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Behavior of Some Olive Cultivars and Genotypes Transplants Under Different NaCl Concentrations

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ABSTRACT

Salinity is a major abiotic constraint limiting olive cultivation in Egypt. This study, conducted in the 2023-2024 seasons at the Horticultural Research Institute, Agricultural Research Center (Giza, Egypt), evaluated the morphological, physiological, and biochemical traits of transplants of two cultivars (Cerasuola and Criolla) and seven genotypes-Giza 32, 48, 55, 91, 92, 99, and 102-subjected to four NaCl levels (2000, 4000, 6000, and 8000 mg/L), compared to a control (tap water).

Results revealed that Giza 48 exhibited the highest tolerance, maintaining survival, stable growth (stem elongation, leaf area, fresh biomass), chlorophyll content, RWC, elevated proline, favorable ion balance (lower Na+ and high K+/Na+ ratio), and low LSHC. Cerasuola showed comparable tolerance, followed by Giza 99. In contrast, Giza 32 was the most sensitive. The other genotypes displayed intermediate responses. Based on growth performance under salinity, Giza 48, Cerasuola and Giza 99 can be categorized as salt-tolerant; Giza 91, Giza 55, Criolla, Giza 92, and Giza 102 as moderately tolerant; and Giza 32 as salt-sensitive.

The anatomical investigations of three olive representatives' genotypes-Giza 48 (tolerant at 8000 mg/L NaCl), Giza 55 (moderately tolerant at 6000 mg/L), and Giza 32 (sensitive at 4000 mg/L)-revealed that Giza 48 exhibited enhanced leaf thickness (upper epidermis, palisade, spongy mesophyll, xylem, and phloem) and well-developed root structures. Giza 32 showed reduced disorganized anatomy, while Giza 55 displayed moderate anatomical plasticity.

In conclusion, Giza 48, Cerasuola, and Giza 99 appeared suitable for cultivation in highly saline reclaimed lands or areas irrigated with saline groundwater.

KEYWORDS: Olive; Salinity; Cultivars; Genotypes, NaCl.

1. INTRODUCTION

Soil salinity is an escalating global concern that significantly impacts agricultural productivity and food security. According to report by the United Nations Food and Agriculture Organization, approximately 1.4 billion hectares of land are currently affected by salinity, with an additional 1 billion hectares at risk. This problem is exacerbated by climate change, inadequate irrigation practices, and rising sea levels, resulting in substantial reductions in crop yields—up to 70% in some regions (FAO, 2024). Furthermore, the increasing salinity in irrigation water, compounded by the decreasing availability of good-quality water, has made the exploration of saline water use in agriculture an urgent necessity (Guo *et al.,* 2020 and Saludo *et al.,* 2023). In areas where freshwater resources are limited or erratic, the use of saline water for irrigation becomes essential, particularly for salt-tolerant crops such as olives (Mousavi *et al.,* 201٥). Salinity is a major abiotic stressor that limits plant growth by disturbing water uptake, nutrient balance, and essential physiological processes such as photosynthesis and respiration, these disruptions can lead to substantial declines in crop yields and restrict the range of cultivable species in affected regions (Atta *et al.,* 2023). Among fruit crops, the olive tree (*Olea europaea* L.) exhibits notable resilience to saline conditions, with many cultivars demonstrating adaptive responses such as osmotic adjustment, selective ion uptake, and morphological modifications. However, while some varieties can tolerate moderate salinity, prolonged or excessive exposure may result in yield reductions, deterioration of fruit quality, and metabolic disorders (Atta *et al.,* 2023; Vergine *et al.,* 2024; Claros *et al.,* 2025).

The olive tree is considered a strategic crop of high economic and nutritional value, primarily cultivated in the Mediterranean basin and increasingly in other regions worldwide. (El Yamani and Cordovilla, 2024; Claros *et al.,* 2025). Its tolerance to salinity is attributed to several physiological and biochemical mechanisms, including the restriction of salt ion translocation to shoots-resulting in ion accumulation in roots - and the maintenance of a favorable K^+/Na^+ ratio in actively growing tissues (Elloumi *et al*., 2024). In addition, olive trees employ osmotic adjustment and reactive oxygen species (ROS) scavenging at the cellular level to mitigate salt stress (Boussadia *et al.,* 2023).

Despite the global expansion of olive cultivation, production remains heavily concentrated in Mediterranean and Middle Eastern regions, which are increasingly challenged by climate change and population pressures. These pressures are leading to declining water availability and quality, accelerating soil and water salinization, and posing a serious threat to agricultural production, including olives and olive oil (El Yamani and Cordovilla, 2024).

One promising strategy for addressing salinity-related challenges is the breeding and development of salt-tolerant olive cultivars. Identifying and selecting genotypes with enhanced tolerance can improve productivity in salt-affected soils. These cultivars can be ranked within a salinity tolerance classification map, guiding their suitability for cultivation under specific environmental constraints. This approach supports the sustainable expansion of olive farming without compromising economic returns (Elloumi *et al*., 2024 and Claros *et al.,* 2025).

In Egypt, both soil and water salinity represent major constraints to agriculture, particularly in arid and semi-arid regions. An estimated 30-40% of irrigated agricultural lands are affected by salinization, mainly due to inadequate drainage systems and seawater intrusion, especially in the Nile Delta (Abdelaziz *et al.,* 2021; Rady and Taha, 2022; Hagage *et al.,* 2024). Climate change and anthropogenic pressures further exacerbate these issues, severely impacting plant growth and reducing productivity (FAO, 2020). Nevertheless, many Egyptian farmers continue to select olive cultivars based mainly on general traits such as oil content or fruit quality, often without considering salinity tolerance. This practice can result in yield losses and reduced profitability in salt-affected areas (Rady and Taha, 2022). Recent studies underscore the importance of developing comprehensive tolerance classification and varietal suitability maps to inform cultivar selection of Egyptian olive genotypes under saline conditions. For instance, genotypes such as 'Giza 61' have shown promising salt tolerance (Fayek *et al.,* 2018). These findings underscore the importance of developing comprehensive salinity tolerance profiles and varietal suitability maps to guide cultivar selection. Such tools can enhance crop resilience, maintain productivity, and promote environmentally sustainable agricultural practices aimed at escalating salinity challenges (FAO, 2020; Rady and Taha, 2022).

Accordingly, this study aims to evaluate selected genetic lines of olive transplants from morphological, physiological, biochemical, and anatomical perspectives to identify the genotypes exhibiting the highest salinity tolerance under climate change conditions. This evaluation contributes to the development of a salinity tolerance classification for olive cultivars, thereby supporting the sustainability and economic viability of olive production in Egypt's salt-affected regions.

2. MATERIALS AND METHODS

Field experiment: The field trial was conducted during the 2023 and 2024 growing seasons at the Horticulture Research Institute, Giza Governorate, Egypt (30.268215N latitude, 30.806534E longitude).

Plant material: Uniform, healthy, one-year-old olive transplants were chosen to assess their salinity tolerance. The plant material included two foreign cultivars (Cerasuola and Criolla) and seven genotypes (Giza 32, 48, 55, 91, 92, 99, and 102) derived through the framework of the Genetic Improvement of Olive Project (CFFC/IOOC project 001), as shown in Table 1.

Table 1. The tested genotypes source according to the project map.

Genotypes	Derived From
Giza 32	Kalamata (open pollination)
Giza 48	Coratina♀ X Toffahi♂
Giza 55	Manzanillo (open pollination)
Giza 91	Manzanillo (open pollination)
Giza 92	Manzanillo (open pollination)
Giza 99	Manzanillo (open pollination)
Giza 102	Manzanillo (open pollination)

Experimental procedure: Tested transplants were pruned to a single main shoot of uniform length, then planted in individual plastic bags (30 cm diameter × 35 cm height) filled with 7.5 kg of sandy soil and irrigated three times a week, with a fixed volume of 750 mL per plant with NaCl saline solutions. Soil moisture was maintained at approximately 25% above field capacity to prevent salt accumulation and avoid salt shock, following the protocol of Moya *et al.,* (2002).

The soil's physical and chemical properties were determined according to the procedures described by Black (1965) and Eaton (1999), as shown in Table 2.

Table 2. Physicochemical properties and moisture characteristics of the soil.

pH (1:2.5)	E.C. dsM^{-1}	SP	O.M. (%)	Soluble cations (meq/L)				Soluble anions (meq/L)			
				Ca^{++}	Mg^{++}	Na^{+}	K^{+}	$CO_3^{=}$	HCO_3^{-}	Cl^{-}	$SO_4^{=}$
7.98	4.50	28.00	0.53	12.10	8.90	23.00	0.50	--	2.30	40.2	2.00

Available nutrients ($mg\ kg^{-1}$)						
N	P	K	Cu	Fee	Mn	Zn
53.00	8.34	415.00	0.04	3.38	1.04	0.31

Soil mechanical analysis				
Soil particle size distribution				Textural classes
Coarse sand %	Fine sand %	Silt %	Clay %	
38.50	40.30	14.20	7.00	Sandy silt

Field capacity	Wilting point	Available water
15.80	8.30	7.50

At the first week of May, 90 of rooted olive plants from each of tested cultivars and genotypes were subjected to five groups including 18 plants per group. The groups were treated with four levels of NaCl solution (2000, 4000, 6000, and 8000 mg/L) and comparing with control treatment (tap water).

After 180 days of salinity treatments in each season, the following measurements were recorded

Biometric determination:

- **Survival percentage (%)**: Calculated as the number of surviving plants divided by the initial number of plants, then multiplied by 100.
- **Stem elongation (cm):** Measured by calculating the difference between the final and initial stem length of each plant.
- **Average leaf area per plant (cm²):** Estimated using the formula proposed by Ahmed and Morsy (1999):

Leaf area (cm^2) = 0.53 (Leaf length (cm) x Leaf width (cm)) +1.66

- **Fresh weight of plant organs (g):** Fresh weights of leaves, shoots, and roots were weighed after removed and washed with deionized water and separated into different parts.
- **Relative water content (RWC %):** Twenty leaf discs from fully expanded leaves (mid-region) were sampled per treatment. Fresh weight (FW) was recorded immediately, then the discs were floated on distilled water in Petri dishes for 24 hours in darkness to attain full turgid weight (TW). Afterward, discs were oven-dried at 70°C for 72 hours to determine dry weight (DW). RWC (%) was calculated following Ben-Ahmed *et al.,* (2008) using the formula:

$$RWC\ (\%) = \frac{FW - DW}{TW - DW} * 100$$

- **Total chlorophyll content:** Chlorophyll a and b contents were determined spectrophotometrically from fresh leaf tissues extracted in 80% acetone, according to A.O.A.C. (2016). Total chlorophyll content was then calculated as follows:

Total chlorophyll (mg/g FW) = chlorophyll a + chlorophyll b

- **Leaf mineral content:** Dried leaf samples were ground, and a 0.1 g subsample was digested and the minerals content were determined as follows:
- **Potassium (K^+) and sodium (Na^+):** Measured using flame photometry (A.O.A.C., 2016).
- **K^+/Na^+ Ratio:** Calculated as the ratio of potassium to sodium content.
- **Calcium (Ca^{2+}):** Measured using an atomic absorption spectrophotometer ("Perkin Elmer–3300") in accordance with (A.O.A.C., 2016).
- **Proline content:** Estimated from fresh leaves (6^{th}-7^{th} basal position) as the following method of (Bates, 1973).
- **Leaf salinity hazard coefficient (LSHC):** Total soluble sugars were determined from dried leaf tissue using the phenol-sulfuric acid method (Dubois *et al.,* 1956), and carotenoids were extracted and quantified following A.O.A.C. (2016) procedures. Based on these measured parameters, the leaf salinity hazard coefficient (LSHC) was calculated using the formula described by Emtithal *et al.,* (1995):

$$LSHC = \frac{Total\ soluble\ sugars}{Clorophyll\ (a+b)} * Carotenoids$$

Anatomical traits:

Anatomical investigations were performed on leaf and root tissues. Samples were collected from fully expanded (mid-region, 1 cm² segments) and intact root systems. Tissues were fixed in FAA (5% glacial acetic acid, 10% formaldehyde, and 85% ethanol 70%), dehydrated through a butyl alcohol series, and embedded in paraffin wax (melting point: 58-60°C). Sections (10-12 µm thick) were prepared using a rotary microtome, stained with Safranin T and Fast Green FCF, cleared in xylene, and mounted in Canada balsam. Prepared slides were oven-dried at 40°C for one week following the technique of Johansen (1940). Microscopic analysis included:

- **Leaf cross-sections:** Measurements of epidermal thickness, palisade and spongy mesophyll layers, midrib anatomy, and vascular bundle dimensions (xylem/phloem).
- **Root cross-sections:** Root diameter, thickness of epidermal and cortical layers, diameter of the vascular cylinder, number of xylem arms, and phloem poles.

The structural stability of anatomical variation under salinity stress was assessed using the relative variation (RV%), calculated as where a represents the value under salinity treatment, and b represents the value under control.

$$RV\ (\%) = \frac{a-b}{b} * 100$$

Statistical analysis

Experimental data were subjected to analysis of variance (ANOVA) using a split-plot design including nine tested cultivars and genotypes, five salinity levels, and three replicates, with six plants per replicate. The obtained data were subjected to variance analysis according to Snedecor and Cochran ,1980. The means were differentiated using the multiple range test at the 0.05 level (Duncan, 1955).

3. RESULTS

3.1. Effect of salinized water on survival percentage:

The survival percentage of the tested olive cultivars and genotypes exhibited a statistically significant response to salinity stress (Table 3). A consistent decline in survival rate was observed as NaCl concentrations increased from 2000 to 8000 mg/L across both growing seasons. The highest survival percentages (100%) were consistently recorded by each of control and the 2000 mg/L NaCl treatment, while the lowest values (63.96 and 65.31%) were observed at the highest salinity level (8000 mg/L) during the 2023 and 2024 seasons, respectively. Genotypes Giza 48, Cerasuola, and Giza 99 maintained high survival rates (98%) in both seasons, indicating strong tolerance to elevated salinity levels, while Giza 32 exhibited the lowest survival (62.80% and 62%). The interaction between genotype and salinity level was also statistically significant. All genotypes exhibited full survival (100%) at 2000 mg/L NaCl, while at 4000 and 6000 mg/L, each of Giza 48, Cerasuola, and Giza 99 sustained complete survival (100%) which declined slightly to 90% under the treatment with 8000 mg/L in both seasons. Conversely, Giza 32 showed minimal survival at 8000 mg/L, recording only 11.11% and 10% in respective seasons. The remaining genotypes demonstrated intermediate survival responses, with significant differences based on both genotype and salinity level. Overall, the results clearly indicate that Giza 48, Cerasuola, and Giza 99 are among the most salt-tolerant genotypes in terms of survival under increasing salinity stress. In contrast, Giza 32 proved to be the most salt-sensitive, exhibiting a marked decline in survival under high salinity conditions.

3.2. Effect of salinized water on morphometric parameters;

Stem elongation, leaf area, and fresh weight of plant organs (leaves, stems, and roots) were evaluated as key morphometric traits to assess the growth responses to olive transplants under increasing the salinity levels. As shown in Tables (4-8), all vegetative growth parameters were significantly affected by both salinity levels and genotype across the two growing seasons. In general, increasing salinity concentrations led to a marked and consistent reduction in all measured growth traits.

Stem elongation:

As presented in Table (4), the extent of stem elongation reduction was clearly dependent on the salinity level applied. Plants irrigated with saline water exhibited progressively reduced stem elongation as NaCl concentration increased, while the greatest elongation was recorded under tap water conditions. The highest stem elongation was maintained by genotype Giza 48 in both seasons, indicating a high level of salt tolerance, followed by Cerasuola and Giza 99 respectively. In contrast, Giza 32 consistently exhibited the lowest stem elongation, whereas the remaining genotypes displayed intermediate values with statistically significant variation. A significant interaction was found, with Giza 48 showing the greatest stem elongation and Giza 32 the least under salinity stress.

Table 3. Effect of salinized water on survival percentage of tested olive cultivars and genotypes in (2023-2024) seasons.

Cultivars & Genotypes / Salt treatments	Cerasuola	Criolla	Giza 32	Giza 48	Giza 55	Giza 91	Giza 92	Giza 99	Giza 102	Mean
					First season; 2023					
Control	100.00a	100.00a	100.00a	100.00a	100.00a	100.00a	100.00a	100.00a	100.00a	100.00A
2000 mg/L	100.00a	100.00a	100.00a	100.00a	100.00a	100.00a	100.00a	100.00a	100.00a	100.00A
4000 mg/L	100.00a	88.89b	69.55d	100.00a	100.00a	100.00a	90.00b	100.00a	83.33c	92.42B
6000 mg/L	100.00a	66.67d	33.33i	100.00a	83.33c	89.99b	66.67d	100.00a	50.00g	76.67C
8000 mg/L	90.00b	54.55f	11.11j	90.00b	70.00d	70.00d	60.00e	90.00b	40.00h	63.96D
Mean	98.00A	82.02C	62.80E	98.00A	90.67B	92.00B	83.33C	98.00A	74.67D	
					Second season; 2024					
Control	100.00a	100.00a	100.00a	100.00a	100.00a	100.00a	100.00a	100.00a	100.00a	100.00A
2000 mg/L	100.00a	100.00a	100.00a	100.00a	100.00a	100.00a	100.00a	100.00a	100.00a	100.00A
4000 mg/L	100.00a	88.89b	66.67f	100.00a	100.00a	100.00a	90.00b	100.00a	83.33c	92.10B
6000 mg/L	100.00a	73.33d	33.33j	100.00a	87.07c	89.99b	70.00e	100.00a	63.33g	79.34C
8000 mg/L	90.00b	61.11gh	10.00k	90.00b	70.00e	72.22de	60.00h	90.00b	44.43i	65.31D
Mean	98.00A	84.67D	62.00F	98.00A	90.81C	92.44B	84.00D	98.00A	78.22E	

Values have the same letter are not significantly different at 5% level using Duncan's Multiple Range Test

Table 4. Effect of salinized water on stem elongation (cm) of tested olive cultivars and genotypes in (2023-2024) seasons.

Cultivars & Genotypes / Salt treatments	Cerasuola	Criolla	Giza 32	Giza 48	Giza 55	Giza 91	Giza 92	Giza 99	Giza 102	Mean
					First season; 2023					
Control	49.17c	36.00lm	44.21f	42.00a	40.67h	38.67i	36.33lm	50.33b	37.00jk	42.71A
2000 mg/L	48.35c	33.50no	34.49m	51.18ab	36.86kl	36.83kl	33.26no	48.25d	33.06o	39.64B
4000 mg/L	43.35f	29.53p	25.50st	45.65e	34.06n	33.26no	29.84p	42.02g	29.17p	34.71C
6000 mg/L	39.69kl	26.00rs	17.67x	40.68h	29.85p	29.06pq	29.57r	37.92ij	24.86t	29.92D
8000 mg/L	33.52no	18.74w	12.03z	35.89lm	22.50v	23.96u	18.92w	26.76r	15.97y	23.14E
Mean	42.22B	28.75G	26.98I	45.08A	32.79D	32.36E	28.98F	41.06C	28.01H	
					Second season; 2024					
Control	50.52b	38.17jk	40.92g	52.68a	38.86ij	39.91h	36.84lm	48.42c	38.56ij	42.76A
2000 mg/L	49.32c	35.39n	33.17o-q	51.78a	35.53n	37.74kl	33.59op	47.42d	32.50q	39.60B
4000 mg/L	44.02f	31.26r	29.17t	45.68e	32.83pq	33.74op	29.39st	41.92g	31.00r	35.45C
6000 mg/L	39.52hi	27.96u	16.87xy	41.18g	29.84st	29.96st	26.00u	37.02lm	27.53u	30.65D
8000 mg/L	34.12o	19.94w	11.77z	36.18mn	22.56v	23.42v	17.50y	30.26rs	18.73x	23.83E
Mean	43.50B	30.54F	26.38H	45.50A	31.92E	32.95D	28.66G	4.01C	29.66G	

Values have the same letter are not significantly different at 5% level using Duncan's Multiple Range Test

3.3.Leaf area:

According to Table (5), the average leaf area declined significantly as salinity levels increased during both seasons. Clear distinctions in leaf area were observed among the different salinity levels. The smallest leaf areas were observed under the highest salinity levels, and the largest under control (tap water) conditions. Among the genotypes, Cerasuola exhibited the highest leaf area (4.63 and 5.00 cm^2 in the first and second seasons, respectively), followed by Giza 102 and Criolla. In contrast, Giza 32 consistently recorded the smallest leaf area (3.71 and 3.86 cm^2), along with Giza 55 and Giza 99 in the first season. The interaction between salinity levels and genotypes was significant, as the extent of leaf area reduction differed markedly among the tested genotypes under increasing salinity. These results indicate that leaf area is highly genotype-dependent, reflecting the role of genetic background in morphological adaptation to salinity stress.

Table 5. Effect of salinized water on leaf area (cm^2) of tested olive cultivars and genotypes in (2023-2024) seasons.

Cultivars & Genotypes / Salt treatments	Cerasuola	Criolla	Giza 32	Giza 48	Giza 55	Giza 91	Giza 92	Giza 99	Giza 102	Mean
	First season; 2023									
Control	5.05a	4.73cd	4.20h-k	4.20h-k	4.08j-n	4.43f-h	4.25g-j	4.04j-o	4.97ab	4.44A
2000 mg/L	4.79bc	4.56d-f	3.93l-r	4.02j-p	3.89m-s	4.27g-j	4.02j-p	3.9m-s	4.70cd	4.23B
4000 mg/L	4.64c-e	4.45e-g	3.67s-u	3.91m-s	3.77q-u	4.17j-l	3.88m-s	3.78p-t	4.53d-f	4.09C
6000 mg/L	4.56d-f	4.37f-i	3.61t-v	3.85n-t	3.69r-u	4.10j-n	3.82o-t	3.71r-u	4.45e-g	4.02D
8000 mg/L	4.11j-m	4.20h-k	3.18w	3.54uv	3.38vw	3.97k-q	3.43v	3.40v	3.99k-q	3.69E
Mean	4.63A	4.46B	3.71E	3.90D	3.76E	4.19C	3.88D	3.76E	4.53B	
	Second season; 2024									
Control	5.36a	5.31a	4.59h-j	4.67f-h	4.75e-h	5.04bc	4.47i-k	4.64g-i	5.45a	4.92A
2000 mg/L	5.11b	4.90c-e	4.18m-o	4.47i-k	4.41j-l	4.73e-h	4.11o-q	4.37k-m	5.01b-d	4.59B
4000 mg/L	5.01b-d	4.86c-f	3.92q	4.37k-m	4.3k-m	4.65g-i	4.06o-q	4.33k-n	4.93b-e	4.50C
6000 mg/L	4.83d-g	4.69f-h	3.67r	4.24l-o	4.19m-o	4.46i-k	3.92q	4.17m-o	4.78e-h	4.33D
8000 mg/L	4.68f-h	4.35k-m	2.94s	4.13n-p	3.95pq	4.24l-o	3.59r	3.93q	4.35k-m	4.02E
Mean	5.00A	4.20C	3.86H	4.38E	4.34EF	4.62D	4.03G	4.29F	4.90B	

Values have the same letter are not significantly different at 5% level using Duncan's Multiple Range Test

3.4.Fresh weight of leaves; stems and roots:

As shown in Tables (6-8), the fresh weight of leaves, stems, and roots were significantly affected by salinity. A clear inverse relationship was observed between salinity concentration and fresh biomass accumulation, consistently across both growing seasons. Regarding genotypic performance, Giza 48, the Cerasuola cultivar and Giza 99 recorded the highest average fresh weights for all organs, respectively. On the other hand, Giza 32 showed the lowest fresh weight values for leaves, stems, and roots. A significant interaction effect was observed, as Giza 48, the Cerasuola cultivar, and Giza 99 maintaining their superiority in fresh weights even under 8000 mg/L. These findings confirm that reductions in growth parameters constitute one of the most severe impacts of salinity stress on olive seedlings. The severity of these reductions increased proportionally with salinity level and varied significantly among genotypes.

3.5.Effect of salinized water on relative water content (RWC):

Relative water content (RWC) in olive leaves was significantly affected by both salinity stress and genotypes in two studied seasons. As shown in Table (9), irrigation with different concentrations of NaCl (2000, 4000, 6000, and 8000 mg/L) resulted in a gradual and significant decrease in RWC compared to the control treatment. This decline reflects the adverse impact of salinity on water uptake and internal water retention within plant tissues. Genotypic variation in RWC values was also statistically significant. Giza 48 consistently

Table 6. Effect of salinized water on fresh weight of leaves (g/plant) of tested olive cultivars and genotypes in (2023-2024) seasons.

Cultivars & Genotypes / Salt treatments	Cerasuola	Criolla	Giza 32	Giza 48	Giza 55	Giza 91	Giza 92	Giza 99	Giza 102	Mean
					First season; 2023					
Control	23.99c	20.15f	19.75f	26.60ab	23.81c	26.63a	21.30e	26.13b	19.78f	23.13A
2000 mg/L	21.87d	17.27j	14.74n-p	23.99c	18.65gh	21.43de	16.49k	21.36e	14.71op	18.95B
4000 mg/L	19.68f	14.95m-o	11.11s	21.87d	15.44lm	18.20hi	13.54q	18.85g	11.09s	16.08C
6000 mg/L	17.24j	12.84r	8.42u	18.12i	12.5r	14.86no	11.04s	15.49l	7.95uv	13.20D
8000 mg/L	14.33p	7.88v	3.40x	15.24l-n	10.40t	12.57r	7.97uv	12.87r	5.03w	9.97E
Mean	19.42B	14.62E	11.48H	21.17A	16.23D	18.74C	14.07F	18.94C	11.71G	
					Second season; 2024					
Control	22.90g	24.83c	20.77i	27.90a	21.17hi	23.55ef	21.21hi	24.22d	22.47g	23.22A
2000 mg/L	23.47f	18.05k	16.46l	26.99b	18.02k	21.57h	15.77m	20.50i	14.42p	19.51B
4000 mg/L	17.88k	13.54q	15.91m	23.97de	18.05k	14.70op	11.69s	19.21j	13.69q	16.52C
6000 mg/L	18.23k	12.16r	8.02v	18.50j	13.94q	14.64op	10.68t	15.10no	8.28v	13.32D
8000 mg/L	15.46mn	9.35u	2.39y	16.84l	8.46v	10.3t	6.83w	11.67s	5.16x	9.62E
Mean	19.59B	15.59F	12.71H	22.91A	15.93E	16.97D	13.24G	18.21C	12.80H	

Values have the same letter are not significantly different at 5% level using Duncan's Multiple Range Test

Table 7. Effect of salinized water on fresh weight of stems (g/plant) of tested olive cultivars and genotypes in (2023-2024) seasons.

Cultivars & Genotypes / Salt treatments	Cerasuola	Criolla	Giza 32	Giza 48	Giza 55	Giza 91	Giza 92	Giza 99	Giza 102	Mean
					First season; 2023					
Control	21.64d	21.78d	19.52l	24.47a	21.60d	20.75e	16.96k	23.51b	18.54h	20.53A
2000 mg/L	19.93f	17.52j	13.33op	22.35c	16.84k	18.17hi	13.96n	19.34g	13.18op	17.18B
4000 mg/L	17.75ij	14.69m	11.88rs	20.06f	14.56m	15.88l	12.79pq	14.55m	11.24t	14.2C
6000 mg/L	13.38o	11.55st	8.98vw	14.27mn	11.49st	11.93rs	9.42uv	12.62q	9.41uv	11.45D
8000 mg/L	10.97t	8.55w	6.81y	12.37qr	8.49w	8.93vw	7.42x	9.62u	5.73z	8.77E
Mean	16.73B	14.82E	11.30H	18.70A	14.60E	15.13D	12.11F	15.93C	11.62G	
					Second season; 2024					
Control	23.06b	23.48b	19.74f	25.37a	23.28b	22.05c	18.26g	25.71a	16.41i	21.93A
2000 mg/L	20.80e	17.40h	12.78o	23.65b	17.34h	19.67f	13.82lm	20.74e	13.19no	17.71B
4000 mg/L	18.49g	14.29kl	9.22u	21.38d	15.09j	17.46h	13.54mn	14.73jk	12.10p	15.14C
6000 mg/L	13.88lm	7.35w	7.48pq	11.58pq	7.29w	10.53rs	5.12x	10.75r	3.98y	8.66D
8000 mg/L	11.60pq	8.35v	3.19z	11.44q	8.94u	10.03st	8.32v	9.93t	7.11w	8.77D
Mean	17.56B	14.17E	10.48G	18.68A	14.39E	15.95D	11.81F	16.37C	10.56G	

Values have the same letter are not significantly different at 5% level using Duncan's Multiple Range Test

Table 8. Effect of salinized water on fresh weight of roots (g/plant) of tested olive cultivars and genotypes in (2023-2024) seasons.

Cultivars & Genotypes / Salt treatments	Cerasuola	Criolla	Giza 32	Giza 48	Giza 55	Giza 91	Giza 92	Giza 99	Giza 102	Mean
	First season; 2023									
Control	23.54d	21.25g	18.21jk	24.47c	26.84b	22.73e	19.87hi	28.48a	18.68j	22.67A
2000 mg/L	21.44g	18.15jk	13.51no	22.03f	20.17h	19.78hi	16.64l	22.05f	14.15n	18.66B
4000 mg/L	19.26i	14.11n	9.73t	19.84h	15.28m	16.58l	13.01op	17.90k	10.51s	15.15C
6000 mg/L	15.77m	12.19qr	7.98v	16.53l	12.01r	13.75n	10.69s	13.58no	8.49uv	12.33D
8000 mg/L	12.77pq	9.79t	4.98x	12.53p-r	9.01u	10.75s	8.69u	10.55s	6.29w	9.48E
Mean	18.56B	15.10D	10.88G	19.10A	16.66C	16.72C	13.78E	18.51B	11.62F	
	Second season; 2024									
Control	23.76d	22.81e	19.81hi	25.67c	28.27b	23.93d	20.87fg	29.28a	20.28gh	23.85A
2000 mg/L	23.34de	20.45fg	12.01rs	23.12e	20.71fg	21.02f	17.29k	22.71e	15.45l	19.57B
4000 mg/L	19.56i	15.81l	10.93tu	20.74fg	15.58l	17.53k	13.24op	17.66k	12.11qr	15.91C
6000 mg/L	14.67m	14.01n	9.68x	18.93j	12.68pq	14.46mn	10.89t-v	13.04op	9.89wx	13.14D
8000 mg/L	13.35o	7.49y	6.08z	13.17op	10.64uv	11.45st	10.29vw	12.75op	7.49y	10.30E
Mean	18.94B	16.11D	11.70G	20.33A	17.58C	17.68C	14.52E	19.09B	13.04F	

Values have the same letter are not significantly different at 5% level using Duncan's Multiple Range Test

Table 9. Effect of salinized water on relative water content of tested olive cultivars and genotypes in (2023-2024) seasons.

Cultivars & Genotypes / Salt treatments	Cerasuola	Criolla	Giza 32	Giza 48	Giza 55	Giza 91	Giza 92	Giza 99	Giza 102	Mean
	First season; 2023									
Control	89.09b	81.11i	75.00m	92.31a	85.71f	88.24c	84.62g	89.09b	80.00j	85.02A
2000 mg/L	86.49e	78.57k	73.23n	87.50d	80.00j	80.955i	80.00j	85.71f	76.92l	81.04B
4000 mg/L	80.00j	72.73o	69.23q	84.62g	76.92l	75.00m	73.33n	80.00j	71.43p	75.92C
6000 mg/L	80.00j	66.67t	61.11v	83.33h	68.42r	73.33n	68.42r	76.92l	63.54u	71.30D
8000 mg/L	73.33n	55.83x	52.21y	76.67l	60.11w	66.92t	60.23w	67.67s	55.92x	63.21E
Mean	81.78B	70.98G	66.16I	84.89A	74.23E	76.89D	73.32F	79.88C	69.56H	
	Second season; 2024									
Control	89.13b	80.43h	78.74k	90.74a	86.49d	87.97c	79.22jk	88.65bc	79.62ij	84.55A
2000 mg/L	88.76b	75.61m	73.34o	90.40a	76.98l	79.67ij	74.80n	86.26d	74.96mn	80.09B
4000 mg/L	84.32e	73.59o	69.76r	83.29f	73.68o	77.57l	72.93op	79.21jk	71.46q	76.15C
6000 mg/L	80.11hi	65.21t	62.42v	82.56g	68.17s	73.38o	64.94t	74.56n	65.49t	70.76D
8000 mg/L	72.35p	58.11w	52.11z	74.96mn	62.03v	63.56u	56.25x	69.86r	54.90y	62.68E
Mean	82.93B	70.59F	67.17I	84.39A	73.47E	76.43D	69.63G	79.71C	69.29H	

Values have the same letter are not significantly different at 5% level using Duncan's Multiple Range Test

maintained the highest RWC levels under salinity stress (84.89 and 84.39 % in the first and the second season, respectively), followed by Cerasuola, and Giza 99. These findings suggest that both of them possess enhanced osmotic adjustment and water retention capacity, indicative of their strong salt tolerance. In contrast, Giza 32-identified as the most salt-sensitive genotype-recorded the lowest significant RWC values (66.16 and 67.17%) at all salinity levels during both seasons. Intermediate RWC values were observed in Giza 91, Giza 55, Giza 92, Criolla, and Giza 102, with statistically significant differences influenced by genotype and salinity treatment. The interaction effect was significant, with Giza 48, Cerasuola and Giza 99 retained higher RWC under severe salinity stress (8000 mg/ L), while Giza 32 showed the weakest performance. Therefore, RWC may serve as a reliable physiological indicator for differentiating salt-tolerant from salt-sensitive olive genotypes.

3.6.Effect of salinized water on leaf total chlorophyll content:

Total chlorophyll content in olive leaves was significantly affected by salinity during both experimental seasons. As shown in Table (10), increasing NaCl concentrations (from 2000 to 8000 mg/L) led to a marked and progressive reduction in total chlorophyll compared to the control, underscoring the high sensitivity of photosynthetic pigments to salt stress.

With regard to the genotyped variation, Giza 48 consistently maintained the highest chlorophyll content under all salinity levels (1.545 and 1.640 mg/ gm F.W. in both seasons, respectively), followed by Cerasuola, Giza 99, while Giza 91, Giza 55, Giza 92, Criolla, and Giza 102 exhibited intermediate values. In contrast, Giza 32 recorded the lowest chlorophyll content across all treatments (0.9401 and 0.9601 mg/ gm F.W.) in both seasons. The statistically significant interaction between genotype and salinity level supports these findings, indicating that Giza 48 exhibited remarkable pigment stability under increasing salinity. The ability of Giza 48 to retain higher chlorophyll levels under salt stress reflects enhanced photosynthetic efficiency and physiological resilience, reinforcing its classification as a highly salt-tolerant genotype. In contrast, Giza 32 showed substantial pigment degradation; the severe reduction in chlorophyll content observed in this genotype indicates limited adaptive capacity and high susceptibility to salt-induced oxidative damage.

Table 10. Effect of salinized water on total chlorophyll (mg/gm F.W.) of tested olive cultivars and genotypes in (2023-2024) seasons.

Cultivars & Genotypes / Salt treatments	Cerasuola	Criolla	Giza 32	Giza 48	Giza 55	Giza 91	Giza 92	Giza 99	Giza 102	Mean
	First season; 2023									
Control	1.795a	1.624d	1.299mn	1.787a	1.601e	1.637d	1.574e	1.665c	1.430i	1.602A
2000 mg/L	1.689b	1.463gh	1.101t	1.658c	1.479g	1.511f	1.415i-k	1.530f	1.283no	1.459B
4000 mg/L	1.512f	1.371l	0.957v	1.523f	1.396k	1.400jk	1.291mn	1.455h	1.214q	1.347C
6000 mg/L	1.418ij	1.253p	0.832x	1.457h	1.267op	1.306m	1.163s	1.353l	1.045u	1.233D
8000 mg/L	1.257p	1.059u	0.510z	1.302mn	1.113t	1.162s	0.937w	1.192r	0.739y	1.030E
Mean	1.534B	1.354F	0.940I	1.545A	1.371E	1.403D	1.278G	1.439C	1.142H	
	Second season; 2024									
Control	1.829a	1.663f	1.139op	1.825a	1.732c	1.644f	1.453j	1.734c	1.412k	1.623A
2000 mg/L	1.741c	1.483i	1.098u	1.771b	1.537gh	1.548g	1.350mn	1.649f	1.312p	1.499B
4000 mg/L	1.681e	1.434j	0.961w	1.705d	1.446j	1.447j	1.219q	1.520h	1.162s	1.397C
6000 mg/L	1.539gh	1.234q	0.875x	1.538gh	1.340n	1.337no	1.034v	1.378l	1.025v	1.256D
8000 mg/L	1.344mn	1.015v	0.548z	1.360lm	1.104tu	1.122t	0.890x	1.196r	0.782y	1.040E
Mean	1.627B	1.366F	0.960I	1.640A	1.432D	1.420E	1.189G	1.496C	1.139H	

Values have the same letter are not significantly different at 5% level using Duncan's Multiple Range Test

3.7. Effect of salinized water on leaf mineral content:

Sodium (Na+):

A presented data in Table (11), increasing the concentration of saline irrigation water from 2000 to 8000 mg/L NaCl resulted in a consistent and significant increase in leaf sodium (Na^+) content across all cultivars and genotypes during both seasons, relative to the control. The salt-sensitive genotype Giza 32 exhibited the highest Na^+ accumulation under salinity stress (0.922 and 1.014 g/ 100 g D.W. in both seasons, respectively), followed by the moderately tolerant genotypes Giza 102, Giza 92, Criolla, Giza 55, and Giza 91. In contrast, the highly salt-tolerant genotypes Giza 48 (0.560 and 0.596 g/ 100 g D.W.) and Cerasuola, (0.560 and 0.621 g/ 100 g D.W.), followed by Giza 99 (0.634 and 0.729 g/ 100 g D.W.), recorded the lowest Na^+ content across all salinity levels and in both seasons, respectively. A significant interaction between genotype and salinity level confirmed substantial genotypic control over Na+ uptake. Notably, Giza 48 demonstrated remarkable salt exclusion capacity, whereas, Giza 32 was the most susceptible to Na^+ accumulation under saline conditions.

Table 11. Effect of salinized water on sodium (g/100 g D.W.) content of tested olive cultivars and genotypes in (2023-2024) seasons.

Cultivars & Genotypes / Salt treatments	Cerasuola	Criolla	Giza 32	Giza 48	Giza 55	Giza 91	Giza 92	Giza 99	Giza 102	Mean
	First season; 2023									
Control	0.111u	0.179r-t	0.224r	0.108u	0.159s-u	0.141s-u	0.152s-u	0.130tu	0.194rs	0.155E
2000 mg/L	0.425q	0.649k-m	0.690jk	0.477p	0.641k-n	0.594m-o	0.630l-n	0.571o	0.655kl	0.592D
4000 mg/L	0.518p	0.831i	0.899gh	0.484p	0.733j	0.629l-n	0.710j	0.587no	0.863hi	0.695C
6000 mg/L	0.834i	1.053e	1.336b	0.830i	0.981f	0.914gh	0.928fg	0.904gh	1.236c	1.002B
8000 mg/L	0.913gh	1.122d	1.461a	0.899gh	1.100de	1.082de	1.091de	0.980f	1.316b	1.107A
Mean	0.560G	0.767C	0.922A	0.560G	0.723D	0.672E	0.702D	0.634F	0.853B	
	Second season; 2024									
Control	0.131tu	0.200r-t	0.214rs	0.120u	0.195r-t	0.161s-u	0.193r-t	0.171r-u	0.237r	0.180E
2000 mg/L	0.463q	0.700n-p	0.756n	0.513q	0.649p	0.717n-p	0.719n-p	0.682op	0.692n-p	0.655D
4000 mg/L	0.750no	0.960g-k	1.251d	0.528q	0.897j-m	0.873lm	0.891k-m	0.689n-p	0.983g-i	0.869C
6000 mg/L	0.849m	1.262d	1.379c	0.903j-m	0.965g-j	0.920i-m	0.933h-l	0.991gh	1.391c	1.066B
8000 mg/L	0.911j-m	1.374c	1.470b	0.918i-m	1.111f	1.008g	1.177e	1.113f	1.544a	1.181A
Mean	0.621G	0.899C	1.014A	0.596G	0.763DE	0.736EF	0.783D	0.729F	0.969B	

Values have the same letter are not significantly different at 5% level using Duncan's Multiple Range Test

Potassium (K^+):

According to Table (12), salinity stress significantly reduced leaf potassium (K^+) content in all tested cultivars and genotypes. As NaCl concentration increased, K^+ content progressively and significantly declined compared to the control, indicating the well-known antagonistic relationship between Na^+ and K^+ uptake under saline conditions. Significant differences among genotypes were detected. Giza 48 consistently maintained the highest K^+ concentrations (0.954 and 0.951 g/ 100 g D.W. in the two seasons, respectively), followed by Cerasuola and Giza 99. Intermediate K^+ values were recorded by Giza 91, Giza 55, Criolla, Giza 92, and Giza 102. In contrast, Giza 32 exhibited the lowest K^+ levels under salinity (0.801 and 0.799 g/ 100 g D.W.). The significant interaction between genotypes and salinity highlighted the superior capacity of Giza 48 for preserve K^+ homeostasis under stress, whereas Giza 32 demonstrated marked K^+ depletion.

Table 12. Effect of salinized water on potassium (g/100 g D.W.) content of tested olive cultivars and genotypes in (2023-2024) seasons.

Cultivars & Genotypes Salt treatments	Cerasuola	Criolla	Giza 32	Giza 48	Giza 55	Giza 91	Giza 92	Giza 99	Giza 102	Mean
	First season; 2023									
Control	1.069ab	1.036bc	0.978d-f	1.100a	1.018cd	1.063ab	0.985d-f	1.054bc	0.971ef	1.030A
2000 mg/L	1.014c-e	0.962fg	0.893i-k	1.038bc	0.950f-h	0.988d-f	0.921g-i	0.986d-f	0.925g-i	0.964B
4000 mg/L	0.922g-i	0.883i-l	0.814n-p	0.949f-h	0.898i-k	0.906h-j	0.848l-n	0.925g-i	0.830m-o	0.886C
6000 mg/L	0.855k-n	0.790o-q	0.710st	0.872j-m	0.781p-r	0.815n-p	0.752q-r	0.824n-p	0.745rs	0.794C
8000 mg/L	0.792o-q	0.710st	0.610v	0.811n-p	0.716s	0.733s	0.670tu	0.754q-s	0.660u	0.717E
Mean	0.930B	0.876D	0.801F	0.954A	0.873D	0.901C	0.835E	0.909C	0.826E	
	Second season; 2024									
Control	1.056a	1.003b-f	0.977c-f	1.032ab	1.018a-c	1.009b-e	1.011b-d	1.025ab	1.007b-e	1.015A
2000 mg/L	1.027ab	0.968d-g	0.900h-k	1.025ab	0.994b-f	0.991b-f	0.966e-g	0.963fg	1.002b-f	0.982B
4000 mg/L	0.998b-f	0.904h-j	0.817mn	1.001b-f	0.919hi	0.935gh	0.893i-k	0.966e-g	0.875j-l	0.923C
6000 mg/L	0.861kl	0.793n-p	0.688u	0.877j-l	0.815mn	0.06no	0.774o-q	0.849lm	0.762p-r	0.803D
8000 mg/L	0.809m-o	0.729r-t	0.615v	0.818mn	0.746q-s	0.765p-r	0.710s-u	0.777n-q	0.691tu	0.740E
Mean	0.950A	0.979D	0.799E	0.951A	0.898C	0.901BC	0.871D	0.916B	0.867D	

Values have the same letter are not significantly different at 5% level using Duncan's Multiple Range Test

Potassium/Sodium (K^+/Na^+) selectivity ratio:

As shown in Table (13), the K^+/Na^+ selectivity ratio was markedly reduced by salinity across all cultivars and genotypes in both growing seasons. The ratio declined significantly with increasing NaCl concentrations, from 7.063 in the control to 0.670 at 8000 mg/L in the first season, and from 5.940 to 0.660 in the second season. Giza 48 and Cerasuola, followed by Giza 99, maintained the highest K^+/Na^+ ratios under salinity stress, reflecting their ability to mitigate ionic imbalance. In contrast, Giza 32 consistently recorded the lowest K^+/Na^+ selectivity ratios, indicating a restricted ability to maintain ion homeostasis. The other genotypes exhibited intermediate values, with significant variation among them in both seasons.

Table 13. Effect of salinized water on K/Na selectivity ratio of tested olive cultivars and genotypes in (2023-2024) seasons.

Cultivars & Genotypes Salt treatments	Cerasuola	Criolla	Giza 32	Giza 48	Giza 55	Giza 91	Giza 92	Giza 99	Giza 102	Mean
	First season; 2023									
Control	9.63b	5.79f	4.37h	10.20a	6.41e	7.55d	6.50e	8.11c	5.01g	7.06A
2000 mg/L	2.39i	1.48m-o	1.29o-q	2.18ij	1.48m-o	1.66l-n	1.46l-n	1.73k-m	1.41n-p	1.68B
4000 mg/L	1.78kl	1.06q-s	0.91s-u	1.96jk	1.23o-r	1.44n-p	1.19p-r	1.59l-n	0.96r-t	1.35C
6000 mg/L	1.03r-t	0.75t-x	0.53w-y	1.05q-s	0.80s-w	0.89s-u	0.81s-v	0.91s-u	0.60v-y	0.82D
8000 mg/L	0.87s-v	0.63u-y	0.42y	0.90s-u	0.65u-y	0.68u-y	0.61v-y	0.77t-x	0.50xy	0.67E
Mean	3.14B	1.94F	1.50H	3.26A	2.11E	2.44D	2.12E	2.62C	1.70G	
	Second season; 2024									
Control	8.08b	5.05f	4.57g	8.76a	5.22e	6.27c	5.24e	6.01d	4.25h	5.94A
2000 mg/L	2.22i	1.38kl	1.19mn	2.00j	1.53k	1.38kl	1.34lm	1.41kl	1.45kl	1.55B
4000 mg/L	1.33lm	0.94o-q	0.65t-w	1.90j	1.02n-p	1.07no	1.00op	1.40kl	0.89o-r	1.13C
6000 mg/L	1.01n-p	0.63u-x	0.50w-y	0.97op	0.84p-s	0.88o-r	0.83p-t	0.86p-r	0.55v-y	0.79D
8000 mg/L	0.89o-r	0.53v-y	0.42y	0.89o-r	0.67s-w	0.76q-u	0.60u-y	0.70r-v	0.45xy	0.66E
Mean	2.71B	1.71E	1.47F	2.90A	1.86D	2.07C	1.80D	2.08C	1.52F	

Values have the same letter are not significantly different at 5% level using Duncan's Multiple Range Test

Calcium (Ca^{2+}):

Leaf calcium (Ca^{2+}) content was significantly affected by both salinity stress and genotype in both seasons. As indicated in Table (14), irrigation with increasing NaCl concentrations (2000-8000 mg/L) led to a gradual and significant decrease in Ca^{2+} levels compared to the control (tap water), which maintained the highest values. All tested cultivars and genotypes followed this decreasing trend, consistent with the pattern observed for K^+ content. Giza 48 maintained the highest Ca^{2+} levels (1.247 and 1.208 g/ 100 g D.W. in both seasons, respectively), followed by Cerasuola and Giza 99. In contrast, Giza 32 recorded the lowest Ca^{2+} values (0.993 and 0.991 g/ 100 g D.W.) across all saline treatments and seasons. Intermediate values were found in Giza 91, Giza 55, Criolla, Giza 92, and Giza 102, with significant differences among them. The interaction effect confirmed the superior performance of Giza 48 in sustaining Ca^{2+} levels under salt stress, while Giza 32 was the least effective. Collectively, the observed patterns of mineral accumulation reinforce the morphological classification of salt tolerance among the tested olive genotypes. Elevated K^+ and Ca^{2+} contents, reduced Na^+ accumulation, and higher K^+/Na^+ selectivity ratios in salt-tolerant genotypes -most notably Giza 48, Cerasuola, and Giza 99- indicate efficient ionic regulation and adaptive capacity under salinity stress. In contrast, the impaired ionic balance in Giza 32 aligns with its pronounced sensitivity to salinity and the corresponding reductions in growth and physiological performance.

Table 14. Effect of salinized water on calcium (g/100 g D.W.) content of tested olive cultivars and genotypes in (2023-2024) seasons.

Cultivars & Genotypes / Salt treatments	Cerasuola	Criolla	Giza 32	Giza 48	Giza 55	Giza 91	Giza 92	Giza 99	Giza 102	Mean
	First season; 2023									
Control	1.402b	1.335d	1.161h-j	1.458a	1.364cd	1.392bc	1.357cd	1.386bc	1.219fg	1.342A
2000 mg/L	1.261e	1.213fg	1.029no	1.348d	1.219fg	1.237ef	1.219fg	1.256e	1.101kl	1.209B
4000 mg/L	1.163hi	1.015op	0.957qr	1.188gh	1.077lm	1.126jk	1.077lm	1.155h-j	0.984pq	1.082C
6000 mg/L	1.101kl	0.993p	0.925r	1.133i-k	1.032no	1.056mn	1.032no	1.078lm	0.956qr	1.034D
8000 mg/L	1.086lm	0.939r	0.892s	1.106kl	0.986pq	1.008op	0.988pq	1.085lm	0.93i-k	1.003E
Mean	1.203B	1.099E	0.993G	1.247A	1.136D	1.164C	1.135D	1.192B	1.040F	
	Second season; 2024									
Control	1.344b	1.196g-i	1.141j-l	1.398a	1.260d-f	1.286c-e	1.220f-h	1.310bc	1.164ij	1.258A
2000 mg/L	1.269c-f	1.184h-j	1.011r-w	1.304b-d	1.233f-h	1.240e-g	1.223f-h	1.239e-g	1.096l-n	1.200B
4000 mg/L	1.169ij	1.014q-v	0.983u-x	1.186h-j	1.048n-s	1.111k-m	1.021p-u	1.157i-k	0.997s-w	1.075C
6000 mg/L	1.065m-q	0.960q-y	0.920yz	1.078m-o	1.055n-r	1.061m-r	0.967v-y	1.066m-q	0.938x-z	1.012D
8000 mg/L	1.041o-t	0.967v-y	0.910z	1.072m-p	1.00r-w	1.010r-w	0.993t-w	1.024p-u	0.928x-z	0.995E
Mean	1.178B	1.064F	0.991H	1.208A	1.121D	1.142C	1.085E	1.159BC	1.024G	

Values have the same letter are not significantly different at 5% level using Duncan's Multiple Range Test

3.8.Effect of salinized water on leaf proline content:

Leaf proline content was significantly affected by salinity treatments across all olive cultivars and genotypes during both growing seasons, as presented in Table (15). Proline accumulation increased progressively with increasing NaCl concentrations from (2000 to 8000 mg/L) compared to the control (tap water). Among the studied cultivars and genotypes, Giza 48 (0.287 and 0.304 µg/g D.W.) and the Cerasuola cultivar (0.280 and 0.290 µg/g D.W.) recorded the highest proline accumulation under salinity stress in both seasons, followed by Giza 99 and Giza 91. Conversely, the salt-sensitive genotype Giza 32 exhibited the lowest proline concentrations (0.224 and 0.247 µg/g D.W.) across all salinity treatments. Intermediate proline levels were observed in Giza 55, Giza 92, Criolla, and Giza 102, with statistically significant differences among them. The interaction effect indicated that

Table 15. Effect of salinized water on proline (µg/g D.W.) content of tested olive cultivars and genotypes in (2023-2024) seasons.

Cultivars & Genotypes / Salt treatments	Cerasuola	Criolla	Giza 32	Giza 48	Giza 55	Giza 91	Giza 92	Giza 99	Giza 102	Mean
	First season; 2023									
Control	0.154st	0.136t-v	0.114w	0.161s	0.143s-u	0.146s-u	0.131u-w	0.149st	0.120vw	0.139E
2000 mg/L	0.264i-n	0.245n-q	0.220r	0.267h-m	0.248m-q	0.253l-p	0.240o-q	0.255l-o	0.235p-r	0.247D
4000 mg/L	0.269h-l	0.258k-o	0.232qr	0.271h-l	0.261j-n	0.262i-n	0.258k-o	0.268h-l	0.252l-p	0.259C
6000 mg/L	0.296fg	0.278g-j	0.253l-p	0.309f	0.281g-i	0.280g-j	0.276h-k	0.284gh	0.279g-j	0.282B
8000 mg/L	0.41a	0.377cd	0.303f	0.425a	0.381c	0.387bc	0.361de	0.400b	0.346e	0.378A
Mean	0.280A	0.259CD	0.224F	0.287A	0.263C	0.266BC	0.253DE	0.271B	0.246E	
	Second season; 2024									
Control	0.15uv	0.141v-y	0.134xy	0.171u	0.1550v-x	0.154u-w	0.139w-y	0.155u-w	0.131y	0.148E
2000 mg/L	0.290l-o	0.268q-s	0.241t	0.296j-m	0.273p-r	0.277n-q	0.257r-t	0.278m-q	0.252st	0.270D
4000 mg/L	0.296j-m	0.277n-q	0.257r-t	0.299i-l	0.286l-q	0.290l-o	0.272o-r	0.292k-n	0.270p-r	0.282C
6000 mg/L	0.321h	0.310h-k	0.288l-p	0.325gh	0.311h-j	0.317hi	0.301i-l	0.317hi	0.302i-l	0.310B
8000 mg/L	0.427ab	0.374ef	0.317hi	0.431a	0.382de	0.397cd	0.360f	0.411bc	0.341g	0.382A
Mean	0.290A	0.274D	0.247F	0.304A	0.280CD	0.287BC	0.266E	0.291B	0.259E	

Values have the same letter are not significantly different at 5% level using Duncan's Multiple Range Test

Giza 48 and the Cerasuola cultivar accumulated the highest proline content under 8000 mg/ L salinity. Overall, proline accumulation was significantly enhanced in response to salinity across all genotypes; however, the magnitude of this response was clearly genotype-dependent. These findings underscore the functional role of proline in osmotic adjustment and highlight its potential utility as a biochemical marker for screening and selection of salt-tolerant olive cultivars and genotypes.

3.9.Leaf salinity hazard coefficient:

The leaf salinity hazard coefficient (LSHC) was significantly influenced by salinity treatments in both experimental seasons, as shown in Table (16). A progressive and statistically significant increase in LSHC was recorded with rising NaCl concentrations (2000-8000 mg/L) across all tested cultivars and genotypes relative to the control (tap water). This trend reflects the enhanced accumulation of toxic salts within leaf tissues under high salinity conditions. Clear genotypic variation in LSHC responses were evident that, Giza 32 consistently exhibited the highest LSHC values at all salinity levels (2.038 and 2.143 in the first and the second seasons, respectively), indicating a greater susceptibility to ionic toxicity and salt-induced cellular damage. In contrast, Giza 48 recorded the lowest LSHC values (1.881 and 1.860), confirming its superior capacity to restrict excessive salt accumulation, followed by Cerasuola and Giza 99. Intermediate LSHC values were observed in, Giza 91, Giza 55, Carolea, Giza 92, and Giza 102, with significant differences among them.

As the interaction effect further confirmed that LSHC is strongly influenced by both salinity level and genetic background. Genotypes such as Giza 48 followed by Cerasuola and Giza 99, demonstrated more efficient salt-exclusion mechanisms, contributing to their enhanced adaptability. In contrast, genotypes like Giza 32 showed limited salt-exclusion capacity, consistent with their heightened sensitivity to salt stress. These findings reinforce the relevance of LSHC as a physiological indicator for assessing salinity tolerance.

Table 16. Effect of salinized water on leaf salinity hazard coefficient of tested olive cultivars and genotypes in (2023-2024) seasons.

Cultivars & Genotypes Salt treatments	Cerasuola	Criolla	Giza 32	Giza 48	Giza 55	Giza 91	Giza 92	Giza 99	Giza 102	Mean
	First season; 2023									
Control	1.785t-v	1.776uv	1.854o-q	1.658x	1.809st	1.823rs	1.795tu	1.766v	1.792tu	1.784E
2000 mg/L	1.845p-r	1.840qr	1.938lm	1.732w	1.824rs	1.866op	1.820rs	1.804st	1.895n	1.840D
4000 mg/L	1.951l	1.981k	1.948l	1.873no	1.855o-q	1.861o-q	1.943lm	1.921m	1.991jk	1.924C
6000 mg/L	1.840qr	2.136ef	2.177cd	2.010ij	2.113fg	2.031i	2.155de	1.957l	2.185c	2.067B
8000 mg/L	2.057h	2.225b	2.270a	2.132ef	2.162cd	2.116fg	2.282a	2.098g	2.211b	2.173A
Mean	1.896G	1.992C	2.038A	1.881H	1.953D	1.939E	1.999C	1.909F	2.015B	
	Second season; 2024									
Control	1.754y	1.853r-u	1.981l	1.750y	1.871qr	1.845t-v	1.820w	1.730z	1.923o	1.836E
2000 mg/L	1.823w	1.926o	1.996kl	1.835u-w	1.847s-v	1.897p	1.860r-t	1.794x	1.928o	1.878D
4000 mg/L	1.836u-w	1.957mn	2.150ef	1.831vw	1.928o	1.941no	2.029j	1.865rs	2.112gh	1.961C
6000 mg/L	1.935o	2.057i	2.131fg	1.924o	2.009k	1.999kl	2.114gh	1.889pq	2.162e	2.044B
8000 mg/L	2.068i	2.108h	2.455a	1.962m	2.155e	2.134f	2.245a	2.216d	2.273b	2.176A
Mean	1.883G	1.980D	2.143A	1.860H	1.962E	1.963E	2.014C	1.99F	2.100B	

Values have the same letter are not significantly different at 5% level using Duncan's Multiple Range Test

3.10. Effect of salinized water on anatomical parameters:

Leaf anatomy:

Anatomical investigations were conducted on three olive genotypes subjected to different salinity levels: Giza 48 (high salt-tolerant at 8000 mg/L NaCl), Giza 55 (moderate salt-tolerant at (6000 mg/L), and Giza 32 (salt-sensitive at 4000 mg/L), during the 2024 season on leaf and root tissues.

Salinity stress induced noticeable structural modifications in the midrib, epidermal layers, mesophyll tissue, and vascular bundles. Statistically significant differences were observed among the genotypes for most measured traits, except for the lower epidermis, which showed no significant changes. Regarding upper epidermis thickness, Giza 48 exhibited a 33.33% increase, suggesting improved protection against water loss. In contrast, Giza 55 and Giza 32 had reductions of 50% and 25%, respectively. Midrib thickness exhibited genotype-specific responses. Giza 48 showed a 36.36% increase, which may reflect stronger support for transport tissues, while Giza 55 and Giza 32 showed reductions of 34.38% and 13.16%, respectively. The mesophyll tissue (including palisade and spongy layers) exhibited clear genotype-dependent plasticity. In Giza 48, palisade thickness increased threefold, while spongy parenchyma thickness increased by 33.33%, indicating enhanced photosynthetic capacity and improved internal gas diffusion. Giza 55 also showed a notable increase in palisade tissue (~2-fold), with a slight decrease in spongy tissue (2.89%). Conversely, Giza 32 presented reductions in palisade (20%) and spongy tissues (16.67%), suggesting a decline in photosynthetic efficiency. The palisade-to-spongy tissue ratio increased in Giza 48 (1.28 vs. 0.70 in the control) and in Giza 55 (0.99 vs. 0.47), reflecting improved mesophyll organization conducive to efficient gas exchange. A slight decline in this ratio was noted in Giza 32 (0.40 vs. 0.42), indicating disorganized internal leaf structure under salt stress. Vascular tissue thickness (xylem and phloem) also varied among genotypes. Giza 48 recorded the most pronounced increases in both xylem (83.33%) and phloem (33.33%) thickness, supporting efficient water and nutrient transport. Giza 55 showed moderate increases (xylem: 57.14%; phloem: 25%), whereas Giza 32 exhibited significant reductions (xylem: 42.86%; phloem: 33.33%), indicating impaired vascular transport capacity under salt stress.

Table 17. Effect of salinized water on leaf anatomical parameters.

Leaf parameters		Upper epidermis thickness (mm)	Lower epidermis thickness (mm)	Midrib thickness (mm)	Palisade thickness (mm)	Spongy thickness (mm)	Palisade /spongy ratio	Xylem thickness (mm)	Phloem thickness (mm)
Genotypes	Salt treatment								
Giza 32	Control	60	30	760	150	360	0.42	210	90
	4000 mg/L	30	30	660	120	300	0.4	120	60
	*RV (%)	-50	0	-13.16	-20	-16.67		-42.86	-33.33
Giza 55	Control	60	30	860	210	450	0.47	210	60
	6000 mg/L	45	30	1160	420	437	0.96	330	75
	RV (%)	-25	0	34.88	100	-2.89		57.14	25
Giza 48	Control	45	30	990	210	300	0.7	180	90
	8000 mg/L	60	30	1350	510	430	1.19	330	120
	RV (%)	33.33	0	36.36	142.86	43.33		83.33	33.33

*RV (%) = ((a-b) / b) x 100, where *a* represents the value under salinity treatment, and *b* represents the value under control.

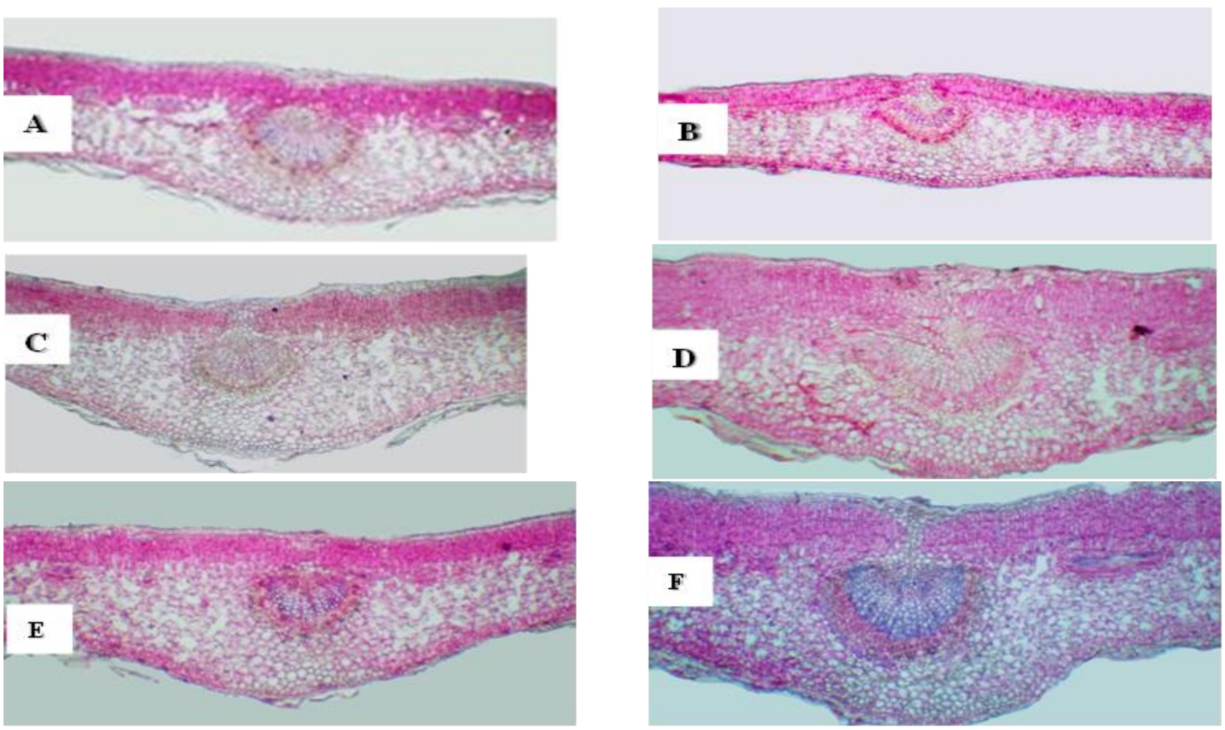

Figure 1. Effect of salinized water on transverse sections in the main vein of olive leaves (10Xs) (A): anatomical structure of Giza 32 leaves under control (without salt treatment); (B): under salinity stress (4000 mg/L NaCl); (C) anatomical structure of Giza 55 leaves under control; (D): under salinity stress (6000 mg/L NaCl); (E) anatomical structure of Giza 48 leaves under control; and (F): under salinity stress (8000 mg/L NaCl).

Root anatomy:

Root anatomical traits exhibited clear genotype-dependent responses to salinity stress. In the salt-tolerant genotype Giza 48, salinity induced increases in root diameter (15.09%) and cortex thickness (19.79%), reflecting enhanced structural development and potential adaptation. Similarly, Giza 55 showed moderate increases in these parameters, while Giza 32 experienced notable declines in root diameter (11.76%) and cortex thickness (37.50%), reflecting impaired development and structural regression under salinity. Epidermal thickness remained relatively unchanged across all genotypes, suggesting that this outer layer was less responsive to salinity stress. In contrast, the vascular cylinder diameter increased significantly in Giza 48 (40.74%) and Giza 55 (36%), highlighting their capacity for anatomical plasticity under salt stress. Although Giza 32 appeared to have a wider vascular cylinder (1200 µm vs. 645 µm), this was accompanied by tissue deformation, indicating anatomical disruption rather than functional improvement. Regarding vascular organization, Giza 48 maintained a stable number of xylem arms and phloem poles (7 in both control and stress conditions), reflecting its ability to preserve vascular structure and transport efficiency under salinity. Giza 55 showed only minor anatomical changes, consisting its moderate tolerance. In contrast, Giza 32 exhibited severe structural damage, with deformed or absent xylem and phloem tissues and disorganized parenchyma, highlighting loss of vascular integrity and reduced capacity to cope with salt stress.

Table 18. Effect of salinized water on root anatomical parameters

Root parameters							
Genotypes	**Salt treatment**	**Root diameter (mm)**	**Epidermis thickness (mm)**	**Cortex thickness (mm)**	**Vascular cylinder diameter (mm)**	**Xylem arms number**	**Phloem pole number**
	Control	2550	30	960	645	6	6
Giza 32	**4000 mg/L**	2250	30	600	1200	0	0
	*** RV (%)**	-11.76	0	-37.5	86.05	-100	-100
	Control	3150	30	900	750	6	6
Giza 55	**6000 mg/L**	3360	30	1010	1020	5	5
	RV (%)	6.67	0	12.22	36	-16.67	-16.67
	Control	2320	30	960	675	7	7
Giza 48	**8000 mg/L**	2670	30	1150	950	7	7
	RV (%)	15.09	0	19.79	40.74	0	0

*RV (%) = ((a-b) / b) x 100, where *a* represents the value under salinity treatment, and *b* represents the value under control.

4. DISCUSSION

Salinity stress is a major abiotic constraint that limits plant growth, physiological function and productivity-particularly in arid and semi-arid regions (Munns and Tester, 2008). The present study aimed to assess the salinity tolerance of two cultivars (Criolla and Cerasuola) and seven olive genotypes (Giza 48, Giza 99, Giza 91, Giza 55, Giza 92, Giza 102, and Giza32) by assessing their growth characteristics, physiological, and biochemical parameters responses to different salinity levels (2000, 4000, 6000, and 8000 mg/L NaCl). The main results concern growth reduction in vegetative characteristic was correlated with increasing NaCl levels from 2000 to 8000ppm. These findings align with previous studies, which have reported that NaCl inhibits plant growth through osmotic inhibition, nutrient imbalance, and ionic toxicity (Chartzoulakis *et al.,* 2002; Garcia- *et al.,* 2018; Boussadia *et al.,* 2023).

Each of Giza 48, Cerasuola, and Giza 99 respectively, was more tolerant to salinity than the other genotypes (Giza 91, Giza 55, Giza 92, Criolla, Giza 102, and Giza32), due to their superior ability to maintain osmotic adjustment and turgor through the accumulation of compatible solutes and preservation of root hydraulic conductivity. In contrast, the reduced growth observed in Giza 32 under salt stress indicates its limited capacity to retain water, as reflected in its lower RWC (Munns *et al*., 2020).

Total chlorophyll content declined significantly in Giza 32 under salinity, which is consistent with NaCl-induced degradation of chlorophyll due to oxidative damage, chloroplast ultrastructure disruption, and pigment-protein complex breakdown (Parida & Das, 2005; El Yamani & Cordovilla, 2024). Conversely,

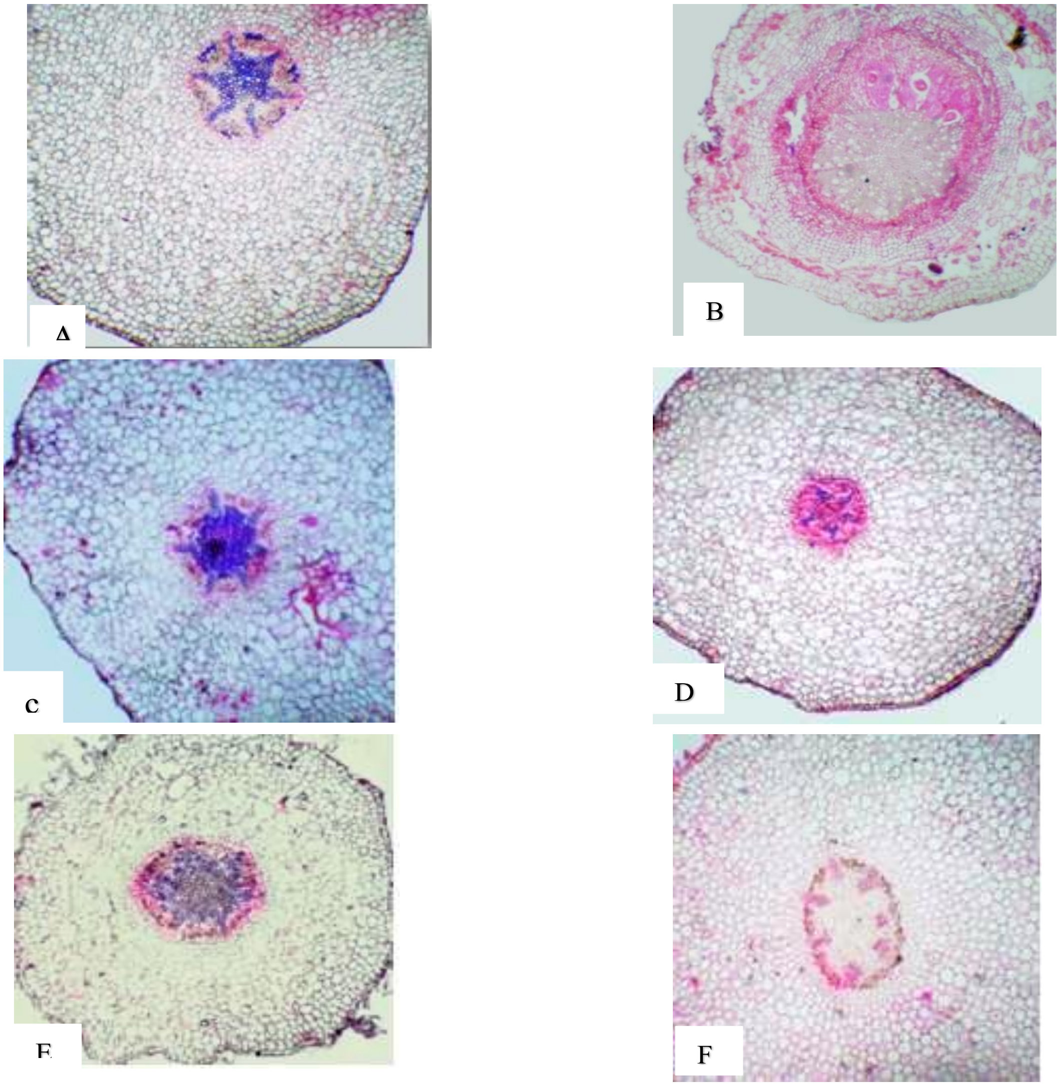

Figure Effect of salinized water on transverse sections in the root of olive leaves (10Xs) (A): anatomical structure of Giza 32 roots under control (without salt treatment); (B): under salinity stress (4000 mg/L NaCl); (C) anatomical structure of Giza 55 roots under control; (D): under salinity stress (6000 mg/L NaCl); (E)anatomical structure of Giza 48 roots under control; and (F): under salinity stress (8000 mg/L NaCl).

the stability of pigment levels in Giza 48 Cerasuola, and Giza 99 suggests enhanced protection against reactive oxygen species (ROS), possibly through increased antioxidant enzyme activity or compartmentalization of Na+ ions, which mitigates cellular toxicity, as noted in salt-tolerant olive cultivars (Gucci et al., 1997; Boussadia et al., 2023).

Proline accumulation was markedly higher in Giza 48, Cerasuola, and Giza 99, highlighting its protective role as osmolyte involved in osmotic adjustment, ROS scavenging, and membrane stabilization (Ashraf & Foolad, 2007; Szabados & Savouré, 2010; Trabelsi *et al*., 2021). In contrast, the low proline level in Giza 32 indicates poor biochemical adaptability under saline conditions.

Mineral analysis further supported these findings. Giza 48, Cerasuola, and Giza 99 accumulated significantly less Na^+ and maintained higher levels of K^+ and Ca^{2+}, along with a favorable K^+/Na^+ selectivity ratio, suggesting efficient ion selectivity and exclusion mechanisms essential for sustaining membrane stability and enzyme activity under salinity (Shabala & Munns, 2012; Azimi *et al*., 2021; El Yamani & Cordovilla, 2024). By contrast, Giza 32 demonstrated poor ion regulation, likely due to passive Na^+ uptake and inadequate compartmentalization. The leaf salinity hazard coefficient (LSHC), a physiological indicator of ion toxicity (Borran, 1989; Emtithal *et al*., 1995), further confirmed genotypic differences. Giza 48 exhibited the lowest LSHC values, indicating efficient exclusion or internal detoxification. while Giza 32 had the highest LSHC, reflecting excessive Na^+ accumulation and impaired defense mechanisms.

Anatomical investigations on Giza 48, Giza 55, and Giza 32 revealed that structural plasticity also play a vital role in salinity tolerance. Giza 48 displayed significant thickening of leaf tissues (upper epidermis, palisade mesophyll, spongy parenchyma, and vascular tissues), which likely enhanced water retention, mechanical stability, and photosynthetic efficiency. These features are consistent with previous studies linking tissue thickening to improved tolerance under salinity (Khayyat *et al*., 2007; Barhoumi *et al*., 2010, Ebtesam, 2018). In contrast, Giza 32 showed mesophyll thinning and vascular disorganization, impairing transport and water conduction. Giza 55 demonstrated intermediate anatomical modifications, consistent with its moderate salt tolerance. Root anatomy also reflecting genotypic variation. Giza 48 exhibited increased cortical thickness, larger vascular cylinder diameter, and a more vascular elements, supporting enhanced water and ion uptake under saline conditions (Yeo *et al*., 1987; Hussein *et al*., 2012). In contrast, Giza 32 showed collapsed root structural and reduced conductivity, further impairing salt stress ability.

Based on overall performance under salinity stress, the tested cultivars and genotypes can be classified as follows: Giza 48, Cerasuola and Giza 99 as highly salt-tolerant; Giza 91, Giza 55, Criolla, Giza 92, and Giza 102 as moderately salt-tolerant; and Giza 32 as salt-sensitive. The classification aligns with previous reports on the relative salt tolerance of olive cultivars (Claros *et al*., 2025).

5. CONCLUSION:

From the obtained results, it could be concluded that, Giza 48, Cerasuola, and Giza 99 might be suitable to be grown in reclaimed or marginal lands affected by salinity or irrigated with saline underground water. Moreover, it may serve as valuable candidates in breeding programs or as a salt-resistant rootstock for grafting. Conversely, the salt-sensitive genotype Giza 32 should be restricted to regions where salinity concentrations do not exceed 4000 mg/L.

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الملخص العربي

سلوك بعض الأصناف والطرز الجينية لشتلات الزيتون تحت تركيزات مختلفة من كلوريد الصوديوم

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تعد الملوحة أحد أبرز العوامل المقيدة لزراعة الزيتون في مصر. أجريت هذه الدراسة خلال موسمي ٢٠٢٣–٢٠٢٤ في معهد بحوث البساتين، التابع لمركز البحوث الزراعية (الجيزة، مصر). بهدف تقييم الصفات المورفولوجية والفسيولوجية والكيميائية الحيوية لشتلات صنفين (سيراسيولا وكريولا) وسبعة من الطرز الجينية، جيزة (٣٢ و ٤٨ و٥٥ و ٩١ و ٩٢ و ٩٩ و ١٠٢) عند تعرضها لأربعة تركيزات من كلوريد الصوديوم (٢٠٠٠، ٤٠٠٠، ٦٠٠٠، ٨٠٠٠ ملغ/لتر)، مقارنة بالكنترول (ماء الصنبور).

أظهرت النتائج أن الطراز " جيزة ٤٨" كان الأكثر تحملا للملوحة، حيث احتفظ بأعلى نسبة بقاء، وثبات مؤشرات النمو (إستطالة الساق و مساحة الأوراق والكتلة الحيوية الطازجة)، ومحتوى الكلوروفيل ونسبة الماء النسبي (RWC) ، وتراكم البرولين، وحقق توازن أيوني جيدا (بانخفاض تركيزالصوديوم وارتفاع نسبة K^+/Na^+)، إلى جانب انخفاض معامل خطورة الملوحة الورقي (LSHC). أظهر الصنف سيراسيولا أداء مشابها، يلية "جيزة ٩٩" . وعلى العكس كان "جيزة ٣٢ الأكثر حساسية، وأظهرت الطرز الجينية الاخرى استجابات متوسطة. وفقا للأداء تحت ظروف الملوحة، صنفت جيزة ٤٨، سيراسيولا، جيزة ٩٩ كأصناف متحملة للملوحة، وجيزة ٩١، جيزة ٥٥، كريولا، جيزة ٩٢، وجيزة ١٠٢كمتوسطة الملوحة، وجيزة ٣٢ كحساس للملوحة.

أظهرت الدراسات التشريحية لثلاثة طرز ممثلة–جيزة ٤٨ (متحمل عند ٨٠٠٠ ملغم/لتر NaCl)، جيزة ٥٥ (متوسط التحمل عند ٦٠٠٠ ملغم/لتر NaCl)، وجيزة ٣٢ (حساس عند ٤٠٠٠ ملغم/لتر NaCl)– أن جيزة ٤٨ تميزبسماكة نسيج الورقة (البشرة العليا، النسيج العمادي، النسيج الأسفنجي، الخشب، واللحاء)، وتطور جيد في الأنسجة الجذرية. بينما عانت جيزة ٣٢ من ترقق وتشوه الأنسجة، وأبدى جيزة ٥٥ مرونة تشريحية متوسطة.

ختاما، تعد جيزة ٤٨، سيراسيولا، وجيزة ٩٩ مناسبة للزراعة في الأراضي المستصلحة عالية الملوحة أو المناطق التى تروى بمياه مالحة.

الكلمات المفتاحية: الزيتون؛ الملوحة؛ الأصناف؛ الطرز الجينية؛ كلوريد الصوديوم.