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ORIGINAL ARTICLES

Effect of medium strength and carbon source on *in vitro* shoot multiplication of two *Ficus carica* cultivars

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ABSTRACT

This work aimed to study the effect of different strengths of Murashig and skoog (MS) medium and carbon sources on *in vitro* multiplication of two fig cultivars i.e. Sultany and Aboudi. For this purpose, double, full, half and quarter strengths of MS medium and different concentrations of both of sucrose and fructose as carbon source were investigated. Shoot tips of the two fig cultivars were cultured on MS medium supplemented with $0.5mgl^{-1}$ BAP for *in vitro* shootlet proliferation. The multiplied shootlets were subcultured on the different multiplication treatments. In concern of MS strength, it was found that double strength obviously enhanced number of leaves and shoot length. However, the highest values of shoot numbers were observed with full MS medium. Otherwise, fructose was better than sucrose as carbon source for multiplication of the two fig cultivars. The highest values of shoot length and shoot number were obtained when 0.2 M/l fructose was added to culture medium**.**

Key words: Carbon sources, medium strength, fig cultivar, Aboudi, Sultany, shoot proliferation.

Introduction

In light of shortage in water resources and agricultural land, Egyptian ministry of agriculture followed a new strategy to maximize utilization of these resources; by focusing on low water requirements crops. One of these crops is *Ficus carica* L., since it is one of the fruit Mediterranean crops has a high nutrient value. Thereby, there is a more interest in increasing fig-cultivated area in Egypt. In order to achieve this target there is a need to get rapid plant cloning for producing sufficient number of fig seedlings. Biotechnology tools can play this role and provide a rapid method for plant mass propagation. Besides, micropropagation has become a reliable and routine approach for large-scale rapid plant cloning, which is based on plant material, type of *in vitro* culture media and hormones combinations under controlled conditions. Pasqual and Ferreira (2007) studied the micropropagation of fig tree and reported that, it is possible to obtain pathogen free plantlets and that is one of the basic requirements for a successful commercial orchard. Furthermore, the technique has the advantage of large-scale production, providing plantlets whenever needed. Beside these advantages of micropropagation, *in vitro* culture has also supported the research on transgenic plants obtained by genetic transformation. Moreover, micropropagation may be utilized in basic research, in production of virus-free planting material, cryopreservation of endangered and elite woody species, applications in tree breeding and reforestation. However, *in vitro* growth and multiplication of *Ficus carica* were affected by many factors (Anwar, *et al*., 2005) these factors included culture medium strength, type and concentration of carbon source.

The composition of the medium is a determining factor for growth. In many of the earlier plant tissue culture experiments, the media designed by White (White, 1963) were employed. These media contained the nutrients; normally required by plant cells and were widely used especially for root culture. However, the amounts particularly of nitrogen and potassium have since been found to be inadequate to sustain maximum growth of callus and cell suspension cultures (Murashige and Skoog, 1962; Murashige 1973). The need for rich mineral salt mixtures was compensated by adding yeast extracts, protein hydrolysates, amino acids, coconut milk or other organic supplements (Risser and White 1964; Reinert and White 1956). Other media have now been designed and are adequate for cell culture without addition of complex substances under most experimental conditions. Successful plant tissue culture depends on the choice of nutrient medium. The cells of most plant species can be grown on completely defined media; the wide use of Murashig and Skoog (MS) medium or modifications. Such media consist of mineral salts, a carbon source (generally sucrose), vitamins and growth regulators. The MS medium designed for tobacco cells is being used very extensively. There are numerous *in vitro* culture media in use. By various criteria, many media were reported adequate but optimum cell growth was often dependent upon the addition of organic and inorganic supplements. The positive response to these additions indicated a requirement by the cells for nitrogen and other nutrients. In many cases, these requirements

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can be provided by increasing concentrations of inorganic salts, particularly nitrogen (Gamborg *et al*., 1976). Andreu and Marín (2005) stated that culture medium composition influenced the multiplication rate of *Prunus* rootstock whereas, QL medium produced a significantly lower number of shoots than on MS or on WPM. Moreover, the obtained results by (Mukherjee *et al*., 2010) showed that MS with half of nitrates (MS-1) medium produced significantly higher rate of shoot proliferation from nodal explants of grape rootstock (deGrasset) in comparison with other three medium compositions, MS, B5 (Gamborg's medium) and WPM (Woody Plant Medium) (Lloyd, G and BH McCown. 1981). In addition, Hassan (2012) showed that full and one-half medium strength of MS medium are more suitable for establishment of leconte pears. Also, Tange *et al*., (2008) found that average shoot regeneration was obtained on leaf section of *pyrus communis* "Bartlett" when cultured on Murashige and Skoog complete medium containing 6 mg/l IBA and 0.1 mg /l NAA. Besides, Baaya (2002) found that full and one-half medium strength succeeded in reducing necrosis while increased shoot development of date palm. However, one-half medium strength was more promising than other medium strengths used in increasing direct regeneration. Meanwhile, Al-Khayri (2003) found that the optimum treatment suitable to produce maximum number of complete plants consists of half strength MS salts supplemented with 0.2 to 0.4 mg/l IBA of date palm. In addition, Jain *et al.,* (2009) showed that of the different media types tested, half-strength Murashige and Skoog (MS) medium reduced shoot-tip necrosis significantly without affecting shoot multiplication and growth in *H. procumbens*.

Regarding to carbon sources, Christian (1983) stated that, culture medium must contain a metabolizable sugar to allow the growth of petunia explant. Vespasiano and Otoni (2003) reported that, plant cell, tissue and organ cultures require a carbohydrate supply in order to satisfy energy demands. They mentioned that, the use of percentage references added an undesirable osmotic variable among different treatments and some authors failed to consider this aspect. Therefore, molar concentrations should be used to carbohydrate concentrations reference because it isolates the osmotic variable influence that acts concomitantly with the nutritional variable. Although there are many available carbon sources, sucrose has been the major one (Petersen *et al*., 2001 and Fuentes *et al*., 2000). In addition, Jain *et al.,* (2009) showed that, sucrose at 0.086 M (3%) was the preferred carbon source in terms of both growth and preventing shoot-tip necrosis of *H. procumbens* compared to glucose, maltose and fructose at equimolar concentrations. Moreover, Abdel-Gawad *et al*., (2010) indicated that all sucrose treatments (20, 30 and 40 g/L) enhanced the proliferation percentage and shoot number compared with mannitol and fructose treatments. Mainwhile, mannitol at 40 g/l improved the shoot length of pineapple (*Ananas comosus*). Al-Khateeb (2008) reported that, fructose produced the highest values of dry weights compared with other carbon sources. In addition, glucose, fructose and maltose were almost equivalently effective as a carbon source for culture of date palm compared with sucrose. In another study, Kadota *et al.,* (2001) stated that different carbon sources (CS) significantly increased the shoot number and fresh mass in Japanese pear, with the best results for shoot proliferation at 20–30 g l^{-1} sorbitol. Moreover, Babbar and Gupta (1986) showed that among the carbohydrates tested, sucrose, mannitol and sorbitol promoted the androgenic response in, *Daturametel* whereas, glucose, fructose, maltose and mannose inhibited it.

This study aims to investigate the effect of different strengths of MS medium and carbon sources on *in vitro* proliferation of two fig cultivars (Sultany and Aboudi).

Material and Methods

This study was carried out at the tissue culture laboratory of Pomology Dept., National Research Centre, during the period from 2011 to 2012. Shoot tips of two fig cultivars (Sultany and Aboudi) were used as starting explants. Explants were washed with tap water and sterilized into the Laminar Flow Hood and cultured individually on Murshige and Skoog medium (1962) as a basal medium supplemented with 0.5 mg\l 6-benzylaminopurine (BAP), 30 g\l sucrose and 6 g\l Difico Bacto agar for establishment stage. The pH of the medium was adjusted to 5.7 and autoclaved at 121°C and 15 Ib\in2 for 20 minutes. The cultured explants were incubated under 16 hours of artificial light (fluorescent light at 30 µM\sec) and 8 hours of darkness at average temperature 23±2°C (Mustafa and Taha , 2012).

In vitro raised clusters were selected with three shoots for each, and cultured on the tested modified media. This investigation was carried out as follows:

1-Effect of medium strength:

Different MS medium strengths (double, full, half and quarter) were tested to find out the best medium strength that encourages the multiplication rate of two fig cultivars.

2- Effect of carbon source and concentration:

Sucrose and fructose were added individually at 0.1 and 0.2 mol/l in MS medium supplemented with BAP at 0.5 mg\l. Shoot proliferation were determined.

At the end of experiments, average shoot number, average leaf number per shoot and average shoot length (cm) per shoot were recorded after 12 weeks of culturing.

Statistical design:

Treatments were arranged in complete randomized design, each treatment was replicated three times, each replicate involved three jars, and each contained three clusters developed *in vitro*. Means were compared according to the method described by Snedecor and Cachran (1989).

Results and Discussion:

1- Effect of medium strength on shootlet proliferation:

Table (1-A) indicated that differences were nil between Sultany and Aboudi fig cultivars when shoot number, leaf number and shoot length parameters were determined. However, Table (1-B) clarified that full medium strength statistically increased shoot number in comparison with the others. Meanwhile, double medium strength was effective in increasing leaf number and shoot length in relation to the other medium strengths.

Whereas, Table (1-c) and fig.(1) show that culturing of fig explants of cv. Aboudi on full medium strength produced the highest number of shoots while, Sultany gave its best result with double-strength MS. In regard to leaf number, aboudi cultivar produced high number of leaves on double medium strength in comparison to other treatments and sultany occupy the second rank in producing leaves on the same medium. Besides, culturing Sultany and Aboudi explants on double strength medium showed a markedly increasing in shoot length. Also, it was obvious from data that the one quarter MS medium gave the lowest results with shoot number, leaf number and shoot length for both cultivars under investigation.

Generally, the obtained results indicated that full and double medium strengths improved most parameters under investigation. This may be due to the high nutrients concentration meet nutrient required for fig explant development and proliferation. These results are in coordination with findings of Andreu and Marín (2005) who demonstrated that culture medium composition influenced the multiplication rate of *Prunus* rootstock. Moreover, the obtained results agree with Hassan (2012) on Leconte pear, Tange *et al*., (2008) on *pyrus communis* "Bartlett", Baaya (2002) on date palm. On the other hand, Fayek *et al*. (2007) stated that shoot tip and nodal segment explants of three female jojoba clones were compared for its potentiality of *in vitro* propagation. The effect of media (2MS, MS, 3\4 MS, 1\2 MS, B5 and N.N) was considered at proliferation. Data indicated that culturing explants on 3\4 strength of Murashige and Skoog nutrient medium is mainly recommended. Finally, these results revealed that fig explants need high nutrient requirements to development and proliferation.

Table (1) Effect of different medium strengths on shoot number, leaf number and shoot length of Sultany and Aboudi fig cultivars.

Table (1-A): Effect of fig cultivars:

parameters / Fig cultivars	Shoot number	Leaf number	Shoot length (Cm)
Sultany	18.52a	4.23a	2.35a
Aboudi	18.31a	4.05a	2.49a

Table (1-B): Effect of different medium strengths:

Parameters / Medium strength	Shoot number	Leaf number	Shoot length (Cm)
Double	18.38b	5.30a	3.50a
Full	23.79a	4.29ab	2.55b
One-half	17.25c	3.89 b	1.95c
One-quarter	14.25d	3.09 c	1.67d

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Table (1-C): Effect of interaction between fig cultivars and different medium strengths:

Parameters	Shoot number		Leaf number		Shoot length (Cm)	
Cultivars / Medium strengths	Sultany	Aboudi	Sultany	Aboudi	Sultany	Aboudi
Double	23.00b	13.75d	4.88 ab	5.72a	3.25a	3.75a
Full	19.23c	28.25a	4.76 abc	3.82c	2.90b	2.20c
One-half	17.75c	16.75d	4.04 bc	3.75c	1.77d	2.13cd
One-quarter	14.00d	14.50 d	3.25 c	2.92 c	1.47d	1.87cd

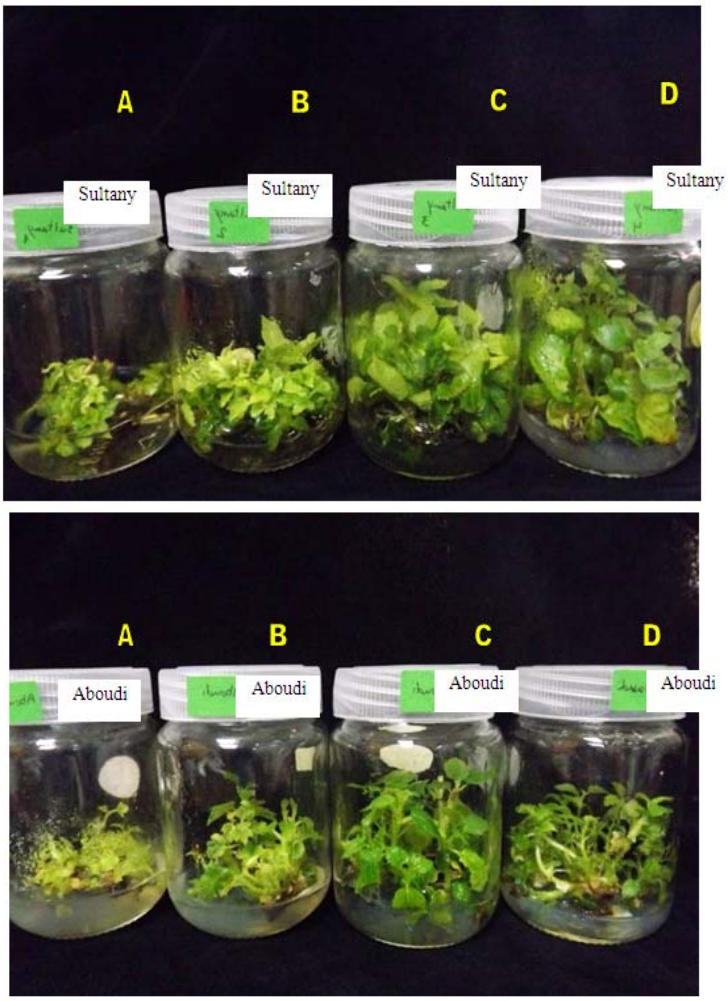

Fig. 1: Effect of medium strength on shoot multiplication of Sultany and Aboudi fig cultivars.
A: :¼ strength B :1/2strength
C :full strength D :double strength

2- Effect of carbon sources and their concentrations on shootlet proliferation:

It is clear from Table (2-A) and fig (2) that shoot number and shoot length of Aboudi fig cultivar significantly surpassed Sultany fig cv. On the other hand, Sultany cv. was significantly superior in leaf number parameter as compared with Aboudi cv. Moreover, table (2-B) reflects that the higher concentration (0.2 M/l) of fructose enhanced significantly shoot proliferation. Besides, fructose at rate of 0.1 M/l enhanced the highest significant increase in shoot length. On the other side, sucrose at 0.1 M/l caused significant increase in leaf number followed by fructose at 0.2 M/l. Meanwhile, table (2-C) explained that, Aboudi cv. Explants cultured on medium containing 0.2 M/l fructose as well as 0.1 M/l fructose significantly increased the shoot number and shoot length in comparison with other combinations. Meanwhile, Sultany cv. with 0.1 M/l sucrose gave the highest leaf number followed by fructose at 0.2 M/l, in relation to the other combinations.

Generally, the above results can recommend that fructose at (0.2 M/l) as a carbon sources was better than sucroses treatments. Besides, fructose at (0.2 M/l) as a carbon sources was better than sucrose treatments in

encouraging shoot number and shoot length. Current results agreed with the findings by Al-Khateeb (2008) who stated that concentrations of 30 and 60 g l^{-1} of sucrose were optimal for qualitative and quantitative date palm shoot growth and fructose produced the highest values of dry weights compared with other carbon sources (sucrose, glucose and maltose). Also, Murdad *et al.,* (2010) stated that protocorms cultured on medium with fructose showed a higher growth index value (537.4 ± 21.7) than those on media with glucose and sucrose (495.0 ± 10.0 and 493.3 ± 32.5, respectively).

On the other hand, Fayek *et al*. (2007) stated that the effect of carbon source (sucrose, fructose and glucose) was considered with proliferation of jojoba clones. Sucrose proved to be the best carbon source for shoot number while, glucose then fructose gave higher increase in shoot length and leaf number.

Surpassing fructose over sucrose in this investigation could be explained with that mentioned by (George, 1993), that sucrose may cause hypoxia and ethanol accumulation in cells due its quick metabolization. Thereby in some cases, sucrose should be replaced totally or partially by other carbon sources.

Table (2):Effect of carbon source and its concentration on shoot number, leaf number and shoot length of sultany and Aboudi fig cultivars.

Table (2-A): Effect of fig cultivars.

Parameters / Fig cultivars	Shoot number	Leaf number	Shoot length (cm)
Sultany	21.36b	4.74a	3.38b
Aboudi	31.91a	3.50b	3.96a

Table (2-B): Effect of carbon source and concentrations.

Parameters / Carbon source concentrations	Shoot number	Leaf number	Shoot length (cm)
Sucrose (0.1M)	22.67c	5.21a	3.63c
Sucrose(0.2M)	10.22d	3.70c	1.71d
Fructose (0.1M)	35.98b	3.31c	4.92a
Fructose(0.2)	37.67a	4.27b	4.43b

Table (2-C): Effect of the interaction between fig cultivars and carbon source and concentration.

Parameters	Shoot number		Leaf number		Shoot length (cm)	
Cultivars / Carbon source concentrations	Sultany	Aboudi	Sultany	Aboudi	Sultany	Aboudi
Sucrose (0.1M)	18.35f	27.00e	6.27a	4.15c	4.74b	2.51e
Sucrose(0.2M)	10.10h	10.33g	4.20c	3.20e	1.43g	1.98f
Fructose (0.1M)	29.00c	42.97b	3.19d	3.43d	4.11c	5.73a
Fructose(0.2)	28.00d	47.33a	5.31b	3.22e	3.23d	5.62a

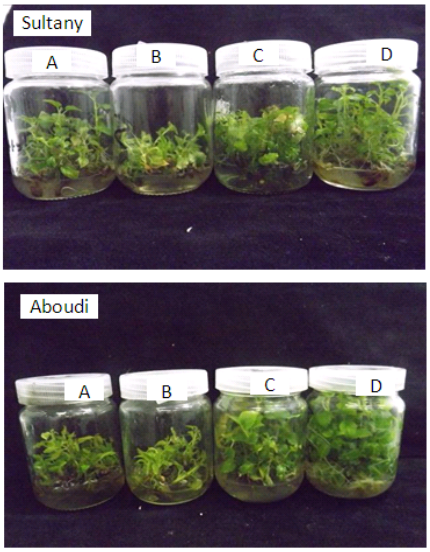

Fig. 2: Effect of carbon sources (sucrose and fructose) at 0.1 and 0.2 mol on shoot multiplication of Slutany and Aboudi: (A: sucrose at 0.1 M/l, B:sucrose at 0.2M/l, C: fructose at 0.1 M/l and D: fructose at 0.2 M/l).

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