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Exploring Salinity Tolerance Mechanisms in Diverse Egyptian Grape Genotypes Based on Morpho-Physiological, Biochemical, Anatomical and Gene Expression Analysis

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Abstract: Viticulture is one of the agricultural sectors with major economic importance in Mediterranean climate zones. Salinity is considered a substantial issue for agricultural sectors in arid and semi-arid regions, where it has the potential to impair production of grape (*Vitis vinifera*), which is categorize as a moderately sensitive species to salinity, and its impact is expected to increase with climate change. As exploiting genetic diversity is one of the most promising strategies to cope with the negative impacts of climate change on viticulture and adapt under the new conditions to maintain grape production and quality, the study aimed to explore salinity tolerance mechanisms in diverse Egyptian grape genotypes based on morpho-physiological, biochemical, anatomical and gene expression analysis. Nine local grape genotypes; Baltim Eswid, Edkawy, Matrouh Eswid, Bez El-Naka, Bez El-Anza, Romy Ahmer, Gharibi, Fayoumi and Romy Abiad, were evaluated for tolerance under saline conditions. Salinity stress was induced in three levels of 2.28, 3.75 and 5.20 ms (using NaCl at 1000, 2000 and 3000 ppm, respectively) comparing to 695 µs irrigation water as control. Results indicated that, the growth of all investigated local grape genotypes was adversely affected by salt treatments in a cultivar-dependent manner. Proposed salt-tolerance mechanisms including; controlling the growth rate, reducing damage resulting from oxidative stress associated with salinity, keep balanced hydric status, structural alterations allowing protection and regulation of ions uptake. It was observed that, Edkawy local grape cultivar is a promising salt-tolerant genotype. On the other hand, Romy Abiad and Gharibi genotypes were classified as the most salt-sensitive comparable with other tested local cultivars. Bez El-Anza genotype which maintained 100% survivability under severe salinity stress condition was characterized by a remarkable decline in vegetative growth accompanied with keeping more leaves with a marked reduction in leaf area and most measurements of certain anatomical features, slight Na uptake, but undisputed oxidative stress indicators and down-regulated expression folds of *AREB2* transcription factor; a sugar accumulation regulatory related gene. Therefore, local genotypes of Egyptian table grapes can be considered a storehouse of germplasm that should be conserved and not threatened with extinction or complete loss because they are adapted to severe environmental conditions and harsh cultural managements.

Keywords: Grape, Salinity, Genotypes, Oxidative Stress, Biochemical, Anatomical, Gene Expression

1. Introduction

Grapes (*Vitis vinifera*), which is one of the most important fruit species of the Vitaceae family, represented the fruit crop with the highest total value of worldwide production according to the report [1]. Viticulture is one of the agricultural sectors with major economic importance in Mediterranean climate zones. For Egypt, grapes are one of the most widely-grown fruit crops, which is second only to citrus, where Egypt had been ranked 4th worldwide in the global production volume of table grapes [2]. Grapevines are among the most suitable fruit crops for sandy soils and newly reclaimed land, as well as for the older Egyptian land traditionally cultivated next to the Nile River [3]. It is worth to be mentioned that, environmental conditions such as soil and climate are determinant key factors affecting grapevine productivity and grape quality [4].

Salinity is considered a substantial issue for agricultural sectors in arid and semi-arid regions, where it has the potential to impair production of grape (*Vitis vinifera*), which is categorize as a moderately sensitive species to salinity, and its impact is expected to increase with climate change. Research on the physiological and molecular changes that occur in salt-affected grapevines has revealed complex osmotic and ionic responses including oxidative stress, water loss, photoinhibition, growth inhibition and necrosis. Accordingly, elevated antioxidant production, hydric regulation and salt exclusion from shoots and berries were proposed as mechanisms of salt tolerance. Unfortunately, it is often hard to disentangle whether observations made experimentally represent an adaptive response to salinity or are a consequence of damage, either from a whole phenotypic or a molecular response perspective [5]. However, there are still significant gaps in knowledge regarding salt tolerance mechanisms for Vitis species. While, a better understanding of the mechanisms that confer salt tolerance in Vitis species is needed to improve the production of new germplasm that are locally adapted and better suited to the challenges of a changing climate.

Exploiting genetic diversity is one of the most promising strategies to cope with the negative impacts of climate change on viticulture and adapt under the new conditions to maintain grape production and quality. Earlier observations indicated that, Egyptian local table grape varieties could be considered as storehouse of germplasm which should not endangered or lost entirely as they are adapted to hard environmental conditions and severe cultural managements [6]. Accordingly, the aim of the study was to explore salinity tolerance mechanisms in diverse Egyptian grape genotypes based on morpho-physiological, biochemical, anatomical and gene expression analysis.

2. Materials and Methods

2.1. Plant Materials and Experimental Design

A pot culture experiment was conducted under shade net house conditions at the experimental orchard of Horticulture Research Institute during 2021 & 2022 seasons. Six months old own rooted healthy transplants of local grape cultivars; Baltim Eswid, Edkawy, Matrouh Eswid, Bez El-Naka, Bez El-Anza, Romy Ahmer, Gharibi, Fayoumi and Romy Abiad, were evaluated for tolerance under saline conditions. Transplants were chosen of similar growth vigor and were pruned to one main shoot before applications by two months. Each transplant was placed in a polyethylene bag with drainage holes and that was full of peatmoss and washed sand (1:2 v/v) mixture. The pots were irrigated with tap water, fertilized as recommended and protected with regular management practices before starting salt treatments. The experimental treatments were arranged in a complete randomized blocks design. Each treatment was represented in three replicates for each treatment and each replicate contained four uniform transplants. Control plants were irrigated three times weekly with 695 µs irrigation water to field capacity. Salinity stress was induced in three levels of 2.28, 3.75 and 5.20 ms (using NaCl at 1000, 2000 and 3000 ppm, respectively). Thirty percent excess water was added for leaching requirements after every two irrigation treatments. Treatments were applied for two months starting mid of July and collecting data start date by mid of September.

2.2. Morpho-Physiological Determinations

2.2.1. Growth Responses

Survivability percentage was calculated as the ratio of the number of survivors divided by the total count of treated plants. Growth parameters of vegetative; plant height (cm), leaves count, leaf area (cm^2) and leaves moisture content (%), and root system; roots count, root length (cm) and root weight (g), were recorded, then leaf samples were collected for further analysis.

2.2.2. Leaf Relative Water Content (RWC)

The technique that is adopted from the study [7] was used. Empty sample tubes were numbered and weighted (tubeW). Six fully expanded flag leaves from randomly chosen plants in each plot were collected. The top and bottom of all the leaves together, and any dead or dying tissue, were removed to leave a 5 cm mid-section, then immediately placed into the pre-weighted tubes and the lid sealed (so that there is no moisture loss/gain from the system). Immediately the tubes were placed into a cooled, insulated container (at around 10°C-15°C; but not frozen), then all sample tubes were weighted (tubeW+FW). One cm of distilled water was added to each tube and the sample tubes were placed in a refrigerator (at 4°C in darkness) for 24h (for leaves to reach full turgor). Afterwards, the leaf samples were taken out of the tube, quickly and carefully blot dry with paper towel, and weighted (TW; turgid weight). Finally, the leaf samples were placed in a labeled envelope and dried at 70°C for 24h, or until constant mass, then reweighted (DW; dry weight).

For calculation, first obtain the fresh weight (FW) of the leaf samples:

$$FW = (tubeW+FW) - tubeW \quad (1)$$

Then calculate the leaf RWC:

$$\text{Leaf RWC (\%)} = [(\text{FW-DW}) / (\text{TW-DW})] \times 100 \quad (2)$$

where: FW = fresh weight; TW = turgid weight; DW = dry weight.

2.2.3. Root Electrolyte Leakage (REL)

The technique that is most often used [8, 9] has changed little from the initial protocol [10]. Roots were first washed in water to remove soil, then in deionized water to remove any surface ions that may be present. A central mass of roots was removed from the plant; a band about 2.5 cm wide running across the mid-section of the root system. Roots with diameter greater than 2 mm were removed from the sample leaving only "fine" roots. Fine roots (500 mg) are placed into a 28 ml glass vessel containing 16 ml of deionized water. The vessel was then capped, shaken, and left at room temperature for about 24 hours. The conductivity of the solution (C_{live}) was measured with a temperature-compensated electrical conductivity meter. The root samples were removed and killed by autoclaving at 100°C for 10 minutes, then cooled to room temperature. The conductivity of the solution surrounding the dead root samples (C_{dead}) was measured. The REL is calculated as the ratio of the EC of the live roots divided by the EC of the dead roots:

$$\text{REL (\%)} = (C_{live}/C_{dead}) \text{ x } 100 \quad (3)$$

2.3. Biochemical Analysis

2.3.1. Photosynthetic Pigments

The content of chlorophylls; chlorophyll a and chlorophyll b, and total carotenoids were determined [11, 12]. Fresh leaves were cut into small pieces and weighted to 0.5 g. Sample was mixed with acetone and homogenized in a mortar. Very small amount of $MgCO_3$ was added before sample homogenization to prevent chlorophyll pheophytinization. Mixtures were filtered, mortar and pestle were washed several times with 10 ml acetone, and the content was quantitatively transferred to the filter (Whatman No. 1). Filtrate was diluted with acetone to a total volume of 25 ml. Finally, the absorbance of the prepared sample was recorded at three wave-lengths viz. 662, 644 and 440 nm using acetone as blank. The absorbance values were recalculated according to the Holm-Wettstein equations in the pigment concentration (mg/l), and the final result of the pigment content was expressed in mg/g:

$$\text{Chlorophyll a} = 9.784\ A_{662} - 0.990\ A_{644} \quad (4)$$

$$\text{Chlorophyll b} = 21.426\ A_{644} - 4.650\ A_{662} \quad (5)$$

$$\text{Chlorophyll a+b} = 5.134\ A_{662} + 20.436\ A_{644} \quad (6)$$

$$\text{Carotenoids} = 4.695\ A_{440} - 0.268\ (\text{chlorophyll a+b}) \quad (7)$$

2.3.2. Ion contents

Potassium and sodium were determined in the dry leaves' tissues using the H_2SO_4 and H_2O_2 [13], against a standard by flame-photometer [14]. For sample preparation, oven-dried plant samples (0.1 g) were entirely digested with H_2SO_4 (98%, 5 ml), at 200°C, supplemented with a few drops of H_2O_2 (30%, v/v). Once digestion was completed, the sample was brought up to 25 ml with distilled-deionized water.

2.3.3. Antioxidant Capacity

(i). Total Phenolic Content

The total phenolic content was determined spectrophotometrically [15]. Briefly, 0.2 ml of the diluted sample ethanolic extract [16] was transferred in tubes containing 1.0 ml of a 1/10 dilution of Folin-Ciocalteu's reagent in water. After waiting for 10 minutes, 0.8 ml of a sodium carbonate solution (7.5% w/v) was added to the sample. The tubes were then allowed to stand at room temperature for 30 min before absorbance at 743 nm was measured. Gallic acid hydrate was used as the reference for the calibration curve.

(ii). Total Antioxidant Activity

The total antioxidant activity was measured by the ferric reducing antioxidant power (FRAP) assay. The aliquots of test sample ethanolic extracts [16] in 1.0 ml of deionized water were mixed with 2.5 ml of (pH 6.6) 0.2 M phosphate buffer and 2.5 ml of (1%) potassium ferricyanide. The mixture was incubated at 50°C in water bath for 20 min. After cooling, aliquots of 2.5 ml of (10%) trichloroacetic acid were added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution 2.5 ml was mixed with 2.5 ml distilled water and a freshly prepared 0.5 ml of (0.1%) ferric chloride solution. The absorbance was measured spectrophotometrically at 700 nm [17]. Increase in absorbance of the reaction mixture indicates increase in reducing power. Ascorbic acid was used as a reference standard, the reducing power of the samples were comparable with the reference standard.

2.3.4. Oxidative Stress Indicators

(i). Lipid Peroxidation

Malondialdehyde (MDA) as a biomarker of lipid peroxidation was determined [18]. Fresh leaf samples (1 g) were homogenized in 5 ml of 0.1% (w/v) trichloroacetic acid (TCA) solution and centrifuged at 10,000 rpm for 15 min. To 1 ml of supernatant, 4 ml of 0.5% thiobarbituric acid (TBA) in 20% TCA was added and heated at 100°C for 30 min before cooled at room temperature and then centrifuged at 10,000 rpm for 15 min. The supernatant was spectrophotometrically monitored at 450, 532 and 600 nm for MDA determination as follows:

$$\text{MDA content} = 6.45 \times (A_{532} - A_{600}) - 0.56 \times A_{450} \quad (8)$$

since A_{450}, A_{532} and A_{600} mean the absorbance at 450, 532 and 600 nm, respectively.

(ii). Hydrogen Peroxide (H_2O_2)

For measuring H_2O_2 content, about 0.5 g fresh weight of leaves was homogenized with 5 ml 0.1% (w/v) trichloroacetic acid (TCA) in ice bath and the mixture was centrifuged at

10,000 rpm for 15 min. Then 1 ml of 10 mM potassium phosphate buffer and 2 ml of 1 M KI were added to 1 ml of the supernatant. The absorbance of supernatant was measured at 390 nm and H_2O_2 content was quantified using a standard curve [19].

2.4. Leaves Anatomical Structure

Anatomical analysis, of the 2nd season samples, was done at the Cairo University Research Park (CURP), Faculty of Agriculture. Specimens from selected treatments; control and the highest stress level, of selected grapevine cultivars were taken from the midrib region of the upper fourth leaf on the main stem. These specimens were prepared according to the method [20]. Specimens were killed and fixed in F. A. A. (10 ml formalin, 5 ml glacial acetic acid, 35 ml distilled water and 50 ml ethyl alcohol 95%) for at least 48 hrs. The selected materials were washed in 50% ethyl alcohol then dehydrated in a normal butyl alcohol series. After that, they were embedded in paraffin wax of melting point of 56°C. Leica RM2125 microtome was used to section them at a thickness of 20 micron, double stained with crystal violet and erythrosine, cleared in xylene and finally mounted in Canada balsam. Slides were analyzed microscopically and photomicrographed.

2.5. Gene Expression Analysis

Total RNA was extracted from the 2nd season leaf samples of selected treatments; control and the highest stress level, for selected grapevine cultivars using "GeneDireX" Total RNA Isolation Kit (Plant) according to the manufacturer protocol. RNA concentration, integrity, and purity were assessed using NanoDrop Spectrophotometry (MicroDigital, Korea).

Differential expression of four genes [21, 22] and VvEF1-α as a reference gene (Table 1) was confirmed by qPCR using Applied Biosystems StepOne™ Real-Time PCR (Thermo Fisher Scientific, USA). Reverse transcription was performed with "Solis BioDyne" FIREScript® RT cDNA synthesis KIT. "Solis BioDyne" HOT FIREPol® EvaGreen® qPCR Mix Plus (ROX) was used for all assays. The thermal cycle conditions were applied for PCR as follows: 10 min of 95°C, 40 cycles with 15 s of 95°C, and 1 min of 60°C; and finally, a climb in increments of 0.05°C from 60 to 95°C for the high-resolution melting curve [23].

Table 1. *Primer sequences of the targeted genes used for expression analysis.*

Name	Forward primer (5'– 3')	Reverse primer (5'– 3')
VvEDS1	ACCAAGAAAAGGCCGAGACT	ACTCGAAAGGGAGGGTTTTC
VvChS	GGAAAGGAGCTTGCAGAGAA	TCCAAAGGTCCTAGCACACA
VvAREB2	TAACCACATTAGCAACTCCC	CATTATGAACGCTGTCCTGC
VvABF1	TGATAAACACATGGCTGACC	TCTTCCAAAGTCATCTCCCC
VvEF1-α	TCTGCCTTCTTCCTTGGGTA	GCACCTCGATCAAAAGAGGA

2.6. Statistical Analysis

Obtained data were statistically analyzed [24] and significant difference was determined using L. S. D. values at P = 0.05.

3. Results

3.1. Morpho-Physiological Responses

3.1.1. Survivability

Data presented in Table 2 shows the effect of saline irrigation water with different NaCl concentrations (1000, 2000 and 3000 ppm) on the survivability (%) of the nine tested local grape genotypes. Results indicated that, lower concentration of NaCl (1000 ppm) didn't affect survival percentage of grape seedlings. Whereas, increasing salinity level up to 3000 ppm NaCl significantly decrease grape seedlings survivability (%). Survival percentage significantly varied according to the cultivars under study. Gharibi gave a significant lowest value of survivability (75%) in the first season, while in the second season both Gharibi and Romy Abiad gave the lowest value in this concern (85%) compared to the other cultivars. A significant effect of the interaction between different local grape cultivars and salinity levels was observed especially at higher concentrations of salinity. The lowest survivability (40%) was obtained with Gharibi in the first season and Romy Abiad in the second season under the highest level of NaCl (3000 ppm).

3.1.2. Vegetative Growth

Generally, all vegetative growth measurements were seriously affected with increasing salinity level regardless of the cultivar of grape seedlings. Plant height (cm), leaves count, leaf area (cm^2), leaves moisture content (%) and leaf relative water content (%) of local grape seedlings were gradually decreased with increasing NaCl concentrations in irrigation water throughout both seasons of the study (Tables 3, 4, 5, 6 & 7). Regarding to cultivars, Edkawy seedlings attained the relatively high values of plant height (39.67 and 40.02 cm) in the first and second season, respectively, as compared with other tested cultivars. On the other hand, Matrouh Eswid and Romy Abiad gave the shortest plants during the two seasons (Table 3). Baltim Eswid and Edkawy gave the highest leaves count (39.67, 39.58 and 38.58, 39.50) in the first and second season, respectively, while Romy Abiad gave the lowest leaves number during the two seasons (Table 4). Edkawy and Matrouh Eswid gave the highest leaf area (59.02, 57.25 and 57.09, 58.18 cm^2) for the two seasons, respectively, while Fayoumi seedling recorded the lowest values in this concern (Table 5). Edkawy and Romy Ahmer cultivars obtained the highest leaves moisture content (75.44, 75.05

and 75.50, 75.02%) in the first and second season, respectively. On the other hand, Gharibi and Romy Abiad cultivars gave the lowest values in this regard (Table 6). Bez El-Naka and Bez El-Anza seedlings were obtained the highest leaf relative water contents (%) and Romy Abiad seedlings gave the lowest values in this concern for both seasons of the study (Table 7). The interaction between cultivars and salt concentrations was also significantly different. There was a remarkable decrease in plant height, leaves count and leaf area of Bez El-Anza, Romy Abiad and Matrouh Eswid seedlings, respectively, compared to control especially at the highest concentration of salinity (3000 ppm NaCl) during the two seasons. The extent of reductions in plant height was lower for Romy Abiad, meanwhile, Edkawy showed the lowest reduction in both leaves count and leaf area under severe salinity than for other cultivars under study (Tables 3, 4 & 5). Meanwhile, in both seasons the lowest reduction in leaves moisture content (%) and leaf relative water content (%) was observed in Matrouh Eswid and Bez El-Naka, respectively (Tables 6 & 7).

Table 2. *Survivability (%) of local grape seedlings as affected by saline irrigation water with different NaCl concentrations (ppm).*

Cultivars	Treatments	First season					Second season				
		Control	1000	2000	3000	Mean	Control	1000	2000	3000	Mean
Baltim Eswid		100.00	100.00	100.00	80.00	95.00	100.00	100.00	100.00	80.00	95.00
Edkawy		100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Matrouh Eswid		100.00	100.00	100.00	80.00	95.00	100.00	100.00	100.00	80.00	95.00
Bez El-Naka		100.00	100.00	100.00	80.00	95.00	100.00	100.00	100.00	80.00	95.00
Bez El-Anza		100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Romy Ahmer		100.00	100.00	100.00	80.00	95.00	100.00	100.00	100.00	80.00	95.00
Gharibi		100.00	100.00	60.00	40.00	75.00	100.00	100.00	80.00	60.00	85.00
Fayoumi		100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Romy Abiad		100.00	100.00	100.00	60.00	90.00	100.00	100.00	100.00	40.00	85.00
Mean		100.00	100.00	95.56	80.00		100.00	100.00	97.78	80.00	
	Cultivars	6.54					5.19				
LSD 0.05	Treatments	4.36					3.46				
	Interaction	13.07					10.38				

Table 3. *Plant height (cm) of local grape seedlings as affected by saline irrigation water with different NaCl concentrations (ppm).*

Cultivars	Treatments	First season					Second season				
		Control	1000	2000	3000	Mean	Control	1000	2000	3000	Mean
Baltim Eswid		36.90	29.50	30.00	26.17	30.64	36.43	29.83	27.67	25.77	29.93
Edkawy		49.50	37.50	37.17	34.50	39.67	51.47	38.77	36.50	33.33	40.02
Matrouh Eswid		37.67	35.67	33.27	15.80	30.60	39.60	35.50	34.57	18.50	32.04
Bez El-Naka		21.00	18.37	15.60	12.83	16.95	20.80	19.37	17.50	13.67	17.83
Bez El-Anza		51.83	36.50	24.67	18.50	32.88	52.77	37.00	24.27	17.50	32.88
Romy Ahmer		40.83	43.67	27.83	25.00	34.33	41.40	43.50	25.77	26.17	34.21
Gharibi		39.50	28.00	18.00	15.00	25.13	39.67	28.00	18.33	15.00	25.25
Fayoumi		28.25	25.50	21.00	21.25	24.00	30.33	23.67	24.00	21.50	24.88
Romy Abiad		19.17	18.63	18.83	14.67	17.83	20.50	22.13	19.17	13.90	18.93
Mean		36.07	30.37	25.15	20.41		37.00	30.86	25.31	20.59	
	Cultivars	3.82					3.96				
LSD 0.05	Treatments	2.55					2.64				
	Interaction	7.64					7.92				

Table 4. *Leaves count of local grape seedlings as affected by saline irrigation water with different NaCl concentrations (ppm).*

Cultivars	Treatments	First season					Second season				
		Control	1000	2000	3000	Mean	Control	1000	2000	3000	Mean
Baltim Eswid		55.67	43.00	30.00	30.00	39.67	53.00	42.00	29.00	30.33	38.58
Edkawy		47.33	36.33	37.67	37.00	39.58	46.33	39.00	36.33	36.33	39.50
Matrouh Eswid		22.67	21.67	18.67	11.33	18.58	21.00	19.33	17.00	10.67	17.00
Bez El-Naka		24.33	20.67	17.33	12.33	18.67	25.00	20.33	18.33	12.00	18.92
Bez El-Anza		46.67	35.00	34.33	33.33	37.33	48.33	35.00	33.33	33.67	37.58
Romy Ahmer		46.00	33.00	27.67	20.00	31.67	46.00	33.67	28.00	20.00	31.92
Gharibi		27.00	27.00	18.00	8.00	20.00	29.00	30.00	17.00	9.00	21.25
Fayoumi		25.00	25.00	12.67	13.00	18.92	25.33	26.00	14.00	13.00	19.58
Romy Abiad		22.67	20.00	16.00	2.33	15.25	24.00	20.67	15.00	2.00	15.42
Mean		35.26	29.07	23.59	18.59		35.33	29.56	23.11	18.56	
	Cultivars	4.06					3.16				
LSD 0.05	Treatments	2.70					2.11				
	Interaction	8.11					6.33				

Table 5. *Leaf area (cm^2) of local grape seedlings as affected by saline irrigation water with different NaCl concentrations (ppm).*

Cultivars	Treatments	First season					Second season				
		Control	1000	2000	3000	Mean	Control	1000	2000	3000	Mean
Baltim Eswid		48.42	43.30	43.95	42.24	44.48	47.42	43.95	41.85	40.77	43.50
Edkawy		60.02	60.08	61.92	54.05	59.02	59.12	57.67	58.27	53.28	57.09
Matrouh Eswid		86.13	72.12	45.60	25.16	57.25	84.90	74.61	47.02	26.17	58.18
Bez El-Naka		64.63	56.93	47.52	39.12	52.05	66.70	56.67	47.32	39.49	52.55
Bez El-Anza		51.13	51.57	35.58	29.32	41.90	52.58	51.05	36.84	29.70	42.54
Romy Ahmer		45.77	42.52	32.80	32.32	38.35	46.46	43.51	33.40	32.13	38.88
Gharibi		52.58	45.95	35.58	27.80	40.48	52.67	45.67	35.92	28.55	40.70
Fayoumi		40.30	29.90	28.05	24.78	30.76	40.25	29.47	28.22	24.91	30.71
Romy Abiad		59.27	50.82	52.82	32.38	48.82	59.56	50.75	50.22	30.35	47.72
Mean		56.47	50.35	42.65	34.13		56.63	50.37	42.12	33.93	
	Cultivars	3.87					4.20				
LSD 0.05	Treatments	2.58					2.80				
	Interaction	7.74					8.40				

Table 6. *Leaves moisture content (%) of local grape seedlings as affected by saline irrigation water with different NaCl concentrations (ppm).*

Cultivars	Treatments	First season					Second season				
		Control	1000	2000	3000	Mean	Control	1000	2000	3000	Mean
Baltim Eswid		74.69	73.93	74.17	70.23	73.26	74.90	74.07	73.97	70.07	73.25
Edkawy		78.47	76.87	75.16	71.25	75.44	78.83	77.00	75.07	71.10	75.50
Matrouh Eswid		72.68	73.00	72.33	70.62	72.16	73.00	72.90	72.10	70.87	72.22
Bez El-Naka		74.89	75.44	72.90	70.31	73.39	75.13	74.97	72.97	70.10	73.29
Bez El-Anza		77.42	73.26	72.05	69.96	73.17	77.13	73.10	72.03	70.00	73.07
Romy Ahmer		79.14	76.21	72.20	72.65	75.05	79.03	76.07	72.90	72.07	75.02
Gharibi		73.33	71.41	69.00	67.00	70.19	73.10	71.13	69.33	67.00	70.14
Fayoumi		76.73	74.87	72.76	70.91	73.82	76.90	74.97	72.93	70.97	73.94
Romy Abiad		71.64	72.59	69.81	68.00	70.51	72.87	71.87	69.93	68.00	70.67
Mean		75.44	74.18	72.26	70.10		75.66	74.01	72.36	70.02	
	Cultivars	1.16					0.58				
LSD 0.05	Treatments	0.77					0.38				
	Interaction	2.32					1.15				

Table 7. *Leaf relative water content (%) of local grape seedlings as affected by saline irrigation water with different NaCl concentrations (ppm).*

Cultivars	Treatments	First season					Second season				
		Control	1000	2000	3000	Mean	Control	1000	2000	3000	Mean
Baltim Eswid		93.48	92.24	93.08	90.65	92.36	93.83	93.03	92.07	90.90	92.46
Edkawy		94.61	93.32	90.21	89.69	91.96	94.87	93.10	89.90	90.07	91.98
Matrouh Eswid		93.20	92.69	89.80	87.32	90.75	92.90	93.07	89.93	87.10	90.75
Bez El-Naka		93.82	93.87	93.74	91.01	93.11	93.97	93.93	93.57	91.33	93.20
Bez El-Anza		95.60	94.11	92.44	91.04	93.30	95.87	94.03	92.13	91.00	93.26
Romy Ahmer		93.39	92.76	93.28	90.29	92.43	92.93	93.13	93.10	90.10	92.32
Gharibi		93.33	93.24	91.25	87.44	91.32	93.07	93.10	91.10	87.17	91.11
Fayoumi		92.58	90.80	91.47	89.30	91.04	92.87	91.83	90.93	89.10	91.18
Romy Abiad		91.96	90.00	88.50	86.91	89.34	92.00	90.00	88.83	86.97	89.45
Mean		93.55	92.56	91.53	89.29		93.59	92.80	91.29	89.30	
	Cultivars	1.13					0.58				
LSD 0.05	Treatments	0.76					0.39				
	Interaction	2.27					1.16				

3.1.3. Root System

In general, increasing NaCl concentration in irrigation water negatively affected root system growth (count, length and weight) as shown in Tables 8, 9 & 10. Bez El-Naka seedling recorded the highest root number (26 and 26.25) and Edkawy recorded the highest root length and root weight (33.42 cm and 10.16 g) in the first season and (33.72 cm and 9.49 g) in the second season, respectively. Under severe salinity (3000 ppm NaCl), Fayoumi seedlings showed no reduction in root number. On the other hand, there was a remarkable decrease in root count of Gharibi seedlings (57.89 and 62.16% relatively to control) in the first and second seasons, respectively (Table 8). Concerning root length and root weight, under the highest salinity level Edkawy cultivar obtained the longest roots (33 and 35 cm) and heaviest roots (8.53 and 7.80 g) for first and second seasons, respectively. Furthermore, Fayoumi seedlings gave lower values in these regards (Tables 9 & 10). Data of root electrolyte leakage (%) clearly revealed that, in both seasons root electrolyte leakage (%) was significantly increased with the increase of salinity level in irrigation water (Table 11). Concerning the interaction, at the highest salinity level (3000 ppm NaCl) there was a remarkable increase in root electrolyte leakage (%) of Edkawy seedlings by 81.29 and 68.41% compared to those irrigated

using tap water for the two seasons, respectively, while Romy Abiad was the least with 12.14 and 4.65% for both seasons, respectively.

Finally, according to the above-mentioned morpho-physiological data, Romy Abiad is classified as a salt-sensitive genotype, and it was excluded from further analysis as it had insufficient leaf samples due to the least leaves count.

Table 8. *Roots count of local grape seedlings as affected by saline irrigation water with different NaCl concentrations (ppm).*

Cultivars	Treatments	First season					Second season				
		Control	1000	2000	3000	Mean	Control	1000	2000	3000	Mean
Baltim Eswid		26.00	19.00	26.00	18.00	22.25	27.00	19.00	25.00	17.00	22.00
Edkawy		23.00	25.00	21.00	19.00	22.00	26.00	24.00	20.00	19.00	22.25
Matrouh Eswid		33.00	16.00	13.00	19.00	20.25	30.00	16.00	13.00	18.00	19.25
Bez El-Naka		34.00	22.00	23.00	25.00	26.00	33.00	24.00	22.00	26.00	26.25
Bez El-Anza		31.00	20.00	20.00	25.00	24.00	30.00	20.00	21.00	24.00	23.75
Romy Ahmer		27.00	20.00	12.00	12.00	17.75	26.00	21.00	14.00	13.00	18.50
Gharibi		38.00	25.00	19.00	16.00	24.50	37.00	23.00	18.00	14.00	23.00
Fayoumi		23.00	18.00	21.00	24.00	21.50	22.00	19.00	23.00	22.00	21.50
Romy Abiad		39.00	18.00	15.00	22.00	23.50	39.00	17.00	14.00	20.00	22.50
Mean		30.44	20.33	18.89	20.00		30.00	20.33	18.89	19.22	
	Cultivars	1.74					1.81				
LSD 0.05	Treatments	1.16					1.21				
	Interaction	3.48					3.62				

Table 9. *Root length (cm) of local grape seedlings as affected by saline irrigation water with different NaCl concentrations (ppm).*

Cultivars	Treatments	First season					Second season				
		Control	1000	2000	3000	Mean	Control	1000	2000	3000	Mean
Baltim Eswid		41.17	33.70	25.75	32.00	33.16	38.60	33.90	25.90	32.00	32.60
Edkawy		35.67	31.50	33.50	33.00	33.42	34.33	31.77	33.77	35.00	33.72
Matrouh Eswid		32.50	34.55	27.25	24.90	29.80	32.27	34.80	27.17	22.30	29.13
Bez El-Naka		47.35	28.00	28.40	26.00	32.44	47.20	27.77	28.53	25.90	32.35
Bez El-Anza		29.55	38.00	35.20	28.00	32.69	29.80	38.50	35.03	27.90	32.81
Romy Ahmer		34.70	33.00	35.00	30.50	33.30	34.87	32.83	35.57	30.27	33.38
Gharibi		25.00	24.00	24.00	21.50	23.63	25.07	24.20	23.87	21.23	23.59
Fayoumi		27.50	28.50	17.00	20.00	23.25	27.77	28.27	18.00	19.00	23.26
Romy Abiad		33.40	36.00	36.00	24.00	32.35	33.20	36.00	36.23	23.77	32.30
Mean		34.09	31.92	29.12	26.66		33.68	32.00	29.34	26.37	
	Cultivars	1.69					1.84				
LSD 0.05	Treatments	1.12					1.23				
	Interaction	3.37					3.69				

Table 10. *Root weight (g) of local grape seedlings as affected by saline irrigation water with different NaCl concentrations (ppm).*

Cultivars	Treatments	First season					Second season				
		Control	1000	2000	3000	Mean	Control	1000	2000	3000	Mean
Baltim Eswid		9.12	8.65	4.57	5.73	7.02	9.39	8.77	5.78	5.73	7.42
Edkawy		11.57	9.67	10.88	8.53	10.16	11.72	9.39	9.06	7.80	9.49
Matrouh Eswid		13.26	10.93	7.13	4.90	9.06	12.00	10.01	7.18	5.30	8.62
Bez El-Naka		8.61	8.55	4.10	5.18	6.61	8.58	8.60	5.22	6.41	7.20
Bez El-Anza		10.20	9.06	7.04	6.62	8.23	9.63	9.10	6.46	6.60	7.95
Romy Ahmer		10.47	6.77	7.33	5.44	7.50	10.55	7.86	7.30	6.39	8.03
Gharibi		8.01	7.72	5.41	3.54	6.17	8.16	7.70	5.40	3.50	6.19
Fayoumi		8.66	7.31	8.45	7.57	8.00	8.76	7.85	8.65	7.60	8.22
Romy Abiad		9.80	7.18	7.89	4.84	7.43	9.26	7.20	7.90	4.80	7.29
Mean		9.97	8.43	6.98	5.82		9.78	8.50	6.99	6.01	
	Cultivars	0.68					0.68				
LSD 0.05	Treatments	0.45					0.45				
	Interaction	1.36					1.36				

Table 11. *Root electrolyte leakage (%) of local grape seedlings as affected by saline irrigation water with different NaCl concentrations (ppm).*

Cultivars	Treatments	First season					Second season				
		Control	1000	2000	3000	Mean	Control	1000	2000	3000	Mean
Baltim Eswid		27.55	26.51	34.57	33.38	30.50	26.63	26.51	33.97	33.38	30.12
Edkawy		23.84	23.52	34.92	43.22	31.38	25.67	26.74	34.93	43.23	32.64
Matrouh Eswid		28.03	32.67	40.08	49.38	37.54	31.32	32.67	40.49	49.38	38.47
Bez El-Naka		24.21	31.71	31.50	40.09	31.88	24.21	31.66	31.56	40.10	31.88
Bez El-Anza		30.78	29.74	35.93	35.81	33.07	32.48	32.43	35.90	35.84	34.16

Cultivars	Treatments	First season					Second season				
		Control	1000	2000	3000	Mean	Control	1000	2000	3000	Mean
Romy Ahmer		25.78	25.39	40.51	40.89	33.14	25.78	27.52	40.50	40.90	33.68
Gharibi		29.49	42.22	40.54	41.24	38.37	29.50	40.76	39.64	41.49	37.85
Fayoumi		33.58	40.03	41.49	40.47	38.89	38.11	40.00	40.87	40.50	39.87
Romy Abiad		25.87	31.26	28.49	29.01	28.66	28.80	29.74	28.59	30.14	29.32
Mean		27.68	31.45	36.45	39.28		29.17	32.00	36.27	39.44	
	Cultivars	1.44					2.01				
LSD 0.05	Treatments	0.96					1.34				
	Interaction	2.88					4.01				

3.2. Biochemical Responses

3.2.1. Leaf Photosynthetic Pigments Content

In both seasons, leaf photosynthetic pigments (total chlorophylls and total carotenoids) gradually decreased with increasing NaCl concentration in irrigation water (Tables 12 & 13). Under severe salinity (3000 ppm NaCl), Romy Ahmer seedlings recorded the highest total chlorophylls and total carotenoids values of 0.93 and 0.49 mg/g in the first season, 0.94 and 0.48 mg/g in the second season, respectively.

Table 12. *Effect of saline irrigation water with different NaCl concentrations (ppm) on leaf total chlorophylls content (mg/g) of local grape seedlings.*

Cultivars	Treatments	First season					Second season				
		Control	1000	2000	3000	Mean	Control	1000	2000	3000	Mean
Baltim Eswid		1.10	0.91	0.82	0.82	0.91	1.11	0.92	0.83	0.82	0.92
Edkawy		1.03	0.84	0.81	0.82	0.88	1.04	0.87	0.82	0.82	0.89
Matrouh Eswid		0.98	0.79	0.78	0.78	0.84	0.97	0.82	0.78	0.78	0.84
Bez El-Naka		1.07	0.97	0.93	0.91	0.97	1.07	0.98	0.93	0.91	0.97
Bez El-Anza		1.16	1.02	0.85	0.80	0.96	1.14	1.02	0.87	0.81	0.96
Romy Ahmer		1.03	1.00	0.96	0.93	0.98	1.02	1.00	0.96	0.94	0.98
Gharibi		0.86	0.87	0.87	0.72	0.83	0.89	0.87	0.84	0.76	0.84
Fayoumi		1.09	1.08	0.96	0.69	0.95	1.09	1.06	0.93	0.76	0.96
Mean		1.04	0.94	0.87	0.81		1.04	0.94	0.87	0.82	
	Cultivars	0.10					0.03				
LSD 0.05	Treatments	0.07					0.02				
	Interaction	0.19					0.06				

Table 13. *Effect of saline irrigation water with different NaCl concentrations (ppm) on leaf total carotenoids content (mg/g) of local grape seedlings.*

Cultivars	Treatments	First season					Second season				
		Control	1000	2000	3000	Mean	Control	1000	2000	3000	Mean
Baltim Eswid		0.53	0.52	0.39	0.39	0.46	0.52	0.50	0.41	0.39	0.46
Edkawy		0.50	0.39	0.39	0.40	0.42	0.50	0.41	0.39	0.40	0.43
Matrouh Eswid		0.51	0.49	0.47	0.40	0.47	0.51	0.49	0.46	0.39	0.46
Bez El-Naka		0.49	0.41	0.40	0.40	0.42	0.49	0.42	0.40	0.40	0.43
Bez El-Anza		0.55	0.45	0.41	0.39	0.45	0.54	0.46	0.41	0.40	0.45
Romy Ahmer		0.48	0.44	0.47	0.49	0.47	0.47	0.45	0.47	0.48	0.47
Gharibi		0.39	0.40	0.39	0.40	0.40	0.41	0.40	0.39	0.40	0.40
Fayoumi		0.53	0.50	0.49	0.37	0.47	0.54	0.50	0.47	0.39	0.48
Mean		0.50	0.45	0.43	0.41		0.50	0.45	0.43	0.41	
	Cultivars	0.04					0.01				
LSD 0.05	Treatments	0.03					0.01				
	Interaction	0.07					0.03				

3.2.2. Leaf Ion Contents

Data in Tables 14 & 15 clearly revealed that, increasing NaCl concentration gradually increased Na content and decreased K content in leaves during both seasons of the study. At the highest level of salinity (3000 ppm NaCl), Bez El-Anza showed lower Na and k contents recording 0.47 and 1.13% in the first season, 0.47 and 1.21% in the second season, respectively. Otherwise, Baltim Eswid gave the highest Na and K contents (0.77 and 2.08% in the first season, 0.70 and 2.04 % in the second season, respectively).

Table 14. *Effect of saline irrigation water with different NaCl concentrations (ppm) on leaf Na content (%) of local grape seedlings.*

Cultivars	Treatments	First season					Second season				
		Control	1000	2000	3000	Mean	Control	1000	2000	3000	Mean
Baltim Eswid		0.44	0.43	0.55	0.77	0.55	0.45	0.51	0.61	0.70	0.57
Edkawy		0.49	0.55	0.55	0.63	0.56	0.48	0.54	0.55	0.61	0.54
Matrouh Eswid		0.45	0.50	0.60	0.65	0.55	0.49	0.54	0.56	0.65	0.56

Cultivars	Treatments	First season					Second season				
		Control	1000	2000	3000	Mean	Control	1000	2000	3000	Mean
Bez El-Naka		0.42	0.48	0.51	0.66	0.52	0.42	0.47	0.53	0.64	0.52
Bez El-Anza		0.38	0.41	0.49	0.47	0.44	0.39	0.42	0.47	0.47	0.44
Romy Ahmer		0.42	0.50	0.58	0.70	0.55	0.41	0.50	0.58	0.68	0.54
Gharibi		0.40	0.49	0.52	0.70	0.53	0.41	0.48	0.55	0.72	0.54
Fayoumi		0.55	0.66	0.61	0.67	0.62	0.54	0.64	0.63	0.66	0.62
Mean		0.44	0.50	0.55	0.66		0.45	0.51	0.56	0.64	
	Cultivars	0.04					0.03				
LSD 0.05	Treatments	0.02					0.02				
	Interaction	0.07					0.05				

Table 15. *Effect of saline irrigation water with different NaCl concentrations (ppm) on leaf K content (%) of local grape seedlings.*

Cultivars	Treatments	First season					Second season				
		Control	1000	2000	3000	Mean	Control	1000	2000	3000	Mean
Baltim Eswid		2.00	1.97	2.08	2.08	2.03	2.01	2.00	2.05	2.04	2.02
Edkawy		2.01	1.99	2.00	1.78	1.94	2.05	1.99	1.98	1.81	1.96
Matrouh Eswid		2.05	1.99	1.71	1.37	1.78	1.87	1.83	1.70	1.43	1.71
Bez El-Naka		1.99	1.57	1.57	1.50	1.66	1.95	1.64	1.56	1.53	1.67
Bez El-Anza		2.00	1.87	1.58	1.13	1.64	2.01	1.84	1.55	1.21	1.65
Romy Ahmer		2.16	2.08	1.87	1.80	1.98	2.11	2.06	1.89	1.81	1.97
Gharibi		1.75	1.40	1.30	1.18	1.41	1.83	1.44	1.30	1.20	1.44
Fayoumi		1.99	2.08	1.35	1.35	1.69	2.00	1.94	1.47	1.39	1.70
Mean		1.99	1.87	1.68	1.52		1.98	1.84	1.69	1.55	
	Cultivars	0.12					0.08				
LSD 0.05	Treatments	0.09					0.05				
	Interaction	0.24					0.15				

3.2.3. Leaf Antioxidant Capacity

Leaf antioxidant capacity, which was estimated as total phenolic content and total antioxidant activity, was greatly influenced by high salinity level (Tables 16 & 17). Regarding to cultivars, Matrouh Eswid and Bez El-Anza seedlings attained the relatively high values of total phenolic content (10.58 and 10.09 mg/g in the first season, 10.78 and 10.09 mg/g in the second season, respectively) and total antioxidant activity (10.08 and 9.72 mg/g in the first season, 9.96 and 9.72 mg/g in the second season, respectively) as compared with other tested cultivars. Meanwhile, Bez El-Anza and Gharibi seedlings showed their highest rates of leaf antioxidant capacity when treated with the highest concentration of NaCl.

3.2.4. Oxidative stress Indicators in Leaves

Generally, increasing NaCl concentration in irrigation water positively affected oxidative stress indicators (malondialdehyde and hydrogen peroxide) as shown in Tables 18 & 19. Significant variations according to the cultivars under study in oxidative stress indicators were reported, but undisputed Bez El-Anza seedlings recorded the highest values in this concern. Under severe salinity condition (3000 ppm NaCl), Bez El-Anza seedlings attained the highest values of malondialdehyde (15.17 and 16.53 µmol/g) and Hydrogen peroxide (5.10 and 4.86 µg/g) for the two seasons, respectively. On the other hand, Edkawy and Romy Ahmer seedlings attended the lowest malondialdehyde values, meanwhile, Edkawy represented the lowest hydrogen peroxide values for both seasons of the study.

Table 16. *Effect of saline irrigation water with different NaCl concentrations (ppm) on leaf total phenolic content (mg/g) of local grape seedlings.*

Cultivars	Treatments	First season					Second season				
		Control	1000	2000	3000	Mean	Control	1000	2000	3000	Mean
Baltim Eswid		9.55	9.86	9.51	9.38	9.58	9.57	9.82	9.24	9.42	9.51
Edkawy		8.20	8.70	9.03	9.12	8.76	8.22	8.64	9.01	9.19	8.76
Matrouh Eswid		10.96	10.68	10.85	9.85	10.58	11.82	10.75	10.56	9.98	10.78
Bez El-Naka		7.44	7.69	7.37	7.76	7.56	8.12	7.73	7.14	7.78	7.69
Bez El-Anza		8.46	9.63	11.12	11.15	10.09	8.43	9.69	11.12	11.14	10.09
Romy Ahmer		8.85	9.38	7.42	7.64	8.32	8.72	9.34	7.43	7.69	8.30
Gharibi		6.28	6.48	7.17	11.60	7.88	6.33	6.65	7.20	11.44	7.90
Fayoumi		6.15	6.32	6.93	6.30	6.42	7.63	6.48	6.13	6.54	6.70
Mean		8.24	8.59	8.67	9.10		8.60	8.64	8.48	9.15	
	Cultivars	0.60					0.34				
LSD 0.05	Treatments	0.42					0.24				
	Interaction	1.19					0.69				

Table 17. *Effect of saline irrigation water with different NaCl concentrations (ppm) on leaf total antioxidant activity (mg/g) of local grape seedlings.*

Cultivars	Treatments	First season					Second season				
		Control	1000	2000	3000	Mean	Control	1000	2000	3000	Mean
Baltim Eswid		7.12	7.77	8.26	6.72	7.47	7.14	7.76	8.24	6.82	7.49
Edkawy		6.43	6.75	7.19	6.77	6.79	6.49	6.76	7.20	6.78	6.81
Matrouh Eswid		10.74	10.88	10.70	7.99	10.08	11.42	10.18	10.45	7.80	9.96
Bez El-Naka		6.10	7.66	6.59	6.42	6.69	6.18	7.56	5.89	6.43	6.51
Bez El-Anza		8.15	8.53	11.08	11.10	9.72	8.15	8.56	11.59	10.59	9.72
Romy Ahmer		9.03	10.75	6.39	6.24	8.10	9.03	10.32	6.43	6.36	8.04
Gharibi		4.41	4.68	5.64	10.83	6.39	4.47	4.61	5.68	10.74	6.37
Fayoumi		5.19	4.97	5.13	4.86	5.04	5.61	4.80	4.55	4.90	4.96
Mean		7.14	7.75	7.62	7.62		7.31	7.57	7.50	7.55	
	Cultivars	0.58					0.38				
LSD 0.05	Treatments	0.41					0.27				
	Interaction	1.16					0.77				

Table 18. *Effect of saline irrigation water with different NaCl concentrations (ppm) on leaf malondialdehyde content (µmol/g) of local grape seedlings.*

Cultivars	Treatments	First season					Second season				
		Control	1000	2000	3000	Mean	Control	1000	2000	3000	Mean
Baltim Eswid		7.32	7.34	7.61	9.64	7.98	7.39	7.46	7.54	9.19	7.89
Edkawy		6.36	6.65	7.15	8.57	7.18	7.27	7.37	7.24	7.70	7.39
Matrouh Eswid		7.17	8.73	9.20	11.83	9.23	7.56	8.34	9.02	10.10	8.76
Bez El-Naka		7.31	8.57	9.30	9.57	8.69	7.87	9.50	9.37	9.15	8.97
Bez El-Anza		9.34	10.56	10.92	15.17	11.50	10.24	11.13	11.03	16.53	12.23
Romy Ahmer		5.45	8.18	7.64	8.22	7.37	6.01	7.46	7.01	8.21	7.17
Gharibi		8.86	9.05	9.60	10.76	9.57	8.16	8.17	9.20	10.47	9.00
Fayoumi		8.73	8.46	9.99	9.91	9.27	8.84	9.27	9.79	10.21	9.53
Mean		7.57	8.44	8.93	10.46		7.92	8.59	8.77	10.19	
	Cultivars	0.67					0.57				
LSD 0.05	Treatments	0.48					0.40				
	Interaction	1.35					1.13				

Table 19. *Effect of saline irrigation water with different NaCl concentrations (ppm) on leaf hydrogen peroxide content (µg/g) of local grape seedlings.*

Cultivars	Treatments	First season					Second season				
		Control	1000	2000	3000	Mean	Control	1000	2000	3000	Mean
Baltim Eswid		1.00	1.16	1.24	1.61	1.25	1.08	1.16	1.26	1.63	1.28
Edkawy		0.93	1.12	1.30	1.33	1.17	1.08	1.17	1.15	1.23	1.16
Matrouh Eswid		1.15	1.27	1.39	1.64	1.36	1.21	1.32	1.53	1.76	1.46
Bez El-Naka		1.05	1.10	1.37	1.56	1.27	1.12	1.22	1.39	1.54	1.32
Bez El-Anza		1.63	2.39	2.29	5.10	2.85	1.70	2.12	2.58	4.86	2.81
Romy Ahmer		1.17	1.38	1.56	1.63	1.44	1.19	1.33	1.46	1.55	1.38
Gharibi		1.27	2.47	2.39	3.56	2.42	1.27	1.88	2.50	3.41	2.26
Fayoumi		1.30	1.38	1.46	1.53	1.42	1.29	1.36	1.48	1.55	1.42
Mean		1.19	1.53	1.63	2.25		1.24	1.44	1.67	2.19	
	Cultivars	0.15					0.14				
LSD 0.05	Treatments	0.10					0.10				
	Interaction	0.29					0.28				

According to the above-mentioned morpho-physiological and biochemical data, Bez El-Anza genotype which maintained 100% survivability under severe salinity stress condition was characterized by a remarkable decline in vegetative growth accompanied with keeping more leaves with a marked reduction in leaf area, slight Na uptake, but undisputed oxidative stress indicators. Wherefore, leaf samples from selected treatments; control and the highest stress level, of phenomenal Bez El-Anza genotype along with the salt-tolerant Edkawy genotype and the salt-sensitive Gharibi genotype were subjected to additional anatomical and gene expression analysis.

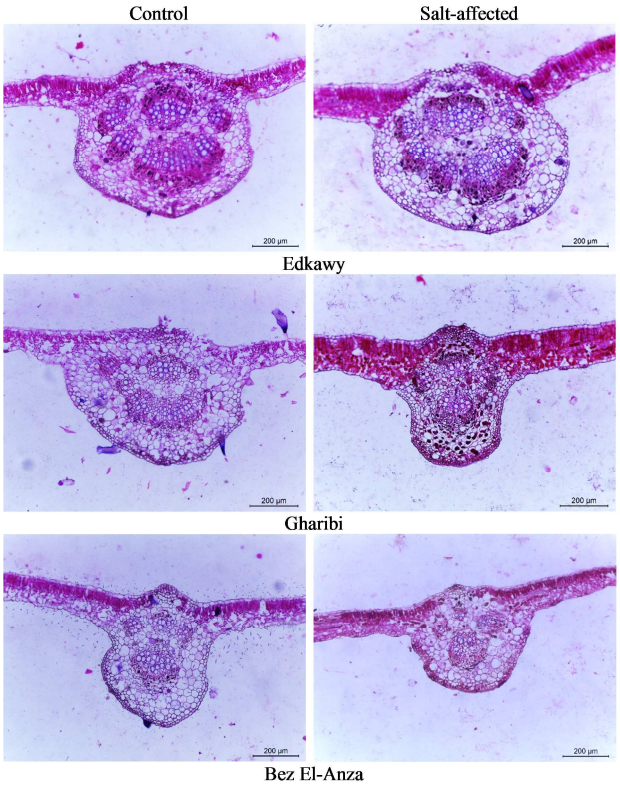
3.3. Leaves Anatomical Structure

The most salt-tolerant Edkawy genotype maintained almost normal thickness of both upper and lower epidermis under severe salt stress condition, while both of Gharibi and Bez El-Anza were got thinner by 18.23, 38.17% for upper and 20.79, 41.11% for lower epidermis, respectively, as compared to controls (Table 20 & Scheme 1). Otherwise, the most salt-sensitive Gharibi genotype recorded a remarkable increment of both palisade and spongy mesophyll thickness by 83.31 and 100.31%, respectively, as affected by high salt concentration comparing to control, followed by Edkawy tolerant genotype which enhanced by 6.67 and 48.77%, while palisade mesophyll thickness of Bez El-Anza genotype reduced by 22.95% under severe salinity stress condition as compared to control. Additionally, the most salt-tolerant Edkawy genotype maintained almost normal diameter of the vascular bundles under severe salt stress condition, while Bez El-Anza was the most negatively affected

by 33.17 and 29.10% of xylem and phloem normal thickness, respectively, 19.03% of overall midvein normal thickness and 33.28% of xylem normal diameter. On the other hand, the most salt-sensitive Gharibi genotype maintained almost normal thickness of midvein under severe salinity stress condition, which was accompanied with a remarkable increment of phloem tissue thickness by 66.48% but a reduction by 27.10 of xylem tissue thickness relatively to control.

Table 20. *Relative changes (%) in measurements of certain anatomical features in transverse sections through the blade of upper fourth leaf on the main stem for selected local grape genotypes grown under severe salinity stress as compared to control.*

Anatomical features	Edkawy	Gharibi	Bez El-Anza
Thickness of Upper Epidermis	+2.60	-18.23	-38.17
Thickness of Lower Epidermis	+0.82	-20.79	-41.11
Thickness of Palisade Mesophyll	+6.67	+83.31	-22.95
Thickness of Spongy Mesophyll	+48.77	+100.31	+14.45
Thickness of Xylem Tissue	+2.34	-27.10	-33.17
Thickness of Phloem Tissue	+0.72	+66.48	-29.10
Thickness of Midvein	+10.44	+2.26	-19.03
Xylem diameter	+16.55	-8.15	-33.28



Scheme 1. *Transverse sections through the blade of upper fourth leaf on the main stem for selected local grape genotypes grown under severe salinity stress as compared to control.*

3.4. Gene Expression

Figure 1 shows the changes in regulation of the targeted genes for selected local grape genotypes grown under severe salinity stress relatively to control. Transcripts of both *VvChS* and *EDS1* genes increased in leaves of the salt-sensitive genotype "Gharibi" under long-term salinity compared to control, while down-regulated in the most tolerant one "Edkawy". *AREB2* showed remarkable positive gene expression folds in the most salt-tolerant Edkawy genotype as affected by severe stress condition relatively to control, while down-regulated in Bez El-Anza genotype. *ABF1* gene transcripts was found to decrease in all selected local grape genotypes (salt tolerant and susceptible) with increasing salinity.

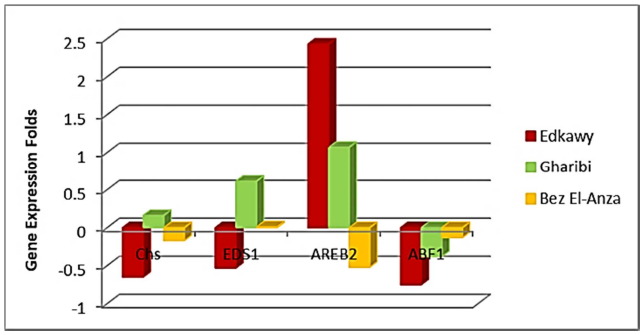


Figure 1. *Changes in regulation of the targeted genes for selected local grape genotypes grown under severe salinity stress relatively to control.*

4. Discussion

Growth responses to salinity are often considered as a basis for evaluation of tolerance [25]. It was observed that, salinity treatments caused a growth reduction of all the tested local grape genotypes with varying degrees. Generally, Edkawy genotype was the least salt-affected with survivability of 100% under severe salinity stress condition, while Romy Abiad and Gharibi were the most sensitive recording 60 and 40%, alternately during both seasons of the study. In accordance, Edkawy genotype was previously reported as a promising salt tolerant local grape cultivar [26]. Edkawy cultivar is a local grapevine, planted on the hill side along the Mediterranean Sea from Edko to Rosetta, Beheira governorate, for long time ago, which is now on the verge of extinction [27].

It was observed that, under long term salt stress condition, changes in cell elongation and cell division lead to slower leaf emergence and smaller final size, and leaf growth is usually more affected than root growth [28, 29]. Accordingly, Romy Abiad is classified as a salt-sensitive genotype, and it was excluded from further analysis as it had insufficient leaf samples due to the least leaves count.

Concerning leaf relative water content (LRWC), Romy Abiad genotype was the most negatively affected by salt treatments. It was previously indicated that, salt-sensitive grape cultivars which revealed the highest loss of growth under salinity stress condition was accompanied with the most reduction of leaf water potential [30]. LRWC has been considered as a substitute evaluation of plant water status, reflecting metabolic activity of the plant [31]. A reduction in LRWC under salinity has been previously recorded [32]. This decline can be attributed to less water accessibility, or to defeat of the plant roots' efficiency to catch up with water throughout a lessening of the absorbing surface [33].

Considering root electrolyte leakage (REL), there was a remarkable increase in root electrolyte leakage (%) of Edkawy seedlings at the highest salinity level (3000 ppm NaCl)

compared to those irrigated using tap water, while Romy Abiad was the least. In general, REL is casually and statistically related with root damage and survivability of seedling. However, it depends on many other factors apart from root damages, and thus it remains a great challenge to improve its reliability [34]. In the same context, it was observed that, high EL values may not be considered a sign of damaged plasma membranes due to salt stress [35], as also reported by the study [36], but related to a naturally high content of potassium, where electrolyte leakage is mainly related to the efflux of K^+, which is abundant in plant cells [37].

According to the obtained results, chlorophyll content reduced gradually with increasing salt concentrations, with maximum rate for Bez El-Anza genotype. In grapevine, chlorophyll fluorescence has been observed to weaken under high NaCl concentration, suggesting an inhibition in the electron transport of photosystem II, and this could be associated with membrane lipid peroxidation due to reactive oxygen species (ROS) [30].

As for leaf Na^+ content, Bez El-Anza genotype markedly was the lowest under severe salt stress condition. Salt exclusion tolerance mechanism has been found in certain Vitis genotypes that, when grafted as rootstocks, can protect fruit-bearing scions from accumulating significant amounts of saline ions from soils, most notably through the presence of specific transport proteins that are involved in regulating the transfer of ions from root to shoot via the xylem [5]. Salt exclusion is a term that commonly refers to the ability of plants to limit uptake of salt (Na^+ and Cl^-) from the surrounding media and/or limit the translocation of these ions from the root xylem to the shoot. So, a greatest area of research focus on grape salt-tolerance involves the mechanisms that limit Cl^- and Na^+ accumulation in shoots.

Long term exposure to severe salt stress led to remarkable increase in both indicators of oxidative stress; malondialdehyde (MDA) and H_2O_2, in Bez El-Anza genotype. In addition, increment of H_2O_2 was also clear in Gharibi but with a relatively less extent. In accordance to a previous study on four grape cultivars under salinity, salt stress increased the rate of lipid peroxidation in all tested varieties, particularly in salt-sensitive grapes, where increment in malondialdehyde (MDA) content is an indication that salinity induces oxidative stress [30]. It has been observed in many plant species that, low levels of reactive oxygen species (ROS) act as a signaling element in response to salinity that can aid salinity tolerance, whereas a higher concentration of ROS can damage tissues by oxidizing and degrading biopolymers including cell wall polysaccharides and nucleic acids [38].

Reactive oxygen species (ROS) detoxification is an important component associated with salt stress, and ultimately damage, which increases over time and can be used to classify tolerance mechanisms [39, 40]. Therefore, another key feature of salt tolerance is the synthesis of antioxidants, which is an essential mechanism for ROS detoxification in grapevine [41, 42]. It is obvious that, both Bez El-Anza and Gharibi genotypes had the maximum phenols content and overall antioxidant activity under severe salinity stress condition, indicating exposure to oxidative stress subsequently activate antioxidant defense mechanisms to combat. Additionally, increment rate of antioxidant potential of Gharibi was so clear with increasing salt concentration to the highest one. Numerous phenolic compounds are stress-induced metabolites in plants [32]. It has been reported that, excessive phenolics accumulation imparts superior radical scavenging activity thus avoiding cellular oxidative rupture [43]. Soluble phenols provide antioxidants since they have mediators that donate electrons and, consequently, alleviate accumulation of extra ROS [44]. This production is likely induced by stimulation of the phenylpropanoid pathway and enhancement gene expression of phenyl-aminolyase (PAL) [45].

Besides physiological and biochemical adaptations, plants adapt to saline conditions by changes in the anatomical structure of leaves and roots as well as changes in morphology [46]. The decline in growth of Bez El-Anza genotype at severe salinity was associated with a marked decrease in leaf area. Salinity induced reduction in growth of Bez El-Anza genotype, which maintained 100% survivability under severe salinity stress condition, may help in better adaptation under high salinity condition by saving photosynthetic energy, reducing protein synthesis and providing more free amino acids for osmotic adjustment [47].

Both halophytes and non–halophytes exhibit marked anatomical alterations when exposed to elevated levels of salinity [48], and most of conspicuous changes are noticeable in the leaf. Since, dehydration avoidance is a vital adaptive strategy against salt stress [49], thick epidermis is therefore a trait of many salt-tolerant terrestrial species [50] and this is one of the most valuable mechanisms relating to xeric adaptation for prevent water loss [51, 52]. The most salt tolerant Edkawy genotype maintained normal thickness of both upper and lower epidermis under severe salt stress condition, while both of Gharibi and Bez El-Anza were got thinner. Accordingly, the epidermal thickness was reduced in the glycophyte *Hordeum vulgare* with increasing salinity [53]. Declined epidermal thickness may be attributed to the limited cell division and growth at high salinity [54]. On the other hand, thick epidermis may help to better adapt under high salinity conditions by maintaining transpiration rate that keeps the water content in mesophyll tissue and improves the water use efficiency of the plant, and also provides additional space for efficient Na^+ sequestration in the leaf epidermis [55].

The most salt sensitive Gharibi genotype recorded a remarkable increment of both palisade and spongy mesophyll thickness as affect by high salt concentration comparing to control. It has been observed that, the ratio of mesophyll surface area to leaf area (A^{mes}/A) increased more rapidly with salinity as species' salt tolerance decreased [56]. On the other hand, palisade mesophyll thickness of Bez El-Anza genotype reduced under severe salinity stress condition as compared to control. Reduction of the palisade tissue at extreme salinity might be an adaptation to minimize the photosynthetic energy utilization under high saline condition, where the palisade

tissues are the chlorenchymatous mesophyll tissue that contains numerous chloroplasts and considered principal site for photosynthesis [47]. Additionally, enhanced spongy mesophyll thickness in salt treated Edkawy tolerant genotype may help in maintaining leaf water content and turgor, which is in accordance with the study [47]. In mulberry, it was demonstrated that, the spongy cell layer increases in all genotypes (salt-tolerant and susceptible) with increasing salinity [57].

The most salt tolerant Edkawy genotype maintained normal diameter of the vascular bundles under severe salt stress condition, while Bez El-Anza was the most negatively affected. Similar results were obtained in barley seedlings under salinity where, the diameter of the vascular bundles of leaves decreased in all studied barley varieties except for the salt-tolerant Asem cv., which had no change in diameter of vascular bundles as affected by salt stress, this means that the tolerant genotypes maintained a larger vascular bundle size and a smaller reduction in interveinal distance as response to NaCl salinity [53]. It was observed that, reducing the diameter of vascular bundles is directly related to decreasing the area of xylem vessels, which are conductive elements that clearly responsible for holding various elements by changing their diameter [58]. Such reduction in size and number of conducting elements of the xylem in response to heavy metals was also reported in pea plants [59]. It was supposed that, reducing the number of conducting elements has been reported in literature as being an adaptive measure to secure water flow [60].

An important step in understanding how a genome functions in a different environmental cue is to determine the pattern of how the expression of the genes is regulated. Various adverse environmental stresses alter the expression pattern of a variety of genes in many plant species [61]. Differential expression of genes in salt-sensitive and tolerant grape genotypes, combined with previous studies of salt-induced responses in specific cultivars [62], provides useful information for salt tolerance in grape [22]; a crop of major economic interest that is exposed to salt stress.

Under stress conditions, plants express several genes as a part of defense responses. Among these genes, *ChS* is commonly induced in different plant species under abiotic stresses [63]. Chalcone synthase is a key enzyme in flavonoid biosynthesis that belongs to the phenylpropanoid pathway, and its content is severely affected by plant growth conditions. *VvChS* gene transcripts increased in leaves of the salt-sensitive genotype "Gharibi" under long-term salinity compared to control, while down-regulated in the most tolerant one "Edkawy". It is possible that, the different expression of *VvChS* in leaves of the examined salt-tolerant and sensitive genotypes under long-term salinity was related to the genotype's stress tolerance, which is means that, sensitive genotypes that showed reduced salt tolerance attempt to activate defense mechanisms by increasing *VvChS* transcription.

In this study, the expression of *VvEDS1* was studied as a defense gene, where *EDS1* and PAD4 are required for the expression of hypersensitive response cell death. *EDS1* gene transcripts increased in leaves of the salt-sensitive genotype "Gharibi" under long-term salinity compared to control, while down-regulated in the most tolerant one "Edkawy". Further studies should be performed to reveal the precise role of *VvEDS1* in grapevine defense pathways, which is in accordance with the study [64]. *EDS1* is also needed to maintain the stress response program after ROS production. Hence, *EDS1* appears to act as a master regulator of cell death in response to stress signals [65]. Stress responses of plants can be caused by the perception of ROS as a signal that activates the genetic stress response program. The *EDS1* protein seems to be involved in controlling the singlet-oxygen–mediated visible stress responses. It was supposed that, *EDS1* may control recovery after plants have been exposed to environmental stress conditions [66].

Recent findings highlighted a strong relationship between the accumulation of specific transcripts and salinity tolerance in grapevine (*Vitis vinifera* L.) [21]. Transcriptional induction of genes in response to salt stress has been recognized as an adaptive mechanism of plants against salinity [67]. AREB/ABFs are key transcription factors that respond to abiotic stresses by regulating downstream stress-responsive genes in ABA-dependent and independent pathways [21]. In the present study, AREB2 showed remarkable positive gene expression folds in the most salt-tolerant Edkawy genotype as affected by severe stress condition relatively to control, while down-regulated in Bez El-Anza genotype. The MdAREB2 transcription factor was found to regulate sugar accumulation by directly binding to the promoters of several genes; that encode sugar transporters and amylases, and activate their expression causing production of more soluble sugars in apple (*Malus domestica*) [68].

5. Conclusions

The study reviewed salinity tolerance mechanisms in diverse Egyptian grape genotypes based on morpho-physiological, biochemical, anatomical and gene expression analysis. Proposed salt-tolerance mechanisms including; controlling the growth rate, reducing damage resulting from oxidative stress associated with salinity, keep balanced hydric status, structural alterations allowing protection and regulation of ions uptake. Accordingly, it could be concluded that, Edkawy local grape cultivar is a promising salt-tolerant genotype. On the other hand, Romy Abiad and Gharibi genotypes were classified as the most salt-sensitive comparable with other tested local cultivars. Bez El-Anza genotype which maintained 100% survivability under severe salinity stress condition was characterized by a remarkable decline in vegetative growth accompanied with keeping more leaves with a marked reduction in leaf area and most measurements of certain anatomical features, slight Na uptake, but undisputed oxidative stress indicators and down-regulated expression folds of *AREB2* transcription factor; a sugar accumulation regulatory related gene. Therefore, local genotypes of Egyptian table grapes can be

considered a storehouse of germplasm that should be conserved and not threatened with extinction or complete loss because they are adapted to severe environmental conditions and harsh cultural managements, which may help to cope with the negative impacts of climate change on viticulture and adapt under the new conditions to maintain grape production and quality.

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Conflicts of Interest

The authors declare no conflicts of interest.

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