



Role of Proline and Silicon in Improvement of Olive Seedlings (*Olea europea* L) Growth under Effect of Irrigation Water Salinity

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Abstract: *Olea europaea* L is considered one of the trees of the Mediterranean basin, which is considered show medium tolerance for salinity and drought. The olive seedlings at the age of one year of cultivar Sourany were used to determine the effect of different levels of proline (200, 100, 0 ml L⁻¹) and silicon (K₂SiO₃) (Si 38%) in three concentrations (150, 75, 0 mg L⁻¹) on growth parameters. Seedlings sprayed with proline 200 ml L⁻¹ and silicon 150 mg L⁻¹ increased in plant height, number of leaves and chlorophyll content and reduced the effects of irrigation water salinity. The treatment of irrigation water salinity (9 ds/m) caused decrease in the vegetative growths and an increase sodium, chlorine and proline in leaves

Keywords: Olive, Sorani, Proline, Silicon, Salinity

The olive (*Olea europea* L.) is cultivated in the Mediterranean regions in dry and semi-arid lands and endures the harsh conditions of high temperatures and lack of moisture and grows in various soils with variable fertility and has medium tolerance to salinity (Regni et al 2019). The tolerance of the olive tree's salinity is mainly related to the salt-elimination mechanisms that operate at the roots and is more sensitive to Na⁺ than Cl⁻ ion in the leaves especially in high salinity and Cl⁻ ion does not cause negative effects if the concentration does not exceed 80 mmol in the cellular tissue juices, as it has been proven that adequate calcium nutrition plays a major role in these mechanisms (Tattini and Traversi 2008). Salinity causes damage to plants growing in the saline medium in two phases, the rapid osmotic stage that prevents the growth of small leaves, and the slower ionic stage that accelerates aging in mature leaves as a result of impairing the absorption of water and nutrients (AL-Taey and AL-Musawi 2019). Salinity also affects the morphological form where leaf cells and roots are reduced and the market increases the thickening of the cell walls and tends towards stiffness as a result of the accumulation of salts in the cells and the manufacture of lignin and increase the activities of manufacturing IAA, SA, ABA and ethylene in plants growing under the salinity stress. The best way to withstand the salinity of plants is to devise new cultivars that tolerate salinity. Due to the difficulty of this method, alternative techniques have been used in order to reduce salinity damage. Silicon element (Si), which is one of the most important beneficial elements and not one of the necessary elements of the plant, but play several roles in physiological processes such as increasing the effectiveness of the roots

to absorb the elements necessary for plant growth, reducing the toxicity of Na⁺ ions, increasing the ratio of, K⁺: Na⁺, improving the efficacy of photosynthesis, increasing the effectiveness of antioxidant enzymes and reducing the toxicity of heavy elements (Adrees et al 2015). In view of the exacerbation of the problem of salinity, the research aims to reduce the damages of salinity on the olive seedlings by spraying with silicon and proline and improving the growth indicators.

MATERIAL AND METHODS

The study was conducted in the lath house in AL-Furat Al-Awsat Technical University during 2019 on olive seedlings cultivar Sourany of one year. The seedlings were transferred to perforated containers filled with 18 kg of river soil and Peat moss in a ratio of 1: 3. The water was used as drainage water and diluted with tap water so that different levels of saline water were obtained (9, 6, 3 ds/m) and irrigation was repeated when 50% of the ready water was lost and the cations and anions in the irrigation water used in the study were estimated (Table 2). Then the seedlings were sprayed with proline acid (200, 100, 0 ml L⁻¹). After a day, sprayed with potassium silicate (Si 38%) (K₂SiO₃) with three concentrations (150, 75, 0 mg L⁻¹) in the early morning until complete wetness with Tween 20 with a concentration of 0.1% as wetting agent. The experiment was in factorial layout. The results were analyzed statistically using the Genstat statistical program. After the experiment ended, the measurements were taken on different parameters. The plant height was measured from the surface of the soil to the growing top and the number of leaves was calculated per

Table 1. Chemical and physical traits of the soil used in the study

K	P	N	Cl ⁻¹	Na ⁺	O. M.	EC	pH	Clay	Soil Texture	Sand	Silt
34.81 mg L ⁻¹	1.42%	4.55%	5.60	6.35	3.37%	3.01	7.12	23.5	Sandy soil	50.3	26.2

Table 2. Important chemical traits of irrigation water

Parameters (Meq l ⁻¹)	Water salinity		
EC*	3	6	9
pH	7.45	7.77	7.74
Ca ⁺²	2.00	3.11	5.89
Mg ⁺²	15.78	5.93	0.50
K ⁺	7.73	12.33	6.77
Na ⁺	10.64	56.98	75.64
Cl ⁻	11.98	31.44	48.45
CO ₃ ⁻²	1.00	1.02	1.43
HCO ₃ ⁻¹	2.10	3.23	4.89
SO ₃ ⁻²	15.44	52.44	70.00
HPO ₄ ⁼	0.29	0.13	0.13

* dsm⁻¹

seedlings. The chlorophyll content was estimated using a direct chlorine pigment severity estimator 502 - SPAD of Minolta Corporation. Proline / leaves content of proline acid (micromole g⁻¹) was estimated according to the method of Bates et al (1973) and sodium and chloride was estimated according to Jones and Steyn (1973).

RESULTS AND DISCUSSION

Seedlings height: The proline at 200 ml L⁻¹ and silicon 150 mg L⁻¹ significantly increased the height (139.41 and 136.38 cm). The salinity of the irrigation water 9 ds/m significantly decreased the height of seedlings (109.56 cm) compared to salinity 3 ds/m (131.26 cm). The interaction between proline, 200 ml L⁻¹ and silicon at 150 mg per 1 liter caused significant increase in height (166.82 cm) compared to the control treatment. The proline 200 ml L⁻¹+ silicon 75 mg L⁻¹+ salinity of 3 ds/m resulted in maximum plant height (184.67 cm) compared to the control (68.91 cm). Spraying with proline increases the osmotic pressure and increases the absorption of water and nutrients, thus increasing photosynthesis that affects cell division and elongation, and act as important because it is an energy source (Farooq et al 2012). The silicon had a positive role in increasing this trait and reducing salinity damage as a result of its contribution to increasing anti-oxidative enzymes and increasing the effectiveness of the root system and reducing transpiration in addition to increasing plant hormones and encouraging the absorption of nutrients such as Ca⁺, K⁺ and reducing concentrations of Cl⁻ ions Na⁺, increasing the K⁺ ratio: Na⁺, thereby

accelerating growth and exposure. The salinity has a harmful effect on the height of the seedlings due to the decrease in the emphysema of the cells and the physiological processes affected by the salt stress through the ionic imbalance and the obstruction of the elongation and growth of the seedlings.

Number of leaves (leaf plant⁻¹): The proline spraying the concentration of 200 ml L⁻¹ and silicon 150 mg L⁻¹ s significantly increased the number of leaves (498.89 and 488.98 leaf plant⁻¹) as compared to the control (393.08 and 404.70 leaf plant⁻¹) (Table 4). The proline and silicon cause an increase in plant height (Table 3) due to increased cell division and increased photosynthesis. The treatment 9 ds/m, resulted in significant decrease in the number of leaves (366.67 leaf plant⁻¹) as compared to salinity 3 ds/m (518.80 leaf plant⁻¹). The proline at a concentration of 200 ml L⁻¹ and silicon 75 mg L⁻¹ significantly excelled and gave 497.21 leaf plant⁻¹ compared to the control (350.15 leaf plant⁻¹). The interaction between the proline and the salinity of irrigation water (proline at 200 ml L⁻¹ + salinity 3 ds/m) gave maximum leaf plant⁻¹(588.91) while silicon 150 mg L⁻¹+ salinity 3 ds/m resulted in 566.78 leaf plant⁻¹, compared to the salinity treatments 9 ds/m and without spraying recorded a decrease in the number of leaves (319.92 and 337.78) leaf plant⁻¹ respectively. The proline 200ml L⁻¹+ silicon 150 mg L⁻¹+ salinity 3 ds/m significantly increased the number of leaves. That use of high levels of salinity of irrigation water led to the inhibition of the number of leaves because the increase in salinity in the medium of growth has negative effects on the growth, development and detection of plants and the decrease in the value of water potential, This resulted in less cell expansion, as well as the closure of stomata, and this is accompanied by a decrease in the efficiency of photosynthesis, and the presence of salts leads to an imbalance in the ionic and hormonal balance (Srivastav 2002)(Table 3).

Chlorophyll contents in leaf (SPAD unit)

Proline a concentration of 200 ml L⁻¹and silicon of a concentration of 150 mg L⁻¹ resulted in a significant increase in the leaf content of chlorophyll amounted to 114.27 and 111.96 spad units, respectively, compared to the both control treatment (92.45 and 96.46 spad). The increase in leaf content of chlorophyll is due to the fact that proline leads to increased absorption of water and nutrients and thus increase photosynthesis units by maintaining the osmotic balance within the cells and that the synthesis of this compound is important because it is a source of energy and

Table 3. Effect of proline and silicon on seedlings height at variable irrigation water salinity

Proline (mg L ⁻¹)	Silicon (mg L ⁻¹)	Irrigation water salinity (d sm ⁻¹)			Proline × silicon
		3	6	9	
0	0	98.54	81.43	68.91	82.96
	75	100.61	99.78	81.46	93.95
	150	107.67	101.98	93.78	101.14
100	0	100.87	99.78	94.67	98.44
	75	145.78	132.76	120.65	133.06
	150	162.65	150.65	151.89	155.06
200	0	101.78	97.84	95.78	98.48
	75	184.67	171.56	144.21	166.82
	150	178.77	145.43	134.65	152.95
LSD 0.05			4.81		3.44
Salinity medium		131.26	120.14	109.56	
LSD 0.05			1.72		
Proline × Salinity					Proline medium
0		102.27	94.40	81.34	92.68
100		136.40	127.73	122.40	128.84
200		155.07	138.29	124.88	139.41
LSD 0.05			3.78		2.89
Silicon × Salinity					Silicon medium
0		100.40	93.03	86.45	93.29
75		143.69	134.70	115.44	131.28
150		149.70	132.66	126.77	136.38
LSD 0.05			4.54		2.45

Table 4. Effect of spraying with proline and silicon acid in improving the number of leaves under the effect of saline irrigation water salinity (leaf plant⁻¹)

Proline (mg L ⁻¹)	Silicon (mg L ⁻¹)	Irrigation water salinity (d sm ⁻¹)			Proline × silicon
		3	6	9	
0	0	401.34	350.76	298.34	350.15
	75	450.56	410.54	310.65	390.58
	150	501.89	444.90	350.78	432.52
100	0	460.67	430.43	323.21	414.77
	75	520.43	489.67	361.93	457.34
	150	567.56	521.89	401.89	497.11
200	0	545.89	439.78	391.68	459.15
	75	589.87	490.98	410.75	497.21
	150	630.98	530.21	450.88	447.32
LSD 0.05			13.89		10.78
Salinity medium		518.80	456.55	366.67	
LSD 0.05			1.72		
Proline × Salinity					Proline medium
0		454.26	402.07	319.92	393.08
100		516.22	480.66	362.34	453.07
200		588.91	486.99	417.78	498.89
LSD 0.05			6.38		4.85
Silicon × Salinity					Silicon medium
0		469.30	406.99	337.78	404.70
75		520.29	463.73	361.12	448.38
150		566.78	499.00	401.15	488.98
LSD 0.05			9.76		6.67

get rid of the stress condition (Farooq, et al 2012). Silicon helps increase the size of chloroplasts and increase the number of grana units (Rangaraj et al 2012), while the salinity treatment 9 ds/m caused a decrease of 97.15 spad units compared to the salinity treatment 3 decimals per m² (112.26 spad units). This is due damage to the photovoltaic system under salt stress (Proietti et al 2012). The bi-interaction treatment proline 200 ml L⁻¹+ silicon 150 mg L⁻¹ significantly increased the chlorophyll (120.96 spad) compared to the control treatment (82.51 spad) while the interactions treatment proline 200 ml L⁻¹+ salinity of 3 ds/m the excelled of salinity treatment with silicon (silicon 150 mg L⁻¹ + Salinity of 3 ds/m. The interaction proline 200 ml L⁻¹ + silicon 150 mg L⁻¹ + salinity of 3 ds/m) resulted in maximum chlorophyll (126.89 units of SPAD) as compared to the treatment 9 ds/m and without spraying that recorded 67.54 SPAD units. The reduce chlorophyll content in olive leaves with higher salinity may be due to a decrease in the stomatal process in the gas exchange process due to the closure of the stomata which leads to a decrease in the chlorophyll manufacturing process or due to the toxic effect of salts in reducing the levels of chlorophyll pigment due to the increased sodium concentration (Table 5) responsible for forming the

chlorophyll molecule (Ben-Rouina et al 2006, Zhu et al 2019).

Sodium content: The treatment proline 200 ml L⁻¹ and silicon 150 mg L⁻¹ excelled in reducing the percentage of sodium in the leaves (0.24 and 0.23%) as compared to both control treatment (0.37 and 0.38 %) (Table 5). The silicon reduces absorption sodium by the roots and transferred to the vegetative system and increases cellulose, lignin and Na⁺ secretion of leaves (Tahir et al 2006). It is highly efficient in controlling osmotic pressure and promotes activity H⁺ ATPase⁻. (Soleimannejad et al 2019). The salinity treatment 9 ds/m recorded an increase of 0.35% compared to the salinity 3 ds/m. The interactions between proline and silicon, (proline 200 ml L⁻¹ + silicon 150 mg L⁻¹) significantly reduced sodium to 0.20% compared to the 0.50%, in proline 200 ml L⁻¹ + salinity of 3 ds/m and silicon 150 mg L⁻¹ + salinity 3 ds/m. The triple interaction treatment (proline 200ml L⁻¹ + silicon 150 mg L⁻¹ + salinity of 3 ds/m) decreased from the percentage of sodium in the leaves to 0.17% compared to salinity of 9 ds/m and without spray (0.58%).

Chlorine contents (%): The spraying with proline 100 ml of 1-liter and silicon of 150 mg L⁻¹, significantly excelled in chlorine contents in leaf (2.82 and 2.72%) as compared to the control treatments (3.33 and 3.25%), respectively. The 9

Table 5. Effect of spraying with proline and silicon acid in improving the leaf content of chlorophyll (spade unit) under the effect of irrigation water salinity

Proline (mg L ⁻¹)	Silicon (mg L ⁻¹)	Irrigation water salinity (d sm ⁻¹)			Proline × silicon
		3	6	9	
0	0	98.23	81.76	67.54	82.51
	75	102.34	97.45	82.23	94.01
	150	107.98	100.34	94.23	100.85
100	0	105.78	101.43	89.34	98.85
	75	112.67	109.12	105.89	109.26
	150	120.12	112.87	109.23	114.07
200	0	115.34	107.89	100.89	108.04
	75	120.98	111.34	109.12	113.81
	150	126.89	120.11	115.89	120.96
LSD 0.05			3.67		2.54
Salinity medium		112.26	104.70	97.15	
LSD 0.05			2.31		
Proline × Salinity					Proline medium
0		102.85	93.18	81.33	92.45
100		112.86	107.81	101.49	107.39
200		121.07	113.11	108.63	114.27
LSD 0.05			2.01		1.88
Silicon × Salinity					Silicon medium
0		106.45	97.02	85.92	96.46
75		112.03	105.97	99.08	105.69
150		118.33	111.11	106.45	111.96
LSD 0.05			1.79		1.93

Table 6. Effect of spraying with proline and silicon acid in the sodium content in leaves (%) under the effect of irrigation water salinity

Proline (mg L ⁻¹)	Silicon (mg L ⁻¹)	Irrigation water salinity (d sm ⁻¹)			Proline × silicon
		3	6	9	
0	0	0.44	0.49	0.58	0.50
	75	0.29	0.27	0.41	0.32
	150	0.22	0.25	0.32	0.26
100	0	0.30	0.35	0.39	0.35
	75	0.25	0.27	0.31	0.28
	150	0.21	0.24	0.28	0.24
200	0	0.24	0.28	0.33	0.28
	75	0.20	0.24	0.28	0.24
	150	0.17	0.20	0.22	0.20
LSD 0.05			0.10		0.04
Salinity medium		0.26	0.29	0.35	
LSD 0.05			0.04		
Proline × Salinity					Proline medium
0		0.32	0.34	0.44	0.37
100		0.25	0.29	0.33	0.29
200		0.20	0.24	0.28	0.24
LSD 0.05			0.09		
Silicon × Salinity					Silicon medium
0		0.33	0.37	0.43	0.38
75		0.25	0.26	0.33	0.28
150		0.20	0.23	0.27	0.23
LSD 0.05			0.09		0.04

Table 7. Effect of spraying with proline and silicon acid in the leaves content of chlorine (%) under the effect of irrigation water salinity

Proline (mg L ⁻¹)	Silicon (mg L ⁻¹)	Irrigation water salinity (d sm ⁻¹)			Proline × silicon
		3	6	9	
0	0	2.21	3.78	4.65	3.55
	75	2.01	3.56	4.34	3.30
	150	1.87	3.43	4.13	3.14
100	0	2.19	3.31	3.89	3.13
	75	2.11	3.19	3.77	3.02
	150	1.89	2.78	2.31	2.33
200	0	2.01	3.24	4.01	3.11
	75	1.91	2.82	3.78	2.84
	150	1.82	2.67	3.60	2.70
LSD 0.05			0.11		0.06
Salinity medium		2.00	3.20	3.83	
LSD 0.05			0.05		
Proline × Salinity					Proline medium
0		2.03	3.59	4.37	3.33
100		2.06	3.09	3.32	2.82
200		1.91	2.94	3.80	2.88
LSD 0.05			0.04		0.06
Silicon × Salinity					Silicon medium
0		2.14	3.44	4.18	3.25
75		2.01	3.19	3.96	3.05
150		1.86	2.96	3.35	2.72
LSD 0.05			0.04		0.06

Table 8. Effect of spraying with proline and silicon acid in leaves content of proline ($\mu\text{g/g f.wt}$) under the effect of irrigation water salinity

Proline (mg L^{-1})	Silicon (mg L^{-1})	Irrigation water salinity (d sm^{-1})			Proline \times silicon
		3	6	9	
0	0	1.39	1.41	1.55	1.45
	75	1.20	1.31	1.41	1.31
	150	1.19	1.24	1.39	1.27
100	0	1.19	1.33	1.49	1.34
	75	0.98	1.20	1.34	1.17
	150	0.88	0.71	0.52	0.70
200	0	0.91	1.01	1.29	1.07
	75	0.82	0.91	0.95	0.89
	150	0.54	0.67	0.89	0.71
LSD 0.05		0.03			0.02
Salinity medium		0.95	1.09	1.20	
LSD 0.05		0.02			
Proline \times Salinity					Proline medium
0		1.26	1.32	1.45	1.34
100		1.20	1.08	1.12	1.13
200		0.75	0.87	1.04	0.89
LSD 0.05		0.01			0.01
Silicon \times Salinity					Silicon medium
0		1.16	1.25	1.44	1.28
75		1.00	1.14	1.23	1.12
150		0.87	0.86	0.93	0.88
LSD 0.05		0.01			0.03

ds/m salinity of the irrigation water recorded an increase in the percentage of chlorine in the leaves (3.83%) compared to the control treatment of 3 ds/m (2.00%). The increase in chlorine, along with the increase in salinity levels, may be due to its increase in the medium of growth, which leads to its absorption and accumulation in the leaves. The saline conditions lead to an increase in the concentration of chlorine in the root zone, which causes a decrease in the absorption of nutrients and low permeability (Tattini et al 1995.). The bi-interactions between proline 200 ml L⁻¹ + silicon 150 mg L⁻¹ significantly excelled in reducing the percentage of chlorine (2.70%) compared to the control (2.21%). The interaction treatment between proline 200 ml L⁻¹ + salinity of 3 ds/m decreased to 1.91% while silicon 150 mg L⁻¹ + salinity of 3 ds/m to 1.86%. The triple interaction proline 200 ml L⁻¹ + silicon 150 mg L⁻¹ + salinity of 3 ds/m excelled in reducing the chlorine content to 1.82% compared to the control treatment (2.21%).

Proline content: The proline a concentration of 200 ml L⁻¹ and silicon 150 mg L⁻¹ caused a decrease in the proline content (0.89 and 0.88 $\mu\text{g/g f.wt}$ respectively), compared with the control treatment (1.34 and 1.28 $\mu\text{g/g f.wt}$ respectively). This may be due to the role of silicon in increasing enzymatic and non-enzymatic antioxidants, which

reduces the effect of damage resulting from the increase in oxygenic compounds (ROS) and then reducing the proline content as a result of its use in physiological processes (Carlos et al 2009). The 9 ds/m recorded an increase in proline (1.20 $\mu\text{g/g f.wt}$) compared to the salinity treatment 3 ds/m (0.95 $\mu\text{g/g f.wt}$). The bi- interactions between proline and silicon was excelled (1.45 $\mu\text{g/g f.wt}$) as compared to proline 200 ml L⁻¹ + silicon 150 mg L⁻¹ which recorded a decrease in the proline content (0.89 $\mu\text{g/g f.wt}$). Proline 200 ml L⁻¹ + salinity 3 ds/m recorded a decrease in the proline (0.75 $\mu\text{g/g f.wt}$). Silicon 150 mg L⁻¹ + salinity 6 ds/m caused a decrease of 0.86 $\mu\text{g/g f.wt}$. Triple interactions between proline 200 ml L⁻¹ + silicon 150 mg L⁻¹ + salinity of 3 ds/m caused a decrease of 0.54 $\mu\text{g/g f.wt}$ proline 0 + silicon 0 + salinity 9 ds/m without spray t recorded an increase of 1.55 $\mu\text{g/g f.wt}$. The increase in proline by increasing salinity is due to its rapid construction and less use, which increases the speed of its accumulation. In addition to inhibiting the effectiveness of the oxidizing enzymes of proline and increasing the demolition of protein and its transformation into amino acids, including proline, the proline works to regulate the osmosis of plant tissue cells and reduces the ionic effect resulting from saline stress and contributes to restriction of toxic elements absorbed under saline

conditions and proline pooling (Hong-Bo et al 2006, Ashraf and Foolad 2007).

CONCLUSIONS

The salinity of irrigation water 9 ds/m has a negative role in influencing growth indicators. The negative effects of salinity as a result of spraying with proline acid at a concentration 200 ml L⁻¹ liter can be reduced. The spraying silicon at 150 mg L⁻¹ contributed in reducing the proline content and in increasing anti-oxidative enzymes, increasing the effectiveness of the root system and reducing transpiration, increasing plant hormones and encouraging the absorption of nutrients such as Ca, K and reducing concentrations Cl⁻, Na⁺ ions and K⁺: Na⁺ ratio increase.

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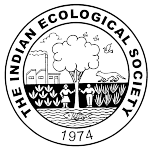
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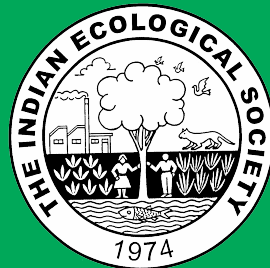
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