Using Salicylic Acid for Alleviating the Adverse Effects of Water Salinity on Growth and Nutritional Status of Mango Cv. Alphonse Seedlings

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Abstract: This study was performed during 2011 and 2012 seasons to test the effect of spraying salicylic acid (SA) at 0.55 ml (1.0 g l^{-1} w) to mango cv. Alphonse seedlings irrigated with water containing NaCl at 10, 20 or 40 mM (i.e. 0.36, 0.72 and 1.44 g NaCl l^{-1} w, respectively). Salinized irrigation water at 10 to 40 mM NaCl caused an inhibition on all growth characters, leaf water content %, plant pigments, total carbohydrates % as well as concentrations and uptake of N, P, K and Mg. However, H_2O_2 content, K^+/ Na^+ as well as concentrations and uptake of Na and Cl tended to promote with salinization conditions. Using SA obviously counteracted the previous adverse effects of saline water on growth and nutritional status of the transplants. It is suggested that the promotive effect of SA on producing vigour transplants of mango cv. Alphonse irrigated with saline water may result in improving salt stress tolerance.

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1. Introduction

Salinity is one of the main problems that negatively affect soil fertility and limit plant production. Soil salinity affects osmotic stress, decreasing water availability, ionic stress, changes in the cellular ionic balance. The effect of salinity was appeared in reducing the uptake of various nutrients **(Afzal *et al*., 2005).** Salt stress increases the formation of reactive oxygen species causing deterioration of membrane functions **(Erdal *et al*., 2010).** Plants have evolved enzymatic and non enzymatic defense mechanisms in order to reduce oxidative damages by detoxifying free radicals. The enzymatic defense system includes peroxidase and catalase. These enzymes detoxify H_2O_2 (an active oxygen species) to H_2O **(Borsani *et al*., 2011).**

Salicylic acid (SA) is recognized as an endogenous signal molecule mainly involved environmental stress tolerance in plants **(Dela- Rosa and Maiti, 1995).** It is synthesized by many plants and is accumulated in the plant tissues under the impact of unfavourable abiotic factors, contributing to the increase of plant resistance to salinization **(Ding *et al*., 2002 and Kang and Saltveit, 2002).** The role of SA in defense mechanisms has been known for several years **(Chiunusamy and Zhu, 2003; Shim *et al*., 2007 and Gunes *et al*., 2007)**. It is a key endogenous signal involved in plant defense response to environmental stressors such as salinity **(Raskin *et al*., 1990)**. SA included increase in the resistance of fruit seedlings to salinity **(Shakirova and Bezrukova, 1997).** Thus, the detrimental effects of high salts on the early growth of crop seedlings may be alleviated by treating seeds with the proper concentration of a suitable hormone **(Darra *et al*., 1973).**

Previous studies showed that supplying mango and banana fruit crops with salicylic acid was beneficial in alleviating the adverse effects of soil and water salinity on growth and nutritional status **(Duran-Zaazo *et al*., 2003 and 2004; Morsy, 2006; Ahmed and Abdelaal, 2007; Hayat and Ahmed, 2007; Josephe *et al*., 2010; Hayat *et al*., 2010 and Al-Wasfy, 2012).**

The target of this study was examining the tolerance of mango cv. Alphonse to water irrigation salinity and the role of salicylic acid on counteracting the adverse effects on growth and nutritional status of the transplants.

2. Material and Methods

This pot trial was conducted in greenhouse located at Faculty of Agriculture, Minia Univ. Minia Governorate during 2011 and 2012 seasons on 240 4-years old Alphonse mango cv. (*Mangifira indica* L.) transplants onto seedling rootstocks. Each seedling was planted in plastic pots 40 cm in diameter and 40 cm. in height, filled with 8.0 kg air dried sandy loamy soil (69.0, 24.2 and 6.8 % of sand, silt and clay, respectively), containing 0.85 % of organic matter 0.05 % of N, 17.6 mg/ kg^{-1} P and 180.0 mg/kg^{-1} K. The soil pH was 7.97 and the EC was 1.41 dS m^{-1}. Each pot was fertilized annually with 10.0 g N, 3.0 P_2O_5 and 8.0 g K_2O (according to **Duran- Zuazo *et al*., 2003).** The plant height (cm.) and stem diameter (cm.) was adjusted to 35.0 cm and 0.90 cm at the start of experiment, respectively (zero measurement).

The present study included the following eight

treatments:-

1. Non water salinization without using salicylic acid (SA).
2. Water salinization at 10 mM NaCl without SA.
3. Water salinization at 20 mM NaCl without SA.
4. Water salinization at 40 mM NaCl without SA.
5. Non water salinization with using SA at 0.55 mM.
6. Water salinization at 10 mM NaCl + SA.
7. Water salinization at 20 mM NaCl + SA.
8. Water salinization at 40 mM NaCl + SA.

Each treatment was replicated three times, ten seedlings per each. Randomized complete design was adopted. The selected plastic pots were kept inside glass greenhouse under natural light. Soil was allowed to equilibrate in the greenhouse for ten days before planting the seedlings. The seedlings were irrigated with non- salinized tap water every four days at field capacity (soil moisture content reached 15 % by weighing). After one month from planting the seedlings were irrigated with saline water (caused by NaCl) at 0.0 (0.0 g^{-1} NaCl), 10.0 (0.36 g^{-1} NaCl), 20.0 (0.72 g^{-1} NaCl) and 40.0 mM (1.44 g^{-1} NaCl). Then, seedlings were separated into two groups, the first was sprayed with salicylic acid at 0.55 mM (0.1 g^{-1} / Iw) and the other one was left without spraying salicylic acid. Salicylic acid was dissolved in distilled water and the pH was adjusted at 6.5 with NaOH. It was applied three times at one month after the addition of saline water and at one month intervals. Irrigation with saline irrigation was done at field capacity as previously mentioned. For preventing salt accumulation in the plastic pots, it is preferable for using non- salinized water irrigation at field capacity in between involved saline water for leaching salt. At the end of experimental (last week of Sept.) the following measurements were recorded:

1. Plant height (cm.) from the surface of soil.
2. Stem diameter 20 cm above ground (cm.).
3. Each seedling was separated into shoots and roots for measuring averages fresh and dry weights of shoots and roots (g/ plant). The ratio between fresh roots and shoots was calculated. Then, the whole plant dry weight (g.) was recorded.
4. Leaf water content % was measured by drying the leaves at 80 ◌̊C for 48 hours and the percentage was calculated as follows: leaf water content = $\frac{F.W \times 100}{F.W}$

   where F.W and d.W were fresh and dry weights of leaf, respectively.
5. H_2O_2 was assayed according to the method of **He *et al.* (2005).** Leaves were homogenized in ice bath with 0.1 % (w/v) TCA. The extract was centrifuged at 12,000 × g for 15 min, after which to 0.5 ml of the supernatant was added 0.5 ml of 10 mM potassium phosphate buffer (pH 7.0) and 1 ml of 1 M KI, and the absorbance was read at 390 nm. The content of H_20_2 was given on a standard curve.
6. Plant pigments namely chlorophylls a & b and total carotenoids were determined according to the procedures of **Arnon (1949) and Wettstein (1957)**.
7. Total carbohydrates % in the leaves by using phenol and sulphoric acid method (**A.O.A.C., 1995**).
8. Visual assessment of the symptoms.

The severity of the plants condition was classified as follows: a) Plants without symptom (WS).
b) Very mild chlorosis (VMC).
c) Partial mild chlorosis (PMC).
d) Burns on the margins and apices of leaves (BMA).
e) Sever damage with leaf drop and plant death (SDD).

9. K^+/ Na^+ was calculated by dividing K^+ by Na^+
10. Percentages of N, P, K, Mg and Na were determined on the dried whole plant (according to **Piper, 1950 and Wilde *et al.*, 1985**) and uptake of these nutrients by each seedling (g/ plant) was calculated by multiplying of each neutral by dry weight of whole plant (g/ plant).

Proper statistical analysis was done using new L.S.D at 5 % (according to **Mead *et al.*, 1993).**

3. Results and Discussion

1- Growth characters:

It is clear from the data in Tables (1 & 2) that the eight growth characters were significantly depressed with using saline water at 10 to 40 mM NaCl with or without spraying salicylic acid (SA) at 0.55 mM in relative to using unsaline water with or without SA. The reduction on these growth traits was significantly associated with increasing water salinity levels. Spraying SA to the seedlings irrigated with saline water was significantly responsible for alleviating the adverse effects of saline water on these growth characters. The tolerance of mango seedling to saline water was significantly enhanced in response to foliar application of SA in terms of increasing all growth characters. Under unsalinized water irrigation, spraying SA gave the best results comparing with non-application of SA at the same conditions. Using saline water at 40 mM NaCl without SA gave the lowest values of growth characters. These results were true during both seasons.

These results are agreement with those obtained by **Al- Wasfy (2012).**

2- Leaf water content %:

As shown in Table (2) using saline water at 10 to 40 mM NaCl regardless SA application significantly reduced leaf water content % in relative to irrigation

with unsalinized water. The degree of reduction was significantly in proportional to the increase in salinity levels. The major reduction was observed on the seedlings that irrigated with saline water at 40 mM. There was a remarkable and significant promotion on leaf water content % in response to foliar application of SA under saline irrigation water conditions. It is appeared from these results that using SA significantly was succeeded in lowering the harmful effects of using saline water on leaf water content %. Using unsalinized irrigation water + spraying SA gave the highest values. The lowest leaf water content % was observed on the transplants irrigated with saline water at 40 nM NaCl. These results were true during both seasons.

These results are in concordance with those obtained by **Al- Wasfy (2012).**

3- H_2O_2 content:

It is worth to mention from the data in Table (2) that irrigation with saline water at 10 to 40 mM NaCl with or without application of SA significantly was accompanied with increasing H_2O_2 content in relative to using unsalinized water with or without spraying SA. Irrigation with unsalinzed water plus spraying SA significantly reduced H_2O_2 content rather than irrigation with unsalinized water without using SA. Spraying SA to the seedlings that irrigated with saline water at 10 to 40 mM NaCl effectively minimized H_2O_2 content as compared with using saline water. The promotion on H_2O_2 alone was significantly associated with increasing NaCl levels in the irrigation water. The maximum values of H_2O_2 (8.8 and 9.1 mol/ μg^{-1} d.w) were presented in the seedlings that irrigated with saline water at 40 mM NaCl without using SA. Using unsaline water along with spraying SA at 0.55 mM gave the lowest values. These results were true during both seasons.

These results are in agreement with those obtained by **Joseph *et al*. (2010) and Hayat *et al*. (2010).**

4- Plant pigments, total carbohydrates % as well as content and uptake of N, P, K, Mg and Cl and K^+/ Na^+:

It is clear from the data in Tables (3 & 4 & 5) that exposing the transplants to saline soil at 10 to 40 mM NaCl regardless SA application significantly resulted in reducing plant pigments namely chlorophyll a, chlorophyll b, total carotenoids and total chlorophylls, total carbohydrates %, K^+/ Na^+ and percentages and uptake of N, P, K, and Mg in relative to using unsalinized water with or without using SA. However, percentages and uptake of Na and Cl were stimulated in response to using saline water. Spraying SA significantly was followed by enhancing plant pigments, total carbohydrates %, K^+/ Na^+ as well as concentrations and uptake of N, P, K, and Mg in seedlings under salinity conditions in relative to irrigation with saline water with saline water or without using SA. Using water free from NaCl gave the maximum values of all parameters except Cl and Na that tended to increase. Using SA has beneficial effect on concentrating the adverse effects of salinity on these characters. These results were true during both seasons.

These results are in harmony with those obtained by **Morsy (2006); Ahmed and Abdelaal (2007) and Al- Wasfy (2012).**

5- Visual assessment of the symptoms:

It can be seen from the data in Tables (3) that plants irrigated with saline water especially at height levels (40 mM NaCl) and without using SA were the most effected by the salt treatments. Irrigation with unsaline water either with or without application of SA produced plants without symptoms (WS). Using SA in the transplants irrigated with saline water at 10 mM NACl produced plants without symptoms (WS). Very mild chlorosis (VMC) transplants were appeared in response to using saline irrigation water at 10 mM NaCl without using SA. Leaves of seedlings treated with saline water at 20 mM (without SA) had mild chlorosis to burns on the margins and apices (BMA) but this symptoms was noticed with using saline water at 40 mM in combined with spraying SA at 0.55 mM. Under saline water conditions at 40 mM without using SA sever damage with leaf drop followed by plant death (SDD) was occurred. Under the same conditions of water salinity but with using SA, the symptoms were mild chlorosis to burns on the margins and apices. Results clearly show that using SA to the plants grown under saline irrigation water considerably prevented the severe damage caused by salinized water especially at the higher levels. These results were true during both seasons.

These results are in approval with those obtained by Duran- **Zuazon *et al*. (2003) and (2004) and Josephe *et al*.(2010).**

4.Discussion

The obtained results concerning the inferior effects of water salinization on growth, plant pigments and nutritional status of mango cv. seedlings could be ascribed on the light of its negative action on photosynthesis, water availability, changes in the nutrient balance with plant tissues, uptake of different nutrients and cell division **(Afzal *et al*., 2005).** The promotive effect of salinity on reactive oxygen species, and H_2O_2 and the deterioration on membrane functions as well as the reduction on the enzymatic defense system **(Erdal *et al*., 2010)** could give another interpretation of the present results. The counteracting effect of salicylic acid on the adverse of water salinity on growth might be attributed to its positive action on increasing cell division, enzymatic and non- enzymatic

defense system, plant pigments and biosynthesis of organic foods **(Raskin *et al*., 1990).** The reducing effect of SA on reactive oxygen species and H_2O_2 **(Ding *et al*., 2000)** could give another explanation.

Conclusion

For alleviating the various inferior effects of salinization water irrigation on growth of mango cv. Alphonse, it is suggested to use salicylic acid via leaves at 0.55 mM.

Table (1): Effect of spraying salicylic acid on some growth characters of Alphonse mango seedlings irrigated with different water salinity levels during 2011 and 2012 seasons.

Treatment	Plant height (cm.)		Stem diameter (cm.)		Shoot fresh weight/ plant		Shoot dry weight (g.)/ plant		Root fresh weight (g.)/ plant		Root dry weight (g.)/ plant	
	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
0.0 mM NaCl without SA	110.7	112.7	1.77	1.82	811.0	830.0	161.0	171.0	276.0	281.0	49.0	50.3
10.0 mM NaCl without SA	101.6	103.3	1.66	1.70	789.0	811.0	151.0	161.0	255.0	260.0	47.0	48.7
20.0 mM NaCl without SA	93.6	95.9	1.40	1.47	741.0	761.9	131.0	141.9	241.0	245.0	40.0	41.6
40.0 mM NaCl without SA	84.3	86.9	1.25	1.30	701.0	721.9	111.0	120.9	211.0	215.0	30.0	31.3
0.0 mM NaCl + 0.55 mM SA	120.7	122.7	1.90	1.95	861.0	882.9	184.0	194.9	296.0	300.9	54.7	55.5
10.0 mM NaCl + 0.55 mM SA	109.0	111.0	1.81	1.87	831.0	852.0	171.0	181.0	271.0	276.0	51.0	52.5
20.0 mM NaCl + 0.55 mM SA	103.7	106.0	1.61	1.66	791.0	811.9	150.0	159.5	251.0	256.0	46.0	47.0
40.0 mM NaCl + 0.55 mM SA	94.7	96.6	1.40	1.45	741.0	761.0	120.9	131.6	221.0	226.0	36.9	37.0
New L.S.D at 5 %	**1.8**	**2.0**	**0.06**	**0.07**	**21.1**	**22.0**	**4.4**	**4.5**	**7.1**	**8.1**	**1.8**	**1.7**

SA = Salicylic acid **1.0 M SA = 180 g^{-1}**

Table (2): Effect of spraying salicylic acid on root/ shoot, plant dry weight, H_2O_2 content and chlorophylls a & b of Alphonse mango seedlings irrigated with different water salinity levels during 2011 and 2012 seasons.

Treatment	Root/ shoot (F.W basis)		Whole plant dry weight (g.)		Leaf water content %		H_2O_2 content (µmol/ g^{-1} dw)		Chl. a (mg/ g^{-1} F.W)		Chl. b (mg/ g^{-1} F.W)	
	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
0.0 mM NaCl without SA	0.34	0.34	210.0	221.3	78.0	79.0	5.1	5.6	9.9	10.5	4.7	5.0
10.0 mM NaCl without SA	0.32	0.32	198.0	209.7	75.9	77.0	5.5	6.1	7.1	7.7	4.0	4.3
20.0 mM NaCl without SA	0.33	0.32	171.0	183.5	73.9	75.0	6.9	7.5	6.6	7.2	3.5	3.8
40.0 mM NaCl without SA	0.30	0.30	141.0	152.2	71.7	72.8	8.5	9.1	6.1	6.7	3.0	3.2
0.0 mM NaCl + 0.55 mM SA	0.34	0.34	238.7	250.4	80.3	81.3	4.0	4.6	12.8	13.4	7.0	7.3
10.0 mM NaCl + 0.55 mM SA	0.33	0.32	222.0	233.5	78.0	79.3	4.5	5.0	10.0	10.5	5.1	5.4
20.0 mM NaCl + 0.55 mM SA	0.31	0.32	196.0	206.5	75.9	77.1	4.8	5.2	9.0	9.5	4.5	4.7
40.0 mM NaCl + 0.55 mM SA	0.31	0.30	157.8	168.7	73.7	74.8	5.0	5.5	8.0	8.4	4.0	4.2
New L.S.D at 5 %	**NS**	**NS**	**10.1**	**11.0**	**2.0**	**1.9**	**0.2**	**0.3**	**0.3**	**0.3**	**0.2**	**0.3**

SA = Salicylic acid **1.0 M SA = 180 g^{-1}**

Table (3): Effect of spraying salicylic acid on total chlorophylls, total carotenoids, total carbohydrates %, symptoms, K^{++}/Na^{+} and percentage of N of Alphonse mango seedlings irrigated with different water salinity levels during 2011 and 2012 seasons.

Treatment	Total chlorophylls (mg/ g^{-1} F.W)		Total carotenoids (mg/ g^{-1} F.W)		Total carbohydrates %		Symptoms		K^{++}/ Na^{+}		N %	
	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
0.0 mM NaCl without SA	14.6	15.5	2.3	2.5	16.0	15.8	WS	WS	12.9	13.4	1.69	1.75
10.0 mM NaCl without SA	11.1	12.0	2.0	2.2	15.5	15.2	PMC	PMC	5.8	5.7	1.55	1.61
20.0 mM NaCl without SA	10.1	11.0	1.7	1.9	13.5	13.3	VMC	VMC	3.7	4.0	1.29	1.34
40.0 mM NaCl without SA	9.1	9.9	1.5	1.6	9.0	8.8	SDD	SDD	2.20	2.2	1.09	1.14
0.0 mM NaCl + 0.55 mM SA	19.8	20.7	3.5	3.7	17.7	17.8	WS	WS	20.0	18.1	1.81	1.87
10.0 mM NaCl + 0.55 mM SA	15.1	15.9	2.6	2.7	17.1	17.2	WS	WS	9.6	9.2	1.67	1.73
20.0 mM NaCl + 0.55 mM SA	13.5	14.2	2.3	2.4	15.0	15.2	VMC	VMC	6.4	6.3	1.40	1.47
40.0 mM NaCl + 0.55 mM SA	12.0	12.6	2.0	2.1	10.9	11.1	VMC	VMC	4.6	4.6	1.20	1.25
New L.S.D at 5 %			**0.2**	**0.2**	**0.5**	**0.4**	**--**	**--**	**1.1**	**1.1**	**0.12**	**0.10**

SA = Salicylic acid 1.0 M SA = 180 g^{-1}

Table (4): Effect of spraying salicylic acid on the percentages of P, K, Mg, Na and Cl in the whole plant and N uptake of Alphonse mango seedlings irrigated with different water salinity levels during 2011 and 2012 seasons.

Treatment	P %		K %		Mg %		Na %		Cl %		N uptake (g/ plant)	
	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
0.0 mM NaCl without SA	0.25	0.28	1.29	1.34	0.71	0.73	0.10	0.10	0.3	0.4	3.5	3.9
10.0 mM NaCl without SA	0.22	0.25	1.22	1.26	0.65	0.67	0.21	0.22	0.5	0.6	3.1	3.4
20.0 mM NaCl without SA	0.14	0.17	1.11	1.15	0.55	0.56	0.30	0.29	0.8	0.9	2.2	2.5
40.0 mM NaCl without SA	0.07	0.10	0.90	0.94	0.45	0.46	0.41	0.43	1.2	1.3	1.5	1.7
0.0 mM NaCl + 0.55 mM SA	0.29	0.34	1.40	1.45	0.82	0.84	0.07	0.08	0.2	0.2	4.3	4.7
10.0 mM NaCl + 0.55 mM SA	0.26	0.30	1.34	1.38	0.74	0.76	0.14	0.15	0.4	0.4	3.7	4.0
20.0 mM NaCl + 0.55 mM SA	0.18	0.22	1.22	1.26	0.61	0.62	0.19	0.20	0.6	0.7	2.7	3.0
40.0 mM NaCl + 0.55 mM SA	0.11	0.13	1.05	1.11	0.52	0.53	0.22	0.24	0.9	0.9	1.9	2.1
New L.S.D at 5 %	**0.03**	**0.03**	**0.07**	**0.09**	**0.05**	**0.06**	**0.04**	**0.04**	**0.2**	**0.2**	**0.3**	**0.3**

SA = Salicylic acid **1.0 M SA = 180 g^{-1}**

Table (5): Effect of spraying salicylic acid on the uptake of P, K, Mg, Na and Cl of Alphonse mango seedlings irrigated with different water salinity levels during 2011 and 2012 seasons.

Treatment	P uptake (g/ plant)		K uptake (g/ plant)		Mg uptake (g/ plant)		Na uptake (g/ plant)		Cl uptake (g/ plant)	
	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
0.0 mM NaCl without SA	0.53	0.62	2.71	2.97	1.49	1.62	0.21	0.22	0.63	0.89
10.0 mM NaCl without SA	0.44	0.52	2.42	2.64	1.29	1.40	0.42	0.46	0.99	1.26
20.0 mM NaCl without SA	0.24	0.31	1.90	2.11	0.94	1.03	0.51	0.53	1.37	1.65
40.0 mM NaCl without SA	0.10	0.15	1.27	1.43	0.63	0.70	0.58	0.65	1.69	1.98
0.0 mM NaCl + 0.55 mM SA	0.69	0.85	3.34	3.63	1.96	2.10	0.17	0.20	0.48	0.50
10.0 mM NaCl + 0.55 mM SA	0.58	0.70	2.97	3.22	1.64	1.77	0.31	0.35	0.89	0.93
20.0 mM NaCl + 0.55 mM SA	0.35	0.45	2.39	2.60	1.20	1.28	0.37	0.41	1.18	1.45
40.0 mM NaCl + 0.55 mM SA	0.17	0.22	1.66	1.87	0.82	0.89	0.36	0.40	1.26	1.52
New L.S.D at 5 %	**0.10**	**0.12**	**0.15**	**0.16**	**0.21**	**0.22**	**0.11**	**0.12**	**0.07**	**0.08**

SA = Salicylic acid **1.0 M SA = 180**

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