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Evaluation of salt tolerance ability in some fig (*Ficus carica L.*) cultivars using tissue culture technique

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ABSTRACT

Ficus carica is one of the most important fruit species of Mediterranean countries. Egypt is one of the top countries in the world for the production of various cultivars of fig. The present work aimed to evaluate *in vitro* tolerance of five fig cultivars (i.e., Achtoy White, Masone Black, Zeiblly Red, Zeiblly Flair, and Shami Stihy) to NaCl on morphological characters and biochemical changes in multiplication stage. The *in vitro* shoots of fig cultivars were subcultured on Murashige and Skoog medium supplemented with 2 or 3 mg/L BAP and 0.5 mg/L 2iP and augmented with different concentrations of NaCl (0.0, 2000, 4000, 6000, 8000, 9000, 10000, 11000, and 12000 ppm) for 5 weeks under *in vitro* culture conditions. Number of newly formed shoots, shoot length, leaves number per shoot, necrosis %, fresh and dry weights, chlorophyll content, and relative water content were recorded at regular intervals. The results revealed that the Masone Black and Shami Stihy cultivars were excellent compared other cultivars, followed by Achtoy White, Zeiblly Red, and Zeiblly Flair cultivars. NaCl concentrations of more than 10000 ppm on Achtoy White and more than 11000 ppm of Zeiblly Red and Zeiblly Flair induced lethal effects. At 12000 ppm, NaCl had no adverse effect on the plantlets of cvs. Masone Black and Shami Stihy. Contents of Na+ and Cl- were increased and loss of K+ ions contents with increasing NaCl levels in all cultivars. In the present study, K+/Na+ ratio was the highest in Masone Black and Shami Stihy while Zeiblly Flair cultivar was the lowest in K+/Na+ ratio when the highest salt stress was applied.

1. INTRODUCTION

Ficus carica belongs to the Moraceae family. It contains more than 140 species categorized in 40 genera [1]. Figs have been cultivated for a long time in different places around the world as edible fruit. It originated in Western Asia and spread to the Mediterranean and some countries of the world. The most productive countries of edible figs are Egypt, Turkey, Morocco, Spain, Greece, California, Italy, Brazil, and other places characterized by mild winter and hot dry summer [2]. The cultivation of fig cultivars depending on the purpose of the use of fruit figs and fruits is eaten fresh, dried, and canned form [3]. *F. carica* originated from the Middle East, which is one of the first cultivated fruit species, and is currently an important crop around the world [4]. Now, the countries of the Mediterranean basin are growing in different varieties of figs. Fig cultivars differ in the morphological form of different leaf and fruit shape. The usual areas of cultivation of fig have decreased significantly; it has been reduced genetic variation due to the disappearance of many cultivars selected in the past. All grown cultivars are maintained by cuttings [5].

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An increase in the salinity of soil water inhibits the germination and root elongation in most plants because it reduces water uptake, water use efficiency, and relative water content and inhibits K, Ca, and NO uptake by plant roots [6,7]. It has been shown that salinity reduces gas exchange, growth traits and yield, reduction in leaf chlorophyll, and photosynthesis [8]. In response to reductions in photosynthetic rate, salinity elevates antioxidant enzymes and proline content as a stress response to deal with increased levels of reactive oxygen species. Most plants under this strain are able to differentiate between K+ ions in saline conditions and accumulate high levels of sodium to the detriment of the necessary K+ and Na+, which leads to loss of function of reaction-dependent K+ leads to the g+-induced toxicity. To obtain the effective use of the salt-affected soils, it is an important to identify saline fig genes and crop production significantly under salinity and benefit from genetic variation present in the genetic material to spread genotypes flexibility or to take advantage of this in the breeding program [9].

Tissue culture is one of the main tools in the field of plant biotechnology and called instead of cells, tissue, and organ culture under laboratory conditions. Salinity and drought-tolerant cultivars can be selected and evaluated using tissue culture [10,11]. Simple screening of plantlets by tissue culture under abiotic or biotic stress provides a study of many plant characteristics and evaluates these plants under specific conditions in a short time [12]. Furthermore, *in vitro* culture provides an important technique for studying the physiological effects of salt

at the cellular level under known environmental conditions [13]. Biotechnology and tissue culture techniques are a powerful tool used to select plants under abiotic or biotic stress and to improve productivity traits in horticultural crops [14]. Meristem culture is the best method to produce large numbers of virus-free plants in a short period of time through *in vitro* propagation [15]. Tissue culture also provides a good advantage of maintained the conserved virus-free plants for different cultivars and easily exchanging them through laboratories between countries [16,17].

The main aim of this study is to establish and examine the response of *in vitro*-propagated shoot tip explants of some cultivars of *F. carica* plantlets for salt tolerance and to determine the feasibility of screening *F. carica* plantlets for salt tolerance.

2. MATERIALS AND METHODS

2.1. Explant Collection and Disinfection

Shoot tip explants were collected from introduced of *F. carica* cvs. Achtoy White, Masone Black, Zeiblly Red, Zeiblly Flair, and Shami Stihy from the Arab Center for the studies of Arid Zones and Dry lands. Explants were washed under running tap water for 3 h. Shoot tip explants (0.5-1.0 cm length) were rinsed several times with sterile antioxidant solution (100 mg/L ascorbic acid and 150 mg/L citric acid) to avoid browning of the tissues. Surface sterilization of the explants was carried out under complete aseptic conditions in the laminar air flow hood. Subsequently, explants were sterilized by immersion for 25 min in sodium hypochlorite 2.5% (v/v). After three subsequent washes in sterile double distilled water, explants were removed and rinsed 3-4 times with sterile antioxdant solution [18].

2.2. Establishment of *In Vitro* Culture

Sterilized shoot tip explants were cultured vertically on murashige and skoog (MS) medium [19] salts and vitamins containing different concentrations of 1.0-2.0 mg/L benzylaminopurine (BAP) combined with 0.05-0.1 mg/L naphthaleneacetic acid (NAA) and/or combined with 0.05-0.1 mg/L IAA to determine the best concentrations of growth regulators combinations for shoot formation. Before autoclaving nutrient media, the pH of the medium was adjusted to 5.8, and the phytagel was added 0.25%. All cultures were transferred to incubation room at 26±2°C and light intensity (50 µmol $m^{-}2S^{-}1$).

2.3. Shoot Multiplication

The shoots (length of 2-3 cm) were separated from establishment stage and then multiplied for eight subcultures on MS media containing different concentrations of N6 BAP alone or in combination with 0.5 mg/L of N6-Δ^2-isopentenyladenine (2ip). Shoots were transferred every 5 weeks on the same media.

2.4. *In Vitro* Root Formation and Acclimatization

Shoots derived from the multiplication stage were cultured on MS medium without hormones for 2 weeks to eliminate any effects of hormone. Then, shoots were transferred to MS medium supplemented with 100 mg/L myoinositol, 30 g/L sucrose fortified with 1.0-2.5 mg/L indole butyric acid (IBA) alone, or combined with 0.0-0.5 mg/L NAA or 0.0-0.5 mg/L IAA with or without 2 g/L activated charcoal. The incubation of cultures under the same conditions used for shoot proliferation for five weeks. Rooted plantlets were removed from the culture medium, and it is washed gently under running tap water to get rid of the phytagel. The plantlets were transferred to plastic pots containing a mixture of peat moss and sand (1:1), and then, plantlets covered with transparent plastic bags under high humidity. After 4 weeks, polyethylene bags were completely opened, and after 4 weeks, more polyethylene bags were removed and plantlets were maintained under greenhouse conditions.

2.5. Effect of Salinity

2.5.1. Growth characters

Shoots (5-10 mm length) were subcultured onto MS medium containing 2.0 or 3.0 mg/L BAP and 0.5 mg/L 2iP and supplemented with different concentration of NaCl at the level of 0.0, 2000, 4000, 6000, 8000, 9000, 10000, 11000, and 12000 ppm, respectively, to study the effect of different concentrations of NaCl on growth and development of *in vitro* shoots. Each treatment consists of four replicates and repeated each experiment twice. The data were calculated on shoot length, number of shoots/explant, and fresh and dry weights after 5-week growing period.

2.5.2. Mineral composition

Plant samples were dried at 500°C for 24 h using Thermolyne Muffle Furnace (6000 Furnace). The samples was heated with 10 ml 2N HCl on 80°C for 10 min. The solution was prepared after filtered using Whatman filter paper #42 and dilute it to 50 ml with distilled water, and then, concentration of Na+ and K+ was determined after calibration with different concentration of Na+ or K+ solutions using Flame photometer 410 [20]. The concentration of Na+, K+, and Cl- was determined by flame emission spectrophotometry.

2.5.3. Chlorophyll content

About 0.5 g fresh weight sample of leaves was taken per replicate after 5-week exposure to salt stress, and the samples were extracted by acetone [21]. Samples were grinded in 2 ml of 80% acetone using a pestle and mortar, and then, the sample extracted was transferred to 2.0 ml Eppendorf tube and centrifuged at 15000 g for 2 min. The supernatant was collected using pipette, and up to 3.0 ml in 10 ml measuring cylinder with an additional 80% acetone. The absorption spectra were measured by spectrophotometer (Smart Spec™ 3000 Bio-Rad) at 510-665 nm absorbance, and chlorophyll *a* and chlorophyll *b* contents were calculated [22].

2.5.4. Proline determination

The proline content was measured in leaves derived from fig plantlets with NaCl treatments by measuring the amount of the colored product from interaction between proline with ninhydric acid [23]. The sample was read at 518 nm using a SmartSpec™ 3000 Bio-Rad spectrophotometer. The proline concentration in the sample was measured from a standard curve and calculated based on fresh weight.

2.6. Statistical Analysis

The data of all the study experiments were statistically analyzed as a factorial experiment. The Randomized Complete Block Design used to find the analysis of variance. Comparisons were made between the means through the least significant differences multiple range tests [24]. The data were analyzed using MSTAT software program.

3. RESULTS AND DISCUSSION

3.1. *In Vitro* Propagation of Fig (*F. carica* L.)

3.1.1. Shoot induction

F. carica cvs. Achtoy White, Masone Black, Zeiblly Red, Zeiblly Flair, and Shami Stihy were efficiently regenerated from shoot tip

explants on MS medium containing different concentrations of BA as a cytokinin and combinations IAA or NAA as auxins.

Data recorded in Table 1 indicate that shoot tip explants of fig cvs. Achtoy White and Shami Stihy grown on MS medium with 1.0 mg/L BA + 0.05 mg/L NAA gave the highest percentage of explants forming shoots (99% and 97%), respectively, compared with the other treatments (42-94%) (Plate 1). However, the percentage of explants forming shoots was reached the highest value 99% of Zeiblly Red cultivar and 100% of Zeiblly Flair cultivar using 2.0 mg/L BA + 0.1 mg/L NAA comparing with the other treatments (38-97%). Furthermore, the percentage of explants forming shoots was reached the highest value 96% for Masone Black cultivar using 2.0 mg/L BA + 0.1 mg/L IAA comparing with the other treatments (59-94%). The mean number of axillary shoots formed explant (2.8 and 2.7) and mean length of the formed axillary (3.0 and 2.6 cm) was the best treatment with 1.0 mg/L BAP + 0.05 NAA mg/L for Achtoy White and Shami Stihy cultivars, respectively. However, the treatment containing 2.0 mg/L BAP + 0.1 NAA mg/L was the best treatment of mean number of axillary shoots (3.2 and 3.4) and mean length of the formed axillary (2.8 and 2.9 cm) for Zeiblly Red and Zeiblly Flair cultivars, respectively. Furthermore, the mean number of axillary shoots formed explant (2.5) and mean length of the formed axillary (2.4 cm) was the best treatment with 2.0 mg/L BAP + 0.1 NAA mg/L for Masone Black cultivar. Similarly, the highest value of mean number of shoots per explant was 2.99, 2.96, and 2.93 of cultivars Black Mission, Brown Turkey, and Brunswick, respectively, when shoot tips were cultured on MS medium containing 1.0 or 2.0 mg/L BAP in the presence of 0.5 or 1.0 mg/L NAA [7]. While, *in vitro* propagation was obtained from shoot tips and nodal explants on a medium based on MS supplemented with NAA, BA, and 2ip in three cultivars in fig cvs. Conadria, Abiad, and Sultani [18].

Plate 1: *In vitro* growth of *Ficus carica* cvs. Achtoy White, Shami Stihy, Masone Black, Zeiblly Red, and Zeiblly Flair on MS medium supplemented with 1.0 mg/L BA + 0.05 mg/L NAA after 4 weeks.

3.1.2. Multiplication of shoot cultures

Shoot cultures were multiplied by subculturing on MS media supplemented with several concentrations of BA even alone or in combination with 2iP were investigated for multiple shoot production in fig cultivars Achtoy White, Masone Black, Zeiblly Red, Zeiblly Flair, and Shami Stihy. The obtained data as summarized on Table 2 showed that shoot multiplication rates were significantly affected by the concentration of BA, as 6.5 shoots/explant were recorded for Masone Black cv., 7.2 shoots/explant for Zeiblly Red cv., and 7.5 shoots/explant for Zeiblly Flair cultivar using 2 mg/L BA and 0.5 mg/L, respectively (Plate 2). While, the mean number of shoots per explant of Achtoy White and Shami Stihy were significantly increased using the medium containing 3 mg/L BA + 0.5 mg/L 2ip; it reached to 7.4-6.8, respectively, than the other treatments (Plate 3). It was observed that the low concentration of BAP in the medium leads to a decrease in shoot multiplication while the low concentration of BA and the combination of 2iP lead to elongation of shoots. The mean of shoots/explant were significantly increased of all cultivars using the medium containing 1 mg/L BA + 0.5 mg/L 2ip; it reached to 2.5, 2.4, 2.7, 2.8, and 2.6 cm for Achtoy White; Masone Black; Zeiblly Red; Zeiblly Flair; and Shami Stihy, respectively, compared than the other treatments. Similarly, the best results for shoot multiplication and the rate of propagation of fig were 4.17, 3.42, and 5.0 over a 5-week period for Conadria, Abaid, and Sultani cultivars, respectively, on MS medium containing 3 mg/L BAP + 0.5 mg/L 2iP [18]. Furthermore,

Table 1: Effect of different concentrations of BAP, NAA, and IAA on shoots formation from shoot tip explants of *F. carica* cvs. Achtoy White, Masone Black, Zeiblly Red, Zeiblly Flair, and Shami Stihy after 5 weeks.

Growth regulators (mg/L)			% of explant forming axillary shoots					Mean number of axillary shoot/ explant					Mean length of axillary shoots (cm)				
BA	NAA	IAA	AW	MB	ZR	ZF	SS	AW	MB	ZR	ZF	SS	AW	MB	ZR	ZF	SS
1	0.05	0	99	55	84	87	97	2.8b	1.0g	1.5e	1.5e	2.7b	3.0a	1.0h	2.0e	2.0e	2.6b
2	0.05	0	94	68	90	91	92	2.0d	1.4e	1.6e	1.6e	2.3c	2.9a	1.5g	1.8f	1.9e	2.5c
3	0.05	0	90	58	87	89	91	2.2d	1.1g	1.1g	1.1g	2.4c	2.7b	1.2h	2.0e	2.0e	2.4c
1	0	0.05	65	80	72	70	76	1.5e	1.8e	1.0g	1.0g	1.0g	2.0e	1.8f	1.7f	1.6f	1.5g
2	0	0.05	75	93	70	68	82	1.2f	2.2d	1.4e	1.2f	1.4e	1.8f	2.0e	1.8f	1.5g	1.7f
3	0	0.05	42	89	65	59	62	0.8h	2.1d	1.2f	1.2f	1.2f	1.5g	2.1d	1.9e	1.3g	1.0h
1	0.1	0	94	59	95	97	89	2.6b	1.7e	2.5c	2.7b	2.2d	2.9a	1.5g	2.7b	2.8b	2.4c
2	0.1	0	92	68	99	100	90	2.3c	1.2f	3.2a	3.4a	2.3c	2.8b	1.3g	2.8b	2.9a	2.3d
3	0.1	0	95	62	97	92	94	2.4c	1.5e	2.7b	2.5c	2.5c	2.0e	1.4g	2.6b	2.5c	2.0e
1	0	0.1	64	89	50	65	77	1.0g	2.4c	1.3f	1.4e	1.0g	1.9e	2.2d	1.5g	1.8f	1.8f
2	0	0.1	68	96	45	72	84	1.3f	2.5c	1.2f	1.3f	1.3f	1.8f	2.4c	1.4g	1.6f	1.7f
3	0	0.1	52	94	38	57	65	1.2f	2.2d	1.0g	1.1g	1.2g	1.7f	2.0e	1.2h	1.3g	1.5g

Mean followed by different letters differs significantly (P<0.05). AW: Achtoy White, MB: Masone Black, ZR: Zeiblly Red, ZF: Zeiblly Flair, SS: Shami Stihy. *F. carica: Ficus carica*

Table 2: Effect of different concentrations of cytokinins on multiplication of *in vitro* proliferation shoots *F. carica* cvs. Achtoy White, Masone Black, Zeiblly Red, Zeiblly Flair, and Shami Stihy after 5 weeks.

Growth regulators (mg/L)		Increase in mean number of axillary shoots/explant					Increase in mean length of axillary shoots/explant (cm)				
BAP	2ip	AW	MB	ZR	ZF	SS	AW	MB	ZR	ZF	SS
1	0	3.5h	2.4i	2.5i	2.8i	3.4h	1.8e	0.5i	0.6i	0.8h	1.5f
2	0	4.8g	3.0h	3.2h	3.7h	4.6g	1.5f	0.9h	1.0g	1.2g	1.7e
3	0	5.4e	4.5g	4.8g	5.0f	5.5e	1.4f	1.0g	1.0g	1.4f	1.2g
4	0	6.0d	5.5e	6.0d	6.2d	5.9e	0.9h	0.5i	0.7h	0.8h	0.8h
1	0.5	4.5g	4.3h	4.7g	4.9g	4.2h	2.5b	2.4c	2.7a	2.8a	2.6b
2	0.5	5.8e	6.5c	7.2a	7.5a	5.4f	2.3c	2.2d	2.4c	2.5b	2.4c
3	0.5	7.4a	6.0d	6.5c	7.0b	6.8b	2.4c	2.0d	2.0d	2.2d	2.1d
4	0.5	6.8b	6.2d	6.7c	6.9b	6.5c	2.0d	1.7e	1.5f	1.8e	1.6e

Mean followed by different letters differs significantly ($P<0.05$). AW: Achtoy White, MB: Masone Black, ZR: Zeiblly Red, ZF: Zeiblly Flair, SS: Shami Stihy. *F. carica: Ficus carica*

Table 3: The efficiency of shoots forming roots of *F. carica* cvs. Achtoy White, Masone Black, Zeiblly Red, Zeiblly Flair, and Shami Stihy after growing on MS nutrient medium supplemented with different auxin concentrations.

Growth regulators (mg/L)		% of shoot forming roots					Mean number of roots/explant					Mean length of roots (cm)				
IBA	NAA	AW	MB	ZR	ZF	SS	AW	MB	ZR	ZF	SS	AW	MB	ZR	ZF	SS
0	0	6	5	7	10	0	1.2j	1.4j	0.5k	0.8k	0	2.4g	2.0h	1.5i	1.7i	0.0k
1	0	78	70	67	69	50	2.4i	2.2i	1.8j	2.0i	1.4j	2.8g	2.2h	2.0h	2.4g	0.9j
1.5	0	62	60	65	67	89	2.7i	3.5h	2.3i	2.5i	1.7j	3.2f	3.0f	3.8f	3.9f	1.5i
2	0	72	65	50	62	72	2.5i	3.3h	2.5i	3.8h	2.5i	3.4f	3.2f	2.4g	3.6f	1.8i
2.5	0	70	68	41	53	68	2.6i	2.5i	2.0i	1.9j	2.2i	2.5g	2.8g	2.0h	2.2h	1.2j
1	0.1	100	96	85	87	93	5.7b	5.5c	5.9b	5.8b	5.0d	6.7a	6.5b	5.0e	5.2d	5.2d
1.5	0.1	93	87	82	90	94	5.2d	5.0d	5.7b	5.6c	6.0b	6.4b	6.0c	5.5d	5.8c	5.9c
2	0.1	92	85	87	89	95	4.5f	4.2g	5.5c	5.0d	5.2d	6.3b	6.2b	5.3d	5.5d	5.6d
2.5	0.1	89	83	80	83	90	4.6f	4.3g	4.9e	4.6f	4.9e	3.6f	5.6d	4.6e	4.8e	5.4d
1	0.5	95	92	93	94	90	5.4c	5.2d	6.0b	5.9b	4.8e	5.8c	6.2b	6.2b	6.8a	5.0e
1.5	0.5	93	90	98	99	92	5.0d	4.9e	6.5a	6.4a	5.0d	6.0c	6.1c	6.8a	7.0a	5.5d
2	0.5	92	88	93	94	94	4.6f	4.5f	5.7b	5.5c	4.7e	6.2b	6.3b	6.5b	6.5b	5.3d
2.5	0.5	90	85	91	92	90	4.8e	4.0g	5.0d	5.2d	4.5f	6.4b	6.5b	6.4b	6.0c	5.1e

Mean followed by different letters differs significantly ($P<0.05$). AW: Achtoy White, MB: Masone Black, ZR: Zeiblly Red, ZF: Zeiblly Flair, SS: Shami Stihy. *F. carica: Ficus carica*

the best shoot multiplication and increase in number of fig shoots were 7.25 and 6.75 for Black Mission and Brunswick cultivars, respectively, cultured on MS medium supplemented with 3 mg/L BAP + 0.5 mg/L 2iP, while cultivar Brown Turkey (6.85) on MS medium containing with 3 mg/L BAP + 0.5 mg/L kinetin [7].

3.1.3. Rooting and ex vitro acclimatization

The data seen in Table 3 explained that the combination of IBA and NAA gave better rooting response than IBA alone. The highest percentage of explants that produced roots (100-96%) were observed on MS medium containing 1 mg/L IBA and 0.1 mg/L NAA with 2 g/L activated charcoal for cultivars Achtoy White and Masone Black, respectively, and the percentage of shoots that formed roots was reached the highest value (98-99%) for cultivars Zeiblly Red and Zeiblly Flair, respectively, using MS phytagel gelled nutrient medium supplemented with 1.5 mg/L IBA, 0.5 mg/L NAA, and 2 g/L activated charcoal (Plate 4). While the percentage of shoots that formed roots and reached the highest value (95%) was observed on MS medium containing 2 mg/L IBA, 0.1 mg/L NAA, and with 2 g/L activated charcoal for Shami Stihy. The mean number of roots (5.7 and 5.5) and mean length of roots formed on shoot

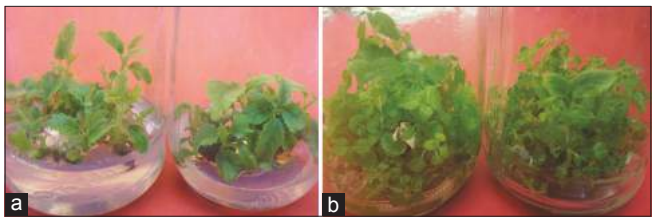

Plate 2: Shoot multiplication of *Ficus carica* cvs. Zeiblly Red and Zeiblly Flair (a): MS medium supplemented with 2 mg/L BAP, and (b): MS medium supplemented with 2 mg/L BAP and 0.5 mg/L 2iP.

(6.7 and 6.5 cm) on MS medium supplemented with 1.0 mg/L IBA and 0.1 mg/L NAA was significantly higher than the other treatments for Achtoy White and Masone Black cultivars, respectively. However, the mean number of roots (6.5 and 6.4) and the mean length of roots formed on the shoot (6.8 and 7.0 cm) treated with 1.5 mg/L IBA and 0.5 mg/L NAA for Zeiblly Red and Zeiblly Flair cultivars, respectively. Furthermore, the mean number of roots (6.0) and the mean length of

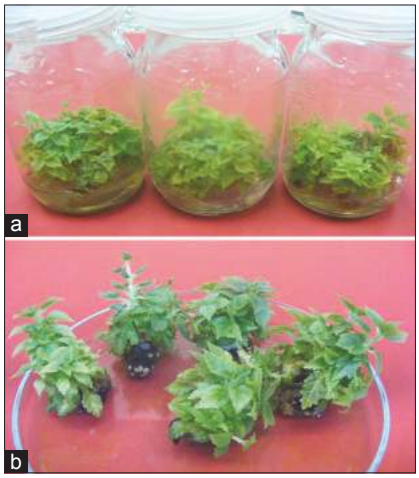

Plate 3: Shoot multiplication of *Ficus carica* L. cvs. Achtoy White, Masone Black, and Shami Stihy on MS medium supplemented with BAP and 2iP. (a): MS medium supplemented with 3 mg/L BAP and 0.5 mg/L 2iP. (b): MS medium supplemented with 2 mg/L BAP and 0.5 mg/L 2iP.

Plate 4: Rooting of *Ficus carica* on MS medium supplemented with1.5 mg/L IBA and 0.5 mg/L NAA. (a): Without activated charcoal, and (b): With 2 g/L activated charcoal.

roots formed on the shoot (5.9 cm) when treated with 1.5 mg/L IBA and 0.1 mg/L NAA for Shami Stihy cultivar compared with the other treatments. Showed the previous work on *in vitro* fig plants that highest percentage of explants that produced roots (80%) for fig shoots were obtained after 1 month in light on MS medium contained with 1 mg/L IBA [25,26]. Furthermore, the percentage of shoots that formed roots was the highest for cultivars black mission and brown Turkey using MS medium supplemented with 1.0 mg/L IBA and 0.5 mg/L NAA and incubated cultures in darkness for 1 week and transferred to light for 3 weeks. While the best rooting (97%) was observed using MS medium contained with 1.5 mg/L IBA and 0.5 mg/L NAA for cultivar Brunswick [7]. Plants produced from the rooting stage were transferred to a greenhouse, and after 8 weeks from transferring to the greenhouse, they were repotted into sterile soil consists equal parts of peat and sand (v/v) (Plate 5). Similarly, acclimatization of *F. carica* plantlets was transplanted into pots containing equal parts of peat and vermiculite (v/v) before transferring to the greenhouse after 4 weeks. After 8 weeks, they were repotted into sterile soil containing equal parts of peat and vermiculite (v/v) [15].

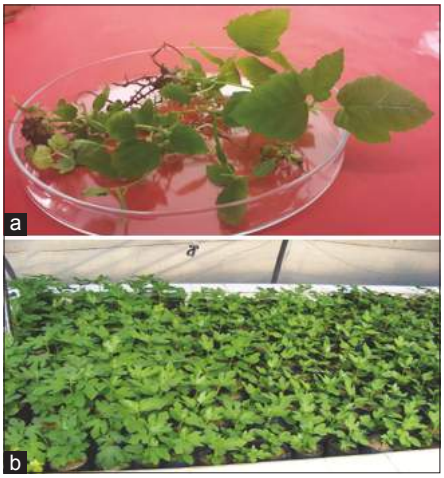

Plate 5: Rooting and *ex vitro* acclimatization of *Ficus carica* (a): Healthy plantlets with normal roots and (b): Acclimatization and establishment of *ex vitro* plants of *Ficus carica* cvs. Achtoy White, Masone Black, Zeiblly Red, Zeiblly Flair, and Shami Stihy under greenhouse conditions.

3.2. Salinity Stress

3.2.1. Growth parameters responses to salinity levels

Microshoots produced from establishment stage were subcultured onto MS proliferation medium containing 2 mg/L BA and 0.5 mg/L 2iP for Masone Black, Zeiblly Red, and Zeiblly Flair cultivars, and MS medium supplemented with 3 mg/L BA + 0.5 mg/L 2ip for Achtoy White and Shami Stihy cultivars containing different concentration of NaCl (0.0, 2000, 4000, 6000, 8000, 9000, 10000, 11000, and 12000 ppm). Data in Fig. 1 indicate that the application of the different concentrations of NaCl to *F. carica* cultivars Achtoy White, Masone Black, Zeiblly Red, Zeiblly Flair, and Shami Stihy after 5 weeks of treatments there were a decrease of in shoot length, shoot number, and number of leaves/shoot when increased salt concentrations in the media. Root length decreased from 2.90 cm compared to the control to 0.75 cm at 10000 ppm NaCl for Achtoy White cultivar, 2.75 and 2.85 cm for the control to 0.40 and 0.50 cm at 11000 ppm NaCl of Zeiblly Red and Zeiblly Flair cultivars, respectively. While root length was decreased from 3.45 and 2.65 cm for the control to 1.35 and 1.00 cm at 12000 ppm NaCl for Masone Black and Shami Stihy cultivars, respectively, and no rooting occurred when shoots were grown on MS medium contained 12000 ppm NaCl for the cultivars Zeiblly Red and Zeiblly Flair and 11000 ppm NaCl for Achtoy White, whereas the number of newly formed shoots of *F. carica* cultivar Achtoy White exhibited a significant decrease at 10000 ppm of NaCl concentration, cultivars Zeiblly Red and Zeiblly Flair exhibited a significant decrease at 11000 ppm of NaCl concentration, and cultivars Masone Black and Shami Stihy exhibited a significant decrease with 12000 ppm of NaCl concentration. However, the highest value was recorded for the control (0.0) and 2000 ppm of all cultivars compared with the other treatments. Results revealed that low level of salinity (control and 2000 ppm) in culture medium significantly enhanced shoot length and the number of newly formed shoots. High salinity levels from 4000 to 12000 ppm NaCl caused a decrease in shoot length and number of shoots. At the concentration of 11000 ppm NaCl, it was lethal for Achtoy White cultivar but Zeiblly Red and Zeiblly Flair survived of that this concentration, and cultivars Masone Black and Shami Stihy were survived at 12000 ppm NaCl. The necrosis rate was increased with increased NaCl in MS medium for most cultivars, but Achtoy

White, Zeiblly Red, and Zeiblly Flair had slightly higher rate of necrosis (100%) compared to Masone Black and Shami Stihy (30 and 32%), respectively, with 12000 ppm of NaCl concentration. Shoot length, shoot fresh, and dry weight significantly decreased under treatment with NaCl in the proliferation medium [27]. The addition of sodium chloride in the media of fig showed a negative effect on the growth of shoots and reduced the appearance of new formed shoots [8]. Similarly, shoot length and the number of newly formed shoots decreased with the increased salt concentration in the medium for three cultivars Black Mission, Brown Turkey, and Brunswick. Plantlets length ranged from 0.13 to 2.98 cm, and the greatest plantlets length was found in 2 g/L for the three cultivars and the smallest value was recorded at 11 g/L compared with other the treatments; the number of newly formed shoots of fig cultivars Brown Turkey and Brunswick exhibited a significant decrease with 12 g/L of NaCl concentration. However, the highest value was recorded for the control (0.0) and 2 g/L the other treatments. The percentage of necrosis increased with increase in NaCl concentration. For Black Mission survived at 12 g/L NaCl, while the death of plantlets at this concentration for the cvs. Brown Turkey and Brunswick both was observed [7].

3.2.2. Fresh and dry weights

The effect of salinity on fresh and dry weights showed significantly increased compared with the control treatment for the five cultivars as compared to all the other treatments. Afterward, a significant gradual decrease in fresh weight and dry weights took place at 4000, 8000, 10000, 11000, and 12000 ppm NaCl where the fresh and dry weight generally decreased with the increase in NaCl level in the medium, from 3.35 to 1.50 g at 4000 ppm NaCl to 1.56 and 0.72 g at 10000 ppm NaCl of cv. Achtoy White, respectively (Table 4), whereas the fresh and dry weights decreased with the increase in NaCl level in the medium, from 3.58 to 1.89 g at 2000 ppm NaCl to 1.85 and 1.04 g at 12000 ppm NaCl of cv. Masone Black, respectively (Table 5). The results also showed that fresh and dry weight decreases when salt concentration increases in the medium, and the highest fresh weight (3.32 and 3.41 g) and dry weight (1.61 and 1.68 g) were obtained at 2000 ppm NaCl, whereas the minimum fresh weight (1.87 and 1.92 g) and dry weight (0.74 and 0.98 g) was obtained at 11000 ppm NaCl for cvs. Zeiblly Red and Zeiblly Flair, respectively (Tables 6 and 7). Furthermore, the fresh and dry weights decreased with the increase in NaCl level in the medium, from 3.55 to 1.80 g at 2000 ppm NaCl to 1.75 and 0.92 at 12000 ppm NaCl for cv. Shami Stihy, respectively (Table 8). Reduced shoots affect fresh and dry weights as a result of increased NaCl level in the medium. Such reductions in shoot fresh and dry weights were also well documented [28].

3.2.3. Chlorophyll content

Chlorophyll a, b, and a+b amounts decreased due to NaCl increased the concentration in the media. Maximum values of chlorophyll a (265.52 and 260.00 g^{-1} FW) and chlorophyll b were 104.62 and 100.54 g^{-1} FW at the control in Masone Black and Shami Stihy cultivars, respectively. While the lowest content values of chlorophyll a and b were 250.75 and 90.00 g^{-1} FW in cv. Zeiblly Red compared with other cultivars. Chlorophyll a/b ratio was decreased in the *F. carica* five cultivars with increased NaCl level. Chlorophyll a/b ratio decrease was 1.35 in Masone Black cv and 1.36 in Shami Stihy cv at 12000 ppm NaCl. Furthermore, chlorophyll a/b ratio decrease was 1.62 when 10000 ppm NaCl in Achtoy White cv., and chlorophyll a/b ratio decrease was 1.30

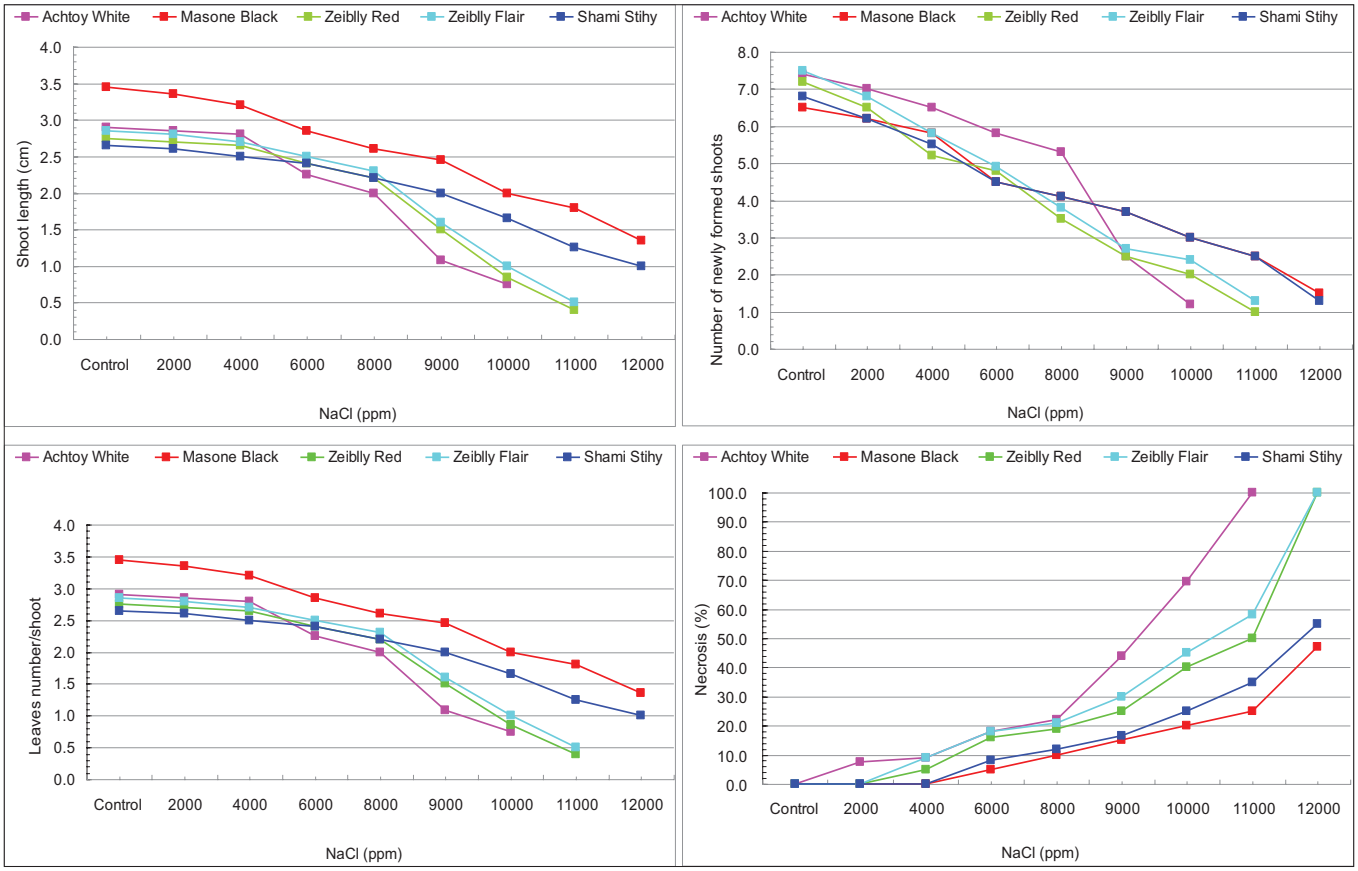

Fig. 1: Effect of different concentrations of NaCl on growth of *in vitro* cultured *Ficus carica* cvs. Achtoy White, Masone Black, Zeiblly Red, Zeiblly Flair, and Shami Stihy after 5 weeks of treatment.

Table 4: Effect of different concentrations of NaCl on fresh weight and dry weight, chlorophyll contents of *in vitro* microshoots of *F. carica* cv. Achtoy White grown on MS medium supplemented with 3 mg/L BAP and 0.5 mg/L 2iP after 5-week growth periods.

NaCl (ppm)	Fresh weight/five explants (g)	Dry weight/five explant (g)	Chl a (µg/g FW)	Chl b (µg/g FW)	Chl a/b ratio
Control	3.55a	1.87a	252.89a	98.85a	2.56a
2000	3.37b	1.55b	242.70b	96.45b	2.51b
4000	3.35b	1.50b	225.35c	90.80c	2.48c
6000	2.87c	1.22c	172.75d	82.40d	2.09d
8000	2.00d	1.10d	125.60e	65.95e	1.90e
9000	1.85e	0.87e	100.40f	60.25f	1.67f
10000	1.56f	0.72f	90.80g	55.95g	1.62g
11000	0.00g	0.00g	0.00h	0.00h	0.00h
12000	0.00g	0.00g	0.00h	0.00h	0.00h

Mean followed by different letters differs significantly ($P<0.05$). *F. carica: Ficus carica*

Table 5: Effect of different concentrations of NaCl on fresh weight, dry weight, and chlorophyll contents of *in vitro* microshoots of *F. carica* cv. Masone Black grown on MS medium supplemented with 2 mg/L BA and 0.5 mg/L 2iP after 5-week growth periods.

NaCl (ppm)	Fresh weight/five microshoots (g)	Dry weight/five mcroshoots (g)	Chl a (µg/g FW)	Chl b (µg/g FW)	Chl a/b ratio
Control	3.75a	1.98a	265.52a	104.62a	2.54a
2000	3.58b	1.89b	249.50b	101.10b	2.47b
4000	2.95c	1.52c	220.60c	90.05c	2.45b
6000	2.50d	1.39d	185.36d	81.30d	2.28c
8000	2.45e	1.30e	170.25e	78.65e	2.16d
9000	2.25f	1.22f	120.50f	69.95f	1.72e
10000	2.15g	1.18g	92.45g	61.75g	1.50f
11000	1.95h	1.09h	85.75h	58.90h	1.45f
12000	1.85i	1.04i	67.40i	49.78i	1.35g

Mean followed by different letters differs significantly ($P<0.05$). *F. carica: Ficus carica*

Table 6: Effect of different concentrations of NaCl on fresh weight, dry weight, and chlorophyll contents of *in vitro* microshoots of *F. carica* cv. Zeiblly Red grown on medium supplemented with 2 mg/L BA and 0.5 mg/L 2iP after 5-week growth periods.

NaCl (ppm)	Fresh weight/five microshoots (g)	Dry weight/five microshoots (g)	Chl a (µg/g FW)	Chl b (µg/g FW)	Chl a/b ratio
Control	3.40a	1.72a	250.75a	90.00a	2.79a
2000	3.32b	1.61b	241.70b	87.50b	2.76a
4000	3.00c	1.45c	225.50c	82.45c	2.73a
6000	2.75d	1.38d	210.90d	80.69d	2.61b
8000	2.52e	1.23e	187.69e	75.80e	2.48c
9000	2.28f	1.05f	148.54f	60.50f	2.45c
10000	2.08g	0.92g	75.50g	55.78g	1.35d
11000	1.87h	0.74h	70.00h	53.50h	1.30d
12000	0.00i	0.00i	0.00i	0.00i	0.00e

Mean followed by different letters differs significantly ($P<0.05$). *F. carica: Ficus carica*

and 1.49 when at 10000 ppm NaCl in cv. Zeiblly Red and Zeiblly Flair, respectively. The chlorophyll a and chlorophyll b amounts measured in the cultivars differed from one cultivar to another. At NaCl 12000 ppm, leaves of Masone Black and Shami Stihy cultivars had the highest amounts of both chlorophyll a (67.40 and 55.89 µg/g FW.) and chlorophyll b (49.78 and 40.98 µg/g FW) while the amounts of chlorophyll a and b were observed in Achtoy White cv (90.80 µg/g FW and 55.95 µg/g FW) at 10000 ppm NaCl, respectively. Moreover, the leaves contained chlorophyll a (70.00 and 82.35 µg/g FW) and chlorophyll b (53.50 and 55.00 µg/g FW) in the medium were observed contained the media 11000 ppm NaCl in Zeiblly Red and Zeiblly Flair cultivars, respectively. The decrease of chlorophyll content is a common symptom under salinity, and the higher ratio of Chla/Chlb was also considered to be the result of the decreased emphasis on light collection in relation to the rates of PSII photochemistry [9].

3.2.4. Mineral analysis

The effect of salinity levels on mineral contents of *F. carica* cvs. Achtoy White, Masone Black, Zeiblly Red, Zeiblly Flair, and Shami Stihy indicated an increasing of Na+, Cl-, and loss of K+ ions contents

Table 7: Effect of different concentrations of NaCl on fresh weight, dry weight, and chlorophyll contents of *in vitro* microshoots of *F. carica* cv. Zeiblly Flair grown on medium supplemented with 2 mg/L BA and 0.5 mg/L after five-week growth periods.

NaCl (ppm)	Fresh weight/five microshoots (g)	Dry weight/five microshoots (g)	Chl a (µg/g FW)	Chl b (µg/g FW)	Chl a/b ratio
Control	3.50a	1.82a	258.38a	94.30a	2.74a
2000	3.41b	1.68b	244.20b	89.70b	2.72a
4000	2.95c	1.53c	230.00c	86.90c	2.65b
6000	2.75d	1.42d	220.30d	83.75d	2.63b
8000	2.64e	1.37e	194.50e	78.60e	2.47c
9000	2.35f	1.25f	160.20f	65.70f	2.43c
10000	2.25g	1.05g	90.25g	59.45g	1.51d
11000	1.92h	0.98h	82.35h	55.00h	1.49d
12000	0.00i	0.00i	0.00i	0.00i	0.00e

Mean followed by different letters differs significantly ($P<0.05$). *F. carica: Ficus carica*

Table 8: Effect of different concentrations of NaCl on fresh weight, dry weight, and chlorophyll contents of *in vitro* microshoots of *F. carica* cv. Shami Stihy grown on medium supplemented with 3 mg/L BAP and 0.5 mg/L 2iP after 5-week growth periods.

NaCl (ppm)	Fresh weight/five microshoots (g)	Dry weight/five microshoots (g)	Chl a (µg/g FW)	Chl b (µg/g FW)	Chl a/b ratio
Control	3.62a	1.95a	260.00a	100.54a	2.59a
2000	3.55b	1.80b	242.30b	98.70b	2.45b
4000	2.82c	1.47c	200.95c	92.68c	2.17c
6000	2.41d	1.28d	179.75d	85.40d	2.10d
8000	2.32e	1.25d	160.85e	81.00e	1.98e
9000	2.05f	1.08e	110.75f	64.65f	1.71f
10000	2.00g	1.05e	89.30g	58.25g	1.53g
11000	1.80h	1.00f	72.50h	52.28h	1.38h
12000	1.75i	0.92g	55.89i	40.98i	1.36h

Mean followed by different letters differs significantly ($P<0.05$). *F. carica: Ficus carica*

with increasing salinity concentrations. When the sodium chloride concentration increased, the level of K+ decreased in the shoots and roots of the studied cultivars. Results can be explained on the basis that the increase of sodium ion is compensated for the loss of potassium ion [29]. The value obtained in the roots was lower than that of the shoots. The study showed that with the increased concentration of NaCl, increased Na+ and Cl- contents in shoots and roots and low K+ content in all cultivars. The analysis of K+content results showed that the treatments at 4000, 6000, 8000, 10000, and 12000 ppm of NaCl were differed significantly from the control treatment. The lowest K+ content was recorded with the highest concentration of NaCl (12000 ppm) with all cultivars. The highest Na+ content was detected at this concentration in all cultivars, and this value differed from one cultivar to another. Na+ content in shoots of Masone Black and Shami Stihy cultivars was significantly lower than Na+ content in the other cultivars. Through these results obtained, it can be concluded that Masone Black and Shami Stihy cultivars were the most salt stress tolerant due to its less Na+ absorption and more K+ accumulation in roots compared with the other cultivars (Fig. 2). In addition, according to results showed in Fig. 3, K+/Na+ ratio was the highest in Masone Black and Shami Stihy cultivars, especially at the highest salt stress, which was 0.14 in shoots and 0.15 in roots of Masone Black cultivar and 0.13 in shoots and 0.14 in roots of Shami Stihy cultivar compared with Achtoy White, Zeiblly Red and Zeiblly Flair cultivars which was lowest in K+/Na+ ratio when the highest salt stress concentration was applied. This trait has a potential value as selection standard for salt stress [30]. In this study, internal concentrations of Na+and Cl- were increased with increasing external NaCl concentration in all fig cultivars, and the selected Masone Black and Shami Stihy cultivars accumulated more Na+ and Cl - than Achtoy White, Zeiblly Red, and Zeiblly Flair cultivars. There are many interactions between these elements, and these interactions depend on the salinity level and composition of salts, the crop species, the nutrient in question, and a number of environmental factors [31]. Similar observations regarding internal Na+and Cl- content for NaCl tolerant were observed with tomatoes [32]. The concentration of salt increases, the ability to exclude salt may become less effective in protecting the plant from salt stress, and other mechanisms, such as osmotic tolerance, may become increasingly important. Furthermore, potassium was lower in NaCl-adapted calli of potato with the increase in the external salt concentration up to 250 mM NaCl exposed to salt than in non-adapted calli grown in standard medium [33].

3.2.5. Proline content

Proline content increased with the increases in NaCl level of all cultivars (Fig. 4). The highest proline content (85.45 and 82.00 µg/g FW**)** was obtained at 12000 ppm NaCl of fig cvs. Masone Black and Shami Stihy, respectively, and was obtained (74.5 and 76.0 µg/g FW) at 11000 ppm NaCl of Zeiblly Red and Zeiblly Flair cultivars, respectively, while cv. Achtoy White was obtained (62.30 µg/g FW) which the lowest in proline content when the highest salt stress concentration was applied. Several studies have shown that treatment of plants with high concentrations of sodium chloride caused an increase in proline content in Phoenix dactylifera callus [34]; Carrizo citrange [35]; *Saccharum* sp. Callus [36]; and *Phaseolus vulgaris* L. callus [37]. Furthermore, the proline content in tissues increased with increased salt stress fig cvs. Black Mission, Brown Turkey, and Brunswick [7].

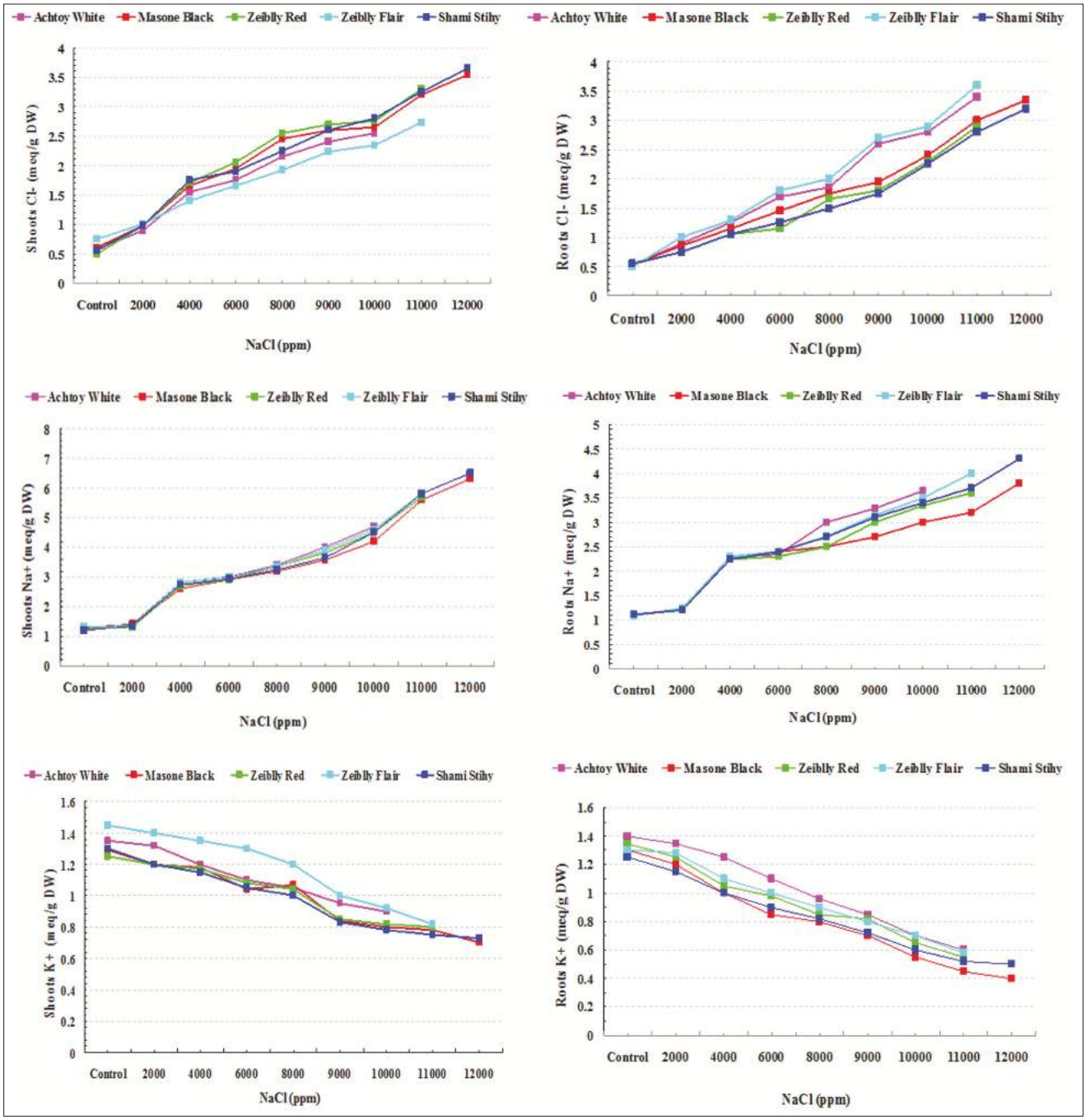

Fig. 2: K+, Na+, and Cl- concentrations (milliequivalents per gram DW) in roots and shoots of five *Ficus carica* cultivars Achtoy White, Masone Black, Zeiblly Red, Zeiblly Flair, and Shami Stihy during 60 days with water containing 0.0, 2000, 4000, 6000, 8000, 10000, 11000, and12000 ppm NaCl.

4. CONCLUSION

We investigated the five *F. carica* cultivars, cultured *in vitro*, to grow under different NaCl concentrations. After treating the plantlets to different concentrations of NaCl for 8 months, morphological and physiological parameters were recorded. The growth of plant on both multiplication and rooting stages was gradually decreased by increasing NaCl levels. As salt stress intensified, there was a reduction in shoot length, formed shoots, and fresh and dry weights. The study showed that with the increase of the NaCl concentration in all studied parameters decrease while the Na+ and Cl- content increases in the all cultivars. Na+ and Cl- in plant tissues of all cultivars increased while K+ decreased with the increase of salt concentrations. Masone Black and Shami Stihy cultivars had a higher tolerance to salinity compared with other cultivars. The results of this study showed the possibility of using tissue culture in the evaluation of fig salt tolerance under certain environmental conditions in a short time.

5. ACKNOWLEDGMENTS

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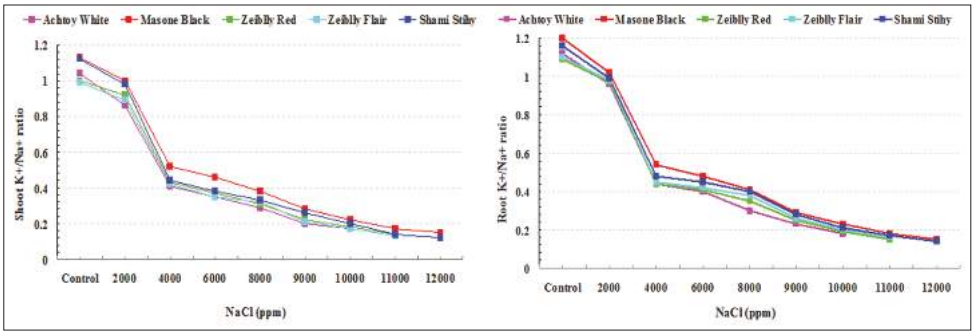

Fig. 3: Effect of NaCl (0, 2000, 4000, 6000, 8000,10000,11000, or 12000 ppm) on shoot and roots K+/Na+ ratio in *Ficus carica* cultivars Achtoy White, Masone Black, Zeiblly Red, Zeiblly Flair, and Shami Stihy.

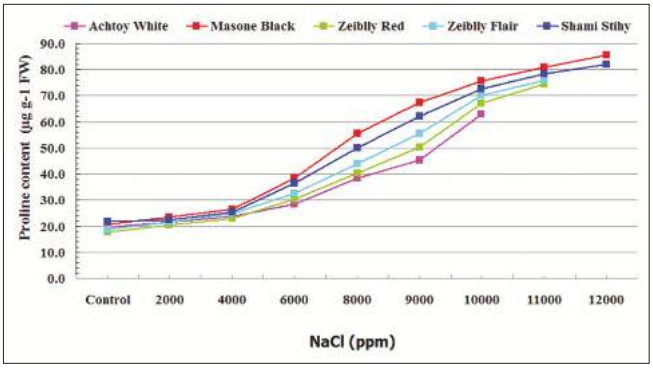

Fig. 4: Effects of different level of NaCl on proline content of grown *Ficus carica* cultivars Achtoy White, Masone Black, Zeiblly Red, Zeiblly Flair, and Shami Stihy during 60 days with water containing 0.0, 2000, 4000, 6000, 8000, 10000, 11000, and12000 ppm NaCl.

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