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# Extended Antioxidants Induced Effects on Mandarine Seedlings (*Citrus reticulata L.*) Growth and Physiological Parameters under Saline Irrigation Conditions

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**Abstract:** Effects of salicylic acid (SA) or ascorbic acid (AsA) at different concentrations (0,0.5,1 and 5 mM/l) on one-year-old mandarin scions (*Citrus reticulate*) budded to C. Volkameriana rootstocks grown under saline water irrigation treatments (0, 25, 50 and 100 mM NaCl) were evaluated. Vegetative growth, leaf total chlorophyll, proline content and $K^+/Na^+$ ratio were evaluated at 4, 8 and 12 weeks of initial growth. The results showed that with increasing of salinity most vegetative parameters were decreased, as shoot length, root length, the amount of total chlorophyll and the $K^+/Na^+$ ratio, but there was increasing in proline amounts. Spraying the plants with a concentration of 1 mM/l SA or AsA reduced the damage of high salinity that was occurred from 100 mM/l NaCl. Plants treated with 1 mM/l SA or AsA level showed significant increasing in growth, whereas interaction between salicylic acid or ascorbic acid and salinity was highly significant for growth parameters i.e. fresh weight, dry matter of shoot and root, diameter and length of stem and root. Total chlorophyll was not affected by application of high concentration of salt at 100 mM/l until 60 days of experiment by using 1 mM/l SA or 0.5 AsA, while, these treatments reduced the chlorophyll contents by 46 and 51%, respectively at 90 days of the experiment. Moreover, proline concentration was directly proportional to salt stress and it was cumulative along with increase in stress. Exogenous application of SA or AsA was able to restore proline concentration to its optimum level, especially at 100 mM/L of NaCl. While the $K^+/Na^+$ ratio was severely affected with salt stress, but this ratio return to the optimum ratio with the application of SA or AsA. These findings open new horizon on enhancing the tolerance of mandarine seedlings to saline conditions by exogenous application of SA and AsA.

**Key words:** Salicylic Acid · Ascorbic Acid · SPAD-502 · Proline · $K^+/Na^+$

## INTRODUCTION

Salt stress can affect physiological processes from seed germination to plant development, causing growth and yield reduction. The elaboration of the damage of salt stress on plant can mainly be due to the fact that salinity causes ionic and osmotic stresses, as well as nutritional imbalance in plant tissues [1]. Salt stress caused by immoderate accumulation of salt in the soil, either directly because of salinization which can be expressed with the effect of $Na^+$ and $Cl^-$ ions, or indirectly because of water lack which can be referred to with osmotic stress [2].

*Citrus* salt tolerance is correlated with both the rootstock's efficiency to restrict accumulation of chloride and/or sodium [3] and the sensitivity to these ions of the scion's vegetative growth [4]. However, most of *Citrus* cultivars and their commercials rootstocks are known as sensitive for salinity, *Citrus* rootstocks vary widely in their salt uptake and translocation to shoots [5].

Salicylic acid (SA) is a monohydroxybenzoic acid, a type of phenolic acid, that can function both as a plant growth regulator and a beta hydroxy acid. It also has a broad wide distribution in plants. However, its minimum levels differ widely among species. It can act as an important signaling molecule and has diverse effects on tolerance in biotic and abiotic stresses by increasing levels of other plant growth regulators in plants. Under abiotic stress, it induces proline metabolic pathway enzymes to form/or regulate defense mechanisms compounds, like, compatible solutes, proline and glycinebetaine (GB). Therefore, SA being an oxidant could be linked to oxidative stress [6, 7].

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Ascorbic acid (AsA) one of the most abundant antioxidants found in plants. Ascorbic acid alleviates salinity effects on plants by enhancing plant growth [8].

Exogenous application of ascorbic acid activates antioxidant enzyme system resulting in reduction of detrimental effects of salinity [9].

Proline was the major amino acid associated with abiotic stresses (salinity, drought, extreme temperatures and heavy metals). Exposure the plants to drought or a high salt content in the soil (both leading to water stress), leads the plants to accumulate high amounts of proline [10].

Proline and hydroxyproline found in specific proteins help overcoming plant stress. Rapid accumulation of free proline in plants is a typical response to a wide range of environmental stresses such as salinity, drought, cold, nutrient deficiency, heavy metals, pathogen infections and high acidity [11].

Leaf chlorophyll concentration is an important parameter that is used as an indicator of chloroplast development, photosynthetic capacity, leaf nitrogen content, or general plant health.

Plants can response to salinity with several strategies; many of them involve interactions of $Na^+$ with $K^+$. Plant processes such as growth, photosynthesis, mineral nutrition, water and ion transport are affected by Na-K interaction. Potassium ($K^+$) uptake by plants is severely affected by the presence of $Na^+$. $Na^+$ competes with $K^+$ in plant uptake specifically through high-affinity potassium transporters (HKTs) and nonselective cation channels (NSCCs). Minimizing $Na^+$ uptake and preventing $K^+$ losses from the cell may help to maintain the $K^+/Na^+$ optimum ratio needed for plant metabolism in the cytoplasm under salt-stress conditions [12].

The aims of this work were (1) to study the effect of salinity stress on growth physiological changes of mandarine seedlings budded to Volkameriana rootstocks and (2) to investigate the effects of salicylic and ascorbic acids on plant growth and development under different concentrations of NaCl. Taking into account the above-mentioned facts about the effects of SA and AsA on plant growth and development, the investigation hypothesized that the stress effects of salinity can be ameliorated by the exogenous application of SA and AsA.

## MATERIALS AND METHODS

**Plant Material and Growth Conditions:** This experiment was conducted in the orchard`s greenhouse of Faculty of Agriculture; Assiut University from June to September 2015. One-year-old mandarin scions grafted on Volkameriana rootstocks were used for this work. Seedlings were planted in pots containing sandy clay soil. The total weight of the soil in each pot wa 4.5 Kg and each pot was irrigated every three days with 200 ml of saline water (0, 25, 50 and 100 mM of NaCl). Two weeks after the start of saline water irrigation four concentration of Salicylic Acid (SA) or Ascorbic Acid (AsA) (0.1% tween 20 [polyoxyethylene sorbitan monolaurate] solution) were applied with a manual pump as foliar spray to the plants (0, 0.5, 1 and 5 mM) for once.

Plant samples were collected at the end of each trial (4, 8 and 12 weeks) to measure the following traits:

**Vegetative Parameters:** Were measured on fresh plant samples, which were later, dried at 75°C for 22 h and 110°C for 2 h. The vegetative parameters were; shoot length (cm), root length (cm), shoot fresh and dry weight (g) and root fresh and dry weight (g).

**Physiological Parameters**

**Total Chlorophyll:** Leaves were collected from the mid-section of branches or seedlings, in order to minimize leaf age variability effects. Chlorophyll was measured with SPAD-502 equipment [13].

**Proline Determination:** A sample of 0.5 g leaf tissue was homogenized in 3% sulphosalycylic acid, filtered to remove debris from the filtrate and then mixed with ninhydrin reagent and acetic acid before heating for one hour in a water bath at100°C. The reddish color complex that formed was mixed with toluene for reading the absorbance at 520 nm at the spectrophotometer for comparison of the absorbance with that of a standard curve of proline concentrations. According to the procedure adopted by Bates *et al*. [14].

**Potassium/sodium Ratio:** The youngest leaves were collected, oven-dried at 75°C for 22 h and 110°C for 2 h and ground. Samples of 0.2 g were digested according to Jackson [15] and analyzed by flame photometry (Digital Flame Analyzer, Cole Parmer, Illinois, USA) for sodium and potassium content.

**Statistical Analysis:** The layout of experimental treatment was in a randomized complete block design (RCBD) with a factorial setup containing 3 replicates. Means ± standard error (SE) were compared using the least significant differences (LSD) at 5% level of probability according to Gomez and Gomez [16].

## RESULTS AND DISCUSSION

This study was carried out to find out the effects of exogenously application of salicylic acid (SA) and ascorbic acid (AsA) on induction tolerance to salinity in Citrus.

Many researches have shown that salt stress shorten plant growth by adversely affecting many biochemical and physiological processes, such as, photosynthesis, nitrogen metabolism and ion homeostasis [17]. According to our study salinity stress significantly reduced growth rate in Mandarin seedlings at both levels 50 and 100 mM NaCl. A numerous deal of researchers suggested that citrus have the genetic potential to be salt-sensitive; yet inheritance studies in citrus are rare.

Zekri and Parsons [18] reported that when using 50 mM of NaCl in irrigation water can cause 50% reduction in about two months. Exogenous SA application promotes oxidative damage [19]. It looks alike that SA ameliorating effects are closely related to applied concentration [20],that is matching with our study, where was found that SA induced increase in growth could be related to SA induced massive enhancement in net photosynthetic rate under salt stress, especially at 1 mM SA level. Also the level of growth increased more in the presence of AsA and it can be able to decrease the effects of salinity stress with all NaCl salinity concentrations compared to control [21], which agrees with our findings as the total growth increased with foliar application of AsA under salinity stress, even at highest level of NaCl in this study (100 mM).

Table (1) showed that shoot length under salinity stress was severely affected; it also noticed that the treatment 100 mM NaCl had less numbers of leaves (data not shown). However when SA and AsA are applied the shoot length maintained its length during the whole time of the experiment. Growth rate was statistically better with concentration 1 mM/ L of SAorAsA at 100 mM NaCl.

Table 1: Averages of shoot length (cm means±SE) in mandarin seedlings affected by different concentrations of SA and AsA combined with (or under) different levels of salinity

| | | Repeated measures (days) | | | |
|---|---|---|---|---|---|
| Treatments | | 30 | 60 | 90 | Mean |
| Control | 0.0 | 121.5±3.2 | 107.0±10.4 | 107.9±12.7 | 112.1 |
| 25 mM NaCl | 0.0 | 100.7±0.7 | 86.0±2.3 | 91.5±0.29 | 92.7 |
| | 0.5 mM SA | 111.6±1.6 | 89.0±2.9 | 105.5±0.29 | 102 |
| | 1.0 mM SA | 120.0±2.3 | 110.0±11.5 | 79.2±1.3 | 103.1 |
| | 5.0 mM SA | 84.7±1.9 | 92.5±2.6 | 99.9±8.9 | 92.4 |
| | 0.5 mM AsA | 107.5±0.87 | 98.0±6.9 | 110.2±16.3 | 105.2 |
| | 1.0 mM AsA | 101.5±7.8 | 89.5±3.8 | 90.8±2.1 | 93.9 |
| | 5.0 mM AsA | 107.5±8.7 | 92.0±4.04 | 87.6±0.92 | 95.7 |
| 50 mM NaCl | 0.0 | 84.0±0 | 93.0±2.3 | 85.7±1.3 | 87.6 |
| | 0.5 mM SA | 112.7±10.2 | 108.5±0.49 | 104.6±0.78 | 108.6 |
| | 1.0 mM SA | 96.5±9.5 | 117.5±4.3 | 98.5±3.5 | 104.2 |
| | 5.0 mM SA | 115.5±2.02 | 103.5±3.8 | 121.0±8.9 | 113.3 |
| | 0.5 mM AsA | 94.0±14.4 | 111.0±9.2 | 129.5±2.1 | 111.5 |
| | 1.0 mM AsA | 111.2±14.6 | 93.5±0.29 | 88.2±7.1 | 97.6 |
| | 5.0 mM AsA | 92.0±2.9 | 104.5±3.2 | 106.5±6.6 | 101 |
| 100 mM NaCl | 0.0 | 110.0±0.8 | 94.7±0.9 | 94.7±2.3 | 99.8 |
| | 0.5 mM SA | 87.5±6.6 | 84.5±4.3 | 128.5±8.3 | 100.2 |
| | 1.0 mM SA | 100.7±4.5 | 95.5±2.6 | 107.5±12.4 | 101.2 |
| | 5.0 mM SA | 92.5±2.02 | 115.5±0.87 | 99.2±13.2 | 102.4 |
| | 0.5 mM AsA | 98.0±6.9 | 85.5±14.7 | 113.7±8.8 | 99.1 |
| | 1.0 mM AsA | 112.0±0.58 | 115.0±0.58 | 119.0±0.58 | 115.3 |
| | 5.0 mM AsA | 105.5±3.2 | 124.0±5.2 | 87.1±8.1 | 105.5 |
| Mean | | 103.05 | 100.5 | 102.6 | |
| LSD 0.05 | Treatments | 8.9 | | | |
| | Time | N.S. | | | |
| | Interaction | 20.11 | | | |

Table 2: Averages of root length (cm means±SE) in mandarin seedlings affected by different concentrations of SA and AsA combined with (or under) different levels of salinity

| Treatments | | Repeated measures (days) 30 | 60 | 90 | Mean |
|---|---|---|---|---|---|
| Control | 0.0 | 36.5±0.87 | 30±0 | 33±0 | 33.2 |
| 25 mM NaCl | 0.0 | 33±1.7 | 31.5±0.29 | 36.5±1.8 | 33.7 |
| | 0.5 mM SA | 33.03±2.1 | 28±0.58 | 33±2.3 | 31.3 |
| | 1.0 mM SA | 39±3.5 | 33.5±1.4 | 29.5±2.6 | 34 |
| | 5.0 mM SA | 37.2±0.72 | 40.5±0.29 | 39.5±2.02 | 39.1 |
| | 0.5 mM AsA | 35±0.58 | 38±0 | 30.7±2.2 | 34.6 |
| | 1.0 mM AsA | 46±0 | 34±4.04 | 35±1.2 | 38.3 |
| | 5.0 mM AsA | 33±2.5 | 31±1.2 | 38.2±0.11 | 34.1 |
| 50 mM NaCl | 0.0 | 36.2±3.9 | 32.5±1.4 | 35.6±1.5 | 34.8 |
| | 0.5 mM SA | 37.7±0.73 | 36.5±1.1 | 41.2±1.5 | 38.5 |
| | 1.0 mM SA | 38.2±0.72 | 36±0 | 29.2±3.03 | 34.5 |
| | 5.0 mM SA | 37±2.9 | 40±2.9 | 31.7±0.72 | 36.2 |
| | 0.5 mM AsA | 33.5±0.29 | 37.5±4.9 | 32.3±1.1 | 34.4 |
| | 1.0 mM AsA | 34±1.2 | 31±0.58 | 31.6±2.1 | 32.2 |
| | 5.0 mM AsA | 35±1.7 | 30.5±1.4 | 32.5±0.29 | 32.7 |
| 100 mM NaCl | 0.0 | 37±2.3 | 34±0 | 33.5±0.87 | 34.8 |
| | 0.5 mM SA | 33.5±1.4 | 31±1.2 | 33.2±1.9 | 32.6 |
| | 1.0 mM SA | 40.5±3.8 | 32±1.2 | 38.1±1.8 | 36.9 |
| | 5.0 mM SA | 31.5±1.4 | 33±0.58 | 37.7±0.69 | 34.1 |
| | 0.5 mM AsA | 37.7±3.9 | 34.5±0.29 | 36.1±1.8 | 36.1 |
| | 1.0 mM AsA | 32.5±1.4 | 39.5±2.02 | 33±0.66 | 35 |
| | 5.0 mM AsA | 33.7±1.6 | 38±2.3 | 34±0.58 | 35.2 |
| Mean | | 35.9 | 34.2 | 34.3 | |
| LSD 0.05 | Treatments | 2.74 | | | |
| | Time | 1.2 | | | |
| | Interaction | 5.6 | | | |

Table (2) referred that, root length (cm) was not severely affected with NaCl concentrations, it even increased at 90 days for the all concentrations of NaCl. While that was matching with Ma³kiewicz *et al*.[22] as they found that the root length of Vilma tomato plants grown under salinity conditions were constituted an exception, where the roots were longer than the control plants, it was contrary to most studies, which reported that salinity reduced the root length and fresh and dry weight [23,24]. On the other hand, salinity did not affect Shoot/Root ratio. Salinity effects on Shoot/Root are not typical in either salt sensitive or tolerant citrus rootstocks [25]. Application of SA and AsA significantly gave longer roots compared to seedlings without the addition of SA and AsA.

The greatest fresh shoot biomass was for the plants under the highest salinity concentration (100 mM NaCl), it recorded high values in shoot fresh weight as it had the highest shoot length (Table 3). Plants treated with 0.5 mM SA under 50 mM/l NaCl gave the highest value with 55 g and it could maintain relative values during 12 weeks of the experiment. However, 1 mM of AsA gave the highest value of shoot biomass under 100 mM of NaCl and it even increased at the 8 weeks of the experiment, but it reduced by 48.2% at the 12 weeks of the experiment compared to the previous one (8 weeks).

In the fresh shoot weight (g), plants treated with 100 mM NaCl recorded the high values in shoot dry weight (g) compared to control and the other two treatments 25 and 50 mM NaCl at the first 4 weeks. SA at 0.5 mM concentration recorded increasing in dry matter at 100 mM of salinity with 50 and 56.5% for 60 and 90 days, respectively

as shown in Table (4). Most of the treatments of SA and AsA could give increasing in dry matter with time.

In Table (5) the fresh root biomass (g) recorded the greatest values in the plants treated with 0.5 mM SA and could maintain relative values when treated with 100 mM/l NaCl. Treatments with 1 mM/l AsA resulted second good values. The plants under 100 mM/l NaCl without SA and AsA treatments recorded high values of fresh root biomass as well as they had high values in root length.

Table 3: Averages of shoot fresh weight (g means ± SE) in mandarin seedlings affected by different concentrations of SA and AsA combined with (or under) different levels of salinity

| Treatments | | Repeated measures (days) 30 | 60 | 90 | Mean |
|---|---|---|---|---|---|
| Control | 0.0 | 35±0 | 36.5±2.02 | 42.3±1.5 | 37.9 |
| 25 mM NaCl | 0.0 | 25±0 | 33.5±3.8 | 35±2.9 | 31.2 |
| | 0.5 mM SA | 19.5±5 | 27.2±1.4 | 34±0.58 | 26.9 |
| | 1.0 mM SA | 40±0 | 52.5±1.4 | 20.6±2.9 | 37.7 |
| | 5.0 mM SA | 27.5±1.4 | 26.5±2.02 | 28.5±0.87 | 27.5 |
| | 0.5 mM AsA | 17.5±1.4 | 35±0 | 37.5±4.3 | 30 |
| | 1.0 mM AsA | 27.5±4.3 | 25±2.9 | 28.5±0.87 | 27 |
| | 5.0 mM AsA | 25±5.8 | 36.5±2.02 | 30±2.9 | 30.5 |
| 50 mM NaCl | 0.0 | 25±2.9 | 35±0 | 22.3±2.7 | 27.4 |
| | 0.5 mM SA | 55±8.7 | 42.5±7.2 | 41.5±1.04 | 43 |
| | 1.0 mM SA | 30±2.9 | 40±2.9 | 34.5±0.8 | 34.8 |
| | 5.0 mM SA | 40±2.9 | 35±2.9 | 39±2.3 | 38 |
| | 0.5 mM AsA | 32.7±3.4 | 42.5±4.3 | 37.5±1.4 | 37.6 |
| | 1.0 mM AsA | 40±2.9 | 32.5±4.3 | 27.5±3.2 | 33.3 |
| | 5.0 mM AsA | 35±2.9 | 35±0 | 35±0 | 35 |
| 100 mM NaCl | 0.0 | 50±2.9 | 32.5±4.3 | 45±2.9 | 42.5 |
| | 0.5 mM SA | 25±2.9 | 32.5±4.3 | 32.5±1.4 | 30 |
| | 1.0 mM SA | 30±2.9 | 22.5±1.4 | 27.5±4.3 | 26.7 |
| | 5.0 mM SA | 31±2.3 | 17.5±1.4 | 24.8±0.88 | 24.4 |
| | 0.5 mM AsA | 41.5±4.9 | 22.5±4.3 | 25±5.8 | 29.7 |
| | 1.0 mM AsA | 42.5±1.4 | 43.5±2.02 | 24.8±0.73 | 37 |
| | 5.0 mM AsA | 26±0.58 | 42.5±1.4 | 22±1.7 | 30.2 |
| Mean | | 32.7 | 34.03 | 31.6 | |
| LSD 0.05 | Treatments | 4.4 | | | |
| | Time | 1.9 | | | |
| | Interaction | 8.9 | | | |

Table 4: Averages of shoot dry weight (gm means ± SE) in mandarin seedlings affected by different concentrations of SA and AsA combined with (or under) different levels of salinity

| Treatments | | Repeated measures (days) 30 | 60 | 90 | Mean |
|---|---|---|---|---|---|
| Control | 0.0 | 22.5±1.4 | 20±2.9 | 31±3.5 | 24.5 |
| 25 mM NaCl | 0.0 | 15±2.9 | 17.5±1.4 | 27.5±1.4 | 20 |
| | 0.5 mM SA | 5.3±0.3 | 10±0 | 27.5±1.4 | 14.3 |
| | 1.0 mM SA | 17.5±1.4 | 23.5±2.02 | 14±0.6 | 18.3 |
| | 5.0 mM SA | 15±0 | 15±0 | 20.8±0.8 | 16.9 |
| | 0.5 mM AsA | 8.5±0.87 | 17.5±1.4 | 25±0 | 17 |
| | 1.0 mM AsA | 12.5±1.4 | 12.5±1.4 | 13.2±0.8 | 12.7 |
| | 5.0 mM AsA | 9.7±2.9 | 12.5±1.4 | 21.9±0.6 | 14.7 |
| 50 mM NaCl | 0.0 | 12.5±1.4 | 12.5±1.4 | 14.4±0.3 | 13.1 |
| | 0.5 mM SA | 28.5±3.8 | 17.5±1.4 | 27.5±1.4 | 24.5 |
| | 1.0 mM SA | 20±2.9 | 22.5±1.4 | 23.6±0.9 | 22.03 |
| | 5.0 mM SA | 22.5±1.4 | 21±2.3 | 30±0 | 24.5 |
| | 0.5 mM AsA | 12.5±4.3 | 22.5±1.4 | 32.5±1.4 | 22.5 |
| | 1.0 mM AsA | 26±0.58 | 15±2.9 | 16.7±0.9 | 19.2 |
| | 5.0 mM AsA | 11±2.3 | 20±0 | 25±0 | 18.7 |
| 100 mM NaCl | 0.0 | 26.5±3.8 | 17.5±1.4 | 30.7±0.4 | 24.9 |
| | 0.5 mM SA | 10±2.9 | 20±0 | 23.1±0.6 | 17.7 |
| | 1.0 mM SA | 17.5±1.4 | 15±2.9 | 16.4±0.8 | 16.3 |
| | 5.0 mM SA | 15±2.9 | 8.5±0.87 | 15.7±1.4 | 13.1 |
| | 0.5 mM AsA | 25±2.9 | 10±2.9 | 14.5±3.3 | 16.5 |
| | 1.0 mM AsA | 25±2.9 | 25±0 | 14.2±0.2 | 21.4 |
| | 5.0 mM AsA | 12.5±1.4 | 25±0 | 10.9±1.6 | 16.1 |
| Mean | | 16.8 | 17.3 | 21.6 | |
| LSD 0.05 | Treatments | 2.82 | | | |
| | Time | 1.1 | | | |
| | Interaction | 5.1 | | | |

Table 5: Averages of root fresh weight (gm means ± SE) in mandarin seedlings affected by different concentrations of SA and AsA combined with (or under) different levels of salinity

| Treatments | | Repeated measures (days) 30 | 60 | 90 | Mean |
|---|---|---|---|---|---|
| Control | 0.0 | 22.5±1.4 | 17.5±4.3 | 27.5±4.3 | 22.5 |
| 25 mM NaCl | 0.0 | 12.5±1.4 | 12.5±1.4 | 12.5±1.4 | 12.5 |
| | 0.5 mM SA | 13.7±1.9 | 12.5±1.4 | 10±0 | 12.9 |
| | 1.0 mM SA | 20±0 | 29±3.5 | 12.2±0.2 | 20.4 |
| | 5.0 mM SA | 15±0 | 17.5±4.3 | 15±2.9 | 15.8 |
| | 0.5 mM AsA | 12.5±1.4 | 15±2.9 | 11±3.5 | 12.8 |
| | 1.0 mM AsA | 15±0 | 10±2.9 | 10±2.9 | 11.7 |
| | 5.0 mM AsA | 10±2.9 | 10±0 | 10±0 | 8.3 |
| 50 mM NaCl | 0.0 | 10±2.9 | 10±0 | 6±0.58 | 8.7 |
| | 0.5 mM SA | 25±2.9 | 17.5±4.3 | 15±2.9 | 19.2 |
| | 1.0 mM SA | 17.5±4.3 | 25±2.9 | 12.5±1.4 | 18.3 |
| | 5.0 mM SA | 32.5±1.4 | 20±0 | 16±0.58 | 22.8 |
| | 0.5 mM AsA | 16.5±2.02 | 21±2.3 | 16.5±2.02 | 18 |
| | 1.0 mM AsA | 22.5±4.3 | 21±2.3 | 15±0 | 19.5 |
| | 5.0 mM AsA | 15.2±1.01 | 15±2.9 | 16±0.58 | 14.5 |
| 100 mM NaCl | 0.0 | 27.8±0.15 | 20±2.9 | 31±3.5 | 26.3 |
| | 0.5 mM SA | 27.5±1.4 | 25±5.8 | 22.5±1.4 | 25 |
| | 1.0 mM SA | 20±2.9 | 23.5±3.8 | 15±0 | 19.5 |
| | 5.0 mM SA | 25±8.7 | 10±0 | 17.5±1.4 | 17.5 |
| | 0.5 mM AsA | 31±2.3 | 21±3.5 | 12.5±1.4 | 21.5 |
| | 1.0 mM AsA | 25±2.9 | 23.5±2.02 | 20±0 | 22.8 |
| | 5.0 mM AsA | 15±0 | 20±0 | 16±0.58 | 17 |
| Mean | | 19.6 | 18.02 | 15.4 | |
| LSD 0.05 | Treatments | 3.5 | | | |
| | Time | 1.68 | | | |
| | Interaction | 7.9 | | | |

On the other hand, Table (6) showed that root dry matter (g) had a significant increasing in the plants treated with 100 mM/l of NaCl, it recorded 43.2, 11.1 and 50% increasing for the 30, 60 and 90 days than the non-saline treated plants. While the plants spraying with 0.5 mM SA and 1 mM AsA at 100 mM NaCl could maintain similar values during the whole experiment time.

In two citrus rootstocks; Cleopatra mandarin (*Citrus reshni* Hort. ex Tan) and Troyer citrange (*Poncirus trifoliata* × *Citrus sinensis*) were irrigated with different concentrations of NaCl (0, 40 or 80 mM) for 12 weeks, significant decreases in net photosynthetic and respiration rates in the leaves, beside the reduction of shoot height, leaf number and fresh weights of the seedlings and relative chlorophyll contents were observed [26]. In our study salinity reduced the growth rate of mandarin seedlings in general, it also caused a significant decrease in chlorophyll, to determine the total chlorophyll in this study we used SPAD-502 meter. The converted SPAD values differ from photometric measurements of solvent- extracted chlorophyll by just ~6% on average [27]. The SPAD-502 readings lower than 40 showed deterioration in photosynthetic process.

Table (7) showed that the plants spraying with AsA and SA reduced effects of saline irrigation water on total chlorophyll and that also was expressed in Gunes *et al.*[28] who reported that SA treatment might mitigate the imposed salt stress, either via osmotic adjustment or by conferring desiccation resistance to plant cells. The highest total chlorophyll values were recorded in the plants treated with 5 mM SA at50 mM NaCl after 30 days, though it recorded a reduction at the 60 and 90 days of the experiment with 10 and 61.2%, respectively. Spraying the plants with 1 mM SA and/or 0.5 mMAsA gave significant increasing in total chlorophyll at 100 mM NaCl at 30 and 60 days of the experiment, though it could not survive these values after 90 days of the experiment and it gave a reduction in total chlorophyll with 63.8 and 67.7%, respectively. Our findings were matching with Melgar *et al.* [29] who found that salinity decreased leaf chlorophyll a content in citrus. Garci´a-Sa´nchez *et al.* [30] also reported that the decrease in leaf chlorophyll content has been described in citrus rootstocks irrigated with high NaCl concentration. It is already known that high accumulation of $Na^+$ in the leaves cause degradation of chlorophyll in sunflower [31], which is in agreement with

Table 6: Averages of root dry weight (gm means ± SE) in mandarin seedlings affected by different concentrations of SA and AsA combined with (or under) different levels of salinity

| Treatments | | Repeated measures (days) 30 | 60 | 90 | Mean |
|---|---|---|---|---|---|
| Control | 0.0 | 10.8±0.5 | 9.6±2.6 | 7.5±1.4 | 9.3 |
| 25 mM NaCl | 0.0 | 5±0 | 5.8±0.8 | 5.8±0.9 | 5.5 |
| | 0.5 mM SA | 4±0.6 | 5±0 | 4.1±0.6 | 4.4 |
| | 1.0 mM SA | 10±1.7 | 15±0 | 3.5±0.9 | 9.5 |
| | 5.0 mM SA | 5±0 | 7.5±1.4 | 3.5±0.9 | 5.3 |
| | 0.5 mM AsA | 5±0 | 9.5±2.6 | 6.4±1.8 | 6.9 |
| | 1.0 mM AsA | 10±0 | 4.6±1.4 | 4.9±1.6 | 6.5 |
| | 5.0 mM AsA | 5±0 | 5±0 | 5±0 | 5 |
| 50 mM NaCl | 0.0 | 4±0.6 | 3±0 | 2.7±0.3 | 3.2 |
| | 0.5 mM SA | 11.1±0.7 | 10±2.9 | 7±1.7 | 9.4 |
| | 1.0 mM SA | 8.6±2.7 | 15±2.9 | 6.5±0.9 | 10 |
| | 5.0 mM SA | 7.5±1.4 | 12.5±1.4 | 10±0 | 13.3 |
| | 0.5 mM AsA | 7.5±1.4 | 10±0 | 7.5±1.4 | 8.3 |
| | 1.0 mM AsA | 14.3±3.3 | 8.5±0.9 | 5±0 | 9.3 |
| | 5.0 mM AsA | 10.1±1.9 | 7.5±1.4 | 7.5±1.4 | 8.4 |
| 100 mM NaCl | 0.0 | 19±0.2 | 10.8±0.9 | 15±2.9 | 14.9 |
| | 0.5 mM SA | 12.5±1.4 | 15±2.9 | 11.4±0.7 | 12.9 |
| | 1.0 mM SA | 10.5±1.02 | 12.5±4.3 | 8.5±0.9 | 10.5 |
| | 5.0 mM SA | 12.5±4.3 | 5±0 | 7.5±1.4 | 8.3 |
| | 0.5 mM AsA | 19.8±1.1 | 14.4±2.5 | 7.5±1.4 | 13.9 |
| | 1.0 mM AsA | 12.5±1.4 | 12.5±1.4 | 10±0 | 11.7 |
| | 5.0 mM AsA | 5±0 | 9.8±0.1 | 5±0 | 6.6 |
| Mean | | 9.9 | 9.5 | 6.9 | |
| LSD 0.05 | Treatments | 2.5 | | | |
| | Time | 0.95 | | | |
| | Interaction | 4.46 | | | |

our result as the total chlorophyll decreased the plants under salinity conditions, that is probably due to the inhibitory effect of the accumulated ions of various salts on the biosynthesis of the different chlorophyll fractions.

The analysis of proline contents in leaves of mandarin seedlings exposed to different concentrations of salinity and different treatments of SA and AsA are presented in Table (8).

Proline accumulation may be a general reaction to salinity stress. Results of this experiment showed that salinity concentrations have a significant effect on proline contents compared to the control. The data showed that with the increasing of salinity from 25-50 to 100 mM NaCl the proline content increased in comparing to control (0 mM NaCl) and that increase was highly significant in the last 90 days of the experiment at 50 and 100 mM NaCl level of salinity. Similar to our results, some reports have shown that salinity stress induces an increase in proline accumulation. Accumulated proline may supply energy to increase salinity tolerance [32] and it is suggested that increasing proline under saline conditions caused less existing glutamate in biosynthesizing chlorophyll, because glutamate is subscriber precursor of chlorophyll and proline biosynthesis and salt leads to more activity of proline synthesis line [33]. Foliar application of SA and AsA affected significantly on proline accumulation. It could remain proline content to similar levels as in seedlings planted in non-saline treatments. In the most of treatments the AsA was more effective than SA in reducing proline amounts under salinity stress. Exogenous application of AsA with 1 or 5 mM caused a reduction in proline amount with 86.5 and 46.5%, respectively at 100 mM NaCl at 90 days of the experiment in comparison with the control plants (untreated with salt). In wheat and barley, Deef [34] reported that proline content was increasing when the plant tissue is injured and suggested that SA with 200mg/l increased the plant growth and gave a better status of the plants under saline stress, damage is less thus proline levels are decreased.

However, a general agreement on the precise role of proline in the response of plants to stress is still lacking and several hypotheses have been proposed on the significance of the accumulation of proline caused by stress.

Table 7: Averages of total chlorophyll (means ± SE) in mandarin seedlings affected by different concentrations of SA and AsA combined with (or under) different levels of salinity

| Treatments | | Repeated measures (days) 30 | 60 | 90 | Mean |
|---|---|---|---|---|---|
| Control | 0.0 | 63.5±0.09 | 58.6±0.8 | 46.4±0.7 | 56.2 |
| 25 mM NaCl | 0.0 | 60.7±0.9 | 43.6±1.6 | 52.2±1.3 | 52.2 |
| | 0.5 mM SA | 61.7±0.94 | 59.5±2.3 | 49.7±1.2 | 56.9 |
| | 1.0 mM SA | 61.7±0.43 | 58.2±2.5 | 60.4±3.7 | 60.1 |
| | 5.0 mM SA | 50.2±0.09 | 62.9±0.09 | 55.3±3.8 | 56.1 |
| | 0.5 mM AsA | 51.6±2.3 | 57.3±1.6 | 31.6±4.2 | 46.8 |
| | 1.0 mM AsA | 49.3±2.6 | 57.4±2.2 | 46.4±7.6 | 51.03 |
| | 5.0 mM AsA | 52±4.9 | 47.5±4 | 44.1±7.9 | 47.9 |
| 50 mM NaCl | 0.0 | 54.7±4.5 | 49.4±0.7 | 31.4±3.6 | 45.2 |
| | 0.5 mM SA | 49.4±0.92 | 61.6±5.4 | 36.7±0.49 | 49.2 |
| | 1.0 mM SA | 59.2±1.2 | 43.4±2.8 | 53.8±0.58 | 52.1 |
| | 5.0 mM SA | 72.1±0.49 | 64.9±2.7 | 28±0.84 | 55 |
| | 0.5 mM AsA | 63.7±0.26 | 48.6±7.9 | 38.1±2.7 | 50.1 |
| | 1.0 mM AsA | 55.8±6.2 | 62.5±2.4 | 36.8±10.5 | 51.7 |
| | 5.0 mM AsA | 56.8±4.5 | 51.7±4.2 | 38.1±6.1 | 48.8 |
| 100 mM NaCl | 0.0 | 54.1±8.1 | 47.1±8.8 | 24.5±1.1 | 41.9 |
| | 0.5 mM SA | 56.2±4.6 | 47.5±6.7 | 17.6±3.1 | 40.4 |
| | 1.0 mM SA | 62.1±3.1 | 59.4±1.2 | 22.5±0.35 | 48 |
| | 5.0 mM SA | 57.2±5.9 | 50.1±7.6 | 16.3±2.4 | 41.2 |
| | 0.5 mM AsA | 64.1±2.3 | 56.3±5.9 | 20.7±1.9 | 47.03 |
| | 1.0 mM AsA | 55.1±6.1 | 37.3±3.4 | 19.5±0.06 | 37.3 |
| | 5.0 mM AsA | 57.1±5.8 | 55.2±1.8 | 25.6±1.4 | 45.9 |
| Mean | | 57.7 | 53.6 | 36.2 | |
| LSD 0.05 | Treatments | 7.7 | | | |
| | Time | 2.2 | | | |
| | Interaction | 10.3 | | | |

Table 8: Averages of proline content (mg/g of dry matter means ± SE) in mandarin seedlings affected by different concentrations of SA and AsA combined with (or under) different levels of salinity

| Treatments | | Repeated measures (days) 30 | 60 | 90 | Mean |
|---|---|---|---|---|---|
| Control | 0.0 | 1.1±0.14 | 1.7±0.12 | 1.7±0.21 | 1.5 |
| 25 mM NaCl | 0.0 | 2.5±1.1 | 1.8±0.23 | 1.7±0.15 | 2 |
| | 0.5 mM SA | 2.7±0.78 | 1.1±0.39 | 2.05±0.38 | 1.95 |
| | 1.0 mM SA | 2.04±0.14 | 1.4±0.26 | 3.2±0.26 | 2.21 |
| | 5.0 mM SA | 0.46±0 | 2.5±0.64 | 2.7±3.1 | 1.89 |
| | 0.5 mM AsA | 0.91±0 | 0.91±0 | 2.5±0.4 | 1.44 |
| | 1.0 mM AsA | 1.4±0.26 | 1.6±0.4 | 1.8±0.23 | 1.6 |
| | 5.0 mM AsA | 1.1±0.14 | 1.6±0.4 | 1.6±0.12 | 1.43 |
| 50 mM NaCl | 0.0 | 1.6±0.12 | 1.1±0.18 | 3.8±0.61 | 2.1 |
| | 0.5 mM SA | 1.6±0.12 | 4.8±0.38 | 2.3±0 | 2.9 |
| | 1.0 mM SA | 2.04±0.38 | 1.8±0 | 2.9±0.15 | 2.25 |
| | 5.0 mM SA | 2.9±0.66 | 1.5±0.12 | 2.04±0.38 | 2.15 |
| | 0.5 mM AsA | 1.6±0.12 | 2.04±0.14 | 2.3±0.26 | 1.98 |
| | 1.0 mM AsA | 2.9±0.38 | 0.91±0 | 2.04±0.91 | 1.95 |
| | 5.0 mM AsA | 0.91±0 | 1.1±0.14 | 1.6±0.4 | 1.2 |
| 100 mM NaCl | 0.0 | 2.04±0.14 | 2.3±0.52 | 2.05±0.38 | 2.13 |
| | 0.5 mM SA | 2.3±0.52 | 2.3±0.26 | 1.1±0.39 | 1.9 |
| | 1.0 mM SA | 1.1±0.14 | 1.6±0.4 | 1.8±0 | 1.5 |
| | 5.0 mM SA | 1.1±0.14 | 3.6±0.26 | 1.8±0.26 | 2.17 |
| | 0.5 mM AsA | 1.8±0 | 5.5±0 | 2.5±0.12 | 3.27 |
| | 1.0 mM AsA | 1.1±0.14 | 2.3±0.52 | 0.23±0.13 | 1.21 |
| | 5.0 mM AsA | 1.8±0.52 | 2.3±0.26 | 0.91±0 | 1.67 |
| Mean | | 1.7 | 2.1 | 2.02 | |
| LSD 0.05 | Treatments | 0.59 | | | |
| | Time | 0.2 | | | |
| | Interaction | 0.95 | | | |

Table 9: Averages of $K^+/Na^+$ ratio (means ± SE) in mandarin seedlings affected by different concentrations of SA and AsA combined with (or under) different levels of salinity

| Treatments | | Repeated measures (days) 30 | 60 | 90 | Mean |
|---|---|---|---|---|---|
| Control | 0.0 | 4.1±0.2 | 3.8±0.2 | 4.6±0.1 | 4.2 |
| 25 mM NaCl | 0.0 | 3.7±0.2 | 0.97±0.1 | 1.6±0.3 | 2.09 |
| | 0.5 mM SA | 2.4±0.3 | 1.7±0.2 | 2.4±0.3 | 2.2 |
| | 1.0 mM SA | 0.66±0.1 | 1.9±0.3 | 0.86±0.1 | 1.1 |
| | 5.0 mM SA | 5.4±0.3 | 5.8±0.4 | 5.5±0.3 | 5.6 |
| | 0.5 mM AsA | 3.7±0.3 | 1.7±0.3 | 2.7±0.3 | 2.7 |
| | 1.0 mM AsA | 1.2±0.1 | 3.2±0.3 | 2.5±0.4 | 2.3 |
| | 5.0 mM AsA | 1.6±0.3 | 4.3±0.3 | 2.3±0.2 | 2.7 |
| 50 mM NaCl | 0.0 | 2.4±0.3 | 1.4±0.2 | 1.3±0.2 | 1.7 |
| | 0.5 mM SA | 1.4±0.3 | 2.5±0.3 | 3.6±0.3 | 2.5 |
| | 1.0 mM SA | 2.5±0.2 | 1.7±0.4 | 1.9±0.2 | 2.03 |
| | 5.0 mM SA | 0.72±0.1 | 1.9±0.3 | 1.1±0.1 | 1.2 |
| | 0.5 mM AsA | 0.44±0.1 | 1.6±0.3 | 1.9±0.3 | 1.3 |
| | 1.0 mM AsA | 4.8±0.3 | 1.9±0.4 | 1.8±0.3 | 2.8 |
| | 5.0 mM AsA | 0.69±0.04 | 0.99±0.1 | 0.8±0.1 | 0.83 |
| 100 mM NaCl | 0.0 | 0.62±0.02 | 1.1±0.1 | 1.7±0.3 | 1.1 |
| | 0.5 mM SA | 3.1±0.1 | 0.55±0.04 | 0.99±0.1 | 1.5 |
| | 1.0 mM SA | 2.4±0.1 | 1.5±0.2 | 0.88±0.1 | 1.6 |
| | 5.0 mM SA | 3.5±0.2 | 1.7±0.3 | 4.3±0.2 | 3.2 |
| | 0.5 mM AsA | 1.6±0.3 | 1.9±0.2 | 1.3±0.2 | 1.6 |
| | 1.0 mM AsA | 2.03±0.1 | 2±0.2 | 0.99±0.1 | 1.7 |
| | 5.0 mM AsA | 5.7±0.2 | 3.6±0.4 | 0.55±0.03 | 3.3 |
| Mean | | 2.5 | 2.2 | 2.1 | |
| LSD 0.05 | Treatments | 0.3 | | | |
| | Measures | 0.12 | | | |
| | Interaction | 0.57 | | | |

Pandey & Srivatsava [35] stated that plants expressed of salinity stress by many ways, one of them by taking up sodium and, therefore, $K^+/Na^+$ ratio in Leaves can be used as an indication for either sodium or potassium uptake. K/Na leaf ratio can be an indicator to distinguish between sodium-includers and sodium-avoiders. High sodium concentrations in the leaf blades indicate includers and if it had high K/Na ratio, it indicates to a high potassium uptake. In contrast, sodium avoiders need less potassium to obtain high K/Na ratios in the leaf blades and therefore have low leaf sodium concentrations. In our investigation the relation between $K^+/Na^+$ was inversely proportional with salinity increasing and that means decreasing in potassium uptake due to sodium uptake under salinity stress as it shown in Table (9) it was at its minimum ratio at 100 mM NaCl at 30 days of the experiment. Typical effect of salt stress was found at Melgar *et al*.[29] who reported that Salinity increased Na content in leaves of *Citrus limonia* Osbeck one-year seedlings at 50 mM of NaCl. Decreased growth was maybe due to a toxic effect of $Cl^-$ and/or $Na^+$ and not due to osmotic stress since citrus was able to osmotically adjust to maintain pressure potential higher than in non-salinized leaves. Also, our results were matching with [36] as they studying the effect of salinity stress for six weeks on the growth of 2-year-old 'Sunburst' mandarin [(*C. reticulata* Blanco)×(*Citrus paradisi* Macf.× *C. reticulata*)] trees grafted on either Cleopatra mandarin (*C. reticulata*) or Carrizo citrange (*Citrus sinensis* L. Osb.× *Poncirus trifoliata* L.) rootstocks grown in a greenhouse and found that salinity increased $Na^+$ and decreased $K^+$ concentrations in leaves of trees on Cleopatra but not in trees on Carrizo. These decreases could be due to the antagonism of $Na^+$ and $K^+$ at uptake sites in the roots and the effect of $Na^+$ on $K^+$ transport into the xylem or the inhibition of uptake processes [37], or to $K^+$ competition with $Na^+$ for binding sites on the plasma membrane which suppressed the influx of Na from the external solution [38]. High $K^+/Na^+$ ratio is more important for many species than simply maintaining a low concentration of $Na^+$[39].

SA and AsA application was significantly affected in keeping $K^+/Na^+$ ratio at its optimum level or near for it, especially at 5 mM SA at 25 mM NaCl, it even gave a higher ratio than the control (untreated plants). Foliar application of SA at 5 mM could maintain the $K^+/Na^+$ ratio at the same level for the whole experiment duration at 100 mM of NaCl. Exogenous application of SA seems to inhibit the $Cl^-$ and $Na^+$ absorption and helps for Mg, Fe, Mn, N and Cu absorption and decreases harmful effect of stress on growth [28]. The antagonistic relation between Na and K as a result of SA treatment implies that, SA could play a role in modifying K/Na selectivity under salt stress, which is reflected in lowering membrane damage and higher water content under salinity conditions [40].

Also, AsA at 5 mM recorded a high ratio of $K^+/Na^+$ (5.7) at the same level of salinity, but it gave a 36.8 and 90.4% reduction at 60 and 90 days, respectively, that was matching with [41], who reported that the application of 100 ppm from vitamins C (AsA) resulted significantly increases of $K^+$, $Ca^{+2}$ and $Mg^{+2}$ contents compared with controls. Ascorbic acid (AsA) protect metabolic processes against $H_2O_2$ and other toxic derivatives of oxygen affected many enzyme activities, minimize the damage caused by oxidative processes through synergistic function with other antioxidants and stabilize membranes [42].

## CONCLUSION

NaCl salt with accumulation of Na and Cl, disorder of osmotic potential and promotes of oxidative stresses, had adverse effects on the mandarin seedlings in vegetative, physiological and biochemical characteristics. The application of exogenous SA and AsA in the salt stressed seedlings could be able to reduce salt adverse effects and increased mandarin seedlings resistance by increasing growth processes, regulation and balance of osmotic potential. One mM of SA and AsA were the most effective concentration to raise the growth rate and the metabolic processes in mandarin seedlings. That would be very useful if it would be applicable on the trees and it could open a new horizons to grow citrus trees in saline soils. That would need more researches before it could be done.

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