdom) at 595 nm.

### 2.3. $H^+$-ATPase hydrolytic activity of tonoplast

$H^+$-ATPase hydrolytic activity of tonoplast was determined by the method of Sayed Hussein Mohamed (2011). The reaction was started by the addition of 3 µg of the prepared samples to a reaction mixture, containing 5 mM disodium-ATP, 5 mM $MgSO_4$, 100 mM KCl, 0.02% (v/v) Triton X-100, 50 mM MOPS-TRIS adjusted to a pH of 7.2, 50 mM $KNO_3$, 0.5 mM $NaN_3$, 0.3 mM; $Na_3VO_4$ and 1 mM $Na^+$-Molybdate. After 30 min incubation at 30 °C, the reaction was stopped with the addition of 1 mL of stopping reagent. The stopping reagent contained 2% (v/v) $H_2SO_4$, 5% (w/v) SDS, and 0.7% (w/v) ($(NH_4)_2MoO_4$). Immediately after the addition of 1 mL of stopping reagent, 100 µL of 10% (w/v) ascorbic acid were added. After 30 min at 30 °C, the absorbance at 820 nm was measured using a spectrophotometer (Dynamica, Halo DB-20 -UV–Visible Spectrophotometer, United Kingdom).

### 2.4. Adenosine triphosphate (ATP)

The total root ATP was measured by the Luciferin-Luciferase assay, using a Luminometer (model: novalum, Charm Sciences, Lawrence, Massachusetts, USA). The ATP was extracted in 4% TCA supplemented with 2 mM EDTA (Larsson and Olssonl, 1979).

### 2.5. Root growth

Dry weights of all root samples were determined after oven drying at 80 °C for 48 h. Specific root length was calculated as root length/root mass.

### 2.6. Cation analysis

The dry shoots (100 mg) and roots (100 mg) were used for determination of sodium ($Na^+$), potassium ($K^+$), calcium ($Ca^{2+}$), magnesium ($Mg^{2+}$), iron ($Fe^{2+}$), zinc ($Zn^{2+}$), copper ($Cu^{2+}$) and