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Ameliorate growth and leaf mineral content of olive seedlings irrigated with saline water by using salicylic acid

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ABSTRACT

The aim of the present investigation was to determine the effect of applying salicylic acid on growth and leaf nutrient content of Picual olive seedlings exposed to saline stress grown under shade house condition. Seedlings subjected during two growth seasons (2019-2020) to salicylic acid (SA) which applied as soil application at three concentrations (0, 200, and 400 ppm) on olive seedlings cv. Picual grown under three levels of salinity (tap water, 2000, 4000 ppm). At the end of each growth season different vegetative growth parameters (plant height increment%, leaves no., shoots no., leaf dry matter % and leaves moist %) were determined. Moreover, leaf nutrient status were been estimated. Obtained results indicated that, vegetative growth parameters of olive seedlings cv. Picual show a negative response when irrigated with saline water up to 2000 ppm and increasing salinity in irrigation water from 2000 to 4000 ppm tended to significantly decrease vegetative growth parameters. Where, exogenous application of salicylic acid with 200 ppm (soil application) on olive seedlings improved most vegetative studied parameters. Also, current study concluded that, exogenous application of salicylic acid on saline stressed Picual olive seedling increased nitrogen, phosphor and potassium content in leaves than untreated once.

Keywords: saline water, salicylic acid, antioxidants, soil application, seedlings, growth performance.

1. Introduction

Olive tree (*Olea europaea* L.) trees is considered as middling salt tolerant and is usually cultivated in dry lands in semi-arid regions with Mediterranean climate, where infrequent and scarce rainfalls lead up to reduction in olive yield. For this reason, using new irrigation programs in olive farms rising fruit yields and income (Orgaz and Fereres, 2004). Increased salinity in agricultural soils around the world considered one of the most destructive stresses and is significantly reduced the area of cultivated land, and fruit quality and quality. World agriculture is under earnest menace because of the growing in human population, and decrease in the land suitable for agriculture (Shahbaz and Ashraf 2013). Also, abiotic stresses as a main factor contributing in decrease productivity by more than 50%. Salinity stress, considered one of the main factors contributing in rapidly increasing reductions in fruit quantity and quality (Shahbaz and Ashraf 2013).

Remarkably, increased salinity in agricultural soils around the world at rate of 10% annually has reached its climax into more than 50% of the arable land be salinized by the year 2050 (Jamil *et al.,* 2011). Salinity influences every aspect of development and growth of plants. Salinity stress effects the antioxidants-mediated metabolism and reactive oxygen species (ROS) in plants (Choudhury *et al.,* 2013; Anjum *et al.,* 2015; Sehar *et al.,* 2019; Jahan *et al.,* 2020). The capability of the plant to tolerate salinity is decided by multiple physiological and biochemical mechanisms, in particular by controlling the readjusting the cell redox state and generation of reactive oxygen species (ROS) readjusting the cell redox state (Gong *et al.,* 2013). Reactive oxygen species negatively affect biological structures, protein and amino acid oxidation, lipid peroxidation and provoking DNA damage (Asada 1999). The reactive oxygen species produced when plants subjected to stress conditions should be excluded and, for this objective, plants have mechanisms to detoxify reactive oxygen species. These can be categorized as

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non-enzymatic or enzymatic. Additionally, phytohormones also are related to detoxify reactive oxygen generation/detoxification processes.

Plant hormones are structurally diverse compounds involved in multiple processes. Consequently, phytohormones have a pivotal function in mediating plant response to abiotic stress. (Fahad *et al.,* 2015). Salicylic acid (SA) considered secondary metabolite which produced in plants. Salicylic acid (SA) acts as plant growth regulator with various roles in plant metabolism (Eraslan *et al.,* 2007; Singh and Gautam 2013).

Salicylic acid (SA) has important organizational functions in plants grow under stress conditions (Dong *et al.,* 2011; Kim *et al.,* 2018; Nie *et al.,* 2018; Wang, *et al.,* 2013). Salicylic acid (SA)has been widely mentioned to preserve plants against abiotic stresses through organizing important physiological processes in plants including nitrogen metabolism, glycine betaine production, photosynthesis, proline metabolism, control plant–water relations and antioxidant defense system (Khan *et al.,* 2015). Salicylic acid (SA) acts as a signaling molecule that induces resistance in stress tolerance of plants (Horváth *et al.,* 2015; Jayakannan *et al.*, 2015; Szepesi, *et al.,* 2009; Wang, *et al.,* 2013). Salicylic acid induced enhancement in the N and S assimilation hence inducing salinity tolerance in plants were reported to involve Salicylic acid increase regulation mediated the activity of non-enzymatic and enzymatic antioxidant systems (Nazar *et al.,* 2011).

Usage of Salicylic acid rushing the uptake of N, K, P, Mg, Ca and efflux of Na+ ions, which increased the growth of Cucumis sativus under salt stress and protected it against salinity effects (Yildirim *et al.,* 2008). In another view, exogenously spraying with salicylic acid (0.5 mM) improved the growth, yield, and gas exchange in salt-stressed Z. mays by regulating the high ratio of K+/Na+ and Ca+/Na+ (Tufail *et al.,* 2013).

Usage strategy of salicylic acid allow plants to tolerate salt stress through modifying several processes including ion compatible, homeostasis, solute accumulation, osmotic adjustments and the regulation of cellular antioxidants. On the other hand, exogenous salicylic acid could improve the salinity-toxicity symptoms in plants by ameliorating photosynthetic capacity, enhanced antioxidant protection through modulating antioxidant defense system components, ROS metabolism, and increasing accumulation of soluble carbohydrates.

In view of all the above reports, the principal objective of the present study was to appraise whether varying levels of exogenously applied salicylic acid could alleviate the adverse effects of salt stress on growth, and leaf nutrient content in Picual olive seedlings.

2. Materials and Methods

This work was carried out in the experimental research shade net house of National Research Centre, Cairo, Egypt during 2019-2020. For this purpose, healthy one-year-old homogeneous self-rooted (40 cm) olive seedlings Picual cv. produced through mist propagation system was used. The seedlings were planted in 10 liter black polyethylene bags with 30 cm diameter fooled with washed sand mixed very well with peat moos (2:1, w/w), Olive seedlings were irrigated twice weekly. Picual olive seedlings were fertilized with recommended dose (1gm/seedling/week) of conventional fertilizers which add as crystalon (20:20:20 NPK). Salicylic acid (SA) applied as soil application at three concentrations (0, 200, and 400 ppm) on olive seedlings cv. Picual grown under three levels of salinity (Tap water, 2000, 4000 ppm) in irrigation water. Salicylic acid applied every 15 day from the beginning of March tell the end of September of growing season. Salt solution comprised from (3 NaCl: 1 (1 MgCl2: 1 CaCl2). Treatments were arranged in randomized complete block design with five replicates for each treatment and each replicate was comprised of three seedlings.

Soil application of salicylic acid treatments:
1- zero ppm (control)
2- 200 ppm
3- 400 ppm

Salinity levels:
4- Tap water (control)
5- 2000 ppm
6- 4000 ppm

At the end of September, plants of each treatment were removed genteelly with their root system to estimate and record the following data:

2.1. Vegetative growth parameters:

1. Seedling height increment % which was calculated monthly after month of each spray and at the end of experiment (at the end of September).
2. Shoots number per seedling.
3. Leaves number per seedling.
4. Leaves dry matter %.
5. Leaves moist %.

2.2. Leaves mineral content:

Nitrogen (N) and phosphorus (P) in leaves were calorimetrically determined according to the methods described by Bremner *et al*. (1982), and Olsen, *et al*. (1982), respectively. Potassium (K) was determined flame photometrically according to the method advocated by Jackson, *et al*. (1970).

2.3. Data Analysis

The average data of the two seasons (2019 - 2020) were subjected to analysis of variance and the method of Duncan's was used to differentiate means (Duncan, 1955).

3. Results

3.1. Effect of soil application of Salicylic acid on vegetative growth parameters of Picual olive seedlings irrigated with saline water:

3.1.1. Seedling height increment percentage

Data in Table (1) represented that interaction between treatments of salicylic acid and salinity levels was significant. Furthermore, the highest Picual olive seedlings were obtained without 200 using salicylic acid treatment and 2000 ppm salinity levels (Table 1). Control treatment (0ppm salicylic acid) gave the highest seedling across all salinity levels. The highest significant value of plant height was obtained with Picual olive seedlings irrigated with 2000 ppm saline water across all salicylic acid treatments. On the other hand, using salicylic acid 200 and 400 ppm as soil application gave the lowest plants height compared with control across all salinity treatments.

Table 1: Effect of Salicylic acid and salinity level and their interaction on seedling height increment percentage of Picual olive seedlings.

Salicylic acid / Salinity	0 ppm	200 ppm	400 ppm	Mean
Without salinity	74.33 bc	75.67 bc	64.33 de	71.44 B
2000 ppm	84.33 a	73.00 bc	71.67 bcd	76.33 A
4000 ppm	68.67 cd	59.00 e	77.67 ab	68.44 B
Mean	75.78 A'	69.22 B'	71.22AB	

Means having the same letters within a column are not significantly different at 5% level.

3.1.2. Leaves number per seedling

No. of leaves per seedling varied according to salicylic acid treatments as well as salinity levels. Table (2) indicates that interaction between salicylic acid treatments and salinity levels was significant. Furthermore, The use of salicylic acid at a concentration of 200 ppm increases the number of leaves of olive seedlings that were not exposed to salt stress (control) with a significant increase, while the seedlings irrigated with 2000 ppm saline water using 400 ppm salicylic acid as soil application gave the best results as it gave a significant increase in the number of leaves. Whereas, seedlings treated with 200 ppm salicylic acid as soil application gave the highest average of number of leaves per seedling across all salinity treatments. On the other hand, Irrigation Picual olive seedlings with tap water (control) or using saline water (2000 and 4000 ppm) not significantly influence number of leaves per seedling across all salicylic acid treatments.

Table 2: Effect of salicylic acid and salinity level and their interaction on leaves number per seedling of Picual olive seedlings.

Salicylic acid / Salinity	0 ppm	200 ppm	400 ppm	Mean
Without salinity	253.00 c	350.00 a	291.67 b	298.22 A
2000 ppm	261.67 bc	271.00 bc	354.00 a	295.56 A
4000 ppm	258.00 bc	262.00 bc	174.67 d	231.67 A
Mean	257.00 B'	294.33 A'	273.44A	

Means having the same letters within a column are not significantly different at 5% level.

3.1.3. Shoots number per seedling

Table (3) revealed that interaction between salicylic acid treatments and salinity levels was significant. Furthermore, the use of salicylic acid at a concentration of 400 ppm increases the number of leaves of olive seedlings irrigated with 2000 ppm saline water .The best value of number of shoots per Picual olive seedling was obtained in Picual olive seedling treated with salicylic acid at 400 ppm concentration across all salinity levels (Table 3). Picual olive seedling irrigated with 2000 ppm saline water recorded the maximum number of shoots per seedling compared to the other salinity level across salicylic acid treatment.

Table 3: Effect of salicylic acid and salinity level and their interaction on number of shoots per seedling of Picual olive seedlings.

Salicylic acid / Salinity	0 ppm	200 ppm	400 ppm	Mean
Without salinity	6.0 d	7.3 cd	11.0 b	8.11 B
2000 ppm	8.0 cd	6.7 cd	14.3 a	9.67 A
4000 ppm	8.3 c	5.3 e	8.0 cd	7.22 B
Mean	7.44 B'	6.44 C'	11.11A	

Means having the same letters within a column are not significantly different at 5% level.

3.1.4. Leaves dry matter percentage

Table (4) shows interaction between salicylic acid treatments and salinity levels showed significant differences. While, the highest significant value obtained from seedlings not subjected to saline condition and treated with 400 ppm salicylic acid as soil application. Whereas, tap water gave the maximum leaves dry weight % compared with other salinity level across all salicylic acid treatments. On the other hand, soil application of salicylic acid with 400 ppm accepts the highest average leaves dry weight percentage across all salinity levels.

Table 4: Effect of salicylic acid and salinity level and their interaction on leaves dry matter percentage of Picual olive seedlings.

Spraying Salicylic acid / Salinity	0 ppm	200 ppm	400 ppm	Mean
Without salinity	64.17 e	71.07 b	74.75 a	70.00A
2000 ppm	64.31 e	65.60 d	67.75 c	65.89 B
4000 ppm	62.75 f	62.74 f	61.37 g	62.29 C
Mean	63.74 C'	66.47 B'	67.96 A	

Means having the same letters within a column are not significantly different at 5% level.

3.1.5. Leaves moisture percentage

Table (5) represented that, there are a significant differences in interaction between salicylic acid treatments and salinity levels. While, the highest significant value obtained from seedlings irrigated with 4000 ppm saline water and treated with 400 ppm salicylic acid as soil application. Moreover, percent of leaves moisture at 4000 ppm salinity level was significantly higher than other salinity levels across all salicylic acid treatments. On the other hand, leaves moisture percentage of Picual olive seedling not treated with salicylic acid accept the highest average leaves dry weight percentage across all salinity levels.

Table 5: Effect of salicylic acid and salinity level and their interaction on leaves moisture percentage of Picual olive seedlings.

Salicylic acid / Salinity	0 ppm	200 ppm	400 ppm	Mean
Without salinity	35.83 c	28.93 f	25.25 g	30.00 C
2000 ppm	35.69 c	34.40 d	32.25 e	34.11 B
4000 ppm	37.25 b	37.26 b	38.63 a	37.71 A
Mean	36.26 A'	33.53 B'	32.04 B'	

Means having the same letters within a column are not significantly different at 5% level.

3.1.6. Leaf nitrogen percent

Data in Table (6) illustrate that the leaf nitrogen percentage of Picual olive seedling drenched with 200 ppm salicylic acid significantly achieved the highest average leaf nitrogen content across all salinity levels. While, seedlings not subjected to saline condition (irrigation with tap water) significantly gave the highest leaf nitrogen percentage across all salicylic acid treatments. Referring to the interaction between salicylic acid and salinity there were significant differences. Results in Table (6) illustrated that, the highest leaf nitrogen content values were obtained with Picual olive seedling not subjected to saline stress (irrigated with tap water) treated with 200 ppm salicylic acid as soil application.

Table 6: Effect of salicylic acid and salinity level and their interaction on leaf nitrogen percent of Picual olive seedlings.

Salicylic acid / Salinity	0 ppm	200 ppm	400 ppm	Mean
Without salinity	1.68 f	2.35 a	2.13 b	2.05 A
2000 ppm	1.80 ef	2.04 bc	1.83 e	1.89 B
4000 ppm	1.85 de	1.88 de	1.96 cd	1.90 B
Mean	1.78 C'	2.09 A'	1.97 B'	

Means having the same letters within a column are not significantly different at 5% level.

3.1.7. Leaf phosphorus percent

Table (7) demonstrated that the effect of different treatments of salicylic acid on leaf phosphorus percentage of Picual olive seedling grow under the three salinity levels was not significant. While, the highest value were obtained with Picual olive seedlings irrigated with 2000 ppm saline water and treated with 400 ppm salicylic acid as soil application. Also data showed that the use of salicylic acid with its different concentrations (200 and 400 ppm) led to an improvement in leaves phosphorous content, and there were no significant differences between the concentrations of the salicylic acid used were detected across all salinity levels. In addition, high leaf phosphorus percentage detected in Picual olive seedling irrigated with 2000 and 4000 ppm saline water with insignificant differences recorded between them across all salicylic acid treatments.

Table 7: Effect of Salicylic acid and salinity level and their interaction on leaf phosphorus percent of Picual olive seedlings.

Salicylic acid / Salinity	0 ppm	200 ppm	400 ppm	Mean
Without salinity	0.033 a	0.049 a	0.042 a	0.041 B
2000 ppm	0.037 a	0.048 a	0.055 a	0.047 A
4000 ppm	0.040 a	0.046 a	0.049 a	0.044 AB
Mean	0.036 B'	0.048 A'	0.49	

Means having the same letters within a column are not significantly different at 5% level.

3.1.8. Leaf potassium percent

Data in Table (8) recorded that Picual olive seedlings drenched with salicylic acid (soil application 200 ppm) achieved the highest leaf potassium percentage across all salinity levels. Whereas Picual olive seedlings not subjected to saline stress (irrigated with tap water) significantly achieved the highest leaf potassium content significantly compared with the other salinity levels (2000 and 4000 ppm) across all salicylic acid treatments. On the other hand, no significances were detected in the interaction between salicylic acid treatments and salinity levels. While, the highest values of leaf potassium content were detected in leaves of Picual olive seedlings not subjected to saline stress (irrigated with tap water) treated with 400 ppm salicylic acid as soil application.

Table 8: Effect of salicylic acid and salinity level and their interaction on leaf potassium percent of Picual olive seedlings.

Salinity / Salicylic acid	0 ppm	200 ppm	400 ppm	Mean
Without salinity	1.11 a	1.24 a	1.31 a	1.22 A
2000 ppm	0.98 a	1.28 a	0.92 a	1.06 B
4000 ppm	0.90 a	0.98 a	1.03 a	0.97 C
Mean	0.99 C'	1.17 A'	1.08 B'	

Means having the same letters within a column are not significantly different at 5% level.

4. Discussion

The present results regarding the effect of foliar application with salicylic acid on vegetative growth as well as leaf mineral content are in line with those of Abd-Alhamid *et al.,* (2019), who found that the increases in number of leaves refer to salicylic acid foliar spraying at a concentration of 300 ppm on Picual olive trees. Furthermore, Abdel Aziz *et al.,* (2017) showed that the highest values of number of new shoots, shoot length, number of leaves/shoot and leaf content of N, P and K were observed on pomegranate trees that received foliar spraying salicylic acid at 200 ppm thrice at growth start. Shakirova *et al.,* (2003) showed that exogenous application of salicylic acid prevented lowering of IAA (Indole Acetic Acid) and cytokinin levels in salinity-stressed plants resulting in improved cell division in root apical meristem, thus increasing growth of plants, these authors also stated that pre-treatment with salicylic acid resulted in ABA (Indole Butyric Acid) accumulation which may have contributed In pre-adaptation of seedlings to salinity stress whereby indole butyric acid stimulates the synthesis of a wide range of anti-stress proteins, thus providing protection to plants. Moreover, the treatment also reduced the level of active oxygen species and thus SOD and POX enzymes activities were also reduced in the roots of young wheat seedlings. These results indicate that the activities of these antioxidant enzymes are indirectly or directly regulated by SA, thus providing protection against salinity-stress (Sakhabutdinova *et al.,* 2004).

Loss of vegetative growth, enzyme activities (nitrate reductase and carbonic anhydrase) and photosynthetic parameters as a result of salinity stress were revived in *Brassica juncea* when salicylic acid was foliar spraying at the 30-day stage. Moreover, the activities of several antioxidant enzymes (POX, CAT and SOD) were increased with a concomitant increase in proline content as a result of exposure to saline or /and salicylic acid treatments, This increases the tolerance of plants to salinity (Yusuf *et al.,* 2008). In addition, these results are in harmony with those obtained by Ahmed and Atheer (2021) on olive trees, they showed that, spraying olive trees with salicylic acid at 200 ppm was very effective in improving average number and length of new branches, number of leaves, dray matter in leaves% as well as enhanced leaf content of nitrogen. Also, the same results are in harmony with those obtained by Jumaa and Zain aldeen (2014) on Ziziphus plant who found that percentage of dry matter in the leaves. However, the branches content of the carbohydrates were positively affected by foliar spraying with salicylic acid at 200 ppm. Moreover, Qaiser *et al.,* (2010) explained that the foliar application of salicylic acid to plants improved the efficiency of the photosynthesis process which reflected in increased vegetative growth parameters of crops, as well as mitigated the harmful effects of environmental stresses such as high temperature, salinity and drought. In addition, the foliar spraying by salicylic acid increased the activity of antioxidant enzymes and physiological and biological properties of plants.

4. Conclusion

Vegetative growth parameters of olive seedlings cv. Picual show a negative response when irrigated with saline water up to 2000 ppm and increasing salinity in irrigation water from 2000 to 4000 ppm tended to significantly decrease vegetative growth parameters. Where, exogenous application of salicylic acid with 200 ppm (soil application) on olive seedlings improved most vegetative studied parameters. In addition, current study concluded that, exogenous application of salicylic acid on saline stressed Picual olive seedling increased nitrogen, phosphor and potassium content in leaves than untreated once.

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