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IN VITRO EVALUATION OF SOME OLIVE CULTIVARS (*OLEA EUROPAEA* L.) GROWN UNDER SALINITY STRESS

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Abstract

The current study was carried out to investigate the effect of salinity stress (NaCl) on three *in vitro* regenerated olive cultivars (Teffahi, Aggizishami and Koroneiki). Olive cultivars were subjected to six saline treatments (0, 2500, 5000, 7500, 10000 and 12500 mg L^{-1} of NaCl). Survival percentage, salinity injury index, shoot length, leaf number, defoliation percentage, shoot fresh and dry weight and chlorophyll content were determined at the end of stress period. The obtained data indicated that, increasing NaCl concentration in growth media significantly decreased survival percentage, shoot growth, chlorophyll content and increased defoliation and salt injury index in the studied olive cultivars. NaCl at 10000 mg L^{-1} considered as the lethal dose as it recorded high mortality rate of the three olive cultivars. The obtained results showed that, Aggizishami and Teffahi *cvs.* were more tolerant to salinity stress as both cultivars showed higher survival percentage and better growth performance compared with Koroneikicv.

Key words: olive, evaluation, tolerance, in *vitro*, salinity.

Introduction

Olive (*Olea europaea* L.) is one of the major fruit tree crops in Mediterranean basin, according to the International Olive Oil Council, Mediterranean countries account for about 97% of the world's olive production (IOOC, 2000). Olive is a moderately salt tolerant tree crop that may grow successfully in saline soils where other fruit trees cannot be grown (Hill *et al.,* 2013). Recent studies suggest that certain olives cultivar are able to tolerant salinity stress up to 5800 mg L^{-1} of salt (Chartzoulakis, 2005; Gucci and Tattini, 1997).

Soil salinity is one of the important environmental factors that limit crop productivity (Ashraf and Foolad, 2005; Pessarakli *et al.,* 1999). In addition, the limited water resources and the increased world population necessitates the use of high salinity water in agriculture (Chartzoulakis, 2005). The situation has worsened over the last 20 years; according to the published statistics of FAO (2019), 20% of global irrigated area (about 62 million hectares) is salt-affected soils, while 35% of irrigated land in Egypt suffers from soil salinization (Fawzi *et al.,* 2011).

The adverse effect of salinity on plant growth results from both osmotic stress and toxic effect of specific elements (Yamaguchi and Blumwald, 2005). Salinity reduces water availability in soil solution as a result of increasing osmotic potential (Zhu, 2001; Meloni *et al.,* 2003). Moreover, plant performance may be adversely affected by salinity-induced nutritional disorders, which result from inhibiting uptake of essential nutrients like K^+ and Ca^{2+} and accumulation of Na^+ and Cl^- to toxic levels within plant cells(Grattana and Grieve, 1998; Xu *et al.,* 1999 and Mazher *et al.,* 2007; Marschner, 2012; Zhu, 2001). The imbalances caused by salinity stress including metabolism alteration (Gao *et al.,* 1998), reduction of enzymes activity (Munns, 1993; Chartzoulakis, 2005), generation of reactive oxygen species (Zhu, 2001; Meloni *et al.,* 2003), degradation of chlorophyll pigments and reduction of photosynthesis activity (Di Martino *et al.,* 2003; Chartzoulakis, 2005).

The salinity tolerance is not a simple trait; it is depend on different physiological parameters, which are difficult to determine (Grattana and Grieve 1998, Munns, 2002 and Qados, 2011). Moreover the plant response to salinity stress can vary depending on plant species climatic and soil conditions. *In vitro* evaluation allow screening of large number of genotype for salt tolerance, insure the uniformity of screening factors and minimize to effect of external environmental conditions (Rai *et al.,* 2011). The

main objective of the current study was investigate the effect of different concentrations of sodium chloride on growth performance of some *in vitro* growing olive cultivars.

Materials and Methods

Micropropagation

Plant material and culture conditions

The current research was carried out during 2018 and 2019 at the laboratory of Pomology Department, Faculty of Agriculture, Cairo University, Giza, Egypt. Active growing shoots were collected from mature olive trees of Teffahi, Aggizishami and Koroneikicultivars (grown at the nursery, of Faculty of Agriculture, Cairo University, Giza, Egypt). Shoots were stripped of leaves, washed with tap water and divided into nodal cuttings. Surface sterilization was performed with commercial bleach (5.25% sodium hypochlorite) for 12 min, followed by Mercury chloride at 1000 mg L^{-1} for 10 min. and washed several times with sterile distillated water.

Multiplication

Olive nodal cuttings were cultured on Rugini Olive medium (Rugini, 1984), supplemented with 2.5mg L^{-1} zeatin, 30 g L^{-1} mannitol and 6 g L^{-1} agar (Hegazi *et al.,* 2018). Media pH was adjusted to 5.8 before adding agar and autoclaved at 121°C for 20 min. All cultures were maintained in growth chamber at 25°C and 16h photoperiod (provided with 40-60µmol m^{-2} s^{-1} cool-white fluorescent lamps).

Evaluation of salinity tolerance of some olive cultivars

In vitro growing olive shoots were sub-culture to OM media supplement with one of the following NaCl concentration (0, 2500, 5000, 7500 and 10000, 12500 mg L^{-1}).

Data collection and analysis

At the end of stress duration (4 weeks) shoots were removed from the culture media gently washed with tap water and the following parameters were recorded;

Table 1: Effect of NaCl concentrations on survival percentage of the studied olive cultivars.

Cultivars	Salinity concentration (mg L^{-1})						Mean
	0	2500	5000	7500	10000	12000	
Koroneiki	100 a	100 a	100 a	33 b	0.0 d	0.0 d	55.50B
Teffahi	100 a	100 a	100 a	100 a	17.0 c	0.0 d	69.50A
Aggizishami	100 a	100 a	100 a	100 a	17.0 c	0.0 d	69.50A
Mean	100 A	100 A	100 A	77.67 B	11.33C	0.0 D	

Means followed by the same letter within each column are not significantly different at 1% concentration.

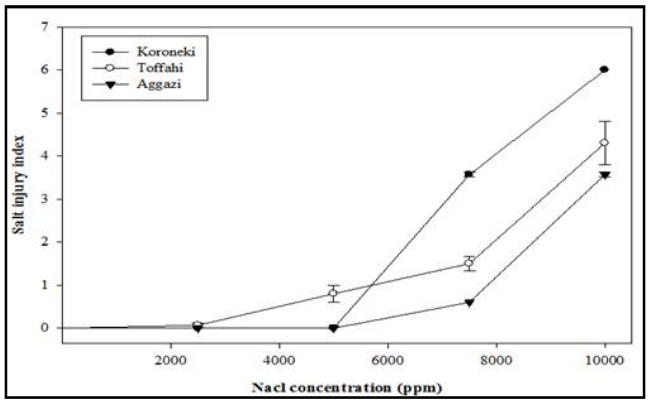

Fig. 1: Effect of NaClconcentrations on salt injury index (SII) of the studied olive cultivars.

survival percentage, shoot length, number of leaves /shoot, defoliation percentage, shoot fresh weight (FW) and shoot dry weight (DW) after drying in oven at 70°c for 72h., total chlorophyll was determined spectrophotometrically using 80% acetone as a solvent (Lichtenthaler and Wellburm, 1983). Salt injury index (SII) calculated according to Erturk *et al.,* (2007), using the equation: SII = Σ (ni* i)/N

Ni = The number of plantlets receiving the mark (i)

N = The total number of plantlets in each salt concentration

Statistical analysis

The experimental treatments were arranged in a complete randomized design (CRD) with 3 replicate for each treatment. Data were subjected to analysis of variance (Snedecor and Cochran 1991) and means were compared according to Duncan multiple range test at 1% (Duncan, 1955).

Results and Discussion

Data presented in table 1 showed, that increasing NaCl concentrations induced negative effect on survival percentage, which were significantly reduced by increasing salinity in the culture medium. The 10000 ppm is considered as lethal dose; as it caused high mortality rate of all cultivars under study. Media contained 0, 2500 and 5000 ppm of NaCl recorded 100% survival percentage while all olive cultivars did not survive at 12000 ppm, Teffahi and Aggizishami cultivars recorded the highest survival percentage (69.5%) as compared with Koroneiki cultivars (55.5%). On the other hand, survival percentage of all olive cultivars under study indicated that 7500 ppm appeared to be the threshold of olive tolerance to salinity stress under *in vitro* conditions.

Data in Fig. 1 showed the effect of NaCl

concentrations on salt injury index of the studied olive cultivars; salinity treatments induce a range of damage symptoms, which become sever under the high NaCl concentration. Salinity damage appeared in yellowing, necrosis subsequentlythe shoot gradually wilted and died. In general increasing NaCl concentration in culture media gradually increased damage symptoms in all cultivars. Koroneiki recorded the highest damages index, while Aggizishami recorded the lowest value.

Data presented in table 2 the obtained data showed that increasing NaCl concentrations induced a negative effect on shoot length, of the studied olive cultivars, the highest shoot length was recorded for Aggizishami cultivar followed by Teffahi as compared with Koroneiki cultivar.

According to the data illustrated in table 3 number of leaves were negatively affected by NaCl concentrations. Aggizishami cultivar recorded the highest number of leaves/shoot followed by Teffahi as compared with Koroneiki cultivar, which recoded the lowest value of number of leaves/shoot.

According to the data illustrated in table 4 defoliation%, was gradually increased in response to salinity stress. The highest defoliation values were recorded for 7500 ppm, Koroneiki recorded the highest value of leaf defoliation (100 %), while Teffahi recorded the lowest value (13.67).

Data in table 5 and 6 showed that shoot fresh and dry weight was negatively affected by NaCl concentrations. Aggizi shami *cv.* recorded the highest fresh weight whileTeffahicv. recorded the highest dry weight compared with other cultivars; Koroneikicv. recoded the lowest value of fresh and dry weight.

The reduction in shoot growth may attribute to the adverse effect of salinity on free water content and nutritional status which in turn disturbed plant physiological and biochemical activity. As previously reported salt stress considered as the most important abiotic stress that creates harmful effects on plant growth and development (Khan *et al.,* 2009; Syeed *et al.,* 2011). Salinity inhibits water uptake, causes ionic imbalance, ionic toxicity and osmotic stress (Grattana and Grieve, 1998; Luo *et al.,* 2005; Munns and Tester 2008). Moreover, salinity inhibiting cell division and elongation (Kasele *et al.,* 1994; Hasegawa *et al.,* 2000) suppress leaf initiation and

Table 2: Effect of NaCl concentrations on shoot length of the studied olive cultivars.

Cultivars	Salinity concentration (mg L^{-1})			
	0	2500	5000	7500
Koroneiki	4.69 ef	4.52 ef	4.36 ef	---
Teffahi	5.58 d	5.00 de	4.49 ef	3.93 f
Aggizishami	11.13 a	10.05 b	7.74 c	6.95 c

Means followed by the same letter within each column are not significantly different at 1% concentration.

Table 3: Effect of NaCl concentrations on number of leaves of the studied olive cultivars.

Cultivars	Salinity concentration (mg L^{-1})			
	0	2500	5000	7500
Koroneiki	19.67 ab	16.67 bcd	15.67 cd	0.00 g
Teffahi	17.67 a-d	15.00 de	14.33 def	12.00 ef
Aggizishami	20.33 a	18.67 abc	16.67 bcd	11.33 f

Means followed by the same letter within each column are not significantly different at 1% concentration.

Table 4: Effect of NaCl concentrations on defoliation percentage of the studied olive cultivars.

Cultivars	Salinity concentration (mg L^{-1})			
	0	2500	5000	7500
Koroneiki	0.00 e	0.00 e	9.66 d	100.0 a
Teffahi	0.00 e	0.00 e	8.33 d	13.67 c
Aggizi shami	0.00 e	0.00 e	0.00 e	32.81 b

Means followed by the same letter within each column are not significantly different at 1% concentration

Table 5: Effect of NaCl concentrations on shoot fresh weight of the studied olive cultivars.

Cultivars	Salinity concentration (mg L^{-1})			
	0	2500	5000	7500
Koroneiki	0.50 cde	0.46 c-f	0.36 ef	0.00 g
Teffahi	0.58 abc	0.53 b-e	0.38 def	0.31 f
Aggizishami	0.73 a	0.69 ab	0.55 bcd	0.50 cde

Means followed by the same letter within each column are not significantly different at 1% concentration.

Table 6: Effect of NaCl concentrations on shoot dry weight of the studied olive cultivars.

Cultivars	Salinity concentration (mg L^{-1})			
	0	2500	5000	7500
Koroneiki	0.073 cd	0.073 cd	0.063 de	0.000 e
Teffahi	0.220 a	0.233 a	0.220 a	0.206 ab
Aggizishami	0.187 ab	0.170 ab	0.173 ab	0.143 bc

Means followed by the same letter within each column are not significantly different at 1% concentration.

Table 6: Effect of NaCl concentrations on total chlorophyll of the studied olive cultivars.

Cultivars	Salinity concentration (mg L^{-1})			
	0	2500	5000	7500
Koroneiki	4.60 bc	4.43 bc	2.59 cd	0.00 d
Teffahi	10.14 a	6.48 ab	4.50 bc	3.90 bc
Aggizi shami	6.74 ab	6.71 ab	4.50 bc	1.73 cd

Means followed by the same letter within each column are not significantly different at 1% concentration.

expansion (Julkowska and Testerink, 2015) and reduce photosynthesis activity (Young and Britton, 1990). Increasing salinity stress in culture media to a toxic concentration decreased plantlet length, leaf number per plantlet, fresh and dry weight, causes leaf senescence and abscission, which can lead to complete defoliation of plants (Benmahioul *et al.,* 2009; Abbas *et al.,* 2014 Karimi and Hasanpour 2014; Yehia *et al.,* 2018). Moreover variation in growth reduction was observed between different plant genotypes growing under salinity stress (Zarei *et al.,* 2016; Yehia *et al.,* 2018).

Table 6 showed effect of NaCl concentrations on total chlorophyll in olive leaves growing under salinity stress. Teffahi recorded the highest significant concentration of chlorophyll under different NaCl concentration while Koroneiki recorded the lowest significant concentration of chlorophyll. In general increasing NaCl concentration in the growth media gradually reduced chlorophyll a percentage in studied cultivars.

There are numerous reports of chlorophylls degradation under water stress conditions (Maroco *et al.,* 2002; Di Martino *et al.,* 2003; Bertamini *et al.,* 2006; Pavlousek, 2011; Haider *et al.,* 2017). Chlorophyll content was significantly decreased by increasing salt concentrations of NaCl in culture medium in different plant species (Zhang *et al.,* 2002; Erturk *et al.,* 2007). This reduction may be related to the activity of proteolytic enzymes which causes chlorophyll degradation (Tuna *et al.,* 2008). Also, the reduction in chlorophyll content in plant leaves may be due to the oxidative effect of salinity on plant tissue (Abd Allatif *et al.,* 2015). The evaluation of chlorophyll content is very important since the reduction in chlorophyll content causes a reduction in photosynthetic activity of the plant.

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