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SALINITY STRESS EFFECTS ON THREE DIFFERENT OLIVE CULTIVARS AND THE POSSIBILITY OF THEIR CULTIVATION IN RECLAIMED LANDS

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Abstract

The use of underground saline water for irrigation became essential in agricultural lands of many countries .Twelve-month-old olive young trees (Olea europaea L. cv Picual, Manzanillo and Aggizi Shami) were exposed to different salinity levels (0, 2000, 3000 and 4000 mg L-1) for 275 days in order to understand the differences among those cultivars, regarding salinity resistance. In specific, the main aim was to select the suitable cultivar for growing in the reclaimed lands in Egypt, which are suffering from poor irrigation sources, forcing the growers to use underground saline water. At the end of this experiment the effects of different salinity concentrations (0, 2000, 3000, 4000 mg L-1), on growth, physiological and biochemical parameters were determined. The obtained results assumed that, the tolerance to salinity stress was as follows: Picual > Manzanillo > Aggizi Shami. The previous order of salt stress tolerance was proven by a lower decrement in vegetative growth parameters (seedlings height, number of leaves and leaf area), relative water content, total chlorophyll content, and greater increment of proline, soluble carbohydrates, and electrolyte leakage. Also the capacity of the elimination procedure of Na+ and Cl_ in the root system.

Keywords: Olea europaea; salt stress; reclaimed land; plant growth

Introduction

Salinity of water is one of the main abiotic stresses that reduce plant improvement and crop production in the world. Salinity stress in most cases decreases vegetative growth and biochemical constitution [Bose and Shabala, 2015; Pandolfi et al., 2017; Hassan I. F et al., 2019]. In the Mediterranean region, especially in the arid regions where olive is mostly grown, there is an increasing request for the water of excellent quality. Hence, the availability of water for irrigation is declining in these regions [Chartzoulakis, 2005]. To supplement the lack of water and satisfy the increasing need for agricultural growth, the use of underground saline water for irrigation is becoming essential in many countries. Very little work is available on the tolerance of Picual and Manzanello under the Egyptian conditions and no work has been conducted on the tolerance of Egyptian olive cultivar Aggizi Shami to salinity stress [Chartzoulakis, 2005; Chartzoulakis et al ., 2002 ; Perica et al., 2008]. In this situation, it is vital to choose suitable cultivars, which will grow well when planted in the newly reclaimed lands irrigated with underground saline water. Most of the Egyptian olive farmers use local varieties of olive, with very few them growing the newly imported varieties of olive, without recognizing their suitability to the Egyptian conditions in general and the high salinity in particular. Hence, we selected three different cultivars namely Picual, Manzanillo and Egyptian Aggizi Shami to compare and select the most suitable cultivar for growing in our reclaimed lands at El Sadat City, Egypt. In most of the cases, water used for irrigation at the reclaimed lands in El Sadat city is saline groundwater. Furthermore, the cultivars grown at these lands are generally imported cultivars. Picual and Manzanillo. Salinity resistance in olive, as in other species, is cultivar-dependent [Chartzoulakis et al., 2002; Perica et al., 2008], there are few studies that compare olive cultivars of different origins to assess the differences among them [Perica et al., 2008]. Olive trees under high salinity conditions are subject to physiological and biochemical alterations, for example, a marked decrease in photosynthesis rate that leads to a reduction in plant growth (mainly shoot growth). Salinity can straightly affect the absorption of nutrient ions absorption, sodium and chloride reduce potassium and nitrate absorption, respectively [Ben Ahmed et al., 2009; Regni et al., 2019; Mousavi et al., 2019]. This study was conducted to explore salinity tolerance of Picual, Manzanillo against the local variety Aggizi Shami to determine which variety is well adapted to grow under saline irrigation conditions of the Egyptian reclaimed lands.

Material and Method

Plant material, growing conditions and salt treatments

The experiment was conducted from 15th February to 15th November 2019 (275 days) on three different olive cultivars (Picual, Manzanillo and Aggizi Shami). One-year-old own-rooted young seedlings similar in trunk diameter and height were selected, and transferred to grow in a slightly shaded and unheated greenhouse located in a private farm at El Sadat City, Egypt. The young seedlings were cultivated in pots of 2 kg capacity in a mixture of sand: clay in 2:1 ratio, each pot had one plant. The young seedlings were kept one month for adaptation to the new growing conditions before starting the experiment. Young olive seedlings were irrigated two times per week by different concentrations (control, freshwater, 2000, 3000 and 4000 mg L-1) of saline solution as shown in table 1, consisted of a mixture of salts according to [Stroganov, 1962] and one time by freshwater to avoid osmotic shock. Every pot watered until the volumetric moisture content which correspond to the field capacity the voultmtric. All the agricultural fertilization programs for olive seedlings, recommended by the Egyptian ministry of agriculture were used.

Table 1. The content of salt solution used for salinization expressed as % of total salt content.

$MgSO_4$	$CaSO_4$	NaCl	$MgCl_2$	$CaCO_3$
%	%	%	%	%
10	1	78	2	9

Plant height, leaf number and leaf area

At the end of the experiment the length of the principal axis (cm) was measured as well as the number of leaves per plant. In addition, leaf area (cm2) was determined by the equation described by [Singh and Snyder, 1984].

Total chlorophyll, proline, soluble carbohydrate, Na+ and Cl- contents

At the end of the experiment, the leaf total chlorophyll content was measured according to [Saini, 2001], by extracting in 85% acetone solution and measuring their absorbance by using a Spectrophotometer at λ= 663 nm and 645 nm. The amount of total chlorophyll was calculated using the following equation:

Total chlorophyll (mg /g fw) = [(20.2×OD 645 nm + 8.02×OD 663 nm) ×V] /(fw ×1000)   (1)

OD is optical density, V is the final solution volume in mL and fw is tissue fresh weight in mg. V is the final solution volume in mL and fw is tissue fresh weight in mg.

Leaf proline was extracted from the 0.5 g samples of young leaves by 3% sulfuric acid and determined by using the ninhydrin reagent, according to the method described by [Bates et al., 1973]. The absorbance of the solution with toluene was determined at 520 nm, using a spectrophotometer (Model UV-120-20, Japan)

The soluble carbohydrate concentration was determined in dried leaf samples according to [Buysse and Merckx, 1993], by extracting 150 mg of dried leaf samples twice with 80% ethanol. The sample was centrifuged at 3500 rpm for 10 min and the volume of the supernatant was adjusted to 25 ml. Then 1ml of supernatant was transferred to a test tube and 1 ml phenol (18%) and 5 ml sulfuric acid were added. The mixture was shaken immediately and its absorption was recorded at 490 nm using a spectrophotometer (Model UV-120-20, Japan).

The shoots and roots were collected at the end of the experiment in order to determine the sodium and chloride percentages according to the method described in [Chapman and Pratt,1961].

Leaf relative water content and electrolyte leakage

The leaf relative water content was estimated according to the method described by [Saini, 2001], performed by incubating 0.2 g of fresh leaf sample in 50 mL of distilled water for 4 h. Then the turgid weights of leaf samples were measured. The leaf samples were oven-dried at 60°C for 48h for dry weight determination. The RWC was calculated using the following equation,

RWC (%) = [(FW-DW) / (TW-DW)] x 100   (2)

Where FW, DW, and TW are fresh, dry and turgid weights.

For the electrolyte leakage ten discs of fresh expanded leaves (0.5 cm diameter) were cut from the fully expanded leaves according to the method of [Saini, 2001]. The leaf discs were washed three times with deionized water to remove dust and put in closed tubes containing 10 ml of deionized water and shake for 30 minutes and left in dark at room temperature for 24 h. The initial electrical conductivity of the solution (EC1) was determined using an electrical conductivity meter. The samples were put in a water bath at 80°C for 20 min to release all the endogenous electrolytes and cooled to 25 °C,. Finally, their electrical conductivity (EC2) was measured.

EL = (EC1/EC2) × 100 (%).   (3)

The solution discharge (EL) was calculated per the subsequent equation:

Experimental design and statistical analysis

The experimental design was split plot design including three cultivars and four salinity concentrations and six replicates. The obtained data were subjected to variance analysis according to [Clarke, and Kempson, 1997]. The means were differentiated using the Rang test at the 0.05 level [Duncan, 1995].

Results

Plant height, leaf number and area, leaf total chlorophyll content

The data presented in Table 3 indicated that, all salinity treatments had a harmful effect on the cultivars vegetative growth (plant length, leaf area, and number of leaves). The effect of salinity on theses measurements showed significant genotypic differences associated with higher concentration of salinity from 0 to 4000 mg L-1 compared with the control plants. The Picual cultivar showed higher values of plant height, number of leaves and leave area compared to Manzanillo and Aggizi Shami as follows. The differences between cultivars were significant.

Table 2. Effect of saline water concentrations on seedlings length (cm), Leaf area (cm) and number of leaves of the three olive cultivars

Characters	Seedlings length (cm^2)				Leaf area (cm^2)				Number of leaves			
Treatments	Cultivars				Cultivars				Cultivars			
Salinity (B)	Picual	Manzanillo	Aggizi Shami	Mean	Picual	Manzanillo	Aggizi Shami	Mean	Picual	Manzanillo	Aggizi Shami	Mean
0 mg L^{-1}	84.33a	80.00bc	76.67d	80.33A	4.10b	4.40a	4.03b-d	4.18A	73.67a	72.00b	71.33bc	72.33A
2000 mg L^{-1}	82.00ab	75.33de	70.67f	76.00B	3.97b-e	4.07bc	3.93c-e	3.99B	70.67c	65.00e	64.00ef	66.56B
3000 mg L^{-1}	79.33c	73.67e	66.33g	73.11C	3.90de	3.90de	3.80e	3.87C	66.67d	62.33g	62.00g	63.67C
4000 mg L^{-1}	75.00c	67.00g	60.00h	67.33D	3.87de	3.80e	3.60f	3.76D	63.67f	60.33h	56.33i	60.11D
Mean	80.17A	74.00B	68.42C		2085B	4.04A	3.84C		68.67A	64.92B	63.42C	

Means marked by the same letter are not significantly different at p = 5% level, using Duncan’s test.

Table 3. Effect of saline water concentrations on total chlorophyll mg g-1 fw , proline µg g-1fw and Soluble sugars mg 100g-1 dw content

	Total Chlorophyll mg g^{-1} fw				Proline µg g^{-1} fw				Soluble sugars mg $100g^{-1}$ dw			
Treatments	**Cultivars**				**Cultivars**				**Cultivars**			
Salinity (B)	**Picual**	**Manzanillo**	**Aggizi Shami**	**Mean**	**Picual**	**Manzanillo**	**Aggizi Shami**	**Mean**	**Picual**	**Manzanillo**	**Aggizi Shami**	**Mean**
0 mg L^{-1}	2.45a	2.39a	2.21b	2.35A	24.00d	22.00e	25.33d	23.78D	26.67g	32.00f	31.00f	29.89D
2000 mg L^{-1}	2.19bc	2.03d	2.08cd	2.10B	27.33c	25.00d	27.33c	26.56C	25.67g	36.00de	37.00cd	32.89C
3000 mg L^{-1}	1.98de	2.01de	1.82f	1.94C	31.67b	28.67c	34.33a	31.56B	35.00e	39.67b	38.00c	37.56B
4000 mg L^{-1}	1.89ef	1.80f	1.80f	1.83D	31.00b	32.67b	35.00a	32.89A	38.33bc	39.67b	43.00a	40.33A
Mean	2.13A	2.06B	1.98C		28.50B	27.08C	30.50A		31.42B	36.83A	37.25A	

Means marked by the same letter are not significantly different at p = 5% level, using Duncan's test.

Table 4. Effects of saline water concentrations on relative water content and electrolyte leakage.

Characters	**Relative water content %**				**Electrolyte leakage %**			
Treatments	**Cultivars**				**Cultivars**			
Salinity (B)	**Picual**	**Manzanillo**	**Aggizi Shami**	**Mean**	**Picual**	**Manzanillo**	**Aggizi Shami**	**Mean**
0 mg L^{-1}	91.00a	84.67b	83.67b	86.44A	10.33j	17.00i	16.33i	14.56D
2000 mg L^{-1}	81.67c	80.00d	81.33c	81.00B	19.67h	23.67f	21.33g	21.56C
3000 mg L^{-1}	80.67cd	75.00f	71.00h	75.56C	21.00g	25.00e	35.00b	27.00B
4000 mg L^{-1}	78.67e	73.33g	69.00i	73.67D	31.67c	26.67d	36.67a	31.67A
Mean	83.00A	78.25B	76.25C		20.67C	23.08B	27.33A	

Means marked by the same letter are not significantly different at p = 5% level, using Duncan's test.

Total chlorophyll, proline and soluble carbohydrate contents

The obtained results presented in Table 4, demonstrated that all the biochemical constitutes of the tested olive cultivars were significantly affected by the increment of water salinity concentrations. The total chlorophyll decreased but soluble sugars and proline contents increased by increasing salinity concentrations compared to control seedlings. The Picual cultivar recorded higher values of total chlorophyll content than Manzanillo and Aggizi Shami, while Aggizi Shami recorded the higher values of proline content and soluble sugars followed by the Picual and Manzanillo. Regarding, the interaction between salinity and cultivars, Picual cultivar recorded higher total chlorophyll content when irrigated with 2000 mg L-1 and lower proline and soluble sugars contents, while the Aggizi Shami cultivar recorded higher proline and soluble sugars when irrigated with 4000 mg L-1 and lower total chlorophyll content than control plants .

Water status: leaf relative water content (%) and electrolyte leakage (%)

The results in Table 5 demonstrated that increasing salinity concentrations gradually decreased, the leaf relative water content in all cultivars compared with the control, while the electrolyte leakage increased by increasing salinity concentrations from 0 to 4000 mg L-1. Leaf water content showed high significant differences among the studied cultivars. Picual cultivar showed higher relative water content (83.00%), while Aggizi Shami (76.25%) showed the lower relative water content Manzanillo showed an intermediate value (78.25%) of relative water content.Ectrolyte leakage percentage varied among the cultivars; the results showed that higher value of electrolyte leakage percentage was recorded by Aggizi Shami (27.33%) and the lower value was recorded by Picual cultivars (20.67%). Concerning the interaction between salinity and cultivars, Picual cultivar recorded higher relative water content when irrigated with 2000 mg L-1 and lower electrolyte leakage percentage, while Aggizi Shami cultivar recorded higher electrolyte leakage percentage when irrigated by 4000 mg L-1 and lower relative water content. Manzanillo ranked in between.

Na+, Cl- and contents in both roots and leaves

The data presented in Figures 1 and 2 illustrated that, both sodium and chloride concentrations in the different seedlings parts (roots and leaves) increased gradually by the increase in salinity concentrations from 0 to 4000 mg L-1. A significant variation was recorded between the leaves and roots in sodium content compared with the control seedlings. Picual cultivar roots accumulated higher sodium content than leaves while Aggizi Shami leaves recorded a higher Na+ content than roots. As for chloride, concentrations both (in leaves and in roots) increased gradually with salinity concentration ranging within 0 to 4000 ppm. Chloride concentrations were higher in roots than in leaves compared with the control seedlings. A remarkable variation was noticed among cultivars, Picual cultivar accumulated higher chloride content in roots , while Aggizi Shami cultivar accumulated lower chloride content and Manzanillo cultivar ranked in between.

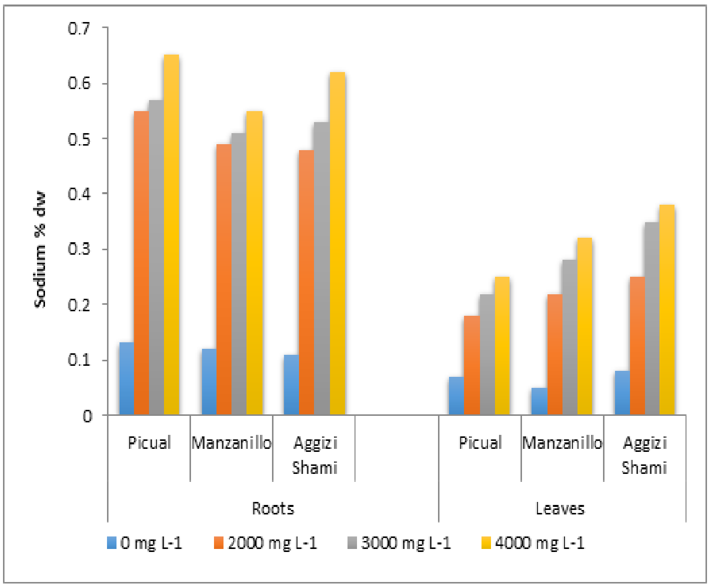

Figure 1. Effects of saline water concentrations on sodium percentage content in both roots and leaves.

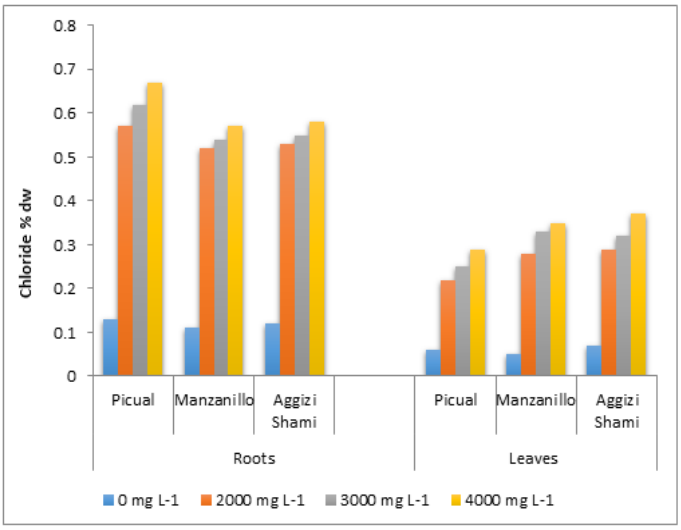

Figure 2. Effects of saline water concentrations on chloride percentage content in both roots and leaves.

Discussion

The results of present research showed a reduction in the vegetative growth parameters namely young tree height, leaf area and number of leaves. In all the three studied cultivars by the increasing of salinity concentrations from 2000 mg L-1 to 4000 mg, L-1 compared to the control plants. The vegetative growth reduction and onset of destruction are further correlated with the Na+ accumulation in leaves that leads to salinity toxicity causing leaf drop [Therios and Misopolinos,1988; Tattini et al., 1992]. Many previous studies reported that shoot growth is more sensitive to salt stress than root growth [Moran et al ., 1994 ;Tattini et al ., 1995; Chartzoulakis et al., 2002]. When using these vegetative growth parameters as the indicators of resistance, Picual can be considered as the most tolerant cultivar to salinity, followed by Manzanillo and Aggizi Shami**.**

A remarkable salt tolerance mechanism was found in Picual cultivar, maybe being associated with the salt accumulation process at the root level, which reduced Na+ accumulation in leaf tissues described by [Therios and Misopolinos,1988; Chartzoulakis]. Indeed, Na+ and Cl- ions concentrations in Picual leaves were lower than the other cultivars while their concentration in the root was higher than the other cultivars. In line with our data, [Chartzoulakis et al., 2002] revealed that salinity resistance in olive cultivars interacted with the efficient performances of ion insularity and limited sodium and chloride concentrations in the roots. Our data proved the occurrence of vital differences between the studied cultivars concerning the expansion and transport of sodium and chloride in roots and leaves**.**

In our experiment, the total chlorophyll content decreased among all the studied cultivars by the increase of salinity concentrations, which may be attributed to various reasons such as the inhibition of chlorophyll biosynthesis by the activation of chlorophyllase deterioration, resulted from salinity-mediated chlorophyll degradation. [Younis et al., 2000; Yasar et al., 2008; Gill and Tuteja, 2010]. Also, the salinity increases ROS which causes the osmotic and hormonal imbalances in plant cells [Gill and Tuteja, 2010; Saha et al., 2010, Din et al., 2011 ; Arjenaki et al., 2012, Feller and Vaseva, 2014]. The accumulation of osmotic protectants plays a vital role in maintaining the intracellular stability and saving cells from the harmful effects of salt and toxicity**.**

The proline and soluble sugars concentrations in the three tested cultivars under salinity conditions varied, higher in Manzanillo and Aggizi Shami and less in Picual compare with control trees. In saline-stressed trees, proline and soluble sugars increments were statistically significant between the three cultivars, these results in harmony with [Parida and Das, 2005; Munns and Tester, 2008 Hayat et al., 2012; Regni et al., 2019]. In this regard, it must be noted that they correlate the trend affecting proline and soluble sugars concentrations with the ability to exclude Na+ and Cl from roots to leaves between the different cultivars. The increase in osmolytes as proline or soluble sugar derived from the metabolisms of protein and carbohydrates is an adaption tool of the plant to reduce the harmful effect of salinity [Parida and Das, 2005; Iqbal et al., 2014; Regni et al., 2019]. Definitely, proline increases the water retention in the cytoplasm and its higher content appears to be a specific mechanism involved by the plants to further tolerate moderate stress conditions [Parida and Das, 2005; Munns and Tester, 2008; Ben Ahmed et al ., 2009; Hayat et al., 2012]

The relative water content declined in all the cultivars exposed to salt stress and the main devaluation was noted in Egizzi Shamii cultivar. The relative water content and electrolyte leakage are important physiological parameters for measuring the water status of the plants. As found in our results the RWC decreased in all the studied cultivars by increasing the salinity from 0 to 4000 ppm, these results were in harmony with [Perica et al., 2008, Mansour et al. 2019 a,b]In general, the salinity stress affected the plant water relations, whereas RWC decrease resulted from high salinity concentrations in the external soil solution caused osmotic stress leading to dehydration and increase in electrolyte leakage values. The increase in electrolyte leakage might be because of the accumulation of ROS because of salinity in plant cells, which induced cell membrane injury [Lutts et al ., 1996 Dionisio-Sese and Tobita ;Cardi , 1998 ; Franklin and Zwiazek, 2004 ; Goreta et al ., 2007 ; Cardi et al ., 2015]The novelty of our research is the long period (275 days) of our experiment whereas most of the former studies have been conducted for short period ranged between 150 and 240 days [Chartzoulakis et al ., 2002 ; Ben Ahmed et al ., 2009; Regni et al., 2019]. However, it could be figured that the difference between the studied cultivars grown in pots may be due to the duration of salinity treatment, which was enough to show the differences under controlled conditions (275 days), to determine which variety is suitable to grow under reclaimed lands conditions. Our results showed that cv Picual olive trees are well suited to reclaimed lands in Egypt and the most tolerant among the studied cultivars to increased salinity conditions from 2000 to 4000 ppm maintaining the greater photosynthetic activity and vegetative growth. However, no toxicity effects were seen at all concentrations. The increments of proline and soluble sugars accumulation under salinity conditions indicated a direct relationship of this osmolyte with salinity tolerance of the Picual olive cultivar in performance with the dehydration prevention mechanisms such as stomata closure to reduce water loss **.**

Conclusion

In conclusion, our results cleared showed a different response to salinity by the tested cultivars. Picual was the most tolerant, showing a lower reduction of growth and ability to limit the increase of leaf Na+ concentration. The growth of 'Aggizi Shami' was highly suppressed by salinity, and together with 'Manzanillo' it showed the higher Na+ accumulation in leaves. The obtained results suggested that Picual cultivar might be the suitable cultivar to be grown in reclaimed lands irrigated by the underground saline water.

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