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The Impact of Sucrose Concentration on Root Growth and Development in Fig *(Ficus carica L.) In Vitro*

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Abstract

With a view to control of ecological condition, in vitro culture is a convenient method to biological studies in plants. Sucrose is a very important component of *in vitro* culture media, serving as a source of carbon and energy. The effect of sucrose concentration was studied under in vitro laboratory culture of Aboudy fig cultivar *Ficus carica L.* Sucrose with three concentrations (1, 3 (control) and 5%) in MS medium supplemented with 0.1mg/l 1-Naphthaleneacetic acid (NAA) were investigated. Results showed that sucrose concentration at 1% gave the best results regarding shoot length (cm) and leaves number, although that sucrose at 3% gave the highest proliferation rate. Where as sucrose at 5% gave the highest rate of root growth estimated as root number (28.66) or root length (2.49 cm).

***Key Words**: Tissue culture, Ficus carica, Aboudy, Micropropagation, Sucrose*

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Introduction

Aboudy *(Ficus carica)* is a well-adapted fig cultivar under Assiut conditions; it belongs to the family Moraceae. It is a typical Mediterranean species and probably the first intentionally grown plant during the Neolithic revolution. Its domestication preceded that of cereals by about a thousand years (Kislev *et al*. 2006). The previous studies reported the hypoglycemic action of fig leaves decoction in type-diabetic I patients. The pharmacological properties are probably in part due to the high content of phenolic compounds in these plant extracts (Martins *et al*., 2006). The source of carbon (sucrose, glucose or fructose) is a very important component of *in vitro* culture media. Vespasiano and Otoni, 2003 reported that, plant cell, tissue and organ cultures need a carbohydrate supply to satisfy energy demands. Although there are many available carbon sources, sucrose is the major one (Fuentes *et al*, 2000 and Petersen *et al*, 2001)

Carbon sources are added to the culture medium because of the light energy deficiency and low CO_2 concentration present in *in vitro* conditions (Tombolato & Costa, 1998). Among non-reducing sugars, sucrose is most common in the phloem sap of many plants (Ahmad *et al*., 2007) and used as energy source in efficient micropropagation studies in many fruit crops (Jain and Babbar, 2003; Faria *et al*., 2004). Sucrose concentrations of 20 and 30 g/L^{-1} are the most commonly used in plant tissue culture studies. The absence of sugar reduces contamination problems in the culture medium and allows plants to grow autotrophically *in vitro*, when sufficient CO_2 is supplied and light intensity is increased. (Deberg 1991) However, the presence of sugar in the culture medium is one of the factors that contribute to a significant increase in the production cost of micropropagated plantlets (Kozai, 1991). Working with strawberry cv.Campinas, (Calvete *et al.* 2002) observed that the concentration of sucrose (45 g/L^{-1}) is ideal for the formation of roots; however, in the absence of this sugar, roots are not formed under *in vitro* cultivation.

The present study was carried out to determine the optimum levels of sucrose, essentially used in the culture medium with the aim of developing a reliable protocol for *in vitro* propagation of Aboudy cultivar *(Ficus Carica)* which is commonly grown in Egypt.

Material and Methods

Plant material:

The plant materials for this study was obtained from Aboudy fig cultivar grown at the fruit orchard of Faculty of Agriculture; Assiut University on fig (*Ficus carica*) trees cv. Aboudy. Shoot tips were collected and washed thoroughly, first with tap water and then with sterile water. 0.5 cm long shoot-tip meristems with 4-5 leaf primordial were excised in a laminar flow hood and then surface sterilized in 2% sodium hypochlorite for 10 min and rinsed 4 times (5 min each) with sterile-distilled water and incubated in glass culture jars, containing MS medium (Murashige and Skoog, 1962) as a basal medium supplemented with 0.5 mg/l 6-benzylaminopurine (BAP), 2 mg/l

Gibberellic acid (GA3), 0.2 mg/l Indole-3-butyric acid (IBA), 0.1g/L myo-instol and 30 g/l sucrose, the medium solidified with 2 g/L gelrite at pH 5.8 and had been sterilized by autoclaving for 20 min at 121°C and 15 Ib\in2. The cultured explants were incubated in a controlled environment (25±1°C, 16 h photoperiod, 2200 lux light intensity, 75±5% humidity) and kept in culture for 8 weeks during the establishment stage.

Proliferation stage:

MS medium supplemented with 1 mg BAP/L, 3% sucrose was used as a multiplication medium.

Rooting stage:

For testing the effect of sucrose on rooting growth and formation, the proliferated plantlets were transferred to medium containing 0.1 mg/l IBA for rooting. The medium was supplemented with 1, 3 (as a control) and 5% sucrose. Scoring system scale (1-4) was used for determining the growth rate of developing roots, where 1 is poor and 4 is very good (Table 1).

Table (1): The scoring system developed for measuring the growth rate of the root length (cm).

Growth Rate	Root length (cm)
1- Poor	0.5-1 cm
2- Medium	1-2 cm
3- Good	2-3 cm
4- Very Good	More than 3 cm

Statistical analysis:

The experiments were set as a complete randomized design with 3 replicates per treatment and the results were evaluated using the method of analysis of variance, and statistically significant differences were indicated in letters and Means were compared according to the method described according to Snedecor and Cochran (1980) and Gomez and Gomez (1984) using the L.S.D. test at $P \leq 0.05$.

Results

1. Effects of different Sucrose Concentrations in rooting medium on Vegetative Growth:

Data presented in Table (2) show that the sucrose concentration had not show significant differences in the number of leaves or proliferation rate. However, sucrose concentration at 1% gave the highest shoot length with a mean 3.6 cm then 5% and 3% with 2.4 and 2.3 cm, respectively.

Table (2): Effect of sucrose concentration on shoots development (Means± SE) of Aboudy fig cv.

Sucrose Concentration	Shoot length (cm)	Leave No.	Proliferation rate
1%	3.6±0.47 A	8.7±0.6 A	2.3±0.33 A
3% (Control)	2.3± 0.39B	7.9±0.4 A	4.7±0.9 A
5%	2.4±0.33 AB	8.4±0.9 A	4.3±0.9 A

Table (3) showed that although there is no significant difference in shoot fresh weight in the three treatments, but there was a significant difference in shoot dry weight as the concentration of 5% gave the highest value with 0.09 g.

Table (3): Effect of sucrose concentration on shoots biomass (Means± SE) of Aboudy fig cv.

Sucrose Concentration	Shoot fresh weight (g)	Shoot dry weight (g)	Shoot water content
1%	0.34±0.02 A	0.06±0 B	0.28±0.02 A
3% (Control)	0.35± 0.05A	0.06±0 B	0.28±0.04 A
5%	0.38±0.05 A	0.09±0A	0.29±0.04 A

2. Effects of different Sucrose Concentrations in rooting medium on Root Development:

Data presented in Table (4) show that the sucrose concentration affected significantly in root growth and formation. The lowest rooting percentage (80%) was at 1% sucrose. Root length (cm) increased proportionally with sucrose concentration, it gave the highest value at 5% sucrose with 2.49 cm then at 3% sucrose with no significant difference between them, but these concentrations were significantly difference of 1% sucrose, which gave 0.82 cm. On the other hand, roots number were significantly difference with different concentrations of sucrose, as it gave the highest value at 5% with a mean 28.67 then 3% and 1% with 19.3 and 9.7, respectively.

Table (4): Effect of sucrose concentration on root development (Means± SE) of Aboudy fig cv.

Sucrose Concentration	Rooting %	Root No.	Root Length (cm)
1%	80	9.7±0.9 C	0.82±0.06 B
3% (Control)	100	19.3±0.9 B	2.15±0.2 A
5%	100	28.67±1 A	2.49±0.3 A

Data in table (5) expressed the root biomass affected by sucrose concentrations. 3% sucrose gave the lowest value in root fresh or dry matter (g). While 5% sucrose gave the highest value in root dry weight (g) with 0.1 g, which was significantly different of 1 and 3%. Root water content showed significant difference at 1 and 5% with 0.34 and 0.29, respectively.

Table (5): Effect of sucrose concentration on root biomass (Means± SE) of Aboudy fig cv.

Sucrose Concentration	Root fresh weight (g)	Root dry weight (g)	Root water content
1%	0.39±0.4 A	0.05±0 B	0.34±0.03 A
3% (Control)	0.2± 0.3 B	0.02±0 C	0.18±0.03 B
5%	0.39±0 A	0.1±0 A	0.29±0 A

Discussion

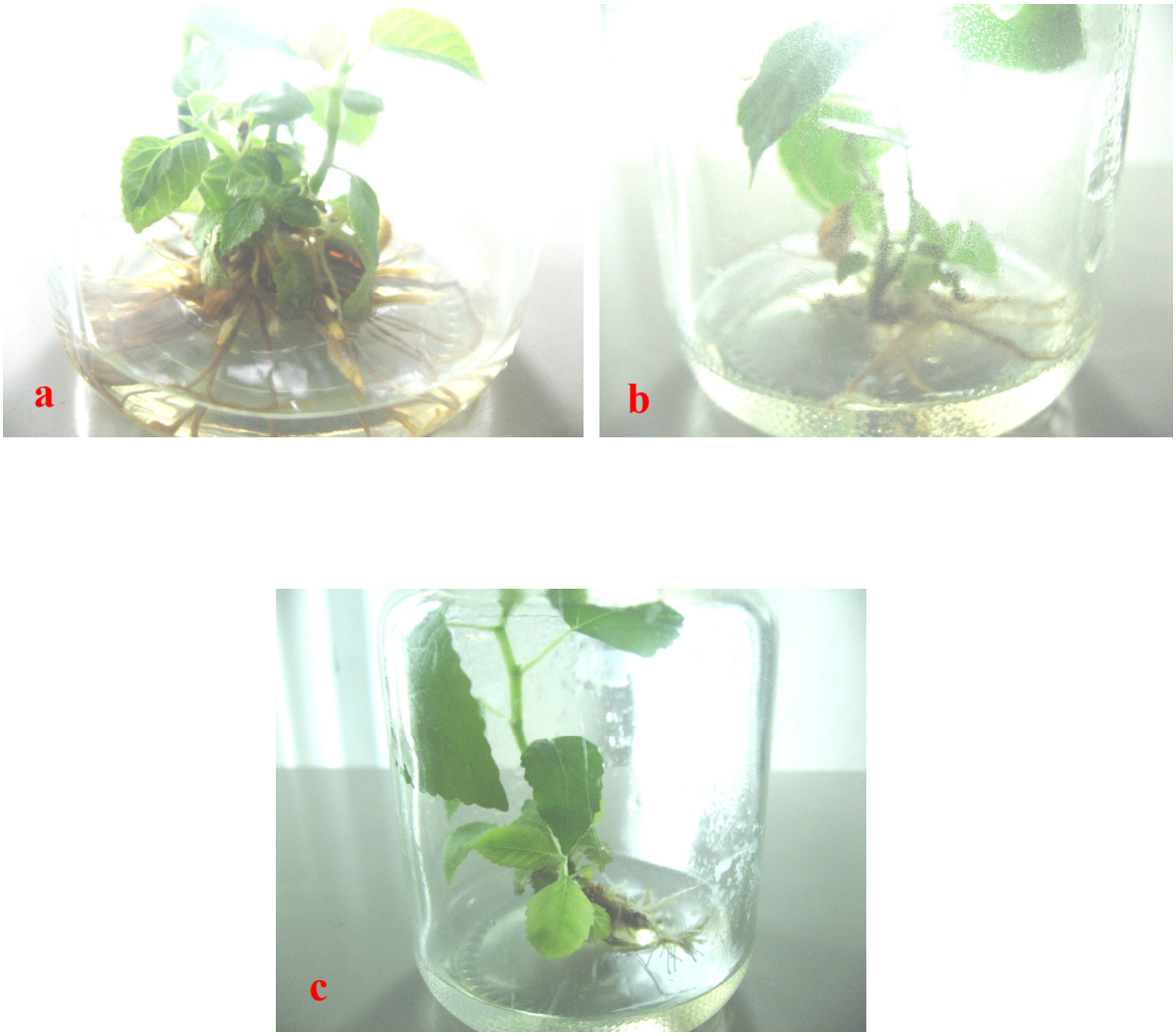
In this study, sucrose concentration did not show significant differences in vegetative growth, i.e. leaves number or proliferation rate but it effected significantly in shoot length (cm) and shoot dry weight (g) which was quite similar to Faria *et al*, 2004 when used different concentrations of sucrose (0, 5, 10, 20, 30 and 60 g/L) they found that the sucrose concentration affected in plantlets height and multiplication, also they found that the Sucrose concentration influenced growth and accumulation of biomass (fresh weight) of Dendrobium plantlets propagated *in vitro*. They also found that the sucrose concentration did not affect in plant rooting, which also was opposed to our result, as we found the sucrose concentration affected significantly in root length (cm) and number of roots. Collins & Dixon, 1992 studied different sucrose concentrations in *in vitro* culturing and observed that for the Australian terrestrial orchid *Diuris longifolia*, 20 g/L^{-1} sucrose plus charcoal had a similar rooting effect as 40 g/L^{-1} sucrose without charcoal.

Usman *et al*, 2012 used different concentrations of sucrose 30, 45 and 60 g/L and they suggested that 45 g/L^{-1} of sucrose as the optimum level of sucrose for better and faster growth and development of guava plants *in vitro*. They concluded that shoot induction, root length and leaf size increased with increase in the concentration of sucrose (45 g/L^{-1}) and at higher concentration of sucrose (60 g/L^{-1}) shoot induction was reduced. That was similar to our result; as the high concentration of sucrose (5%) showed low values of shoot length (cm) and number of leaves compared to 1% sucrose, also, our result was the same with Usman *et al*, 2012 as we found that the root growth and development increased proportionally with increasing of sucrose concentration.

Enhancing sucrose concentration significantly enhanced the shoot induction, number of shoots and leaf area as reported in crops like black plum (Jain and Babbar, 2003), apple

rootstock (Yaseen *et al*., 2009) and in jujube, with shoot length and fresh weight of shoots in apple (Yaseen *et al*., 2009) compared to other disaccharide carbon sources. Increase in sucrose concentration reduced hyperhydricity in black plum and jujube.

The increase in the amount of sucrose in the culture should be taken with caution and should not be progressive, because high sucrose concentrations in *in vitro* cultures favor carbohydrate accumulation and hinder photosynthesis (Cappellades *et al*, 1991) and (Hdider & Desjardins, 1994).



Pic. (1): Effect of sucrose concentrations on plant growth and root formation. a) 5% sucrose b) 3% sucrose c) 1% sucrose

Conclusion:

It could be concluded that using MS medium in rooting stage and sucrose as a carbon source at 5% for fig (*Ficus carica*) explants cv. Aboudy gave he highest root growth and development.

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تأثير تركيز السكروز على نمو وتكوين الجذور فى التين معمليا

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الملخص:

يعتبر السكروز أهم مكون فى البيئات المستخدمة معمليا حيث تعتبر كمصدر كربون وطاقة للنباتات المزروعة معمليا. تم اجراء البحث فى معمل زراعة الأنسجة بقسم البساتين(فاكهه) كلية الزراعة جامعة أسيوط على التين صنف عبودى النامى تحت ظروف أسيوط بشكل جيد.

العمل التجريبى:

تم أخذ نموات خضرية طرفية بطول ١- ١،٥ سم من أفرع التين وذلك خلال فصل الربيع وزُرعت تحت ظروف التعقيم فى المعمل ثم نُقلت الى بيئة تفريع تحتوى على ١ مجم/لتر بنزايلامينوبيورين. ولدراسة تأثير التركيزات المختلفة من السكروز على النمو الخضرى والجذرى للنبيتات تم أضافة تركيزات ١% ، ٥% بالاضافة لتركيز ٣% (كنترول) من السكروز على بيئة التجذير المحتوية على ٠،١جم /لتر من نفثالين حمض الخليك.

وكانت أهم النتائج المتحصل عليها:

ان التركيزات المختلفة من السكروز لم تؤثر على النمو الخضرى والتفريع فى النباتات المزروعة معمليا. بينما أدى استخدام تركيز ٥% من السكروز الى الحصول على أعلى عدد من الجذور (٢٨،٦٦) وكذلك أكبر اطوال للجذور (٢،٤٩ سم) مقارنة بالتركيزات الأخرى. ولذا ينصح باستخدام تركيز ٥% سكروز فى بيئات التجذير للحصول على عدد أكبر من الجذور ذات الأطوال الأكبر لصنف التين العبودى.