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In vitro evaluation of four fig cultivars grown under salinity stress

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The current study was carried out to investigate the effect of sodium chloride (NaCl) on four *in vitro* regenerated fig cultivars (Black Mission, Aboudy, Conadria and Sultani). Seven NaCl concentrations (1000, 2000, 3000, 4000, 5000, 6000 and 7000 mg L^{-1}) were used for salinity stress. Shoot length, leaf number, chlorophyll, proline and leaf mineral contents were determined. The obtained data indicated that the four fig cultivars were negatively affected by salt stress at different concentrations. The addition of NaCl significantly decreased shoot growth, leaf content of N, K and total chlorophyll in all cultivars. Cl^- and Na^+ as well as proline concentration increased as increasing NaCl concentrations up to 6000 mg L^{-1}. NaCl at7000 mg L^{-1} considered as the lethal dose as it caused high mortality rate of all fig cultivars under investigation. Sultani fig cultivar accumulated more content of N and K and had high chlorophyll and proline concentration under high salinity stress compared with other fig cultivars.

Keywords: *Ficus carica*, NaCl, Proline, Mineral content, Chlorophyll.

INTRODUCTION

Salinity is one of the major abiotic stresses, which adversely affect crop productivity and quality, especially in arid and semiarid regions of the world, salinity affects nearly 19.5% of the total irrigated lands (FAO, 2000). In addition, the limited water resources and the increased world population necessitates the use of high salinity water in agriculture (Chartzoulakis, 2005). The adverse effect of salinity on plant growth results from both osmotic and toxic effects of some elements (Yamaguchi and Blumwald, 2005). Crop response to salinity depends on several factors; especially their genetic structure (Goldack et al., 2011). Hence, selection of tolerant genotypes that will survive under saline conditions is a potential solution to overcome salinity problem (Ashraf and Harris, 2004). Tissue culture has been used to evaluate plant tolerance to different abiotic stresses including salinity in several plants species (Munns and Tester, 2008). Fig (*Ficus carica* L.) is deciduous fruit species, which grows in a wide range of soil types (Vemmos et al., 2013). It has the ability to grow under water deficit and moderate salinity conditions (Golombek and Lüdders, 1993). Testing salinity tolerance in different fig cultivars will help us to expect their growth behavior under field conditions. The imbalance caused by salinity stress affects protein synthesis, photosynthesis activity, and degradation of chlorophyll (Di Martino et al., 2003). Proline is a compound that tends to accumulate in plant tissue under salt stress (Sakr et al., 2012). Proline content increases as NaCl increase in culture media (Metwali et al., 2014). Salinity increases Na^+ and Cl^- and decrease K^+ content of fig cultivars (Abdolineyad and Shekafandeh, 2014). The main objective of the present study was to investigate the effect of different concentrations of sodium chloride on plant growth, total chlorophyll, proline and leaf mineral content of *in vitro* grown four fig cultivars

MATERIALS AND METHODS

Plant materials and salinity treatments

The current study was carried out at the Biotechnology Laboratory of Pomology Dept., National Research Center, Dokki, Giza, Egypt during the period from 2015 to 2017 on *in vitro* proliferated shoots from the 3rd subculture of four fig (*Ficus carica* L.) cultivars (Black Mission, Aboudy, Conadria and Sultani), micro propagation of the studied cultivars was performed according to protocol described by Mustafa and Taha, (2012). The studied cultivars were subjected to different salinity treatments to verify the critical level of salinity concentration for each cultivar. Shoots of different fig cultivars under investgation was cultured on MS medium (Murashige and Skoog, 1962) containing different concentrations of NaCl *i.e.* 1000, 2000, 3000, 4000, 5000, 6000 and 7000 mg L^{-1}. All culture media combinations supplemented with 30 g L^{-1} sucrose and 6.5 g agar L^{-1}, media pH was adjusted to 5.8 before adding agar and autoclaved at 121°C for 15 min. The cultured explants were incubated under 16 hours of artificial light (fluorescent light at 40-60μmol m^{-2} s^{-1}) at average temperature 23±2 °C. After 6 weeks of exposure to the NaCl treatments, fig shoots were gently washed from the growth medium with tap water and the following parameters were recorded; survival percentage (%), shoot length (cm), and number of leaves/shoot. Chlorophyll was measured by chlorophyll meter (Minolta- SPAD-502, Japan); the data were expressed as SPAD units (Markwell et al., 1995). Free proline content was determined using the ninhydrin reaction according to the method of Bates *et al*. (1973). Since the 7000mg L^{-1} is considered as a lethal dose, chemical analysis included NaCl treatments up to 6000mg L^{-1} only. Leaf samples were dried in an oven at 70°C for 72 hours then digested with sulphoric acid and hydrogen peroxide. Total nitrogen was estimated by the modified Micro-Kjeldahl method (Paech and Tracey, 2013); Na^+ and K^+ determined by Flame apparatus according to Temminghoff and Houba (2004) and leaf chloride content (Cl^-) was determined by titration method (Eaton et al., 1995).

Statistical analysis

The treatments were arranged in a complete randomized design with five replicates for each treatment, data were subjected to analysis of variance according to Snedecor and Cochron (1991) and the means were compared according to Duncan's multiple range tests at 1% level (Duncan 1955).

RESULTS

1. Survival and plant growth.

Data in Table (1) indicated that NaCl concentrations had an obvious effect on survival percentage of different fig cultivars; Sultani recorded the highest survival percentage (67.22%), while Aboudy recorded the lowest value (62.14%). In general increasing NaCl concentration in the growth media gradually reduced survival percentage in all cultivars. The obtained data indicated that 5000 mg L^{-1} appeared to be the threshold value of fig tolerance to salinity stress, while 7000 mg L^{-1}considered as lethal dose; as it caused high mortality rate of all fig cultivars under investigation.

Data illustrated in Table (2 and 3) showed that shoot length and number of leaves/shoot were negatively affected by NaCl concentrations; a great reduction in growth parameters was associated with the increase of NaCl concentration in growth medium. Sultani cultivar recorded the highest shoot length and number of leaves/shoot compared with other cultivars, while Aboudy recoded the lowest value of both shoot length and leaf number of leaves/shoot.

The reduction in shoot growth may attribute to the adverse effect of salinity on free water content and nutritional status which in turn induced a negative effect on growth parameters. As previously reported osmotic stress decrease cell division and enlargement (Kasele et al., 1994), suppress leaf initiation and expansion (Julkowska and Testerink, 2015), and reduce photosynthesis activity (Young and Britton, 1990). Moreover variation in growth reduction was observed between fig cultivars growing under salinity stress (Zarei et al., 2016).

Table (1) Effect of NaCl concentrations on survival percentage of the studied fig cultivars.

Cultivar	NaCl concentration (mg L^{-1})							Mean
	1000	2000	3000	4000	5000	6000	7000	
Black Mission	95.0 a	95.0 a	95.0 a	90.0 b	70.0 c	20.0 g	0.0 j	**66.50 B**
Aboudy	95.0 a	95.0 a	95.0 a	90.0 b	50.0 e	10.0 h	0.0 j	**62.14 C**
Condria	95.3 a	95.4 a	95.0 a	90.0 b	50.0 e	10.0 h	0.0 j	**62.24 C**
Sultani	95.6 a	95.0 a	95.0 a	90.0 b	60.0d	30.0 f	5.0 i	**67.22 A**
Mean	**95.25 A**	**95.08 A**	**95 A**	**87.5 B**	**57.5C**	**17.5 D**	**1.25 E**	

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

Table (2) Effect of NaCl concentrations on shoot length (cm) of the studied fig cultivars.

Cultivar	NaCl concentration (mg L^{-1})							Mean
	1000	2000	3000	4000	5000	6000	7000	
Black Mission	5.33 bc	5.00 b-e	4.67 c-f	4.33 efg	2.83 i	1.30 j	0.00 k	**3.35 B**
Aboudy	3.67 gh	3.17 hi	2.50 i	2.40 i	2.67 i	1.06 j	0.00 k	**2.06 C**
Condria	5.40 bc	5.17 bcd	4.47 d-g	4.10 fg	2.17 j	1.17 j	0.00 k	**3.29 B**
Sultani	6.27 a	5.83 ab	5.33 bc	4.96 b-e	3.83 gh	2.00 j	1.06 j	**4.18 A**
Mean	**5.16A**	**4.79 B**	**4.24 C**	**3.94 D**	**2.77 E**	**1.38 F**	**0.265 G**	

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

Table (3) Effect of NaCl concentrations on number of leaves /shoot of the studied fig cultivars.

Cultivar	NaCl concentration (mg L^{-1})							
	1000	2000	3000	4000	5000	6000	7000	mean
Black Mission	7.50 a	6.60 b	5.70 cd	5.10 de	4.0 fg	2.6 h	0 j	**4.50 A**
Aboudy	6.27 bc	5.37 d	4.37 ef	3.37 g	2.37 h	1.37 i	0 j	**3.30 C**
Condria	6.50 b	5.50 d	4.50 ef	3.47 g	2.47 h	1.2 i	0 j	**3.38 B**
Sultani	7.60 a	6.80 b	5.33 d	4.86 ef	3.6 g	2.8 i	1.25 i	**4.61 A**
mean	**6.97 A**	**6.07 B**	**4.98 C**	**4.20 D**	**3.11 E**	**1.99 F**	**0.31 G**	

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

Table (4) Effect of NaCl concentrations on leaf nitrogen (%) of the studied fig cultivars.

Cultivar	NaCl concentration (mg L^{-1})						Mean
	1000	2000	3000	4000	5000	6000	
Black Mission	1.10 ab	0.91 abc	0.90 abc	0.84 abc	0.62 abc	0.37 bc	**0.79 C**
Aboudy	1.16 ab	0.90 bc	0.87 abc	0.74 abc	0.52 abc	0.33 bc	**0.75 D**
Condria	1.19 ab	1.10 abc	0.92 abc	0.62 abc	0.58 abc	0.37 bc	**0.80 B**
Sultani	1.51 a	1.17 ab	1.00 bc	0.78 abc	0.74 abc	0.37 bc	**0.93 A**
mean	**1.24 A**	**1.02 B**	**0.92 C**	**0.75 D**	**0.62 E**	**0.36 F**	

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

Table (5) Effect of NaCl concentrations on leaf potassium (%) of the studied fig cultivars.

Cultivars	NaCl concentration (mg L^{-1})						Mean
	1000	2000	3000	4000	5000	6000	
Black Mission	1.24 acd	1.14a-e	0.93 b-g	0.70 d-g	0.63 efg	0.48 g	**0.85 C**
Aboudy	1.10 a-f	0.99 a-g	0.83 c-g	0.67 efg	0.59 efg	0.48 g	**0.78D**
Condria	1.29 abc	1.08 a-f	0.99 a-g	0.88 b-g	0.79 c-g	0.57 fg	**0.93B**
Sultani	1.54 a	1.40 ab	1.06 a-f	0.91 b-g	0.83 c-g	0.66 efg	**1.07 A**
mean	**1.30 A**	**1.15 B**	**0.95 C**	**0.79 D**	**0.71 E**	**0.55 F**	

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

Table (6) Effect of NaCl concentrations on leaf sodium (%) of the studied fig cultivars.

Cultivar	NaCl concentration (mg L^{-1})						Mean
	1000	2000	3000	4000	5000	6000	
Black Mission	0.243 l	0.290 jk	0.336 i	0.365 gh	0.476 e	0.613 b	**0.387 B**
Aboudy	0.211 m	0.246 l	0.287 k	0.346 hi	0.383 g	0.481 de	**0.326 D**
Condria	0.243 l	0.258 l	0.312 j	0.359 h	0.420 f	0.590 c	**0.364 C**
Sultani	0.260 l	0.298 jk	0.343 hi	0.406 f	0.501 d	0.646 a	**0.409 A**
mean	**0.239 F**	**0.273 E**	**0.319 D**	**0.369 C**	**0.445B**	**0.582 A**	

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

2. Leaf mineral content

Data in Table (4) indicated that Sultani cultivar recorded the highest significant percentage of nitrogen contents followed by Aboudy cultivar, while Black Mission cultivar recorded the lowest percentage. Moreover, Sultani cultivar recorded the highest significant leaf potassium content compared with the other fig cultivars used in our investgation (Table 5).

Salinity may damages plant growth through accumulation of toxic ions (Na^+ and Cl^-) in plant tissues, the toxic effects of both Na^+ and Cl^- may cause alteration in nutritional status of plants (Grattan and Grieve, 1998). The obtained results are in agreement with the findings of AbdoliNeyd and Shekafandeh (2014) in fig cultivars; they reported that salinity increased Na^+ and Cl^- concentrations and depressed K^+. In addition, the differences in cultivar response were previously reported (Metwali et al., 2014). Na^+ accumulation may be due to the limited ability of sensitive cultivars to control sodium uptake (Saker et al., 2012). Although Sultni cv. had accumulated high Na^+ compared with the other cultivars, the potassium content still relatively high; this balance may have a role in protecting the plant tissue from salinity damage (Delfine et al., 1998). Moreover, in the tolerant cultivate, most of Na^+ may be moved to the cell vacuoles (Parida and Jha, 2010)

3. Total Chlorophyll and Proline content

Data in Table (8) indicated that Sultani fig cultivar recorded the highest significant level of total chlorophyll followed by both Aboudy and Conadria cultivars as compared with Black Mission. Moreover, total chlorophyll was gradually decreased when concentration of NaCl was increased in the culture medium.

These results are in line with the findings of Metwali et al (2014) on fig cultivars. They found that increasing NaCl concentrations had an adverse effect on total chlorophyll. This may be is due to the activity of proteolytic enzymes such as chlorophyllase, which is responsible for the chlorophyll degradation (Tuna et al., 2008). Furthermore, the reduction in chlorophyll content in plant leaves may be due to the oxidative effect of salinity on plant tissues (Abd Allatif et al., 2015).

Table (7) Effect of NaCl concentrations on leaf chloride (%) of the studied fig cultivars.

Cultivar	NaCl concentration (mg L^{-1})						mean
	1000	2000	3000	4000	5000	6000	
Black Mission	0.204 j	0.245 i	0.343 ef	0.343 ef	0.357 de	0.435 b	**0.32 B**
Aboudy	0.245 i	0.332 fg	0.357 de	0.408 c	0.4503b	0.506 a	**0.38 A**
Condria	0.216 j	0.249 i	0.298 h	0.324 fg	0.356 de	0.406 c	**0.30 C**
Sultani	0.208 j	0.256 i	0.315 gh	0.345 ef	0.371 d	0.445 b	**0.32 B**
mean	**0.21 F**	**0.27 E**	**0.32 D**	**0.35 C**	**0.38 B**	**0.44 A**	

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

Table (8) Effect of NaCl concentrations on leaf total chlorophyll content (SPAD value) of the studied fig cultivars.

Cultivar	NaCl concentration (mg L^{-1})						Mean
	1000	2000	3000	4000	5000	6000	
Black Mission	55.27 e	49.27 h	42.53 k	31.40 n	22.44 q	11.20 u	**35.35 C**
Aboudy	60.43 d	52.97 g	47.56 j	38.77 m	28.13 p	18.20 s	**41.01 B**
Condria	60.00 d	53.64 f	48.36 i	42.67 k	29.73 o	14.47 t	**41.48 B**
Sultani	70.61 a	67.30 b	62.13 c	52.91 g	40.47 l	20.97 r	**52.40 A**
Mean	**61.58 A**	**55.80 B**	**50.15 C**	**41.44 D**	**30.19 E**	**16.21 F**	

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

Table (9). Effect of NaCl concentrations on leaf proline (mg 100g $^{-1}$ FW) content of the studied fig cultivars.

Cultivar	NaCl concentration (mg L^{-1})						Mean
	1000	2000	3000	4000	5000	6000	
Black Mission	14.6 p	19.6 l	69.5 g	74.23 d	77.7 c	78.63 b	**55.71 B**
Aboudy	14.50 q	17.8 n	60.0 j	65.0 i	67.53 h	74.0 e	**49.80 D**
Condria	13.46 r	19.0 m	70.93 fg	71.33 f	74.2 d	76.99 c	**54.31 C**
Sultani	15.00 o	20.0 k	71.0 f	76.0 cd	81.0 b	90.06 a	**58.84 A**
mean	**14.39 F**	**19.1 E**	**67.85 D**	**71.64 C**	**75.10 C**	**79.92 A**	

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

Date illustrated in Table (9) indicated that Sultani cultivar had the highest significant proline content compared with Aboudy cultivar which recorded the lowest value. Supplementation of the culture medium with high concentrations of NaCl caused a significant increase in total proline content. Accumulation of proline in plant tissue is one of most frequently reported mechanism of salt tolerance (Ashraf and Foolad, 2007). Proline plays important role in osmotic adjustment which reduces cellular damage (Hasegawa et al., 2000) and maintains water uptake and photosynthesis activity (Serraj and Sinclair, 2002).

CONCLUSION

It could be concluded from the obtained results, that salinity had an adverse effects on different fig cultivars under investigation. Sultani may be considered as relatively tolerant cultivar to salinity due to their performance under salinity conditions compared with the other fig cultivars under investigation

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CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

All authors contributed equally in all parts of this study.

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