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# MICROPROPAGATION AND SALT TOLERANCE EVALUATION IN SOME GRAPE CULTIVARS
## II- SALT TOLERANCE EVALUATION :

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## ABSTRACT

The present investigation was carried out during three successive years (2000- 2002), in order to evaluate three grape cultivars namely, Early Superior, Flame seedless and Thompson seedless, for salt tolerance under *in vitro* conditions.

The main results can be summarized in the following points:

**A. Effect of Cultivars**

1- Early Superior gave significantly the highest shoot multiplication percentage and average number of new proliferated shoots per original shoot, in the 1st, 2nd and 3rd subcultures, followed by Flame seedless and Thompson seedless. In the meantime, Early Superior achieved significantly the highest leaf P, K, Na, Mg, total chlorophyll contents and peroxidase activity, followed by Flame seedless and Thompson seedless. Moreover, Early Superior recorded significantly the highest leaf N and proline contents, as compared with Flame seedless and Thompson seedless. On the contrary, Thompson seedless exhibited significantly the highest shoot injury percentage and leaf Ca content, followed by Flame seedless and Early Superior. Also, Flame seedless cv. exhibited significantly the highest average length of new proliferated shoot.

2- Early Superior produced significantly the highest rooting percentage and average number of roots per shoot, after 4 weeks of shoot culture, followed by Flame seedless and Thompson seedless. While, Early Superior and Flame seedless at the same period gave significantly the highest average root length, as compared with Thompson seedless.

**B. Effect of Salinity Treatments**

1- In 1st, 2nd and 3rd subcultures, a high decrease in shoot multiplication percentage, average number of new proliferated shoots per original shoot and average length of new proliferated shoot and a high increase in shoot injury percentage joined the increase in salinity concentrations from zero to 4000 ppm. Moreover, leaf N, Na, Cl and proline contents showed a tendency for positive responses to salinity treatments. On the contrary, leaf P, K, Ca, Mg, total chlorophyll contents and peroxidase activity showed a tendency for negative responses to salinity treatments.

2- After 4 weeks of shoot culture, rooting percentage, average number of roots per shoot and average root length showed a tendency for negative responses to salinity treatments, and the differences among salinity treatments were statistically significant.

It is evident that, Early Superior was the most salt-tolerant cultivar, while Thompson seedless appeared to be the most salt-sensitive cultivar in this respect.

Finally, 77%, 70% and 55% of the obtained plants of Early Superior, Flame seedless and Thompson seedless, respectively were successfully transplanted to soil. These plants were healthy and vigorously growing under the greenhouse conditions.

# INTRODUCTION

The grapes (*Vitis vinifera* L.) are considered as one of the most important fruit crop in the world and the second in Egypt. The area devoted for grape culture in Egypt is about 148406 feddan producing about 1078912 ton of fruits according to the statistics of the Ministry of Agriculture and Land Reclamation, Cairo, 2001.

New grape cultivars have been introduced to Egypt such as Flame seedless and Early Superior. It is important to study the possibility of growing them under the Egyptian conditions, and to evaluate these cultivars under different stresses. This might help to spread out their cultivation area to include the new reclaimed lands.

The identification and evaluation of salt tolerant cultivars could help in solving salinity problems in such new reclaimed areas. Tissue culture technique is recently used for the evaluation of such new cultivars towards environmental stresses. This technique offers the control of environmental, pathogenic and nutritional factors. So, the evaluation for stress tolerance would be more valuable and reliable.

The objectives of this investigation were to evaluate salt tolerance of three grape cultivars under *in vitro* conditions. Factors considered were the effects of salinity on the physiological and biochemical aspects in the tested grape cultivars, Early Superior, Flame seedless and Thompson seedless.

# MATERIALS AND METHODS

The present investigation was carried out during three successive years (2000 – 2002), in order to study the responses of Early Superior, Flame seedless and Thompson seedless grapevines to salt stress under *in vitro* conditions. In other words, salt tolerance degree of these grape cultivars (grown *in vitro*) was evaluated in this part of the current study.

## 1. Shoot Multiplication Under Salt Stress Conditions

Experiments were performed to study the possibility of *in vitro* shoot multiplication under salt stress conditions.

### 1.1. Plant Material

Proliferated shoots of the tested grape cultivars that were produced from the original shoot cultures were used after having the results of the shoot multiplication experiments (in the first part of this study). The multiplicated shoots were considered the explant mother stock for salt tolerance evaluation *in vitro*.

### 1.2. Culture Media

Due to the results of the first part of this study, it was cleared that the best shoot multiplication medium was the Murashige and Skoog (1962) (MS) medium at full strength supplemented with sucrose $30g1^{-1}$, 1.0 $mgl^{-1}$ BA + 0.5 $mg1^{-1}$ zeatin. Solidification of this medium was achieved by $7g1^{-1}$ agar. For salt tolerance evaluation, this medium was supplemented with zero, 1000, 2000 or 4000 ppm of salt mixture of sodium chloride (NaCl), calcium chloride

($CaCl_2$) and magnesium chloride ($MgCl_2$) at the ratio of 3 [NaCl]:1 [2 ($CaCl_2$) + 1 ($MgCl_2$)] according to Ibrahim and El-Kobbia (1986). The pH of shoot multiplication media (under salt stress conditions) was adjusted to 5.7 before adding agar. These media were autoclaved at 121°C for 20 min., then left to cool and harden for 24 hrs., before being used.

**1.3. Culture Procedures**

The original proliferated shoots (longer than 2 cm) were excised (individually) under aseptical conditions and cultured vertically on the best cytokinin combination and concentrations (due to the results appeared in the first part of this study).

Routine subculture of axillary shoots was carried out every 4 weeks up to three subcultures. One original shoot was cultured in each glass jar (120 × 70 mm) containing 50 ml of the culture media. At the end of each subculture (after 4 weeks), the new proliferated shoots were used as a mother cultures for the subsequent subculture, individually separated and transferred to fresh multiplication medium. In other words, the resultant proliferated shoots from each subculture were used as a mother stock explants for the next subculture.

The shoot multiplication percentage, average number of new proliferated shoots per original shoot explant and average length of new proliferated shoot were recorded under salt stress conditions for three successive subcultures.

The shoot multiplication rate = number of new proliferated shoots per original shoot in shoot cultures at the end of each subculture. The new proliferated shoots were used as mother cultures for the subsequent rooting experiments under salt stress conditions.

**1.4. Culture Conditions**

The shoot cultures were incubated at 24 ± 2°C, under 16 hrs. light period from fluorescent lamps (2 lamps per shelf), followed by 8 hrs. dark period, for 4,8 and 12 weeks.

After the first (4 weeks), second (8 weeks) and third (12 weeks) subculture, the following data were recorded:

**1.5. Shoot Injury Percentage**

The new proliferated shoots derived from original shoot cultures under salt stress conditions were considered good multiplicated when the stems were green in tips and more than 50% of leaves were still completely green and healthy.

The multiplicated shoots under salt stress conditions also were assessed according to the shoot injury percentage as in the shoot injury index:

Shoot injury index ≥ 50% of leaves and stems were burned.

**1.6. Leaf Mineral Composition**

Leaf samples were collected and oven-dried at 85 °C for 24 hrs. The dried leaf samples (50 mg each) were digested with sulfuric acid and hydrogen peroxide, as outlined by Evenhuis and DeWaard (1980). Suitable aliquots were then taken for the determination of mineral elements. Nitrogen (N) and phosphorus (P) were determined colorimetrically according to Evenhuis (1976) and, Murphy and Riley (1962), respectively. Potassium (K) and

sodium (Na) were determined by E El Flame photometer, calcium (Ca) and magnesium (Mg) by Perkin Elmer Atomic Absorption Spectrophotometer. Chloride (Cl) was determined as described by Cotlove (1965). The data were expressed as percent on dry weight basis.

**1.7. Biochemical Responses**

**1.7.1. Leaf Proline Content**

Free proline was extracted from dried leaf material and determined according to the method of Bates *et al.*,(1973) using pure proline as standard. The data were expressed as percent on dry weight basis.

**1.7.2. Leaf Chlorophyll Content**

In the fresh leaves of each sample, total chlorophyll was determined according to the method of Torrecillas *et al.*,(1984). The value of total chlorophyll content was expressed as mg per 100 $cm^2$ of leaf area.

**1.7.3. Leaf Peroxidase Activity**

Peroxidase (EC 1.11.1.7) activity was spectrophotometrically determined in the fresh leaf samples, according to the method suggested by Chance and Maehly (1955). The data were expressed as changes in optical density (O.D.) at 470 nm for 1- 2 minutes.

**1.8. Statistical Analysis**

The experiments of salt tolerance evaluation during three successive subcultures of shoot multiplication were comprised of 12 treatments (3 grape cultivars × 4 salinity concentrations), three replicates each and with 6 original shoot explants per replicate. All data were statistically analyzed as a factorial experiment in a completely randomized design, according to Steel and Torrie (1980).

**2. Rhizogenesis Under Salt Stress Conditions**

**2.1. Plant Material**

These experiments were carried out on proliferated shoots of the three grape cultivars derived from *in vitro* shoot multiplication under salt stress conditions. The multiplicated shoots considered the explant mother stock for *in vitro* rooting under salt stress conditions.

**2.2. Culture Media**

Due to the results of rooting experiments which mentioned previously (in the first part of this study), it was cleared that the best rooting medium was the Murashige and Skoog (1962) at half strength (designated 1/2 MS medium) amended with 2% sucrose + 0.2 $mg1^{-1}$ IBA. Solidification of the rooting medium was achieved by 0.6% agar. For salt tolerance evaluation, this medium was supplemented with zero, 1000, 2000 and 4000 ppm of salt mixture (as previously described).

The pH of rooting media under salt stress conditions was adjusted to 5.7 before adding agar. The rooting media were dispensed in glass culture tubes (180 × 25 mm) with 15 ml (each), closed with cotton, capped with aluminum foil. The rooting media were autoclaved at 121°C for 20 min., then left to cool and harden for 24 hrs., before being used.

**2.3. Culture Procedures**

At the end of each subculture, uniformity, vigorously growing and healthy proliferated shoots (≥ 2 cm) were excised individually under aseptical conditions and cultured vertically into culture tubes containing rooting media. One proliferated shoot cultured in each culture tube.

**2.4. Culture Conditions**

The shoot cultures were maintained on racks in growth culture room at 24 ± 2°C, with 16 hrs photoperiod provided by white fluorescent lamps, followed by 8 hrs dark period for 4 weeks.

**2.5. Statistical Analysis**

The experiments of rooting under salt stress conditions were comprised of 12 treatments (3 grape cultivars × 4 salinity concentrations), three replicates each and with 6 shoot explants per replicate. All data were statistically analyzed as a factorial experiment in a completely randomized design, according to Steel and Torrie (1980).

Rooting percentage, average number of roots per shoot and average root length per shoot were recorded after 4 weeks of shoot culture.

**3. Transplanting of Plants to Soil**

The obtained plantlets 7 to 10 cm long (healthy and vigorously) of the studied cultivars were potted in a sterilized mixture of vermiculite and peat moss mixed with soil (2:1) and kept under high air humidity for 3 weeks. Irrigation was carried out every four days with saline water (3000 ppm of salt mixture that mentioned above) with the addition of appropriate volume of nutrient 1/2 MS medium without sucrose and gradually exposed to open air (lowering the humidity) during 2 to 3 weeks. Then, they were transplanted and transferred to the greenhouse. Observations on survival and growth were recorded.

All the experiments were repeated for three years.

## RESULTS AND DISCUSSION

**1. Shoot Multiplication Under Salt Stress Conditions**

**1.1.Shoot Multiplication Percentage**

Regarding the effect of cultivars on shoot multiplication percentage, after the first, second and third subcultures, irrespective the effect of salinity treatments, the data of Tables (1 to 3) showed that, Early Superior gave significantly the highest shoot multiplication percentages (70.833%), (66.668%) and (62.500%), followed by Flame seedless (69.443%), (65.277%) and (61.110%), and Thompson seedless (48.613%), (44.445%) and (40.278%), respectively.

As for the effect of salinity treatments on shoot multiplication percentage, after the first, second and third subcultures, regardless of the effect of cultivars, the data in the same Tables revealed, a high decrease in shoot multiplication percentages joined the increase in salinity concentrations from zero to 4000 ppm. Shoot multiplication percentages were 85.183, 68.520 , 55.557 and 42.590% (in the first subculture), 85.183, 62.963,

50.000 and 37.040% (in the second subculture) and 85.183, 57.407, 44.447 and 31.480% (in the third subculture) when salinity concentrations were used at zero, 1000 , 2000 and 4000 ppm, respectively. The differences among salinity treatments were statistically significant.

**Table (1): Effect of four salinity treatments on shoot multiplication percentage, average number of new proliferated shoots per original shoot and average length (cm) of new proliferated shoot of grape cultivars Early Superior, Flame seedless and Thompson seedless after the first subculture.**

| Cultivar | Salinity in ppm | Shoot multiplication (%) | Av. no. of new proliferated shoots/ original shoot | Av. length (cm) of new proliferated shoot |
|---|---|---|---|---|
| Early Superior | 0000 | 83.33 | 4.07 | 2.53 |
| | 1000 | 72.22 | 3.54 | 2.40 |
| | 2000 | 66.67 | 3.33 | 2.18 |
| | 4000 | 61.11 | 3.18 | 1.82 |
| | Mean | 70.833 A* | 3.530 A | 2.233 B |
| Flame seedless | 0000 | 94.44 | 4.53 | 2.70 |
| | 1000 | 77.78 | 3.64 | 2.51 |
| | 2000 | 61.11 | 3.09 | 2.22 |
| | 4000 | 44.44 | 2.25 | 1.65 |
| | Mean | 69.443 B | 3.378 B | 2.270 A |
| Thompson seedless | 0000 | 77.78 | 3.50 | 2.38 |
| | 1000 | 55.56 | 2.70 | 2.08 |
| | 2000 | 38.89 | 2.00 | 1.70 |
| | 4000 | 22.22 | 1.50 | 1.23 |
| | Mean | 48.613 C | 2.425 C | 1.848C |
| Allover effect | 0000 | 85.183 A | 4.033 A | 2.537 A |
| | 1000 | 68.520 B | 3.293 B | 2.330 B |
| | 2000 | 55.557 C | 2.807 C | 2.033 C |
| | 4000 | 42.590 D | 2.310 D | 1.567 D |
| L.S.D. | | | | |
| Cultivars | 0.05 | 0.013 | 0.028 | 0.017 |
| Salinity concentrations | 0.05 | 0.015 | 0.032 | 0.019 |
| Cultivars× Salinity conc. | 0.05 | 0.025 | 0.055 | 0.033 |

***Values followed by the same letters between cultivars or between salinity treatments in the same column significantly are not differed at the 0.05 level of probability.**

### 1.2.Average Number of New Proliferated Shoots

Concerning the effect of cultivars on average number of new proliferated shoots per original shoot, after the first, second and third subcultures, irrespective the effect of salinity treatments, the data in the same Tables indicated that, Early Superior recorded significantly the highest averages number of new proliferated shoots per original shoot (3.530), (3.345) and (3.118), followed by Flame seedless (3.378), (3.150) and (2.960), and Thompson seedless (2.425), (2.143) and (2.043), respectively.

Regarding the effect of salinity treatments on average number of new proliferated shoots per original shoot, after the first, second and third subcultures, regardless of the effect of cultivars, the data in the same Tables revealed a high decrease in average number of new proliferated shoots per

original shoot accompanied with the increase in salinity concentrations from zero to 4000 ppm. Averages number of new proliferated shoots per original shoot were 4.033, 3.293, 2.807 and 2.310 (in the first subculture), 4.097, 2.963, 2.457 and 2.000 (in the second subculture) and 4.163, 2.707, 2.213 and 1.743 (in the third subculture) when salinity concentrations were used at zero, 1000 , 2000 and 4000 ppm, respectively. The differences among salinity treatments were statistically significant.

**Table (2): Effect of four salinity treatments on shoot multiplication percentage, average number of new proliferated shoots per original shoot and average length (cm) of new proliferated shoot of grape cultivars Early Superior, Flame seedless and Thompson seedless after the second subculture.**

| Cultivar | Salinity in ppm | Shoot multiplication (%) | Av. no. of new proliferated shoots/ original shoot | Av. length (cm) of new proliferated shoot |
|---|---|---|---|---|
| Early Superior | 0000 | 83.33 | 4.13 | 2.55 |
| | 1000 | 66.67 | 3.25 | 2.38 |
| | 2000 | 61.11 | 3.00 | 2.15 |
| | 4000 | 55.56 | 3.00 | 1.78 |
| | Mean | 66.668 A* | 3.345 A | 2.215 B |
| Flame seedless | 0000 | 94.44 | 4.59 | 2.73 |
| | 1000 | 72.22 | 3.31 | 2.47 |
| | 2000 | 55.56 | 2.70 | 2.19 |
| | 4000 | 38.89 | 2.00 | 1.62 |
| | Mean | 65.277 B | 3.150 B | 2.253 A |
| Thompson seedless | 0000 | 77.78 | 3.57 | 2.40 |
| | 1000 | 50.00 | 2.33 | 2.03 |
| | 2000 | 33.33 | 1.67 | 1.65 |
| | 4000 | 16.67 | 1.00 | 1.18 |
| | Mean | 44.445 C | 2.143 C | 1.815 C |
| Allover effect | 0000 | 85.183 A | 4.097 A | 2.560 A |
| | 1000 | 62.963 B | 2.963 B | 2.293 B |
| | 2000 | 50.000 C | 2.457 C | 1.997 C |
| | 4000 | 37.040 D | 2.000 D | 1.527 D |
| L.S.D. | | | | |
| Cultivars | 0.05 | 0.487 | 0.078 | 0.013 |
| Salinity concentrations | 0.05 | 0.562 | 0.090 | 0.015 |
| Cultivars× Salinity conc. | 0.05 | 0.973 | 0.156 | 0.027 |

*** Values followed by the same letters between cultivars or between salinity treatments in the same column significantly are not differed at the 0.05 level of probability.**

### 1.3.Average Length of New Proliferated Shoot

As for the effect of cultivars on average length of new proliferated shoot, after the first, second and third subcultures, irrespective the effect of salinity treatments, the data in the same Tables indicated that, Flame seedless gave significantly the highest average length of new proliferated shoot (2.270 cm), (2.253 cm) and (2.253 cm), followed by Early Superior (2.

233 cm), (2.215 cm) and (2.210 cm), and Thompson seedless (1.848 cm), (1.815 cm) and (1.803 cm), respectively.

Concerning the effect of salinity treatments on average length of new proliferated shoot, after the first, second and third subcultures, irrespective the effect of cultivars, the data in the same Tables also indicated that a high decrease in average length of new proliferated shoot joined the increase in salinity concentrations from zero to 4000 ppm. Averages length of new proliferated shoot were 2.537, 2.330, 2.033 and 1.567 cm (in the first subculture), 2.560, 2.293, 1.997 and 1.527 cm (in the second subculture) and 2.580, 2.280, 1.983 and 1.510 cm (in the third subculture) when salinity concentrations were used at zero, 1000 , 2000 and 4000 ppm, respectively. The differences among salinity treatments were statistically significant.

**Table (3): Effect of four salinity treatments on shoot multiplication percentage, average number of new proliferated shoots per original shoot and average length (cm) of new proliferated shoot of grape cultivars Early Superior, Flame seedless and Thompson seedless after the third subculture.**

| Cultivar | Salinity in ppm | Shoot multiplication (%) | Av. no. of new proliferated shoots/ original shoot | Av. Length (cm) of new proliferated shoot |
|---|---|---|---|---|
| Early Superior | 0000 | 83.33 | 4.20 | 2.57 |
| | 1000 | 61.11 | 2.91 | 2.37 |
| | 2000 | 55.56 | 2.80 | 2.14 |
| | 4000 | 50.00 | 2.56 | 1.76 |
| | Mean | 62.500 A* | 3.118 A | 2.210 B |
| Flame seedless | 0000 | 94.44 | 4.65 | 2.76 |
| | 1000 | 66.67 | 3.08 | 2.47 |
| | 2000 | 50.00 | 2.44 | 2.18 |
| | 4000 | 33.33 | 1.67 | 1.60 |
| | Mean | 61.110 B | 2.960 B | 2.253 A |
| Thompson seedless | 0000 | 77.78 | 3.64 | 2.41 |
| | 1000 | 44.44 | 2.13 | 2.00 |
| | 2000 | 27.78 | 1.40 | 1.63 |
| | 4000 | 11.11 | 1.00 | 1.17 |
| | Mean | 40.278 C | 2.043 C | 1.803 C |
| Allover effect | 0000 | 85.183 A | 4.163 A | 2.580 A |
| | 1000 | 57.407 B | 2.707 B | 2.280 B |
| | 2000 | 44.447 C | 2.213 C | 1.983 C |
| | 4000 | 31.480 D | 1.743 D | 1.510 D |
| L.S.D. | | | | |
| Cultivars | 0.05 | 0.544 | 0.013 | 0.027 |
| Salinity concentrations | 0.05 | 0.628 | 0.015 | 0.031 |
| Cultivars×Salinity conc. | 0.05 | 1.088 | 0.026 | 0.054 |

*** Values followed by the same letters between cultivars or between salinity treatments in the same column significantly are not differed at the 0.05 level of probability.**

Regarding the forecited results, it was cleared that in the 1st , 2nd and 3rd subcultures, Early Superior gave significantly the highest shoot

multiplication percentage and average number of new proliferated shoots per original shoot, followed by Flame seedless and Thompson seedless. Such results were confirmed by Shahin (1997) who reported that Early Superior exhibited the highest survival percentage, followed by Flame seedless, while King Ruby showed the lowest survival percentage, after 4 or 12 weeks from culturing on saline media.

The same results indicated that, shoot multiplication percentage, average number and length of new proliferated shoots per original shoot of the studied cultivars were decreased with increasing salinity concentrations (from zero to 4000 ppm) and salt treatment period (from 1st to 3rd subculture). These findings also agreed with those reported by Shahin (1997) who found that shoot survival percentages of the cultivars Flame seedless, King Ruby and Early Superior were significantly decreased with increasing salt concentrations in MS medium. In this concept, Sivritepe and Eris (1998) mentioned that, under *in vitro* conditions, shoot proliferation and growth of grapevine rootstocks decreased with increasing NaCl concentration and treatment period. The same authors (1999) found that shoot proliferation and growth of explants were decreased due to an increase in NaCl concentration in the culture medium and length of treatment period.

**2. Shoot Injury Under Salt Stress Conditions**

Regarding the effect of cultivars on shoot injury percentages, after the first, second and third subcultures, irrespective the effect of salinity treatments, the data in Table (4) indicated that, Thompson seedless exhibited significantly the highest shoot injury percentage (37. 500 %), (42.860%) and (48.215%) followed by Flame seedless (26. 468 %), (30.880%) and (35.292%), then Early Superior (14.995%), (19.993% ) and (24.995%), respectively.

Concerning the effect of salinity treatments on shoot injury percentages, after the first, second and third subcultures, regardless of the effect of cultivars, the data in the same Table revealed a high increase in shoot injury percentage joined the increase in salinity concentrations from zero to 4000 ppm. Shoot injury percentages were 00.000, 19.847, 35.093 and 50.343% (in the first subculture), 00.000, 26.413, 41.660 and 56.903% (in the second subculture), and 00.000, 32.973, 48.220 and 63.477% (in the third subculture) when salinity concentrations were used at zero, 1000, 2000 and 4000 ppm, respectively. The differences among salinity treatments were statistically significant.

According to Table (4), it was obvious that, Thompson seedless exhibited significantly the highest shoot injury percentage, followed by Flame seedless and Early Superior. The results in the same Table also indicated that shoot injury percentage of the studied cultivars increased with increasing salinity concentrations (from zero to 4000 ppm) and salt treatment period (from 1st to 3rd subculture). These results were in harmony with those found by Sivritepe and Eris (1998). They stated that salt treatments caused loss of viability and injuries at different levels in explants, depending on NaCl concentration, treatment period and rootstock.

## 3. Leaf Mineral Content Under Salt Stress Conditions

### 3.1. Nitrogen

As for the effect of cultivars on leaf nitrogen content, after the first, second and third subcultures, irrespective the effect of salinity treatments, the data in Tables (5 to 7) indicated that, Early Superior achieved significantly the highest leaf nitrogen content (2.238%), (2.210%) and (2.180%), followed by Flame seedless (2.163%), (2.125%) and (2.080%), then Thompson seedless (2.145%), (2.078%) and (2.030%), respectively.

Concerning the effect of salinity treatments on leaf nitrogen content, after the first, second and third subcultures, regardless of the effect of cultivars, the data in the same Tables revealed that the leaf nitrogen content showed a tendency for positive responses to salinity treatments. Leaf nitrogen contents were 2.007, 2.103, 2.260 and 2.357% (in the first subculture), 1.977, 2.047, 2.210 and 2.317% (in the second subculture) and, 1.930, 2.017, 2.160 and 2.280% (in the third subculture) when salinity concentrations were used at zero, 1000, 2000 and 4000 ppm, respectively. The differences among salinity treatments were statistically significant.

**Table (4): Effect of four salinity treatments on shoot injury percentage[z] in grape cultivars Early Superior, Flame seedless and Thompson seedless after the first , second and third subcultures.**

| Cultivar | Salinity in ppm | 1st subculture | 2nd subculture | 3rd subculture |
|---|---|---|---|---|
| Early Superior | 0000 | 00.00 | 00.00 | 00.00 |
| | 1000 | 13.33 | 19.99 | 26.66 |
| | 2000 | 19.99 | 26.66 | 33.32 |
| | 4000 | 26.66 | 33.32 | 40.00 |
| | Mean | 14.995 C* | 19.993 C | 24.995 C |
| Flame seedless | 0000 | 00.00 | 00.00 | 00.00 |
| | 1000 | 17.64 | 23.53 | 29.40 |
| | 2000 | 35.29 | 41.17 | 47.06 |
| | 4000 | 52.94 | 58.82 | 64.71 |
| | Mean | 26.468 B | 30.880 B | 35.292 B |
| Thompson seedless | 0000 | 00.00 | 00.00 | 00.00 |
| | 1000 | 28.57 | 35.72 | 42.86 |
| | 2000 | 50.00 | 57.15 | 64.28 |
| | 4000 | 71.43 | 78.57 | 85.72 |
| | Mean | 37.500 A | 42.860 A | 48.215 A |
| Allover effect | 0000 | 00.000 D | 00.000 D | 00.000 D |
| | 1000 | 19.847 C | 26.413 C | 32.973 C |
| | 2000 | 35.093 B | 41.660 B | 48.220 B |
| | 4000 | 50.343 A | 56.903 A | 63.477 A |
| L.S.D. | | | | |
| Cultivars | 0.05 | 0.487 | 0.018 | 0. 487 |
| Salinity concentrations | 0.05 | 0.562 | 0.020 | 0.562 |
| Cultivars× Salinity conc. | 0.05 | 0.973 | 0.035 | 0.973 |

[z] **Shoot injury index ≥ 50% of leaves and stems were burned.**

* **Values followed by the same letters between cultivars or between salinity treatments in the same column significantly are not differed at the 0.05 level of probability.**

### 3.2. Phosphorus

Regarding the effect of cultivars on leaf phosphorus content, after the first, second and third subcultures, irrespective the effect of salinity treatments, the data in Tables (5 to 7) showed that, Early Superior exhibited significantly the highest leaf phosphorus content (0.398%), (0.393%) and (0.383%), followed by Flame seedless (0.320%), (0.318%) and (0.310%), then Thompson seedless (0.268%), (0.260%) and (0.268%), respectively.

As for the effect of salinity treatments on leaf phosphorus content, after the first, second and third subcultures, regardless of the effect of cultivars, the data in the same Tables indicated that the leaf phosphorus content showed a tendency for negative responses to salinity treatments. Leaf phosphorus contents were 0.410, 0.360, 0.307 and 0.237% (in the first subculture), 0.410, 0.357, 0.297 and 0.230% (in the second subculture) and, 0.413, 0.350, 0.290 and 0.227% (in the third subculture) when salinity concentrations were used at zero, 1000, 2000 and 4000 ppm, respectively. Significant differences were found between the salinity treatments.

### 3.3. Potassium

Concerning the effect of cultivars on leaf potassium content, after the first, second and third subcultures, regardless of the effect of salinity treatments, the data in Tables (5 to 7) indicated that, Early Superior produced significantly the highest leaf potassium content (0.965%), (0.975%) and (0.998%), followed by Flame seedless (0.678%), (0.685%) and (0.695%), then Thompson seedless (0.548%), (0.550%) and (0.535%), respectively.

As for the effect of salinity treatments on leaf potassium content, after the first, second and third subcultures, irrespective the effect of cultivars, the data in the same Tables revealed that the leaf potassium content showed a tendency for negative responses to salinity treatments. Leaf potassium contents were 0.887, 0.783, 0.687 and 0.563% (in the first subculture), 0.907, 0.793, 0.673 and 0.573% (in the second subculture) and, 0.900, 0.810, 0.683 and 0.577% (in the third subculture) when salinity concentrations were used at zero, 1000, 2000 and 4000 ppm, respectively. The differences among salinity treatments were statistically significant.

### 3.4. Calcium

Regarding the effect of cultivars on leaf calcium content, after the first, second and third subcultures, irrespective the effect of salinity treatments, the data in Tables (5 to 7) indicated that, Thompson seedless gave significantly the highest leaf calcium content (1.193%), (1.248%) and (1.285%), followed by Flame seedless (1.145%), (1.150%) and (1.170%), then Early Superior (1.105%), (1.080%) and (1.085%), respectively.

As for the effect of salinity treatments on leaf calcium content, after the first, second and third subcultures, regardless of the effect of cultivars, the data in the same Tables revealed that the leaf calcium content showed a tendency for negative responses to salinity treatments. Leaf calcium contents were 1.240, 1.197, 1.127 and 1.027% (in the first subculture), 1.230, 1.200, 1.143 and 1.063% (in the second subculture) and, 1.253, 1.213, 1.167 and 1.087% (in the third subculture) when salinity concentrations were used at zero, 1000, 2000 and 4000 ppm, respectively. The differences among salinity treatments were statistically significant.

### 3.5. Sodium

Concerning the effect of cultivars on leaf sodium content, after the first, second and third subcultures, regardless of the effect of salinity treatments, the data in Tables (5 to 7) showed that, Early Superior produced significantly the highest leaf sodium content (0.628%), (0.658%) and (0.688%), followed by Flame seedless (0.553%), (0.565%) and (0.575%), then Thompson seedless (0.535%), (0.520%) and (0.520%), respectively.

**Table (5): Effect of four salinity treatments on leaf mineral content of grape cultivars Early Superior, Flame seedless and Thompson seedless after the first subculture.**

| Cultivar | Salinity in ppm | (%) on dry weight basis | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | N | P | K | Ca | Na | Cl | Mg |
| Early Superior | 0000 | 2.11 | 0.46 | 1.06 | 1.15 | 0.54 | 0.58 | 1.10 |
| | 1000 | 2.19 | 0.42 | 1.00 | 1.12 | 0.60 | 0.64 | 1.00 |
| | 2000 | 2.27 | 0.38 | 0.96 | 1.10 | 0.65 | 0.70 | 0.93 |
| | 4000 | 2.38 | 0.33 | 0.84 | 1.05 | 0.72 | 0.75 | 0.81 |
| | Mean | 2.238 A* | 0.398 A | 0.965 A | 1.105 C | 0.628 A | 0.668 A | 0.960 A |
| Flame seedless | 0000 | 1.98 | 0.40 | 0.86 | 1.26 | 0.50 | 0.62 | 0.94 |
| | 1000 | 2.07 | 0.36 | 0.73 | 1.21 | 0.54 | 0.65 | 0.88 |
| | 2000 | 2.24 | 0.32 | 0.60 | 1.08 | 0.57 | 0.68 | 0.76 |
| | 4000 | 2.36 | 0.20 | 0.52 | 1.03 | 0.60 | 0.71 | 0.70 |
| | Mean | 2.163 B | 0.320 B | 0.678 B | 1.145 B | 0.553 B | 0.665 A | 0.820 B |
| Thompson seedless | 0000 | 1.93 | 0.37 | 0.74 | 1.31 | 0.49 | 0.65 | 0.80 |
| | 1000 | 2.05 | 0.30 | 0.62 | 1.26 | 0.52 | 0.67 | 0.78 |
| | 2000 | 2.27 | 0.22 | 0.50 | 1.20 | 0.55 | 0.69 | 0.66 |
| | 4000 | 2.33 | 0.18 | 0.33 | 1.00 | 0.58 | 0.70 | 0.60 |
| | Mean | 2.145 C | 0.268 C | 0.548 C | 1.193 A | 0.535 C | 0.678 A | 0.710 C |
| Allover effect | 0000 | 2.007 D | 0.410 A | 0.887 A | 1.240 A | 0.510 D | 0.617 D | 0.947 A |
| | 1000 | 2.103 C | 0.360 B | 0.783 B | 1.197 B | 0.553 C | 0.653 C | 0.887 B |
| | 2000 | 2.260 B | 0.307 C | 0.687 C | 1.127 C | 0.590 B | 0.690 B | 0.783 C |
| | 4000 | 2.357 A | 0.237 D | 0.563 D | 1.027 D | 0.633 A | 0.720 A | 0.703 D |
| L.S.D. | | | | | | | | |
| Cultivars 0.05 | | 0.015 | 0.017 | 0.051 | 0.013 | 0.015 | 0.014 | 0.050 |
| Salinity concentrations 0.05 | | 0.018 | 0.019 | 0.059 | 0.015 | 0.017 | 0.016 | 0.058 |
| Cultivars ×Salinity conc.0.05 | | 0.030 | 0.033 | 0.102 | 0.026 | 0.029 | 0.028 | 0.101 |

***Values followed by the same letters between cultivars or between salinity treatments in the same column significantly are not differed at the 0.05 level of probability.**

Regarding the effect of salinity treatments on leaf sodium content, after the first, second and third subcultures, irrespective the effect of cultivars, the data in the same Tables indicated that the leaf sodium content showed a tendency for positive responses to salinity treatments. Leaf sodium contents were 0.510, 0.553, 0.590 and 0.633% (in the first subculture), 0.517, 0.563, 0.603 and 0.640% (in the second subculture) and, 0.510, 0.573, 0.627 and 0.667% (in the third subculture) when salinity concentrations were used at zero, 1000, 2000 and 4000 ppm, respectively. The differences among salinity treatments were statistically significant.

### 3.6. Chloride

Concerning the effect of cultivars on leaf chloride content, after the first, second and third subcultures, regardless of the effect of salinity treatments, the data in Tables (5 to 7) indicated that the differences among the studied cultivars were not significant (in the first subculture). In the second subculture, Thompson seedless gave significantly the highest leaf

chloride content (0.705%), followed by Early Superior (0.683%) and Flame seedless (0.660%). While, in the third subculture, Thompson seedless and Early Superior achieved significantly the highest leaf chloride contents (0.700% and 0.698%, respectively), followed by Flame seedless (0.668%).

**Table (6): Effect of four salinity treatments on leaf mineral content of grape cultivars Early Superior, Flame seedless and Thompson seedless after the second subculture.**

| Cultivar | Salinity in ppm | (%) on dry weight basis | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | N | P | K | Ca | Na | Cl | Mg |
| Early Superior | 0000 | 2.08 | 0.47 | 1.08 | 1.12 | 0.56 | 0.56 | 1.08 |
| | 1000 | 2.16 | 0.43 | 1.03 | 1.10 | 0.64 | 0.65 | 1.02 |
| | 2000 | 2.24 | 0.37 | 0.92 | 1.07 | 0.68 | 0.72 | 0.95 |
| | 4000 | 2.36 | 0.30 | 0.87 | 1.03 | 0.75 | 0.80 | 0.80 |
| | Mean | 2.210 A* | 0.393 A | 0.975 A | 1.080 C | 0.658 A | 0.683 B | 0.96 3 A |
| Flame seedless | 0000 | 1.96 | 0.41 | 0.87 | 1.23 | 0.51 | 0.61 | 0.96 |
| | 1000 | 2.03 | 0.34 | 0.75 | 1.20 | 0.55 | 0.65 | 0.85 |
| | 2000 | 2.20 | 0.30 | 0.62 | 1.11 | 0.59 | 0.67 | 0.73 |
| | 4000 | 2.31 | 0.22 | 0.50 | 1.06 | 0.61 | 0.71 | 0.68 |
| | Mean | 2.125 B | 0.318 B | 0.685 B | 1.150 B | 0.565 B | 0.660 C | 0.80 5 B |
| Thompson seedless | 0000 | 1.89 | 0.35 | 0.77 | 1.34 | 0.48 | 0.67 | 0.81 |
| | 1000 | 1.95 | 0.30 | 0.60 | 1.30 | 0.50 | 0.70 | 0.76 |
| | 2000 | 2.19 | 0.22 | 0.48 | 1.25 | 0.54 | 0.72 | 0.69 |
| | 4000 | 2.28 | 0.17 | 0.35 | 1.10 | 0.56 | 0.73 | 0.62 |
| | Mean | 2.078 C | 0.260 C | 0.550 C | 1.248 A | 0.520 C | 0.705 A | 0.72 0 C |
| Allover effect | 0000 | 1.977 D | 0.410 A | 0.907 A | 1.230 A | 0.517 D | 0.613 D | 0.95 0 A |
| | 1000 | 2.047 C | 0.357 B | 0.793 B | 1.200 B | 0.563 C | 0.667 C | 0.87 7 B |
| | 2000 | 2.210 B | 0.297 C | 0.673 C | 1.143 C | 0.603 B | 0.703 B | 0.79 0 C |
| | 4000 | 2.317 A | 0.230 D | 0.573 D | 1.063 D | 0.640 A | 0.747 A | 0.70 0 D |
| L.S.D. Cultivars | 0.05 | 0.013 | 0.015 | 0.015 | 0.015 | 0.015 | 0.013 | 0.01 4 |
| Salinity concentrations | 0.05 | 0.015 | 0.018 | 0.017 | 0.017 | 0.017 | 0.015 | 0.01 6 |
| Cultivars ×Salinity conc. | 0.05 | 0.025 | 0.030 | 0.029 | 0.029 | 0.029 | 0.027 | 0.02 8 |

*** Values followed by the same letters between cultivars or between salinity treatments in the same column significantly are not differed at the 0.05 level of probability.**

As for the effect of salinity treatments on leaf chloride content, after the first, second and third subcultures, irrespective the effect of cultivars, the data in the same Tables revealed that the leaf chloride content showed a tendency for positive responses to salinity treatments. Leaf chloride contents were 0.617, 0.653, 0.690 and 0.720% (in the first subculture), 0.613, 0.667, 0.703 and 0.747% (in the second subculture) and, 0.600, 0.667, 0.720 and 0.767% (in the third subculture) when salinity concentrations were used at zero, 1000, 2000 and 4000 ppm, respectively. Significant differences were found between the salinity treatments.

## 3.7. Magnesium

Regarding the effect of cultivars on leaf magnesium content, after the first, second and third subcultures, irrespective the effect of salinity treatments, the data in Tables (5 to 7) revealed that, Early Superior achieved significantly the highest leaf magnesium content (0.960%), (0.963%) and (0.983%), followed by Flame seedless (0.820%), (0.805%) and (0.828%), then Thompson seedless (0.710%), (0.720%) and (0.740%), respectively.

Concerning the effect of salinity treatments on leaf magnesium content, after the first, second and third subcultures, regardless of the effect of cultivars, the data in the same Tables indicated that the leaf magnesium content showed a tendency for negative responses to salinity treatments. Leaf magnesium contents were 0.947, 0.887, 0.783 and 0.703% (in the first subculture), 0.950, 0.877, 0.790 and 0.700% (in the second subculture) and, 0.967, 0.897, 0.810 and 0.727% (in the third subculture) when salinity concentrations were used at zero, 1000, 2000 and 4000 ppm, respectively. The differences among salinity treatments were statistically significant.

**Table (7): Effect of four salinity treatments on leaf mineral content of grape cultivars Early Superior, Flame seedless and Thompson seedless after the third subculture.**

| Cultivar | Salinity in ppm | (%) on dry weight basis | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | N | P | K | Ca | Na | Cl | Mg |
| Early Superior | 0000 | 2.03 | 0.48 | 1.10 | 1.14 | 0.57 | 0.54 | 1.09 |
| | 1000 | 2.12 | 0.41 | 1.06 | 1.11 | 0.67 | 0.67 | 1.03 |
| | 2000 | 2.23 | 0.35 | 0.95 | 1.09 | 0.72 | 0.75 | 0.98 |
| | 4000 | 2.34 | 0.29 | 0.88 | 1.00 | 0.79 | 0.83 | 0.83 |
| | Mean | 2.180 A* | 0.383 A | 0.998 A | 1.085 C | 0.688 A | 0.698 A | 0.983 A |
| Flame seedless | 0000 | 1.92 | 0.40 | 0.85 | 1.25 | 0.50 | 0.61 | 0.98 |
| | 1000 | 2.00 | 0.35 | 0.76 | 1.21 | 0.56 | 0.64 | 0.88 |
| | 2000 | 2.14 | 0.29 | 0.64 | 1.13 | 0.61 | 0.69 | 0.75 |
| | 4000 | 2.26 | 0.20 | 0.53 | 1.09 | 0.63 | 0.73 | 0.70 |
| | Mean | 2.080 B | 0.310 B | 0.695 B | 1.170 B | 0.575 B | 0.668 B | 0.828 B |
| Thompson seedless | 0000 | 1.84 | 0.36 | 0.75 | 1.37 | 0.46 | 0.65 | 0.83 |
| | 1000 | 1.93 | 0.29 | 0.61 | 1.32 | 0.49 | 0.69 | 0.78 |
| | 2000 | 2.11 | 0.23 | 0.46 | 1.28 | 0.55 | 0.72 | 0.70 |
| | 4000 | 2.24 | 0.19 | 0.32 | 1.17 | 0.58 | 0.74 | 0.65 |
| | Mean | 2.030 B | 0.268 C | 0.535 C | 1.285 A | 0.520 C | 0.700 A | 0.740 C |
| Allover effect | 0000 | 1.930 D | 0.413 A | 0.900 A | 1.253 A | 0.510 D | 0.600 D | 0.967 A |
| | 1000 | 2.017 C | 0.350 B | 0.810 B | 1.213 B | 0.573 C | 0.667 C | 0.897 B |
| | 2000 | 2.160 B | 0.290 C | 0.683 C | 1.167 C | 0.627 B | 0.720 B | 0.810 C |
| | 4000 | 2.280 A | 0.227 D | 0.577 D | 1.087 D | 0.667 A | 0.767 A | 0.727 D |
| L.S.D. | | | | | | | | |
| Cultivars | 0.05 | 0.050 | 0.015 | 0.013 | 0.013 | 0.011 | 0.009 | 0.013 |
| Salinity concentrations | 0.05 | 0.058 | 0.017 | 0.015 | 0.015 | 0.013 | 0.011 | 0.015 |
| Cultivars ×Salinity conc. | 0.05 | 0.101 | 0.029 | 0.025 | 0.026 | 0.022 | 0.019 | 0.025 |

*** Values followed by the same letters between cultivars or between salinity treatments in the same column significantly are not differed at the 0.05 level of probability.**

Referring to the obtained results in Tables (5 to 7), it is evident that, in the 1st, 2nd and 3rd subcultures, Early Superior achieved significantly the highest leaf P, K, Na and Mg contents, followed by Flame seedless and Thompson seedless. In contrast, Thompson seedless presented significantly the highest leaf Ca content, followed by Flame seedless and Early Superior. The results in the same Tables also indicated that leaf N, Na and Cl contents in the studied cultivars increased with increasing salinity concentrations (from zero to 4000 ppm). On the contrary, leaf P, K, Ca and Mg contents in the studied cultivars decreased with increasing salinity concentrations (from zero to 4000 ppm). Such findings agreed with those reported by Troncoso *et al.*, (1999). They found that increasing salt concentrations significantly reduced the leaf K content and, to a smaller extent, the leaf P and Ca contents. Sodium and Cl were accumulated to a greater extent in tolerant plants. The tolerance to NaCl of *in vitro* grown grape rootstocks seemed to be due to their capacity to accumulate salt, to increase K concentration in their tissues and to maintain a high water content.

Recently, El-Gazzar *et al.*, (2002) found that the NaCl – tolerant callus of sour orange had higher K content, as compared with the NaCl – sensitive callus. On the contrary, the NaCl – tolerant callus had lower Cl, Ca and Mg contents, as compared with NaCl – sensitive callus.

**4. Biochemical Responses Under Salt Stress Conditions**

**4.1. Leaf Proline Content**

Concerning the effect of cultivars on leaf proline content, after the first, second and third subcultures, regardless of the effect of salinity treatments, the data in Tables (8 to 10) indicated that, Early Superior gave significantly the highest leaf proline content (0.123%), (0.148%) and (0.163%), followed by Flame seedless (0.090%), (0.103%) and (0.085%), then Thompson seedless (0.078%), (0.088%) and (0.078%), respectively.

As for the effect of salinity treatments on leaf proline content, after the first, second and third subcultures, irrespective the effect of cultivars, the data in the same Tables revealed that the leaf proline content showed a tendency for positive responses to salinity treatments. Leaf proline contents were 0.063, 0.083, 0.110 and 0.130% (in the first subculture), 0.067, 0.097, 0.130 and 0.157% (in the second subculture) and, 0.057, 0.087, 0.133 and 0.157% (in the third subculture) when salinity concentrations were used at zero, 1000, 2000 and 4000 ppm, respectively. The differences among salinity treatments were statistically significant.

**4.2. Leaf Chlorophyll Content**

Regarding the effect of cultivars on leaf chlorophyll content, after the first, second and third subcultures, irrespective the effect of salinity treatments, the data in Tables (8 to 10) indicated that, Early Superior exhibited significantly the highest leaf chlorophyll content (1.368 mg/ 100 $cm^2$), (1.398 mg/100 $cm^2$) and (1.413 mg/100 $cm^2$), followed by Flame seedless (1.220 mg/ 100 $cm^2$), (1.278 mg/100 $cm^2$) and (1.263 mg/100 $cm^2$), then Thompson seedless (1.100 mg/ 100 $cm^2$), (1.148 mg/100 $cm^2$) and (1.180 mg/100 $cm^2$), respectively.

**Table (8): Effect of four salinity treatments on leaf proline, total chlorophyll contents and peroxidase activity of grape cultivars Early Superior, Flame seedless and Thompson seedless after the first subculture.**

| Cultivar | Salinity in ppm | Proline (%) on dry weight basis | Total chlorophyll (mg/ 100 $cm^2$) | Peroxidase activity (% of control) |
|---|---|---|---|---|
| Early Superior | 0000 | 0.08 | 1.48 | 100.00 |
| | 1000 | 0.10 | 1.40 | 92.87 |
| | 2000 | 0.14 | 1.32 | 83.16 |
| | 4000 | 0.17 | 1.27 | 70.56 |
| | Mean | 0.123 A* | 1.368 A | 86.648 A |
| Flame seedless | 0000 | 0.06 | 1.37 | 100.00 |
| | 1000 | 0.08 | 1.30 | 85.17 |
| | 2000 | 0.10 | 1.21 | 76.64 |
| | 4000 | 0.12 | 1.00 | 65.15 |
| | Mean | 0.090 B | 1.220 B | 81.740 B |
| Thompson seedless | 0000 | 0.05 | 1.23 | 100.00 |
| | 1000 | 0.07 | 1.18 | 74.22 |
| | 2000 | 0.09 | 1.10 | 69.18 |
| | 4000 | 0.10 | 0.89 | 56.10 |
| | Mean | 0.078 C | 1.100 C | 74.875 C |
| Allover effect | 0000 | 0.063 D | 1.360 A | 100.000 A |
| | 1000 | 0.083 C | 1.293 B | 84.087 B |
| | 2000 | 0.110 B | 1.210 C | 76.327 C |
| | 4000 | 0.130 A | 1.053 D | 63.937 D |
| L.S.D. | | | | |
| Cultivars | 0.05 | 0.012 | 0.015 | 0.013 |
| Salinity concentrations | 0.05 | 0.014 | 0.018 | 0.015 |
| Cultivars × Salinity conc. | 0.05 | 0.024 | 0.031 | 0.027 |

*** Values followed by the same letters between cultivars or between salinity treatments in the same column significantly are not differed at the 0.05 level of probability.**

As for the effect of salinity treatments on leaf chlorophyll content, after the first, second and third subcultures, regardless of the effect of cultivars, the data in the same Tables revealed that the leaf chlorophyll content showed a tendency for negative responses to salinity treatments. Leaf chlorophyll contents were 1.360, 1.293, 1.210 and 1.053 (mg/ 100 $cm^2$) in the first subculture, 1.377, 1.317, 1.253 and 1.150 (mg/100 $cm^2$) in the second subculture, 1.373, 1.333, 1.280 and 1.153 (mg/100 $cm^2$) in the third subculture when salinity concentrations were used at zero, 1000, 2000 and 4000 ppm, respectively. The differences among salinity treatments were statistically significant.

### 4.3. Leaf Peroxidase Activity

Regarding the effect of cultivars on leaf peroxidase activity, after the first, second and third subcultures, irrespective the effect of salinity treatments, the data in Tables (8 to 10) reveled that, Early Superior presented significantly the highest leaf peroxidase activity (86.648% of control), (88.783% of control) and (89.898% of control), followed by Flame seedless (81.740% of control), (83.280% of control) and (84.868% of control), then Thompson seedless (74.875% of control), (76.655% of control) and (78.795% of control), respectively.

Concerning the effect of salinity treatments on leaf peroxidase activity, after the first, second and third subcultures, regardless of the effect of cultivars, the data in the same Tables indicated that the leaf peroxidase activity showed a tendency for negative responses to salinity treatments. Leaf peroxidase activities were 100.000, 84.087, 76.327 and 63.937 (% of control) in the first subculture, 100.000, 85.947, 79.403 and 66.273 (% of control) in the second subculture and, 100.000, 87.693, 80.823 and 69.563 (% of control) in the third subculture when salinity concentrations were used at zero, 1000, 2000 and 4000 ppm, respectively. The differences among salinity treatments were statistically significant.

**Table (9): Effect of four salinity treatments on leaf proline, total chlorophyll contents and peroxidase activity of grape cultivars Early Superior, Flame seedless and Thompson seedless after the second subculture.**

| Cultivar | Salinity in ppm | Proline (%) on dry weight basis | Total chlorophyll (mg/ 100 $cm^2$) | Peroxidase activity (% of control) |
|---|---|---|---|---|
| Early Superior | 0000 | 0.09 | 1.50 | 100.00 |
| | 1000 | 0.12 | 1.43 | 93.98 |
| | 2000 | 0.17 | 1.36 | 88.10 |
| | 4000 | 0.21 | 1.30 | 73.05 |
| | Mean | 0.148 A* | 1.398 A | 88.783 A |
| Flame seedless | 0000 | 0.06 | 1.38 | 100.00 |
| | 1000 | 0.09 | 1.32 | 87.30 |
| | 2000 | 0.12 | 1.26 | 78.22 |
| | 4000 | 0.14 | 1.15 | 67.60 |
| | Mean | 0.103 B | 1.278 B | 83.280 B |
| Thompson seedless | 0000 | 0.05 | 1.25 | 100.00 |
| | 1000 | 0.08 | 1.20 | 76.56 |
| | 2000 | 0.10 | 1.14 | 71.89 |
| | 4000 | 0.12 | 1.00 | 58.17 |
| | Mean | 0.088 C | 1.148 C | 76.655 C |
| Allover effect | 0000 | 0.067 D | 1.377 A | 100.000 A |
| | 1000 | 0.097 C | 1.317 B | 85.947 B |
| | 2000 | 0.130 B | 1.253 C | 79.403 C |
| | 4000 | 0.157 A | 1.150 D | 66.273 D |
| L.S.D. | | | | |
| Cultivars | 0.05 | 0.011 | 0.051 | 0.012 |
| Salinity concentrations | 0.05 | 0.013 | 0.059 | 0.014 |
| Cultivars × Salinity conc. | 0.05 | 0.022 | 0.102 | 0.024 |

*** Values followed by the same letters between cultivars or between salinity treatments in the same column significantly are not differed at the 0.05 level of probability.**

From the mentioned results in Tables (8 to 10), it was obvious that, in the 1st, 2nd and 3rd subcultures, Early Superior achieved significantly the highest leaf chlorophyll content and peroxidase activity, followed by Flame seedless and Thompson seedless. Also, in the 1st, 2nd and 3rd subcultures, Early Superior gave significantly the highest leaf proline content, as compared with Flame seedless and Thompson seedless.

**Table (10): Effect of four salinity treatments on leaf proline, total chlorophyll contents and peroxidase activity of grape cultivars Early Superior, Flame seedless and Thompson seedless after the third subculture.**

| Cultivar | Salinity in ppm | Proline (%) on dry weight basis | Total chlorophyll (mg/ 100 $cm^2$) | Peroxidase activity (% of control) |
|---|---|---|---|---|
| Early Superior | 0000 | 0.08 | 1.49 | 100.00 |
| | 1000 | 0.13 | 1.46 | 94.06 |
| | 2000 | 0.20 | 1.38 | 89.18 |
| | 4000 | 0.24 | 1.32 | 76.35 |
| | Mean | 0.163 A* | 1.413 A | 89.898 A |
| Flame seedless | 0000 | 0.05 | 1.36 | 100.00 |
| | 1000 | 0.07 | 1.31 | 88.16 |
| | 2000 | 0.10 | 1.28 | 80.24 |
| | 4000 | 0.12 | 1.10 | 71.07 |
| | Mean | 0.085 B | 1.263 B | 84.868 B |
| Thompson seedless | 0000 | 0.04 | 1.27 | 100.00 |
| | 1000 | 0.06 | 1.23 | 80.86 |
| | 2000 | 0.10 | 1.18 | 73.05 |
| | 4000 | 0.11 | 1.04 | 61.27 |
| | Mean | 0.078 B | 1.180 C | 78.795 C |
| Allover effect | 0000 | 0.057 D | 1.373 A | 100.000 A |
| | 1000 | 0.087 C | 1.333 B | 87.693 B |
| | 2000 | 0.133 B | 1.280 C | 80.823 C |
| | 4000 | 0.157 A | 1.153 D | 69.563 D |
| L.S.D. | | | | |
| Cultivars | 0.05 | 0.009 | 0.012 | 0.016 |
| Salinity concentrations | 0.05 | 0.011 | 0.014 | 0.019 |
| Cultivars × Salinity conc. | 0.05 | 0.018 | 0.024 | 0.033 |

*** Values followed by the same letters between cultivars or between salinity treatments in the same column significantly are not differed at the 0.05 level of probability.**

The results in the same Tables also indicated that, leaf proline content in the studied cultivars increased with increasing salinity concentrations (from zero to 4000 ppm) and salt treatment period (from 1st to 3rd subculture). In contrast, leaf chlorophyll content and peroxidase activity in the studied cultivars were decreased with increasing salinity concentrations and treatment period. These findings agreed with those reported by Sivritepe and Eris (1998). They found that leaf chlorophyll content of grape rootstocks decreased with increasing NaCl concentration and treatment period. In addition, the same authors (1999) stated that leaf chlorophyll content of explants decreased due to increases in NaCl concentration and length of treatment period. They also reported that salt tolerant cultivars were better to avoid metabolic disorders such as chlorophyll deficiency under salt stress conditions. In this concept, Shahin (1997) and wafaa *et al.*,(1999) reported that increasing the salt concentration led to an increase in leaf proline content in all studied cultivars.

Recently, El-Gazzar *et al.*, (2002) found that proline content increased in salt- tolerant callus as compared to salt- sensitive callus (control). Accumulation of proline in the salt- tolerant callus of sour orange has been shown to be an adaptive mechanism for resistance to salt stress.

**5. Rhizogenesis Under Salt Stress Conditions**

Regarding the effect of cultivars on rooting percentage and average number of roots per shoot, after 4 weeks of shoot culture, irrespective the effect of salinity treatments, the data in Table (11) indicated that, Early Superior exhibited significantly the highest rooting percentage and average number of roots (63.890% and 5.118, respectively), followed by Flame seedless (58.333% and 4.623, respectively), then Thompson seedless (38.887% and 2.643, respectively).

**Table (11): Effect of four salinity treatments on rooting percentage of proliferated shoots, average number of roots per shoot and average root length of grape cultivars Early Superior, Flame seedless and Thompson seedless after 4 weeks of shoot culture.**

| Cultivar | Salinity in ppm | Rooting (%) | Av. no. of roots/ proliferated shoot | Av. root length (cm) |
|---|---|---|---|---|
| Early Superior | 0000 | 77.78 | 8.21 | 4.48 |
| | 1000 | 66.67 | 5.50 | 3.32 |
| | 2000 | 61.11 | 4.09 | 3.00 |
| | 4000 | 50.00 | 2.67 | 1.98 |
| | Mean | 63.890 A* | 5.118 A | 3.195 A |
| Flame seedless | 0000 | 88.89 | 9.13 | 5.63 |
| | 1000 | 61.11 | 5.92 | 3.42 |
| | 2000 | 50.00 | 2.11 | 2.60 |
| | 4000 | 33.33 | 1.33 | 1.27 |
| | Mean | 58.333 B | 4.623 B | 3.230 A |
| Thompson seedless | 0000 | 72.22 | 6.62 | 2.97 |
| | 1000 | 44.44 | 1.75 | 1.32 |
| | 2000 | 27.78 | 1.20 | 1.00 |
| | 4000 | 11.11 | 1.00 | 0.51 |
| | Mean | 38.887 C | 2.643 C | 1.450 B |
| Allover effect | 0000 | 79.630 A | 7.987 A | 4.360 A |
| | 1000 | 57.407 B | 4.390 B | 2.687 B |
| | 2000 | 46.297 C | 2.467 C | 2.200 C |
| | 4000 | 31.480 D | 1.667 D | 1.253 D |
| L.S.D. | | | | |
| Cultivars | 0.05 | 0.344 | 0.028 | 0.056 |
| Salinity concentrations | 0.05 | 0.398 | 0.032 | 0.064 |
| Cultivars×Salinity conc. | 0.05 | 0.688 | 0.055 | 0.112 |

*** Values followed by the same letters between cultivars or between salinity treatments in the same column significantly are not differed at the 0.05 level of probability.**

Flame seedless and Early Superior exhibited significantly the highest average root length (3.230 and 3.195 cm, respectively), followed by Thompson seedless (1.450 cm).

As for the effect of salinity treatments on rooting percentage, average number of roots and average root length, after 4 weeks of shoot culture, regardless of the effect of cultivars, the data in the same Table revealed that, the rooting percentage, average number of roots and average root length, showed a tendency for negative responses to salinity treatments. The rooting percentages were 79.630, 57.407, 46.297 and 31.480%, averages number of roots per shoot were 7.987, 4.390, 2.467 and 1.667, and averages root length were 4.360, 2.687, 2.200 and 1.253 cm when salinity concentrations were used at zero, 1000, 2000 and 4000 ppm, respectively. The differences among salinity treatments were statistically significant.

As shown in the previous results in Table (11), it was obvious that, after 4 weeks of shoot culture, Early Superior produced significantly the highest rooting percentage and average number of roots per shoot, followed by Flame seedless and Thompson seedless. While, Early Superior and Flame seedless gave significantly the highest average root length, as compared with Thompson seedless.

The results in the same Table also indicated that, rooting percentage, average number of roots per shoot and average root length of the studied cultivars decreased with increasing salinity concentrations from zero to 4000 ppm. These findings agreed with those reported by Shahin (1997) who found that rooting percentage and average roots number per shoot of the three grape cultivars were significantly decreased with increasing salinity levels.

**6. Transplanting of Plants to Soil**

The obtained plants of the studied cultivars were transferred to greenhouse conditions (after acclimatization in growth chamber). The plants of Early Superior cultivar demonstrated morphologically identical to the parent vines, uniformity, vigorously growing and healthy appearance under the greenhouse conditions, survived and showed no damage and increased in growth at 3000 ppm of salt mixture (as previously described), followed by Flame seedless cultivar compared to Thompson seedless cultivar which showed sever leaf burn, progressive wilting and decreased growth under salt stress conditions. These results were in accordance with those obtained by Mohamed (1996) who evaluated grape cultivars Thompson seedless, Flame seedless, Early Superior and King Ruby, to salt stress *in vivo*.

It is worthy to mention that, 77%, 70% and 55% of the obtained plants of Early Superior, Flame seedless and Thompson seedless, respectively were successfully transplanted to soil. These findings agreed with those obtained by Compton and Gray (1995) who reported that approximately 90% of plantlets of "Southern Home" hybrid grape survived acclimatization to ambient conditions. In addition, Biasi *et al.*, (1998) reported that for acclimatization plants of Jales grape rootstock were kept in closed containers for 3 weeks, then under intermittent mist for 4 more weeks, until being transferred to the open air.

It is evident that, Early Superior was the most salt-tolerant cultivar, followed by Flame seedless which considered salt-moderate, while Thompson seedless cutlivar appeared to be the most salt- sensitive cultivar in this respect. These results agreed with those reported by Shahin (1997) who found that Early Superior was the most salt- tolerant cultivar, followed by Flame seedless.

Finally, the present study gives a very detailed protocol for salt tolerance evaluation of the three grape cultivars Early Superior, Flame seedless and Thompson seedless under *in vitro* conditions. In other words, the use of tissue culture technique as a tool for the salt tolerance evaluation in the three tested grape cultivars achieved in this study.

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# الإكثار المعملى الدقيق والتقييم لتحمل الملوحة فى بعض أصناف العنب
## ٢- التقييم لتحمل الملوحة

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أجرى هذا البحث خلال ثلاث سنوات متتالية (٢٠٠٠- ٢٠٠٢) بغرض تقييم ثلاثة أصناف من العنب هى إيرلى سوبيريور وفليم سيدلس والبناتى لتحمل الملوحة وذلك باستخدام تقنية زراعة الأنسجة.
ويمكن تلخيص النتائج الرئيسية لهذه الدراسة فى النقاط التالية:

أ- تأثير الأصناف:

١- إيرلى سوبيريور أعطى أعلى قيم وذلك بصورة جوهرية لكل من النسبة المئوية لإكثار الأفرخ ومتوسط عدد الأفرخ الناتجة الجديدة بالنسبة للفرخ الأصلى خلال النقلة الأولى والثانية والثالثة، يليه فليم سيدلس والبناتى. وفى نفس الوقت أحرز إيرلى سوبيريور أعلى قيم وذلك بصورة جوهرية لكل من محتوى الورقة من الفوسفور والبوتاسيوم والصوديوم والماغنسيوم والكلوروفيل الكلى ونشاط إنزيم البيروكسيديز يليه فليم سيدلس والبناتى. بالإضافة إلى ذلك فقد أعطى الصنف إيرلى سوبيريور أعلى محتوى للورقة من النيتروجين والبرولين وذلك بصورة جوهرية مقارنة بفليم سيدلس والبناتى. وعلى العكس من ذلك فإن البناتى أظهر أعلى نسبة مئوية لضرر الأفرخ وأعلى محتوى للورقة من الكالسيوم وذلك بصورة جوهرية يليه فليم سيدلس وإيرلى سوبيريور. أيضا أظهر الصنف فليم سيدلس أعلى متوسط طول للفرخ الناتج الجديد وذلك بصورة جوهرية يليه إيرلى سوبيريور والبناتى.

٢- إيرلى سوبيريور أنتج أعلى قيم وذلك بصورة جوهرية لكل من النسبة المئوية للتجذير ومتوسط عدد الجذور بالنسبة للفرخ بعد أربعة أسابيع من زراعة الأفرخ، يليه فليم سيدلس والبناتى. بينما أعطى إيرلى سوبيريور وفليم سيدلس أعلى متوسط لطول الجذر وذلك بصورة جوهرية مقارنة بالبناتى.

ب- تأثير معاملات الملوحة:

١- خلال النقلة الأولى والثانية والثالثة، لوحظ انخفاض كبير فى قيم كل من النسبة المئوية لإكثار الأفرخ ومتوسط عدد الأفرخ الناتجة الجديدة بالنسبة للفرخ الأصلى ومتوسط طول الفرخ الناتج الجديد ولوحظ زيادة مرتفعة فى النسبة المئوية لضرر الأفرخ مصاحبا للزيادة فى تركيزات الملوحة من الصفر إلى ٤٠٠٠ جزء فى المليون. بالإضافة إلى ذلك لوحظ أن محتوى الورقة من كل من النيتروجين والصوديوم والكلوريد والبرولين يتجه إلى الاستجابات الموجبة نحو معاملات الملوحة. وعلى العكس من ذلك، لوحظ أن محتوى الورقة من كل من الفوسفور والبوتاسيوم والكالسيوم والماغنسيوم والكلوروفيل الكلى ونشاط إنزيم البيروكسيديز يتجه إلى الاستجابات السالبة نحو معاملات الملوحة.

٢- بعد أربعة أسابيع من زراعة الأفرخ، لوحظ أن النسبة المئوية للتجذير ومتوسط عدد الجذور بالنسبة للفرخ ومتوسط طول الجذر تتجه إلى الاستجابات السالبة نحو معاملات الملوحة. وإن الاختلافات بين معاملات الملوحة كانت إحصائيا اختلافات بصورة جوهرية.

من الواضح أن إيرلى سوبيريور كان أكثر الأصناف تحملا للملوحة بينما الصنف البناتى فكان الأكثر حساسية للملوحة.

أخيرا، ٧٧%، ٧٠%، ٥٥% من النباتات المتحصل عليها لإيرلى سوبيريور وفليم سيدلس والبناتى على التوالى تم نقلها إلى التربة بنجاح وكانت هذه النباتات سليمة وتنمو بقوة تحت ظروف الصوبة.