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Response of anatomical traits to environmental stresses in the leaves of local orange seedlings (*Citrus sinensis* L)

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This study explores the effects of salinity and drought stress on the anatomical characteristics of local orange seedlings. The research was conducted at the Agricultural Research Station of the College of Agriculture, University of Basra, during the 2022-2023 agricultural season. Salinity stress was imposed using three concentrations of irrigation water (0, 3, and 6 ds.m^{-1}), while drought stress was implemented by varying irrigation frequency (daily, weekly, and monthly). The anatomical traits examined included cuticle layer thickness, epidermal cell thickness, mesophyll cell diameter, vascular bundle diameter, tannin cell diameter, and vascular bundle sheath thickness. A recent study found that salinity stress in irrigation water and drought have a negative impact on the anatomical characteristics of local orange seedlings. Salinity (6 ds/m) and drought (monthly irrigation) led to an increase in the thickness of the cuticle layer (2.50 and 2.03 µm) and the diameter of the tannin cells (7.74 and 6.67 µm). These treatments also caused a decrease in the thickness of the epidermal cells, the diameter of the cells of the mesophyll tissue, the vascular bundle, and the thickness of the sheath of the vascular bundle. The study also found that the interaction between salinity stress and drought led to an even greater decrease in the anatomical characteristics of the seedlings. The interaction between salinity (6 ds/m) and drought (monthly irrigation) caused a decrease in the thickness of the epidermal cells, the diameter of the cells of the mesophyll tissue, the vascular bundle, and the thickness of the sheath of the vascular bundle to 2.57, 5.40, 33.23, and 20.11 µm, respectively.
Keywords: Anatomical characteristics, drought, environmental stresses, orange seedlings, salinity.

INTRODUCTION

Orange plants, scientifically known as *Citrus sinensis* L., belong to the Rutaceae family and are vital in global agriculture due to their production and quality. In Iraq, orange production is relatively low compared to the number of fruit trees, partially due to environmental stresses such as salinity and drought. Salinity and drought are major factors impacting fruit tree cultivation, causing a decline in orange production in Iraq. Salinity, in particular, has been exacerbated by water scarcity and degradation of water quality. This study aims to investigate how these environmental stresses affect the anatomical traits of orange seedlings.
Iraq produces 375 thousand tonnes of oranges, which is low for this number of trees. First in fruit production, oranges produce 66 million tonnes annually (F.A.O, 2005; Agha et al., Baldwin, 1993; 1991).
Most citrus-producing regions endure hot, dry summers with insufficient water and low-quality irrigation water due to high salt regimes. These factors reduce citrus tree output and quality. Climate change worsens salinity and drought stress in citrus-growing regions (Garcia-Sanchez and Syvertsen 2014). The fall in orange output in Iraq is due to salt and drought, which are major variables in fruit tree cultivation and productivity, and a shortage of farmed land (Ahmed *et al.*, 2013). Iraq has the most salinity space in Arab and Asian countries. Lack of water, water resource degradation, poor management, and rising groundwater levels have worsened Iraq's salinity problem in irrigated parts in central and southern Iraq (Taha, 2011). It affects almost 20% of the world's irrigated lands and worldwide agriculture, mainly in arid and semi-arid regions (Munns and Tester, 2008). The average tree production is modest, and the fruit quality has declined in recent years due to its tiny size and lack of juice. Most of Iraq's mineral elements are exposed to many factors that limit their solubility in soil solution and their readiness for the plant due to high salinity and pH and competition and interaction between ions, which decreases the activities of positive and negative ions that the growing plant benefits

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from. Increasing the concentration of some of them also increases salinity and pH.

Plants grown under a variety of environmental and natural conditions have growth restrictions. Plants have evolved morphological and anatomical features to survive these harsh conditions (Hopkins and Muner 2008).

This study aims to investigate how these environmental stresses affect the anatomical traits of orange seedlings.

MATERIALS AND METHODS

Samples of the wheat crop grown in the agricultural season One-year-old local orange seedlings were planted using a peat moss and river sand mixture. The study included two stress factors: salinity stress (with three NaCl concentrations) and drought stress (with varying irrigation frequencies). Anatomical sections were prepared using the paraffin technique, and measurements were taken with a light microscope.

The seedlings were treated for a full year as follows:

1- Salinity stress Salinity parameters: Irrigation of seedlings with water containing NaCl with three concentrations (0, 3 and 6 $ds.m^{-1}$).
2- Drought pressure Treatments for irrigation: Irrigating seedlings three times a month, one irrigation per week, and one irrigation per day.

The paraffin technique was employed to produce the anatomical sections in accordance with the (Willey, 1971) method, which makes use of both molten and solid paraffin wax. With a light microscope, measurements were taken and the slices were captured in pictures.

Statistical Analysis: The experiment followed a randomized complete block design (RCBD) with factorial analysis. The data were analyzed using GenStat, and mean differences were compared using the Least Significant Difference Test (L.S.D.) at a significance level of 0.05 (Al-Rawi and Khalaf Allah 2000; Bashir, 2003).

RESULTS AND DISCUSSION

Thickness of the cuticle layer: Table (1) showed that delay in harvest date harms grain The salinity treatment (6 $ds.m^{-1}$) was significantly better than the salinity treatment (3$ds.m^{-1}$) and recorded the highest rate (2.50 mµ), while the salinity treatment (6$ds.m^{-1}$) recorded the lowest rate (1.15 mµ). The results of the study are displayed in Table (1) and panels (1, 2) and show the effect of irrigation water salinity stress and drought stress on the thickness of the cuticle layer in the leaves of orange seedlings. The length of the irrigation periods also significantly affected how thick the cuticle layer was on the leaves. The rest of the treatments were significantly affected by monthly irrigation, which produced the greatest rate of (2.03 mµ), while the cuticle layer thickness in the leaves was lowest (1.60 mµ) in the daily irrigation treatment.

The salinity treatment (6 $ds.m^{-1}$) and the monthly irrigation treatment were found to interact with the salinity factor and irrigation period factor, respectively. The highest rate of skin thickness in the leaves was (2.91 mµ), significantly different from the other interactions, and the interaction with the highest rate of skin thickness was between water salinity was recorded. Irrigation (0 $ds.m^{-1}$) and daily irrigation, the lowest rates of epidermal thickness in the leaves were (0.99 µm).

Table 1. Effect of some environmental stresses on the thickness of the cuticle layer of the leaves of orange seedlings (mµ).

Salinity coefficients $ds.m^{-1}$	Irrigation treatments daily	weekly	Monthly	Salinity effect rate
0	0.99	1.12	1.35	1.15
3	1.71	1.76	1.82	1.76
6	2.11	2.47	2.91	2.50
irrigation effect rate	1.60	1.78	2.03	to overlap = 0.41
L.S.D.	for salinity =0.37		for irrigation =0.37	

Thickness of the epidermal cells: The results of Table (2) and panels (1, 2) show the effect of irrigation water salinity stress and drought stress on the thickness of epidermal cells in the leaves of orange seedlings. With the untreated plants (control treatment) and the treatment of low concentration of salinity (3 $ds.m^{-1}$), as the thickness of the epidermal cells in the treatment of high salt concentration was (3.51 µm), with a significant difference from the control treatment, which recorded the highest thickness of the epidermal cells amounted to (7.73 µm) As for the effect of irrigation periods, drought stress (monthly irrigation) significantly reduced the thickness of the epidermal cells and recorded the lowest value (4.63 µm), while the daily irrigation treatment recorded the highest mean of the thickness of the epidermal cells (6.26 µm).

Table 2. Effect of some environmental stresses on the thickness of epidermal cells of orange seedlings leaves (µm)

salinity coefficients $ds.m^{-1}$	irrigation treatments daily	weekly	Monthly	salinity effect rate
0	8.61	8.11	6.46	7.73
3	5.95	5.44	4.86	5.42
6	4.23	3.73	2.57	3.51
irrigation effect rate	6.26	5.76	4.63	to overlap =0.49
L.S.D.	for salinity =0.31		for irrigation =0.31	

According to the findings of the statistical analysis, the interaction between the salinity stress and drought stress treatments had a substantial impact on the thickness of the epidermal cells. (2.57 µm), but, in contrast to the other interactions, the interaction between the salinity treatment (0 ds.m^{-1}) and the daily watering treatment recorded the greatest value of epidermal cell thickness (8.61 µm).

***Diameter of mesophyll cells*:** The salinity treatment (0 ds.m^{-1}) was significantly better than the other salinity treatments and recorded the highest A value of (11.98 mµ), whereas salinity stress (6 ds.m^{-1}) had a significant effect on reducing the diameter of mesophyll cells and the lowest v value. The results of the study are shown in Table (3) and panels (1, 2), which show the effect of irrigation water salinity stress and drought stress on the diameter of the meso Drought stress (monthly irrigation) had a considerable impact on the average effect of irrigation periods. The rate that reduced the mesophyll cells' diameter at the slowest rate was (8.51 mµ). while the daily irrigation treatment recorded the highest rate of the mesophyll cells diameter was (10.53 µm).

There was also a significant effect of the interaction between salinity stress and water stress treatments on the diameter of mesophyll cells, the interaction between salinity treatment (6ds.m^{-1}) and monthly irrigation of orange seedlings had a significant effect on reducing the diameter of mesophyll cells and the lowest rate was (5.40 µm), while the interaction between the salinity treatment (0 ds.m^{-1}) and the daily irrigation treatment recorded the highest average diameter of the mesophyll tissue cells (12.86µm), with a significant difference from the rest of the interactions.

Table 3. Effect of some environmental stresses on the diameter of mesophyll cells of orange seedling leaves (µm)

Salinity coefficients ds.m^{-1}	Irrigation treatments			Salinity effect rate
	Daily	Weekly	Monthly	
0	12.86	12.17	10.91	11.98
3	10.34	9.91	9.23	9.83
6	8.38	7.61	5.40	7.13
irrigation effect rate	10.53	9.90	8.51	to overlap =0.64
L.S.D.	for salinity =0.54		for irrigation =0.54	

Diameter of the vascular bundle: The information in Table (4) and panels (1, 2) demonstrates how the width of the vascular bundle in the leaves of orange seedlings is affected by both drought and salinity stress. With regard to the average effect of irrigation periods, drought stress (monthly irrigation) had a significant effect on reducing the diameter of the vascular bundle (mean diameter of the vascular bundle: 39.16 µm, significant difference from treatment of salinity (0 ds.m^{-1}), whose samples recorded the highest mean of the diameter of the vascular bundle: 72.05 µm); however, with regard to the treatment of salinity (0 ds.m^{-1}), The diameter of the vascular bundle had the lowest average (49.59 µm) and the highest rate (60.30 µm) for the daily irrigation treatment.

Through the interaction between salinity stress and water stress treatments, it was found that there is a significant effect on the diameter of the vascular bundle. (33.23 µm), while the interaction between the salinity treatment (0 ds.m^{-1}) and the daily irrigation treatment recorded the highest average vascular bundle diameter (77.22 µm), with a significant difference from the rest of the interactions.

Table 4. Effect of some environmental stresses on the diameter of the vascular bundle of orange seedlings leaves (µm)

salinity coefficients ds.m^{-1}	irrigation treatments			salinity effect rate
	daily	weekly	monthly	
0	77.22	71.60	67.33	72.05
3	59.25	52.31	48.20	53.25
6	44.43	39.81	33.23	39.16
irrigation effect rate	60.30	54.57	49.59	to overlap =5.59
L.S.D.	for salinity =4.14	for irrigation =4.14		

***Diameter of the tannin cells*:** It was observed through microscopic examination in panels (1, 2) and the results shown in Table (5) the effect of irrigation water salinity stress and drought stress on the characteristic of the diameter of tannin cells in the leaves of orange seedlings, as the salinity treatment (6 ds.m^{-1}) was significantly superior to The salinity treatment (3 ds.m^{-1}) recorded the highest rate (7.74 µm), While the lowest rate (4.69 µm) was obtained with the salinity treatment (0 ds.m^{-1}).

Table 5. Effect of some environmental stresses on tannin cell diameter of orange seedling leaves (µm).

salinity coefficients ds.m^{-1}	irrigation treatments			salinity effect rate
	daily	weekly	Monthly	
0	4.22	4.72	5.12	4.69
3	5.62	6.11	6.72	6.15
6	7.28	7.58	8.17	7.74
irrigation effect rate	5.71	6.20	6.67	to overlap =0.55
L.S.D.	for salinity =0.38	for irrigation =0.38		

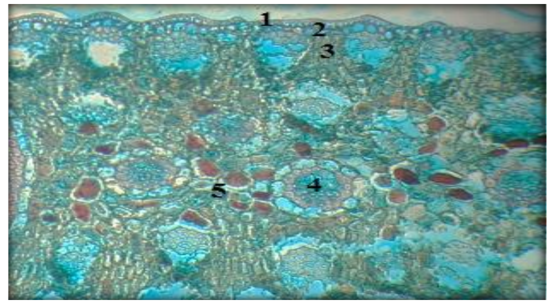
The results of the same table also revealed that the monthly irrigation treatment, which was much better than the other treatments and recorded the greatest rate of (6.67 µm), had the highest diameter of the Tannin cells. The diameter of tannin cells in the leaves measured (5.71 mµ), with the daily watering treatment recording the lowest rate. The interaction between salinity and irrigation periods showed a significant effect. The interaction between the salinity treatment (6 ds.m^{-1}) and the monthly irrigation treatment recorded the highest rate of tannin cell diameter in the leaves, with a While the interaction between salinity was seen, there was a substantial variance from the other interactions, which was (8.17 µm). The diameter of the tannin cells in the leaves was (4.22 µm); the lowest rates for irrigation water (0 ds.m^{-1}) and daily irrigation.

Thickness of the vascular bundle sheath: Table 6 and panels (1, 2) show the effect of irrigation water salinity stress and drought stress on the thickness of the vascular bundle sheath in the leaves of orange seedlings. (50.72 µm), while salinity stress (6 ds.m^{-1}) had a significant effect on reducing the thickness of the vascular bundle sheath, and the lowest rate was (24.24 µm). As for the average effect of irrigation periods, drought stress (monthly irrigation) had a significant effect on the thickness of the vascular bundle sheath was reduced and the lowest rate was (32.20 µm), while the daily irrigation treatment recorded the highest rate of the vascular bundle thickness which was (41.87 µm).

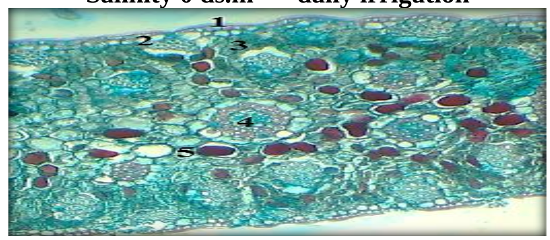
There was also a significant effect of the interaction between the treatments of salinity stress and water stress on the thickness of the vascular bundle sheath. (20.11 µm), while the interaction between the salinity treatment (0 ds.m^{-1}) and the daily irrigation treatment recorded the highest rate of vascular bundle sheath thickness (57.20 µm), with a significant difference from the rest of the interactions

Table 6. Effect of some environmental stresses on the thickness of the vascular bundle sheath of the leaves of orange seedlings (mµ).

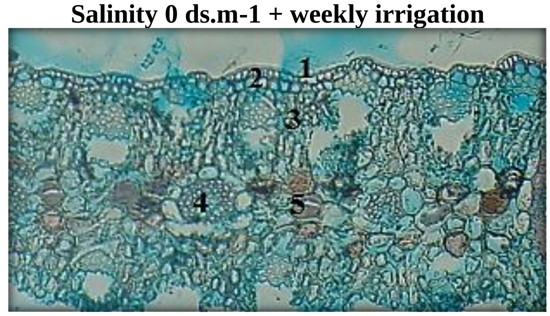
salinity coefficients ds.m^{-1}	irrigation treatments			salinity effect rate
	daily	weekly	Monthly	
0	57.20	50.60	44.35	50.72
3	40.12	36.45	32.14	36.24
6	28.28	24.32	20.11	24.24
irrigation effect rate	41.87	37.12	32.20	to overlap =6.45
L.S.D.	for salinity =4.44		for irrigation =4.44	



Salinity 0 ds.m^{-1} + daily irrigation



Salinity 0 ds.m-1 + weekly irrigation



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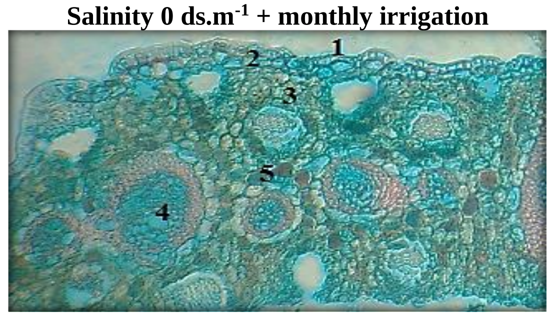

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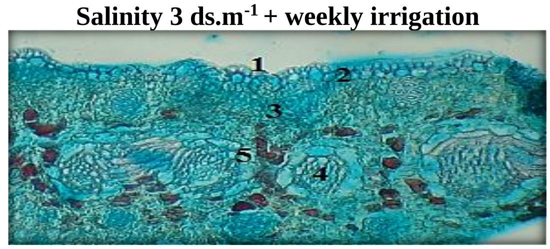
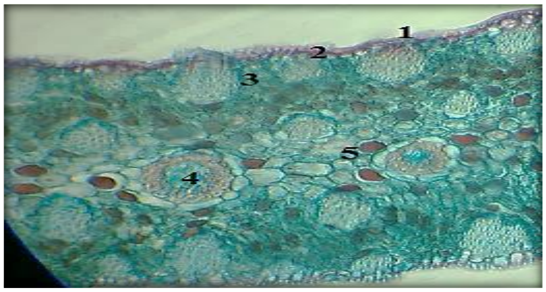
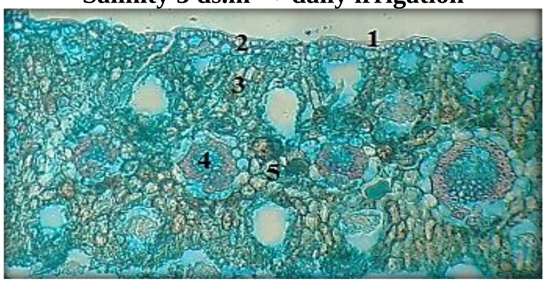


Salinity 3 ds.m^{-1} + monthly irrigation

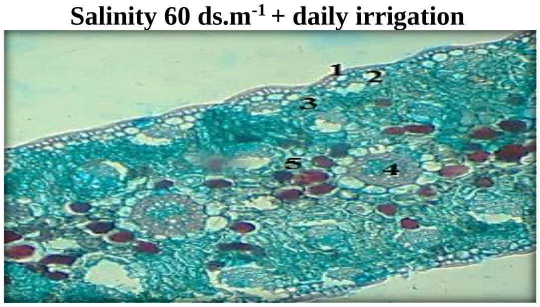
Panel 1. The effect of some environmental stresses on the anatomical characteristics of the leaves of orange seedlings (1-cuticle 2- epidermis 3- mesophyll cell 4- vascular bundle 5- Tannin cell 6-bundle sheath cell)



Salinity 3 ds.m^{-1} + daily irrigation



Salinity 60 ds.m^{-1} + daily irrigation



Salinity 60 ds.m^{-1} + monthly irrigation

Panel 2. The effect of some environmental stresses on the anatomical characteristics of the leaves of orange seedlings (1-cuticle 2- epidermis 3- mesophyll cell 4- vascular bundle 5- Tannin cell 6-bundle sheath cell)

The results show that there is a significant effect of each of salt stress and drought stress on the anatomical characteristics of the leaves of orange seedlings. Drought stress (also known as water stress) is the second type of stress (Al-Najjar and Al-Ibrsim, 2018). Plants that grow in unsuitable environments experience morphological (phenotypical) and anatomical changes as a result of fluctuations in environmental and natural factors, which are specific factors for plant growth and development. This is how salinity has a direct effect on plants., and despite the harsh conditions in these environments, plants have developed some Phenotypic and anatomical characteristics that enabled them to survive under unfavorable conditions (Challa and Van, 2004; Abdul-Wahid, 2003). Water-deficit conditions (drought stress) are a major environmental factor, often determining the growth and productivity of important crop species (Barnabas *et al.*, 2008). High salt concentrations reduce the ability of the plant to absorb water, and this has affected the increase in the thickness of the cuticle layer to reduce water loss under such stress conditions. They are represented by the smallness of the leaves, the lack of the number of stomata per unit leaf area, In times of environmental stress, cuticle layer thickness increases, waxy materials are deposited, and these adaptations together play a significant role in saving water for plant growth (Sagi *et al.*, 1997; Dickison, 2000). Moreover, the impact of stress The thickness of epidermal cells, mesenchymal cells, the width of the vascular bundles, and the thickness of the vascular bundle cover were all significantly impacted by salinity and drought stress.. The reason may be attributed to a decrease in the water content of the plant under the stress of salinity and drought, which was negatively reflected on the efficiency of photosynthesis in the cells of the bundle sheath and the lack of filling of the tissue cells. The leaf where the longitudinal growth decreases in the root and vegetative system with an increase in the salt concentration in the growth medium, and this is reflected in their content of nutrients and thus the limitation of tissue growth and development (Ramoliya and Pandey, 2003; Mafakheri *et al.*, 2010; Shafqat *et al.*, 2019), while increasing water uptake (control treatment and daily irrigation) led to the ability of plants to maintain the growth and development of roots and maintain the water content of cells compared with those growing under saline and drought conditions. This in turn led to an increase in the activity of cell division and an improvement in the seedlings' growing conditions as reflected by the anatomical features of their leaves (*Nawazish et al.*, 2006; Trifilo *et al.*, 2007). The results of this study agreed with the results of the study (Dolatabadian *et al.*, 2011) on the effect of salts on some anatomical characteristics of soybeans, and with the results of the study of Gabash and Auda ,(2023), to understand the impact of various soil conditioners and the caliber of irrigation water on the anatomical traits of the date palm and agreed with the findings of Shafqat *et al.* (2021) who demonstrated the impact of drought stress on a number of anatomical traits of leaves of several citrus species.

***Conclusion*:** This study highlights the adaptability of orange seedlings to environmental stress through changes in anatomical traits. The thickness of the cuticle layer, epidermal cells, mesophyll cells, vascular bundles, and vascular bundle sheath were all significantly affected by stress conditions. These anatomical traits serve as valuable indicators of the environmental conditions in which the plant thrives.

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