Effect of Sucrose Concentration on Growth and Root Formation of Aboudy fig cultivar *(Ficus carica L.) In Vitro*

Doaa, Sh. Elazab and Mokhtar M. Shaaban

Horticulture Dept., Fac. of Agriculture, Assiut University, Assiut, Egypt

Abstract:

Sucrose is a very important component of *in vitro* culture media, serving as a source of carbon and energy. In this experiment, the rooting and growth of Aboudy fig cultivar (*Ficus carica*), was studied by culturing the new shoots on MS medium supplemented with 0.1 NAA (mg/L) at pH 5.8 and different concentrations of sucrose (1, 5 and 3% as a control treatment). Sucrose concentration in the culture medium had no effect on plant shooting and growth, but sucrose concentration at 5% gave the highest rate of root production estimated as root numbers or root length.

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 (Martins *et al*., 2006). The chloroform extract was obtained also from a decoction of *F. carica* leaves, to decrease the cholesterol levels of diabetic rats (Martins *et al*., 2006). The pharmacological properties are probably in part due to the high content of phenolic compounds in these plant extracts. The source of carbon (sucrose, glucose or fructose) is a very important component of *in vitro* culture media. 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). Sucrose concentrations of 20 and 30 g/L^{-1} are the most commonly used in plant tissue culture studies. According to Deberg (1991), 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. 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 on 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.

Objectives:

- To find out the concentration of sucrose required in the culture medium for optimum vegetative and rooting development.
- To understand the effect of sucrose on plant growth and root formation.

Material and Methods:

In spring, 5-10 cm long shoots, which were still actively developing, were taken from fig (*Ficus carica*) trees cv. Aboudy, grown at the experimental orchard of Faculty of Agriculture; Assiut University and washed thoroughly, first with tap water and then with sterile water. Shoot tips (1.0-1.5 cm long) were excised from these shoots and then surface sterilized in 2% sodium hypochlorite for 10 min and rinsed 4 times (5 min. each) with sterile-distilled water. For proliferation stage, 0.5 cm long shoot-tip meristems with 4-5 leaf primordial were excised in a laminar flow hood and then incubated in glass culture jars, containing MS (Murashige and Skoog, 1962) basal medium supplemented with 1 mg Benzyle Amino Purine (BAP)/L, 3% sucrose, 0.1g/L myo-instol and solidified with 2 g/L gelrite at pH 5.8 and had been sterilized by autoclaving for 20 min at 1.5 kPA. The proliferated plantlets were transferred to medium containing 0.1 mg/l IBA for rooting. For testing the effects of sucrose, the medium was supplemented with 1, 3 and 5% sucrose. 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. 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). Mean shoot number per explant and the mean growth rate of the shoots were recorded at the end of the experiment.

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

The experiments were set as a complete randomized design with 3 replicates per treatment. Statistical analysis was carried out according to Snedecor and Cochran (1980) and Gomez and Gomez (1984). The means were compared using Duncan multiple range and L.S.D. test at 5% level.

Results:

1-Effects of Sucrose Concentrations on Vegetative Growth:

Data presented in Table (2) and Figure (1, 2, 3 and 4) show that the sucrose concentration had not significant differences on vegetative growth development with the except of plantlets height. Sucrose concentration at 1% gave the highest length in plantlets shoots with a mean of 3.6 cm and then 5% and 3% which recorded 2.4 and 1.94 cm, respectively.

Table (2): Effect of sucrose concentration on shoots development

	Vegetative Growth Characteristics				
Sucrose Concentration	**Plantlet height (cm)**	**Leave No.**	**Proliferation rate**	**Fresh weight (g)**	**Dry weight (g)**
1%	3.6 A	9.3 A	2.3 A	0.34 A	0.06 A
3% (Control)	1.94 B	6.52 A	7.6 A	0.34 A	0.06 A
5%	2.4 AB	8.4 A	3 A	0.39 A	0.09 A

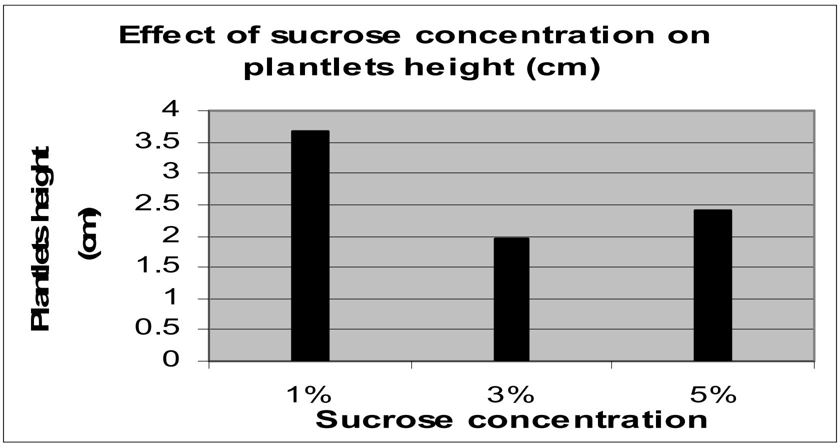


Fig (1): Effect of sucrose concentration on plantlet height (cm)

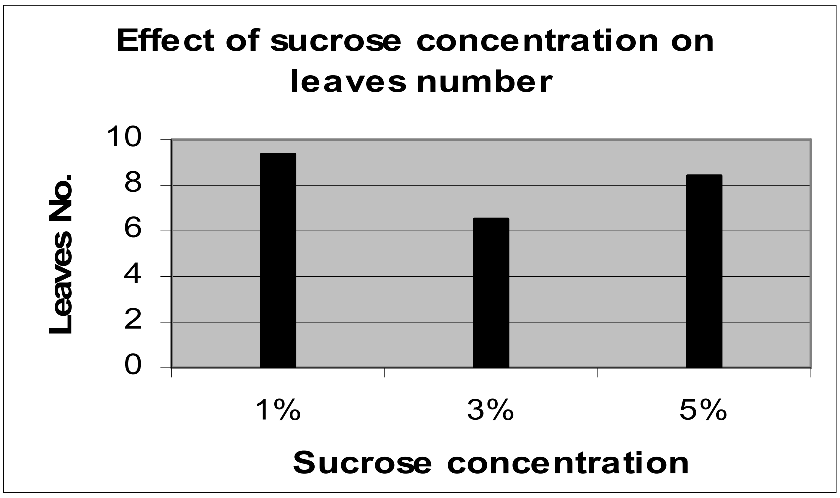


Fig (2): Effect of sucrose concentration on number of leaves

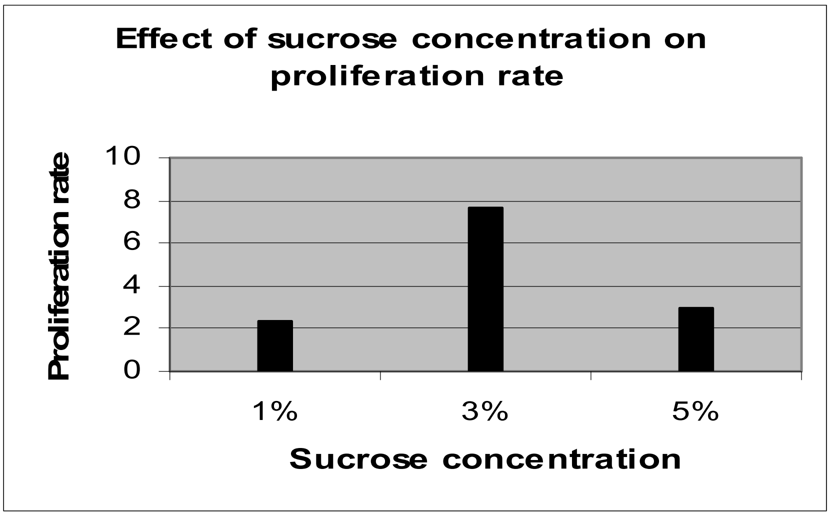


Fig (3): Effect of sucrose concentration on proliferation rate

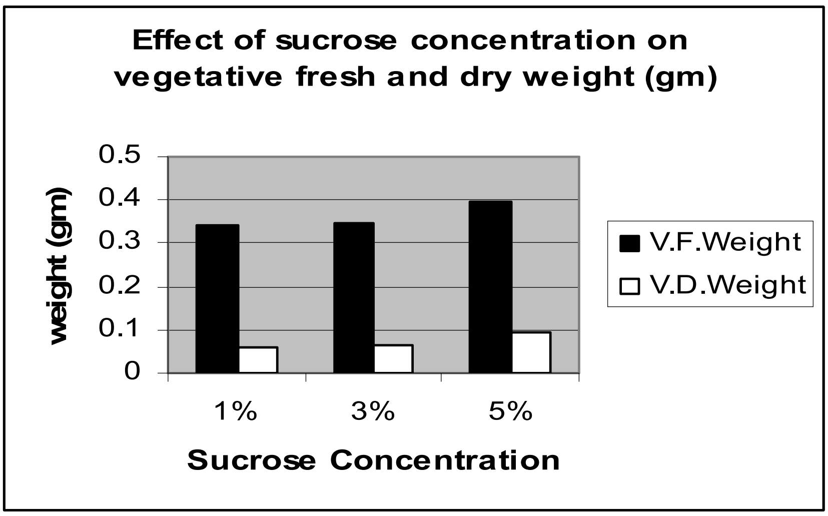


Fig (4): Effect of sucrose concentration on vegetative fresh and dry weight (gm)

1-Effects of Sucrose Concentrations on Root Development:

Data presented in Table (3) and Figure (5, 6, 7 and 8) show that the sucrose concentration had not significant differences on rooting growth and formation with the except of root length. Sucrose concentration at 5% gave the highest length in roots with a mean of 2.16 cm and then 3% and 1% which gave 1.96 and 0.82 cm, respectively.

Table (3): Effect of sucrose concentration on root development

	Rooting Growth Characteristics				
Sucrose Concentration	**Rooting %**	**Roots No.**	**Roots Length (cm)**	**Fresh Weight (g)**	**Dry Weight (g)**
1%	80%	11.33 A	0.82 B	0.45 A	0.05 A
3% (Control)	100%	19.33 A	1.96 A	0.17 A	0.02 A
5%	100%	28.66 A	2.16 A	0.41 A	0.07 A

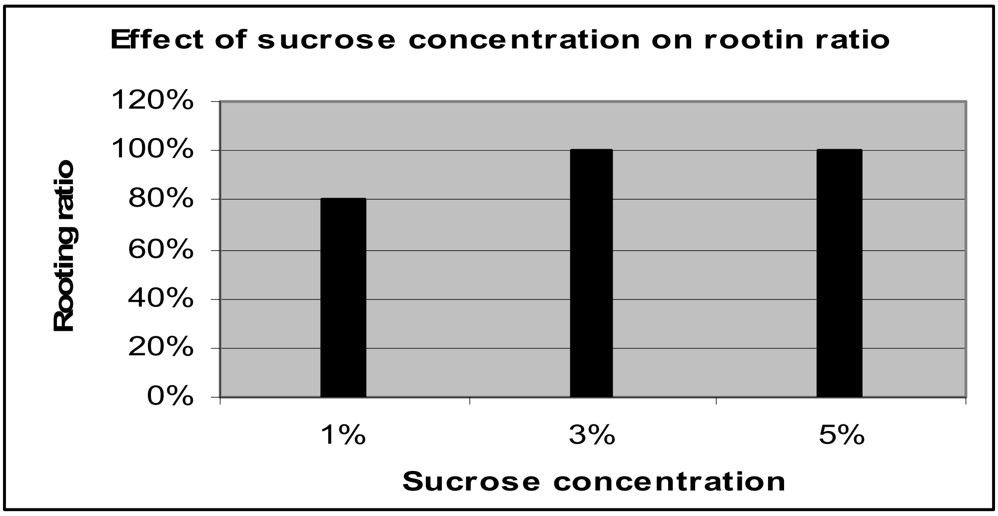


Fig. (5): Effect of sucrose concentration on rooting %

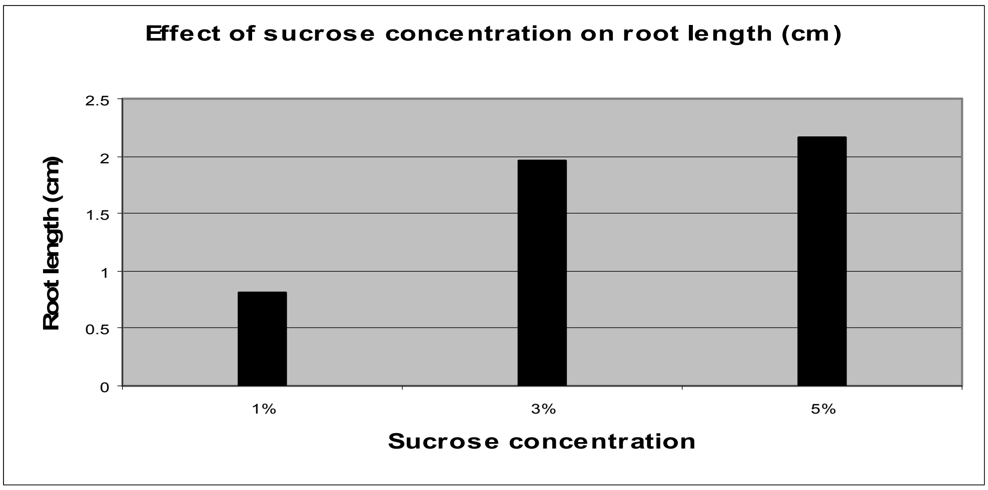


Fig. (6): Effect of sucrose concentration on root length (cm)

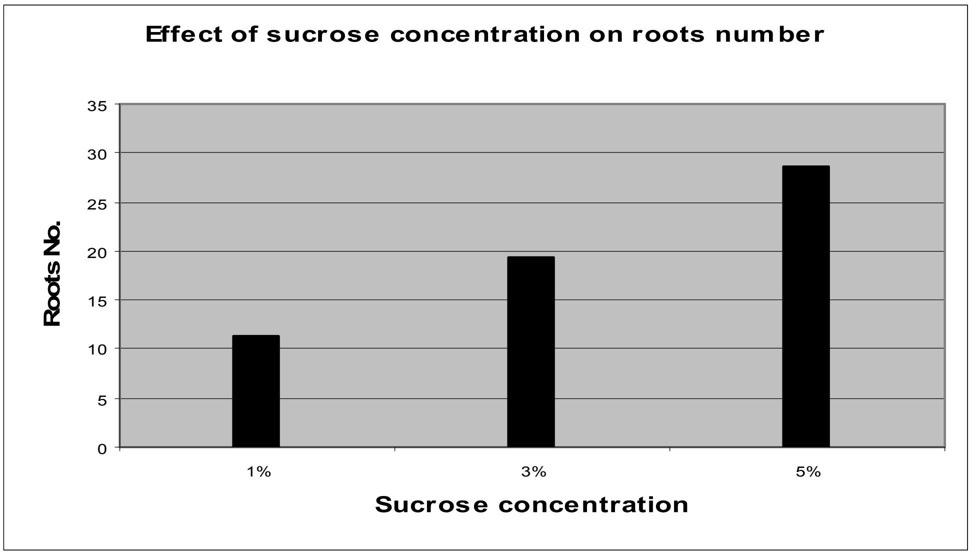


Fig (7): Effect of sucrose concentration on root number

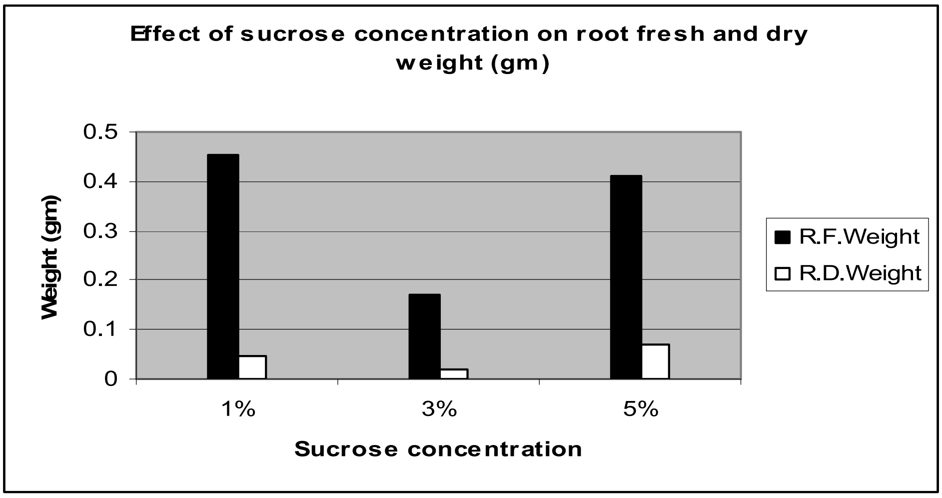


Fig (8): Effect of sucrose concentration on roots fresh and dry weight (gm)

Discussion:

Sucrose concentration influenced in length of shoots. The plantlets height increased at 1% concentration with 3.6 cm followed by sucrose at 5% with 2.4 cm. A positive relationship between the increase in sucrose concentration and root length (cm) was verified (2.16, 1.96 and 0.82 cm) for 5%, 3% and 1%, respectively.

Faria *et al.*,(2004), used different concentrations of sucrose (0, 5, 10, 20, 30 and 60 g/L) and found that the sucrose concentration affected in plantlets height and multiplication, without any affect on plant rooting. They also found that the Sucrose concentration influenced growth and accumulation of biomass (fresh weight) of Dendrobium plantlets propagated *in vitro*. The presence of 60 g/L^{-1} sucrose in the culture medium was the most efficient treatment for increasing height and fresh weight of plants cultured *in vitro*. The current study revealed that, sucrose concentration at 50 g/L increased the vegetative fresh weight (0.39 g) and dry weight (0.09 g).

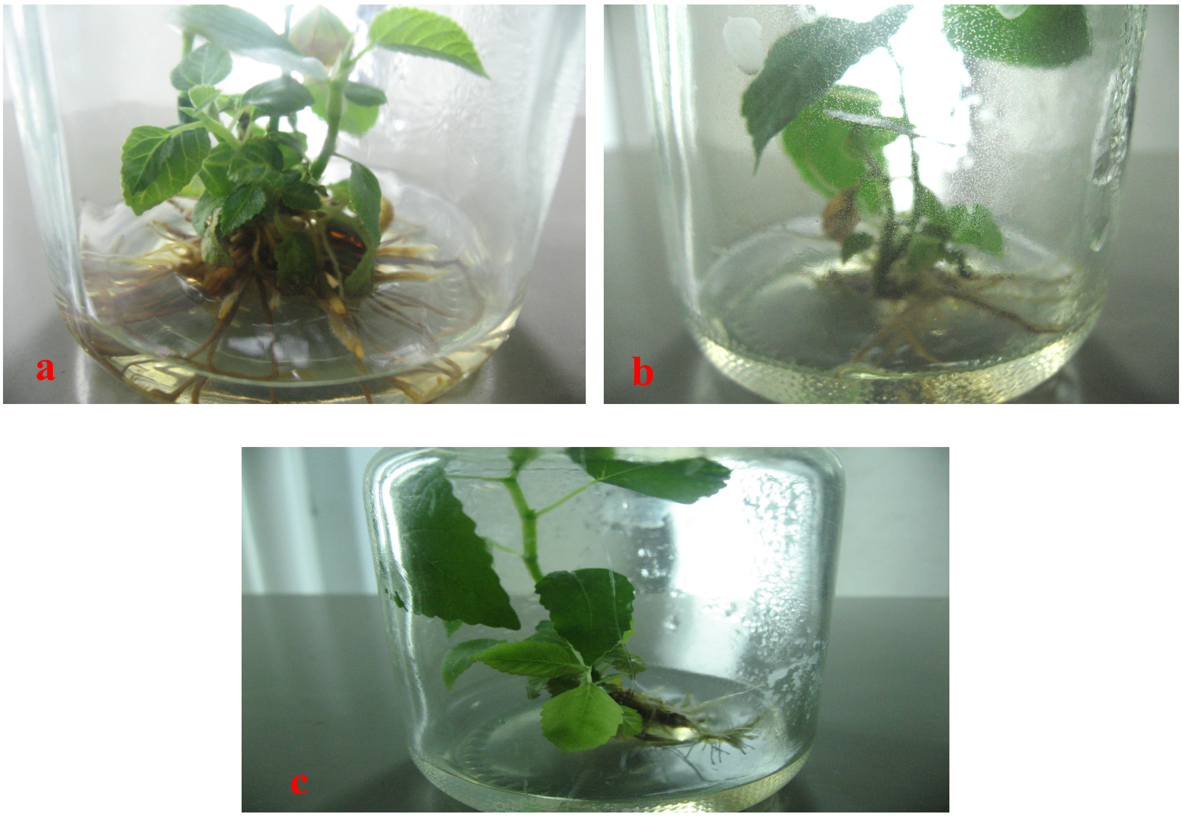
Collins & Dixon, (1992) studied different sucrose concentrations in *in vitro* culturing and observed that for the Australian terrestrial orchid *Di-*

uris 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 is 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 increasing the concentration of sucrose (45 g/L^{-1}) and at higher concentration of sucrose (60 g/L^{-1}) shoot induction was reduced.

Among non-reducing sugars, sucrose is the 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). Increasing the sucrose concentration significantly enhanced the shoot induction, number of shoots and leaf area as reported in crops like black plum 80% (Jain and Babbar, 2003), apple rootstock (Yaseen *et al*., 2009) and 80% 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, according to (Cappellades *et al*., 1991) and (Hdider & Desjardins, 1994), high sucrose concentrations in *in vitro* cultures favor carbohydrate accumulation and hinder photosynthesis.



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

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تأثير تركيز السكروز على نمو وتكوين الجذور فى صنف التين عبودي معمليا

دعاء شحاته العزب ، مختار ممدوح شعبان
قسم البساتين (فاكهه) – كلية الزراعة– جامعة اسيوط

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 1– 1,5 سم من أفرع التين وذلك خلال فصل الربيع وزُرعت تحت ظروف التعقيم فى المعمل ثم نقلت الى بيئة تفريع تحتوى على 1 مجم/لتر بنزايلامينوبيورين. ولدراسة تأثير التركيزات المختلفة من السكروز على النمو الخضرى والجذرى للنبيتات تم أضافة تركيزات 1% ، 5% بالاضافة لتركيز 3% (كنترول) من السكروز على بيئة التجذير المحتوية على 0,1جم /لتر من نفثالين حمض الخليك.

وكانت أهم النتائج المتحصل عليها:

ان التركيزات المختلفة من السكروز لم تؤثر معنويا على النمو الخضرى والتفريع فى النباتات المزروعة معمليا.بينما أدى استخدام تركيز 5% من السكروز الى الحصول على أعلى عدد من الجذور (66,28) وكذلك أكبر اطوال للجذور (16,2 سم) مقارنة بالتركيزات الأخرى. ولذا ينصح باستخدام تركيز 5% سكروز فى بيئات التجذير للحصول على عدد أكبر من الجذور ذات الأطوال الأكبر لصنف التين العبودى.