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Full Length Research Paper

Effect of different concentrations of carbon source, salinity and gelling agent on *in vitro* growth of fig (*Ficus carica* L.)

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Three *in vitro* experiments were conducted to study the effect of different concentrations of carbon source, salinity and gelling agent on number of new shoots, shoot length, fresh weight, and dry weight of fig (*Ficus carica* L.) to develop an efficient *in vitro* propagation method. The results show that there were no significant differences between the three carbon sources regarding number of newly formed shoots and their shoot lengths. However, there were some differences regarding fresh and dry weights. On the other hand, increased salinity concentrations reduced the number of the newly formed shoots, as well as, their shoot lengths. In addition, liquid media produced more shoots compared with the three solidifying agents used. However, there were significant differences among the concentrations of solidifying agents used.

Key words: Carbon source, salinity, gelling agent, fig (*Ficus carica* L.).

INTRODUCTION

Ficus carica L. is considered an ancient fruit tree that is relatively small in size. Fig trees are deciduous and are mainly grown in the Mediterranean region. The Food and Agricultural Organization of the United Nations (FAO, 2006) estimated that figs are harvested from 427 000 ha, producing more than one million metric tons per year. *F. carica* cultivars are propagated by cuttings, grafting, layering, and micropropagation. However, seeds are used mainly for breeding purposes (Hartmann et al., 2011) and some cultivars produce non-viable seeds (Kumar et al., 1998). Moreover, propagation by cuttings result in low rooting percentages and only 20 to 30% of the cuttings survive (Kumar et al., 1998).

Tissue culture is the basic method that allows the propagation of plant material with high multiplication rates, and hence, provides sufficient plant material for long-term storage of germplasm (Shatnawi et al., 2007). Tissue culture presents the great advantage that, conserved material can be maintained (disease-free) *in vitro*, easily exchanged between countries (Shatnawi et al., 2011a,b), and produce rejuvenated mature *F. carica* plantlets. Simple screening of plantlets by tissue culture provides a unique opportunity for studying many aspects of plant growth and development under well-defined conditions (Shatnawi et al., 2004). Moreover, tissue culture provides an important tool for studying the physiological effects of salt at the cellular level under controlled environment (Olmos et al., 1994; Shibli et al., 2000; Shatnawi et al., 2011a).

Locally, there is no literature regarding the performance of fig under saline water or soil conditions. During the last two decades effluent and saline water reuse, particularly for irrigation purposes, has increasingly become a

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common practice worldwide. Cell and tissue culture systems have been considered for selection of plant tolerance to salinity, drought, and other stresses (Luttus et al., 1999; Shatnawi et al., 2009; Shibli et al., 2007; Zattimeh et al., 2007). In spite the earlier attempts for micropropagtation that have been carried out (Fráguas et al., 2004; Hepaksoy and Aksoy, 2006), still efforts are required to make it more practical. Therefore, the objective of this study was to develop an efficient *in vitro* growth media by studying the effect of different carbon source, salinity, and gelling agent of *in vitro* grown fig (*F. carica* L.).

MATERIALS AND METHODS

Explant sources, sterilization and culture establishment

In vitro grown *F. carica* L. were obtained from Plant Tissue Culture Laboratory, Al-Balqa' Applied University, Al-Salt, Jordan. Shoot tips were isolated from three year old trees. The shoot tips were rinsed in tap water for 5 min, then were rinsed with 70% ethanol for 30 s, and finally were rinsed with 3% sodium hypochlorite solution for 10 min under a laminar flow cabinet. After each step of sterilization, the explants were washed with distilled water. The explants were inoculated on half Murashige and Skoog (MS) (1962) medium. After inoculation in the half MS medium, the cultures were incubated at 24 ± 2°C with white fluorescent lamps at 16 h daily photoperiod (8 h dark) and a photosynthetic photon flux density (PPFD) of 45 to 50 µmol $m^{-2}s^{-1}$. For shoot proliferation purposes, micro shoots were sub-cultured to a MS media supplemented with 0.4 mg/L benzyl amino purine (BAP) and 0.05 mg/L naphthalene acetic acid (NAA). The medium pH was adjusted to 5.8 prior autoclaving. Sub-culturing was performed every four weeks to establish a massive mother stock culture before conducting the experiments.

Effect of carbon sources on *in vitro* growth

Micro shoots, 15 mm in length, were sub-cultured in Erlenmeyer flasks (250 ml) containing 60 ml of solid MS media. Sucrose, glucose, and fructose were studied at different concentrations of 0, 15, 30, and 45 g/L. Micro-shoots were incubated using the same previously mentioned conditions regarding photoperiod and PPFD. Number of newly formed shoots, shoot length, fresh weight, and dry weight were recorded after six weeks growth periods.

Effect of NaCl concentrations on *in vitro* growth

To investigate the effect of different NaCl concentrations on micro-shoots proliferation, micro-shoots, 15 mm, were subjected to 0, 50, 100, 150, and 200 µM NaCl added to the MS medium. 60 ml of the medium were dispensed into 250 ml flasks. Each treatment was replicated 16 times. Number of newly formed shoots, shoot length, fresh weight, and dry weight were recorded after six weeks growth periods.

Effect of various solidifying agents on *in vitro* growth

The experiment consisted of seven solidifying agents: agar, gelatin, and phytagel each at half and full, and the liquid control medium. Similarly, 60 ml of the medium were dispensed into 250 ml flasks and each treatment was replicated 16 times. The same previously mentioned parameters were recorded after six weeks growth periods.

Statistical analysis

All statistical analyses were performed using SAS/STAT Version 9.2 and analysis of variance was conducted by the PROC GLIMMIX procedure. The experiments were designed as a completely randomized design (CRD). Means were compared to Fisher's Protected Least Significant Difference Test (LSD) at p=0.05 level of probability.

RESULTS

Effect of various concentrations of carbon source on *in vitro* growth of *F. carica*

Table 1 shows that there were no significant differences among and between the three carbon sources and their concentrations regarding the number of newly formed shoots, as well as, shoot lengths except with using fructose at 30 mg/L. Using fructose at 30 mg/L significantly produced less number of newly formed shoots although, the newly formed shoots were significantly longer than those produced when sucrose and glucose were used (Table 1). However, differences can be observed among the carbon source concentrations regarding fresh and dry weights. Regarding fresh weight, except for fructose at 45 g/L, there were no significant differences between the carbon sources and their concentrations compared to the control (Table 1). Heaviest fresh weight was obtained when fructose was used at 45 g/L and the lightest fresh weight was obtained when fructose was used at 30 g/L (Table 1). On the other hand, dry weights of sucrose at 45 g/L and fructose at 45 g/L were significantly different from the control (Table 1). Maximum and minimum dry weights were found when fructose was used at 45 and 30 g/L, respectively (Table 1). Dry weight when fructose was used at 45 g/L was significantly different from the control. On the other hand, fructose at 30 g/L was not significantly different from the control.

Effect of various NaCl concentrations on *in vitro* growth of *F. carica*

In our study, it seems that addition of NaCl reduces the number of newly formed shoots. In this regard, the highest NaCl concentration (200 µM) caused a significant reduction in the number of newly formed shoots as compared to the control (Table 2). In addition, shorter newly formed shoots were formed in response to the increase in NaCl concentration. Shortest newly formed shoots were obtained when NaCl was 150 µM. Those shoot lengths were not significantly different with the remaining NaCl concentrations (50, 100, and 200 µM) but were significantly different from the control. Regarding the fresh weights of the five treatments, significant differences were detected among the treatments (Table 2). The maximum and minimum values of fresh weights were obtained using NaCl at 50 and 200 µM, respectively. However, using NaCl at 50 µM did not

Table 1. Effect of various carbon source concentrations on shoot length, number, fresh, and dry weight of *Ficus carica* after six weeks growth period.

Carbon source (g/L)	Number of newly formed shoots	Shoot length (mm)	Fresh weight (mg)	Dry weight (mg)
Sucrose				
0	2.4± 0.40^{a}	21.1±1.50^{b}	614.0±254.54^{b}	67.0±29.00^{c}
15	2.3± 0.38^{a}	21.8±1.10^{b}	718.33±150.10^{ab}	111.0±15.95^{bc}
30	2.4± 0.29^{a}	19.8±0.81^{b}	611.33±86.44^{b}	96.33±15.40^{bc}
45	2.4± 0.38^{a}	20.1±1.40^{b}	940.0±282.80^{ab}	195.0±48.95^{ab}
Glucose				
15	2.58± 0.42^{a}	19.8±0.93^{b}	572.0±72.90^{b}	87.0±7.02^{bc}
30	2.92± 0.34^{a}	21.2±1.03^{b}	897.0±88.10^{ab}	165.7±6.70^{abc}
45	2.25± 0.31^{a}	18.3±0.41^{b}	471.0±129.70^{b}	149.6±34.90^{abc}
Fructose				
15	2.8± 0.37^{a}	19.7±0.51^{b}	437.7±51.70^{b}	78.7±14.67^{bc}
30	1.3± 0.23^{b}	31.5±13.97^{a}	329.7±88.60^{b}	61.7±16.68^{c}
45	2.8± 0.46^{a}	25.6±2.53^{ab}	1553.7±256.0^{a}	261.7±9.62^{a}

Means followed by the same superscript(s) within the column are not significantly different according to LSD at $P \leq 0.05$.

Table 2. Effect of different NaCl concentrations on shoot number, shoot length, and fresh and dry weights of *F. carica* after six weeks growth period.

NaCl concentration (µM)	Number of newly formed shoots	Shoot length (mm)	Fresh weights/ four explant (mg)	Dry weights/ four explant (mg)
0	2.8±0.55^{a}	21.4±1.04^{a}	932.3±190.3^{a}	158.7±27.7^{a}
50	2.3±0.4^{ab}	21.8±1.1^{ab}	1069±291.6^{a}	178.7±39.5^{a}
100	1.3±0.2^{b}	18.5±1.1^{ab}	569±81.7^{b}	113.7±12.61^{b}
150	1.6±0.3^{ab}	18.0±0.55^{b}	600.3±40.3^{b}	90.0±7.0^{ab}
200	1.3±0.2^{b}	19.3±0.37^{ab}	381.7±16.91^{c}	70.7±2.3^{c}

Means followed by the same superscript(s) within the column are not significantly different according to LSD at $P \leq 0.05$.

significantly affect the fresh weights compared with the control. This was not the case when NaCl was used at 200 µM (Table 2). Dry weights of the four plants show similar trend and similarly, there were no significant differences between the control and NaCl at 50 µM. Again, this was not the case when comparing the control with NaCl at 200 µM which was significantly different.

Effect of different solidifying agent on the *in vitro* growth of *F. carica*

The effect of solidifying agents on shoot number, shoot length, and fresh and dry weights after a six week growth periods on MS medium supplemented with 0.3 mg BA are reported in Table 3. Minimum number of newly formed shoots was obtained by using a half Gelatin solidifying agent (Table 3), which was highly and significantly different from the control. No significant differences were detected between the half and full Gelatin agent as well as the half and full Phytagel agent (Table 3). On the other hand, both half and full Gelatin were significantly different when compared to the control (Table 3). Moreover, using a half agar resulted in a lower number of newly formed shoots compared to the control although the reduction was not significantly different (Table 3). On the other hand, using a full agar agent was significantly different from the control with regards to number of newly formed shoots (Table 3). Differences regarding shoot length were observed among solidifying agents (Table 3). Longest and shortest shoots were achieved when the half agar and the half gelatin solidifying agents were used, respectively (Table 3). However, there were no significant differences in the fresh weights among the solidifying agents except when the half Agar agent was used (Table 3). All fresh weights produced from the six treatments were significantly different from the control (Table 3). Dry weights did show

Table 3. The effect of different solidifying agents on shoot number, shoot length, and fresh and dry weights of *F. carica* after six weeks growth period.

Solidifying agent (g/L)	Number of newly formed Shoots	Shoot length (mm)	Fresh weight/four explants (mg)	Dry weight/four explants (mg)
Agar				
0	5.75± 0.33^{a}	43.92±3.98^{ab}	11219.0±1042.62^{a}	1178.30±206.85^{a}
½ Agar	5.50± 0.36^{a}	55.83±5.36^{a}	7094.7±1448.33^{b}	720.33±32.67^{b}
Full Agar	2.83± 0.32^{b}	24.08±2.04^{c}	904.33±215.17^{d}	145.33±16.19^{d}
Gelatin				
½ Gelatin	0.50± 0.15^{c}	19.42±0.48^{d}	298.7±54.4^{d}	47.70±6.30^{d}
Full Gelatin	1.70± 0.50^{bc}	23.25±2.05^{c}	1043.0±309.9^{d}	109.30±28.70^{d}
Phytagel				
½ Phytagel	4.92± 0.63^{a}	32.5±2.30^{bc}	3373.3±250.4^{c}	416.70±35.60^{c}
Full Phytagel	5.30± 0.48^{a}	35.0±2.9^{b}	3073.0±415.33^{c}	390.3±43.20^{b}

Means followed by the same superscript(s) within the column are not significantly different according to LSD at $P \leq 0.05$.

a similar trend to fresh weights (Table 3). The half agar was significantly different from the control and both gelatin and phytagel were significantly different from the control (Table 3).

DISCUSSION

In vitro growth and development of *F. carica* L. was minimally affected by the various carbon sources (sucrose, fructose, and glucose) and the concentrations used in this study (Table 1). Hepaksoy and Aksoy (2006) show that the plant growth regulators added to the MS medium effected rooting more than number of shoots. Al-Khaybari (2008) in his study, reported that using 30 g/L of sucrose results in a significant increase in the fig root development and its growth parameters. No significant differences were detected among the treatments compared to the control regarding number of newly formed shoots and shoot length (Table 1). The maximum values of fresh and dry weights were obtained when fructose was used at 45 g/L and these values were significantly different compared to the control (Table 1). Utilizing *in vitro* propagation, number of newly formed shoots was more essential for the propagator than fresh and dry weights. Adding NaCl showed a negative impact on the growth and development of *F. carica* L. It significantly reduced the number of newly formed shoots and resulted in producing shorter shoots (Table 2). Same results were obtained by Benmahioul et al. (2009) where they found that pistachio plantlet growth decreased as well as the fresh and dry weights with salinity concentrations.

Regarding the importance of testing salinity in fig micro-propagation, obtaining water free from salts is difficult in arid Mediterranean regions and countries such as Jordan. Testing salinity *in vitro* will help us expect the performance of fig under field conditions where fig is irrigated with similar water. The reduction in growth and shortness in shoot length is considered a drawback for *in vitro* propagation. Our findings show that Gelatin is not a suitable solidifying agent for fig. However, it seems that using a liquid media can be adopted for a fig *in vitro* protocol. This was not the case in Hepaksoy and Aksoy (2006) study, where they found that some fig clones show better growth percentages in a solid media. Meanwhile, our findings coincide with the general statement that liquid cultures may provide increased shoot multiplication (Hartmann et al., 2011). Other studies show that banana did exhibit marked effects observed utilizing different gelling agents (Kaçar et al., 2010). In their study, phytagel compared with agar and agar gel resulted in the highest shoot weight, length, and number.

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