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EFFECTS OF SALICYLIC ACID APPLICATION ON GERMINATION, GROWTH AND DEVELOPMENT OF ROUGH LEMON (*Citrus jambhiri* Lush.) UNDER SALT STRESS

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ABSTRACT

Rough lemon (*Citrus jambhiri* Lush.) – RL, is used as a rootstock for citrus plants in saline conditions. NaCl causes an osmotic stress on plants mainly preventing the water uptake by the roots and thus reducing the plant growth. The objective of this study was to determine the effects of salicylic acid (SA) on germination of seeds and the growth and development of seedlings of RL rootstock under salt stress. For seed germination, a study was conducted in a completely randomized design in a 4 × 4 factorial scheme (SA at 0.00, 0.25, 0.50, or 1.00 mM and NaCl at 0, 50, 100, or 200 mM) with 4 repetitions, totaling 64 plots, of 25 seeds per plot. RL seeds were incubated in SA solutions for 24 h. Then, they were treated with NaCl-containing water in Petri dishes and incubated in the growth chamber at 25°C. For greenhouse experiment, a study was conducted in a randomized complete block in a 4 × 4 factorial scheme (SA at 0.0, 0.5, 1.0, or 2.0 mM and NaCl at 0, 50, 100, or 150 mM) with 3 repetitions, totaling 48 plots, of 2 plants per plot. Some morphological and physiological characteristics were determined. While germination time was extended, germination ratio and radicle extension were decreased in seeds under salt stress compared to control. Moreover, in these conditions the leaf membrane permeability and leaf falling were increased. In turn, plant height, diameter, root and shoot dry weight, leaf relative water content, and leaf chlorophyll were decreased in seedlings. Salt stress had negatively affected seed germination from 97.5% in control to 23.5% in 200 mM NaCl. However, SA treatments significantly decreased plant height to 67.8 cm in 2.0 mM compared to 80.1 cm in control in RL rootstock.

Key words: *Citrus jambhiri*, salinity, seed germination, plant growth and development, stress tolerance

INTRODUCTION

Citrus species are the most produced (158,490, 986 t for 2020) fruit crop in the world, including oranges, mandarins, lemons and limes, and grapefruit, is the major fresh fruit group. While China produced 44,124,954 t (1st rank), Turkey produced 4,348, 742 t (8th rank) citrus fruit in 2020 [FAOSTAT 2020]. Citrus fruits have high value, because they are rich in vitamins and minerals and therefore valuable in the human diet.

Salinity is one of the major abiotic factors causing crop loss in the world [Yıldız et al. 2014]. It is expected to cause more than 50% of agricultural product losses by 2050 due to salinity. The main negative impact of NaCl on plants is an osmotic stress induction due

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to preventing the water uptake by the roots and thus reduction the plant growth [Mir et al. 2021]. Salt stress reduces growth, development, and water use efficiency in plants [Syvertsen and Garcia-Sanchez 2014]. It was found that *Vitex trifolia* Linn. var. *simplicifolia* Cham died when the NaCl concentration reached 450 mM (20 days after salt stress) [Yin et al. 2021]. In rocket (*Eruca sativa* L.), excessive salinity reduced stoma length, pore length, and relative water content (RWC) but increased leaf dry matter (LDM), chlorophyll, and cell membrane stability (CMS) [Kaya 2021]. On modern fruit growing, fruit trees are propagated by budding or grafting. Bud-union is composed of scion (the above-ground portion) and rootstock (the below-ground portion) of the plant. Citrus plants are sensitive even to relatively low salt concentrations [Hepaksoy 2000]. In a study using 7 citrus rootstocks with −0.10, −0.20, and −0.35 MPa, at the −0.10 MPa salinity level, sour orange, rough lemon, and Milam lemon could not exclude either Na^+ or Cl^- from their leaves [Zekri and Parsons 1992]. According to Cole [1985], four salinity-level treatments (range from 2 to 5 meq L^{-1} Cl^-) were applied to mature Washington Navel orange trees grown on RL. The fruit yield reduction started after 4 meq L^{-1} Cl^- treatment. When uniform seedlings of RL and Swingle citrumelo (SC) were grown with or without 50 mM NaCl for 42 days, salinity reduced leaf chlorophyll and plant transpiration rate (Ep) more in RL than SC [Gonzalez et al. 2012, Ziogas et al. 2021]. In turn, citrus rootstocks were ordered from the most salt tolerant to the least salt tolerant is as follows: Cleopatra mandarin, Rangpur lime, Volkameriana, sour orange, Swingle citrumelo, rough lemon (RL), Carrizo, Troyer, citron [Boman et al. 2005]. RL (*Citrus jambhiri* Lush.), native to northeastern India, performs well under deep sandy soil. The root system can reach 4.6 m in depth. Additionally, mature trees on RL have good drought tolerance. RL is considered having an intermediate salt tolerance [Davies and Albrigo 1994, Al-Yassin 2004].

Salicylic acid (SA) is distributed in the whole plant kingdom and is an endogenous plant growth regulator of phenolic nature that possesses an aromatic ring with a hydroxyl group or its functional derivative [Hayat et al. 2010]. An alternative approach is to enhance salt tolerance through exogenous application of SA has been studied [Yıldız et al. 2014, Mohamed et al. 2020]. SA plays an important role in seed germination. However, a variability of results has been reported [Hernández et al. 2017]. SA treatments advanced seed germination by reducing the NaCl-induced oxidative damage in saline conditions [Lee et al. 2010, Mir et al. 2021]. SA treatments as seed priming in maize (*Zea mays*) indicated that SA was more effective at early spring sowing dates. It is suggested that SA can be taken up by the seeds and then is converted to bound forms [Szalai et al. 2016, Janda et al. 2017]. Seeds primed with SA showed increased germination speed index, reduced mean germination time, and increased leaf relative water content, as well as seedling fresh and dry weight compared to non-primed seeds. Seedling vigor index was increased by 23.4% in seeds primed with 0.2 mM SA in grass pea (*Lathyrus sativus*) [Siavash Moghaddam et al. 2020]. While SA can inhibit seed germination in Arabidopsis [Borsani et al. 2001], pea [Barba-Espín et al. 2011], and tomato [Galviz-Fajardo et al. 2020]; it can also promote seed germination, plant growth and development under saline conditions in wheat [Shakirova et al. 2003, Fardus et al. 2018], pepper [Korkmaz 2005], broad bean [Soliman et al. 2016, Anaya et al. 2018], marigold [Afzal et al. 2017], sesame [Ahmad et al. 2019], soybean [Aalam et al. 2019] and onion [da Silva et al. 2019]. The role of SA in the response of plants to salt stress and related resistance mechanisms has yet to be solved [Hernández et al. 2017].

To the best of our knowledge, there are limited studies conducted to determine the tolerance of RL to salt stress. In this manner, determining the salt tolerance of RL is very important for effective growing as well as breeding studies. The objective of this study was to determine the effect of SA on germination and growth characteristics of of RL seeds exposed to salt stress.

MATERIAL AND METHODS

Plant material. Two experiments were conducted in the Department of Horticulture, Ortaca Vocational School, Muğla Sıtkı Koçman University (located at 36°50′55.0″ N 28°44′39.4″ E, with an altitude of 28 m), using seeds from a single tree of RL. The study comprised in 2 sections. First, seed germination trial was conducted in a growth chamber in the laboratory in February–March 2018. Then, seedling growth and development trial was conducted in a greenhouse in February 2018–April 2019.

Laboratory experiment. RL seeds were extracted from mature fruit. Seeds were surface sterilized with 70% ethanol for 1 min followed by 2.5% sodium hypochlorite for 20 min. Then, seeds were rinsed 4 times with sterile water. The seeds were divided in 4 batches including 400 seeds in total. The batches were treated with 0.00, 0.25, 0.50, and 1.00 mM SA in 100 mL solution for 24 h. Access water was removed by using sterile blotting paper. One hundred seeds were sown in 4 replicates containing 25 seeds in each replicate. The seeds were sown in 9-cm diameter sterile Petri dishes. Shortly after seeds were sown, 0, 50, 100, and 200 mM doses of NaCl in 30 mL solution were applied to the Petri dishes located at 25°C in an incubator (Digitech Senkro Hi-tech-DG08) in the dark. The Petri dishes were irrigated with 10 mL sterile H2O every 15 days.

Seed germination was recorded when radicle appears as 1 cm in length. Germination ratio (GR) and the mean number of days to germination (MDG) were calculated according to Rouse and Sherrod [1996]. The final seed germination was determined 55 days after seeds were sown. In the 28th day of the experiment, the radicle length (mm) was measured by a digital electronic caliper (Asimeto–AS-307).

The experimental design was completely randomized, in a 4×4 factorial scheme (SA concentrations of 0.00, 0.25, 0.50, 1.00 mM and NaCl concentrations of 0, 50, 100, 200 mM), with 4 repetitions, totaling 64 plots, of 25 seeds per plot.

Greenhouse experiment. In February 2018, seeds of RL were sown in peat : perlite (1 : 1, v : v) mixture in containers. Seedlings were transferred to 8 L polyethylene pots including peat : perlite (1 : 1, v : v) mixture 3 months after seed sowing. Each pot was irrigated with 500 mL water consisting of 18–18–18 (N : 18, P2O5 : 18, K2O : 18, B : 0.01, Cu : 0.01, Fe : 0.05, Mn : 0.02, Mo : 0.001, Zn : 0.02, 250g 100 L^{-1}, EC: 2000 µS cm^{-1}) fertilizer solution once a month. Salt and SA were incorporated in irrigation water with respected doses. Experiment was conducted in a complete randomized block (SA at 0.0, 0.5, 1.0, or 2.0 mM and NaCl at 0, 50, 100, or 150 mM) with 3 repetitions, totaling 48 plots, of 2 plants per plot. Control plants were irrigated with tap water (pH = 7.83, E.C. = 0.65 mS cm^{-1}).

At the end of the experiment, plant height (cm) and shoot diameter (mm) measurements were made using digital electronic caliper. Membrane conductivity (%), leaf relative water content (RWC, %), chlorophyll (PlantPen NDVI300 PSI, Photon System Instruments, spol. s.r.o., Drasov, Czech Republic) were measured. The PlantPen model NDVI300 measures relative units of Normalized Difference Vegetation Index (NDVI), which is an important indicator of chlorophyll content in plants. Roots and shoots were cut. Root and shoot dry weight (g) were measured separately. Root and shoot samples were dried at 65°C for 72 h in an incubator. Defoliation ratio (%) was also calculated.

The experimental design was a randomized complete block, in a 4×4 factorial scheme (SA concentrations of 0.0, 0.5, 1.0, 2.0 mM and NaCl concentrations of 0, 50, 100, 150 mM), with 3 repetitions, totaling 48 plots, of 2 plants per plot.

For statistical analysis, the data obtained from both experiments were subjected to 2-way analysis of variance (ANOVA) by F-test [SAS Institute 1989]. In cases of significance, the least significant difference – LSD ($p \leq 0.05$) was used to compare differences between means.

RESULTS

Laboratory experiment. The NaCl treatment was found to have significant ($p \leq 001$) effect to RL seed germination which was decreased with the increased salt concentrations from 97.5% in the control to 92.4% in 50 mM NaCl, 66.2% in 100 mM NaCl, and 23.5% in 200 mM NaCl (Tab. 1, Fig. 1a). Increasing salt doses had significant inhibitory effect on seed germination. However, neither NaCl × SA interaction, nor SA application were statistically significant.

Increasing salt application doses significantly increased the MDG from 12.5 in the control to 21.4 in 50 mM NaCl, 28.8 in 100 mM NaCl, and 46.3 in 200 mM NaCl treatments (Fig. 1b). The effect of SA and NaCl × SA on the MDG was not significant.

Interaction between NaCl × SA in relation to the radicle length was a statistically significant ($p \leq 0.05$). While the longest root was observed as 2.32 mm in 0 mM NaCl × 0.25 mM SA, the shortest of that was measured as 0.35 mm in 100 mM NaCl × 0.25 mM SA. The radicle length was significantly ($p \leq 0.01$)

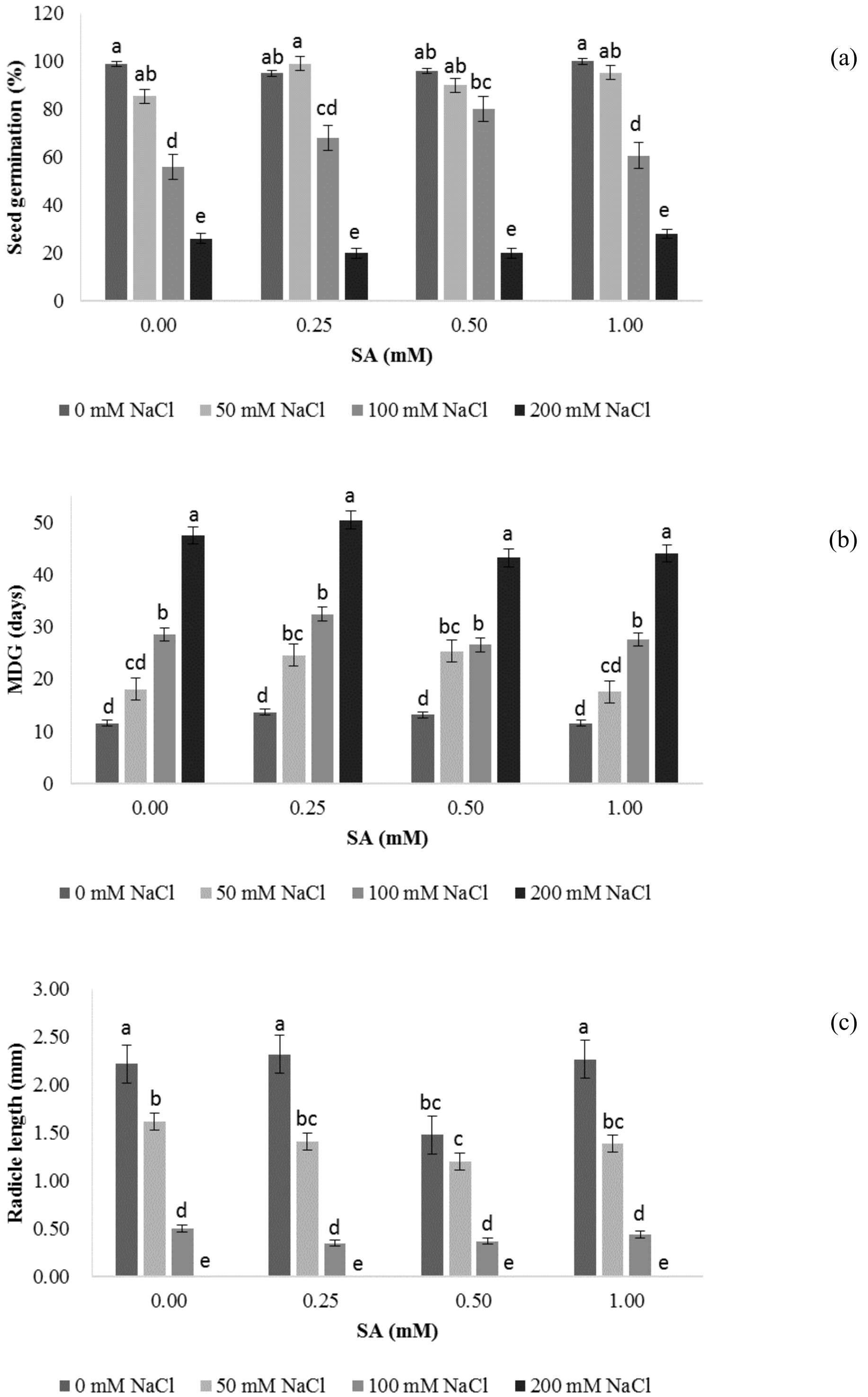


Fig. 1. a) The effect of salicylic acid (SA) on seed germination ratio (%), **b)** the mean number of days to germination (MDG), and **c)** radicle length (mm) in rough lemon grown under salt stress in the laboratory conditions

Table 1. Summary of analysis of variance for the variables of seed germination, the mean number of days to germination, and radicle length of rough lemon (*Citrus jambhiri* Lush.) grown at different salicylic acid cocentrations and subjected to different levels of salt stress in the laboratory conditions

Sources of variation	Degrees of freedom	Pr > Fc		
		seed germination (%)	the mean number of days to germination (days)	radicle length (mm)
NaCl	3	<0.0001**	<0.0001**	<0.0001**
SA	3	0.664^{ns}	0.087^{ns}	0.001**
NaCl × SA	9	0.257^{ns}	0.694^{ns}	0.011*
Error	48	–	–	–
Coefficient of variation (%)		17.50	20.88	23.57

*, **, and ^{ns} – significant at $p \leq 0.05$, $p \leq 0.01$, and not significant, respectively

decreased with the increasing of salt concentrations from 2.1 mm in control to 1.4 mm in 50 mM NaCl, and 0.4 mm in 100 mM NaCl alone. There was not any radicle growth in 200 mM NaCl. The effect of SA was significantly ($p \leq 0.01$) decreased radicle length from 1.5 mm in control to 1.4 mm in 0.25 SA, and 1.0 mm in 0.50 mM SA alone (Fig. 1c).

Greenhouse experiment. There was not a statistically significant ($p \leq 0.05$) interaction between NaCl × SA in plant height. Increasing salt application doses significantly ($p \leq 0.01$) decreased plant height from 84.3 cm in control to 74.3 cm in 50 mM NaCl, 67.1 cm in 100 mM NaCl, and 73.3 cm in 150 mM NaCl treatments (Tab. 2, Fig. 2a, Fig. 3). Also SA negatively ($p \leq 0.05$) affected plant height from 73.9 cm in 0.25 mM SA, 77.2 cm in 0.50 mM SA, 67.8 cm in 2.0 mM, compared to 80.1 cm in control in RL (Fig. 2a).

There was not statistically significant ($p \leq 0.05$) interaction between NaCl×SA in shoot diameter. Increasing salt application doses significantly ($p \leq 0.01$) decreased shoot diameter in RL from 7.4 mm in control to 5.7 mm in 50 mM NaCl, 5.4 mm in 100 mM NaCl, and 5.2 mm in 150 mM NaCl. SA application doses did not affect this parameter (Fig. 2b).

There was not a statistically significant ($p \leq 0.05$) interaction between NaCl×SA in shoot dry weight. Increasing salt application doses significantly ($p \leq 0.01$) decreased shoot dry weight in RL from 25.3 g in control to 10.2 g in 50 mM NaCl, 4.9 g in 100 mM NaCl, and 5.7 g in 150 mM NaCl. Hovewer, separate treatment with SA did not affect shoot dry weight (Fig. 2c). However, there was a significant ($p \leq 0.05$) interaction between NaCl and SA treatments in root dry weight. While the highest root biomass was observed in the control plants (17.2 g), the lowest (1.8 g) was noted in 150 mM NaCl × 2.00 mM SA treatment. Increasing salt application significantly ($p \leq 0.01$) decreased root dry weight in RL from 14.0 g in control to 9.4 g in 50 mM NaCl, 4.6g in 100 mM NaCl, and 4.8 g in 150 mM NaCl. SA application also significantly ($p \leq 0.01$) affected root dry weight from 10.0 g in control to 7.5 g in 0.50 mM SA, 7.4 g in 1.00 mM SA, and 7.9 g in 2.00 mM (Fig. 2d).

There was not a statistically significant ($p \leq 0.05$) interaction between NaCl × SA in the defoliation rate. Increasing salt application doses significantly ($p \leq 0.01$) increased the ratio of defoliation in RL from in 0.0% in the control to 71.6% in 50 mM NaCl, 92.4% in 100 mM NaCl, and 99.3% in 150 mM NaCl. In turn, SA applied separately did not affect the value of this parameter (Fig. 2e).

The leaf membrane permeability was significantly ($p \leq 0.05$) affected by NaCl and NaCl × SA treatments. While the highest value of this parameter was observed as 43.7% in 150 mM NaCl × 0.50 mM SA, the lowest of that was measured as 18.0% in 0 mM NaCl × 2.00 mM SA treatment. Increasing salt application increased leaf membrane permeability in RL from 19.2% in control to 30.6% in 50 mM NaCl, 35.1% in 100 mM NaCl, and 40.8 mM in 150 mM NaCl. On the other hand, the effect of SA was not significant (Fig. 2f).

The interaction between NaCl and SA treatments exerted a significant ($p \leq 0.05$) influence on leaf RWC. While the highest value was observed as 74.2% in

Table 2. Summary of analysis of variance for the variables of plant height, shoot diameter, shoot dry weight, root dry weight, defoliation rate, membrane permeability, relative water content, and chlorophyll content of rough lemon (*Citrus jambhiri* Lush.) grown at different salicylic acid concentrations and subjected to different levels of salt stress in the greenhouse conditions

Sources of variation	Degrees of freedom	Pr > Fc					
		plant height (cm)	shoot diameter (mm)	shoot dry weight (g)	root dry weight (g)	defoliation (%)	membrane permeability (%)
NaCl	3	0.002**	<.0001**	<.0001**	<.0001**	<.0001**	<.0001***
SA	3	0.026*	0.533^{ns}	0.182^{ns}	0.007**	0.236^{ns}	0.360^{ns}
NaCl × SA	9	0.144^{ns}	0.229^{ns}	0.164^{ns}	0.015*	0.369^{ns}	0.033*
Error	30	–	–	–	–	–	–
Coefficient of variation (%)		12.97	16.95	32.72	23.67	16.91	17.31
		relative water content (%)	chlorophyll (young leaf underside) (NDVI)	chlorophyll (young leaf upperside) (NDVI)	chlorophyll (mature leaf underside) (NDVI)	chlorophyll (mature leaf upperside) (NDVI)	
NaCl	3	0.002**	0.003**	0.001**	0.000**	<.0001**	
SA	3	0.776^{ns}	0.434^{ns}	0.278^{ns}	0.838^{ns}	0.931^{ns}	
NaCl × SA	9	0.002**	0.577^{ns}	0.694^{ns}	0.828^{ns}	0.784^{ns}	
Error	30	–	–	–	–	–	
Coefficient of variation (%)		15.81	13.05	11.53	14.59	13.72	

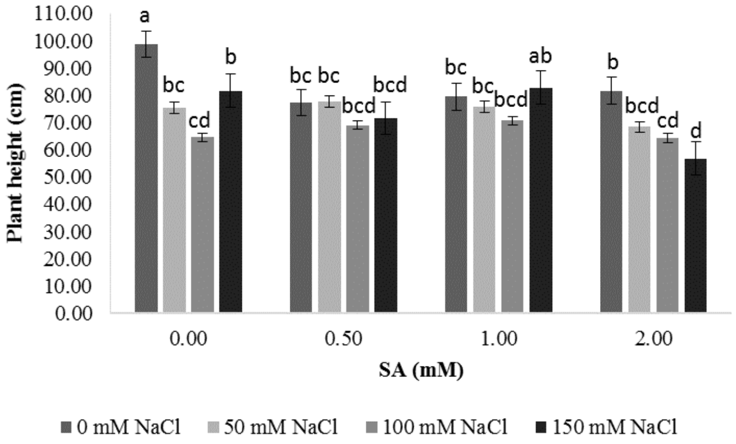
*, **, and ^{ns} – significant at $p \leq 0.05$, $p \leq 0.01$, and not significant, respectively

0 mM NaCl × 0.00 mM SA, the lowest of that was measured as 38.5% in 150 mM NaCl × 0.00 mM SA. The aplication of SA under severe salt stress (150 mM NaCl) significantly increased leaf RWC. Moreover, the effect of salt concentration on RWC was significant ($p \leq 0.01$) and RWC value decreased from 67.7% in control to 57.6% in 50 mM NaCl, 51.5 in 100 mM NaCl, and 58.8% in 150 mM NaCl (Fig. 2g).

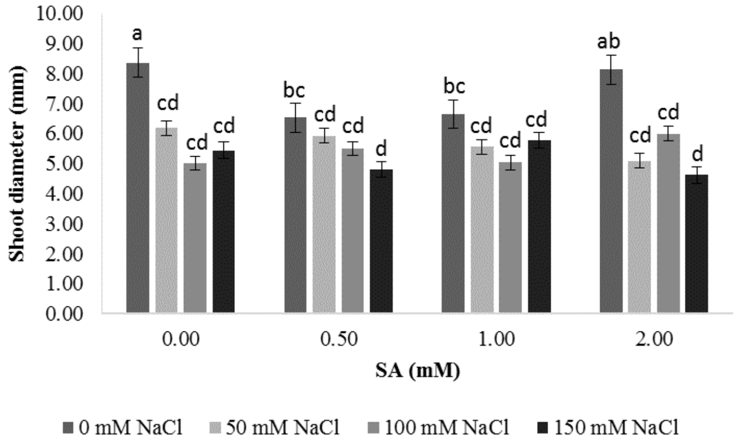
Chlorophyll level can be changed due to leaf age and developmental stage. Therefore, chlorophyll content was seperately measured on abaxial (underside) and adaxial (upperside) surface of young and mature leaves. Since all leaves were fallen off from the RL plants in 100 and 150 mM NaCl concentrations, chlorophyll level was not determined in those doses (Fig. 2h–k). The efffect of SA and NaCl × SA combinations on the chlorophyll was not significant. In turn, NDVI level decreased in underside of young leaves from 0.52 in control to 0.42 in 50 mM NaCl, upperside of young leaves from 0.54 in control to 0.44 in 50 mM NaCl, underside of mature leaves from 0.53 in control to 0.39 in 50 mM NaCl, and upperside of mature leaves from 0.57 in control to 0.40 in 50 mM NaCl in RL. In general, chlorophyll content was higher in the upperside than the underside of the leaves.

DISCUSSION

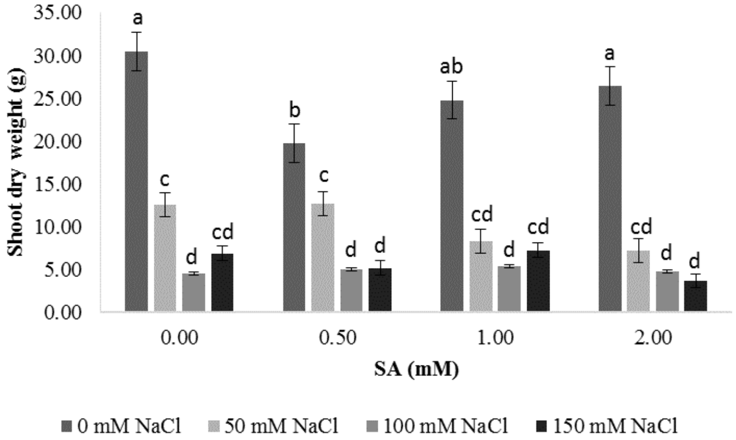
In the laboratory experiment, the effect of SA on seed germination and MDG value was not significant. It was found that RL seeds can tolerate salt stress at least to 50 mM NaCl. The similar results were obtained from seed germination studies conducted with RL by Sharma et al. [2013] and Kaushal et al. [2013]. In these experminents RL seeds were sown on MS medium solidified by 7.5 g l^{-1} agar supplemented with 30 g l^{-1} sucrose using 0.0–0.9% NaCl concentrations. Significant decrease in seed germination was recorded with increasing salt concentration. Moreover, salt treatments increased the level of Na^+ and Cl^- ions in the RL seedlings and also resulted in a decrease of K^+/Na^+ ratio [Sharma et al. 2013, Kaushal et al. 2013].


Plant height (cm)
110.00
100.00
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
a
bc
cd
b
bc
bc
bcd
bcd
bc
bc
bcd
ab
b
bcd
cd
d
0.00
0.50
1.00
2.00
SA (mM)
0 mM NaCl
50 mM NaCl
100 mM NaCl
150 mM NaCl

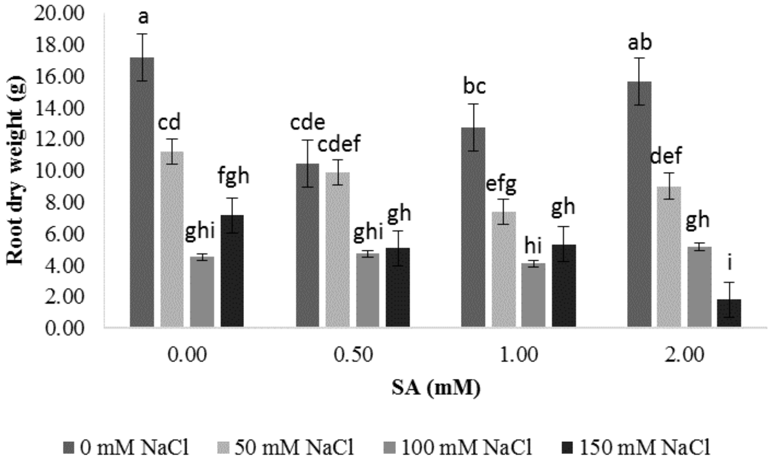
(a)


Shoot diameter (mm)
10.00
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
a
cd
cd
cd
bc
cd
cd
d
bc
cd
cd
cd
ab
cd
cd
d
0.00
0.50
1.00
2.00
SA (mM)
0 mM NaCl
50 mM NaCl
100 mM NaCl
150 mM NaCl

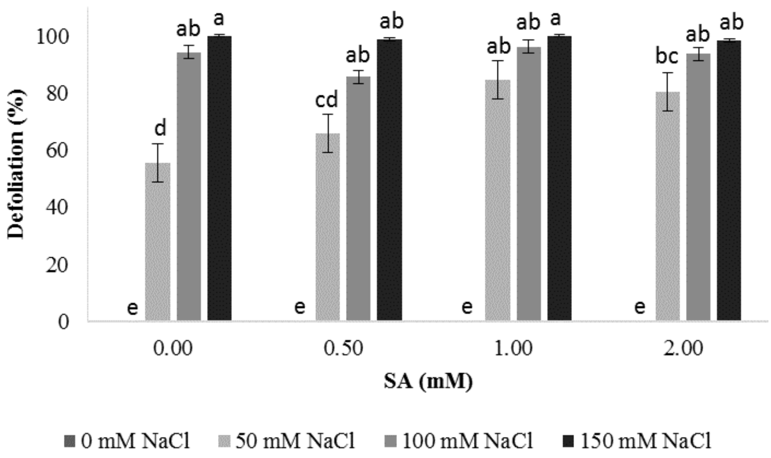
(b)


Shoot dry weight (g)
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
a
c
d
cd
b
c
d
d
ab
cd
d
cd
a
cd
d
d
0.00
0.50
1.00
2.00
SA (mM)
0 mM NaCl
50 mM NaCl
100 mM NaCl
150 mM NaCl

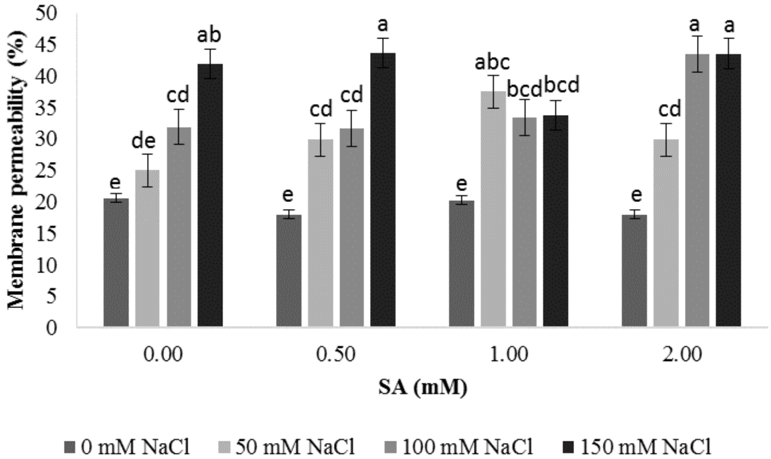
(c)


Root dry weight (g)
20.00
18.00
16.00
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
a
cd
ghi
fgh
cde
cdef
ghi
gh
bc
efg
hi
gh
ab
def
gh
i
0.00
0.50
1.00
2.00
SA (mM)
0 mM NaCl
50 mM NaCl
100 mM NaCl
150 mM NaCl

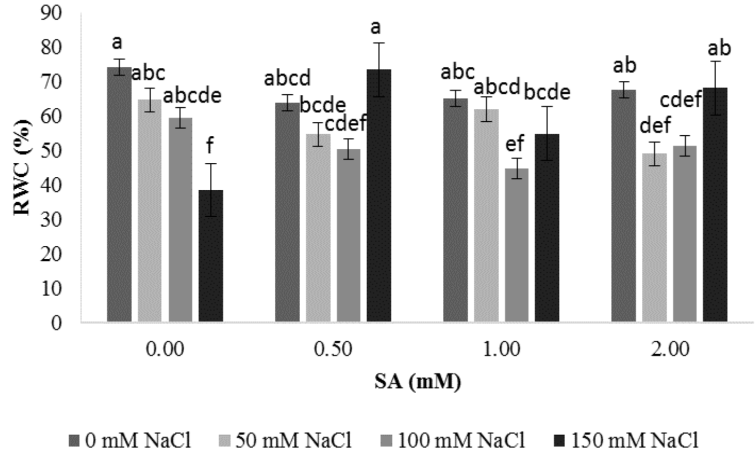
(d)


Defoliation (%)
100
80
60
40
20
0
e
d
ab
a
e
cd
ab
ab
e
ab
ab
a
e
bc
ab
ab
0.00
0.50
1.00
2.00
SA (mM)
0 mM NaCl
50 mM NaCl
100 mM NaCl
150 mM NaCl

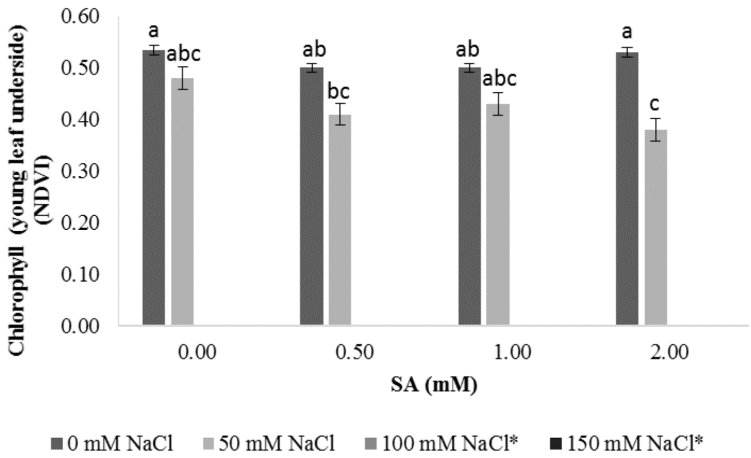
(e)


Membrane permeability (%)
50
45
40
35
30
25
20
15
10
5
0
e
de
cd
ab
e
cd
cd
a
e
abc
bcd
bcd
e
cd
a
a
0.00
0.50
1.00
2.00
SA (mM)
0 mM NaCl
50 mM NaCl
100 mM NaCl
150 mM NaCl

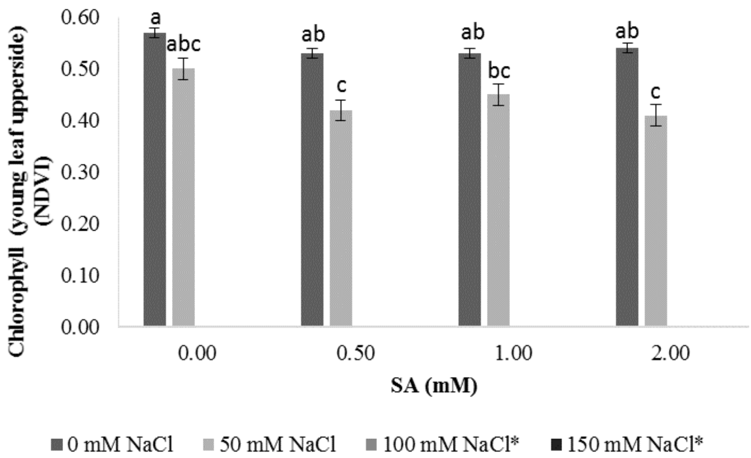
(f)


RWC (%)
SA (mM)
0.00
0.50
1.00
2.00
0 mM NaCl
50 mM NaCl
100 mM NaCl
150 mM NaCl

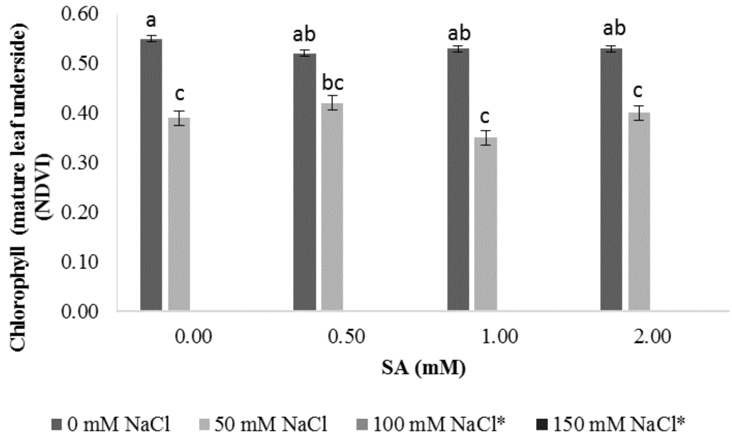
(g)


Chlorophyll (young leaf underside) (NDVI)
SA (mM)
0 mM NaCl
50 mM NaCl
100 mM NaCl*
150 mM NaCl*

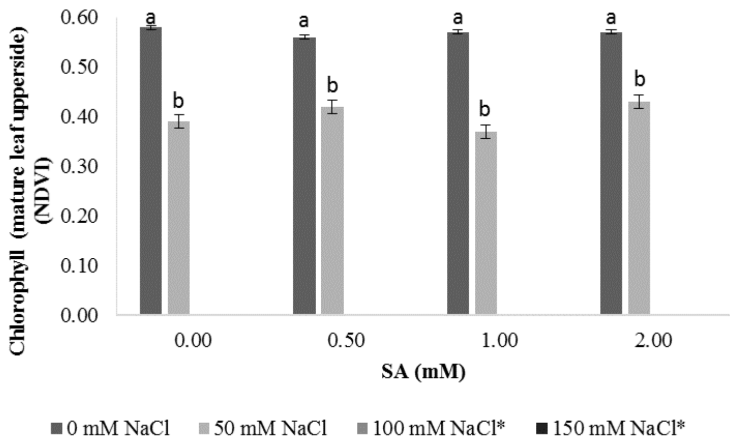
(h)


Chlorophyll (young leaf upperside) (NDVI)
SA (mM)
0 mM NaCl
50 mM NaCl
100 mM NaCl*
150 mM NaCl*

(i)



(j)



(k)

Fig. 2. a) The effect of salicylic acid (SA) on plant height (cm), **b)** shoot diameter (mm), **c)** shoot dry weight (g), **d)** root dry weight (g), **e)** defoliation ratio (%), **f)** membrane permeability (%), **g)** relative water content (RWC, %), chlorophyll content: **h, j)** underside and i, **k)** upperside of: h, **i)** young and **j, k)** mature leaves, of rough lemon rootstock grown under salt stress in the greehouse experiment. NDVI – Normalized Difference Vegetation Index. * – data could not be obtained because the leaves were fallen

In turn, Turgutoğlu et al. [2009] found lower seed germination (89.0%) of RL comparing to our results of 96.8%. Results obtained in the present study are similar to these of sour orange seed germination when increasing salt treatment decreased seed germination. The exposition to 15.6 and 23.4 dS m^{-1} established with NaCl decreased seed germination percentage (85.7% and 46.9%, respectively). The lowest seed germination was observed in the highest salt treatment (23.4 dS m^{-1}) [Shiri and Bakhshi 2011].

In *Salvia przewalskii*, osmopriming reduced the time of germination of stored and non-stored seeds. The highest germination rate was recorded in the seeds primed with –0.8 MPa established with PEG and stored at 16°C. Priming caused the acceleration of RNA and protein synthesis and improved the activity of free radical scavenging enzymes such as superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) [Bilińska et al. 2022]. Rouse and Sherrod [1996] found the MDG value between 9 and

Fig. 3. Rough lemon plants grown at different concentrations of NaCl in the greehouse experiment

28 days at 30°C in RL seeds. In turn, Turgutoğlu et al. [2009] found higher MDG (19.3 days) comparing to our results of 14.4 days. It can be suggested that decreasing MDG value could be used as important breeding criteria in citrus rootstock breeding against salt stress. Lee et al. [2010] stated that the differences in the effect of SA on seed germination depends on plant genotype and experimental conditions. The effect of NaCl × SA interaction on radicle length was significant. While the root length was decreased in 50 mM NaCl as 87.0% and 74.1% in 0.25 mM and 0.50 mM SA, respectively, compared to control, the minor decrease was observed at 100 mM NaCl as 70.0% and 74.0% in 0.25 mM and 0.50 mM SA, respectively. Moreover, the application of 0.5 mM SA at 100 mM NaCl significantly increased seed germination.

In the greenhouse experiment, increasing salt concentrations decreased plant height. This result can be attributed to lack of obtaining adequate water and thus osmotic stress as well as ion toxicity to plants [Sharma et al. 2013, Al-Hayany et al. 2017]. NaCl showed more negative effect on shoots than roots. With an increase in salt concentration, both total fresh and dry weight of seedlings decreased [Sharma et al. 2013]. Moreover, the higher amounts of salt delayed seedling emergence [Zekri 1993] and decreased the accumulation of dry matter in citrus seedlings [Zekri and Parsons 2017, Sá et al. 2017]. In strawberry, 4 mM SA treatment increased root tissue density [Aras and Eşitken 2019]. The effect of defoliation of RL to salt stress resistance was also mentioned in previous studies [Boman et al. 2005]. The reason for decreasing leaf relative water content probably is large leaf width of RL. It is thought that restriction to water flow probably caused this water deficiency due to salt stress. Similar results on negative impact of salt stress were reported in sour orange, Volkamer lemon, and Macrophylla lemon grown in greenhouse conditions [Ibrahim et al. 2018].

It was found that SA treatment decreased Na and Cl accumulation in the leaves. Therefore, the negative effects of salt stress could at least partially reduced by exogenous SA applications [Ibrahim et al. 2018]. High amounts of salt could cause disintegration of cell membrane in plants [Guo et al. 2019]. Because abscisic acid (ABA) is ensuring the development of anti-stress reactions maintained by free proline accumulation, SA-induced increase in ABA concentration might contribute to the pre-adaptation of plants to stress conditions [Bandurska and Stroinski 2005]. Moreover, SOD and peroxydase (PER) enzymes contribute to the protective effect of SA on plants under saline conditions [Sakhabutdinova et al. 2004]. SA reduces NaCl stress by lowering lipid peroxidation levels, H2O2 (hydrogen peroxide) and ROS (reactive oxygen species) accumulation, and also enhancing the activity of CAT, APX, DHAR (dehydroascorbate reductase) enzymes, and GSH (reduced glutathione) concentration [Lee et al. 2010]. In our study the exogenous aplication of SA under severe salt stress (150 mM NaCl) caused significant increase in leaf RWC. On the other hand, at 50 mM NaCl the higher concentrations of SA induced increase in defoliation rate. Therefore, the effect of SA under salt stress is not unequivocally positive or negative and largely depends on the tested parameter, the level of salinity and the concentration of SA applied. In dwarf (‘Mina’) and pole (‘Beyza’) beans (Phaseolus vulgaris) grown under salinity stress conditions, the lipid peroxidation level, SOD, APX, and CAT activities, and proline content was increased compared to the control. While chlorophyll level was stable at the 0,25, and 50 mM NaCl doses, it was significantly decreased at 100 mM salt application [Çirka et al. 2022]. In turn, in ‘Zard’ and ‘Roghani’ olives (*Olea europaea*), simultaneous SA and kaolin applications mitigated the harmful effects of water shortage on plants by enhancing chlorophyll accumulation, antioxidant activity, and yield compared to the control treatment [Azizifar et al. 2022].

CONCLUSIONS

1. Increasing NaCl application significantly decreased seed germination, radicle length, plant height, shoot diameter, shoot dry weight, root dry weight, leaf relative water content, and chlorophyll level in RL.

2. Increasing NaCl application significantly increased the mean number of days to germination, defoliation ratio, leaf membrane permeability in RL compared to control.

3. Increasing SA application significantly decreased plant height in RL rootstock. This application can be used in dwarfing effect for orchard management. For more practical purpose, field performance of cultivar-budded RL rootstocks for salt tolerance needs to be evaluated in the future.

4. The influence of SA treatment on RL grown under salt stress is not unequivocally and depends on the tested parameter, development stage of plant, the level of salinity, and the concentration of SA.

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