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EFFECT OF DIFFERENT CARBON SOURCES AND CONCENTRATIONS ON *IN VITRO* BUD AND SHOOT FORMATION OF STRAWBERRY (*Fragaria X ananassa* DUCH)

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ABSTRACT

Influences of carbon sources, sucrose, glucose, fructose, maltose and sorbitol on shoot proliferation of strawberry were compared at relatively wide range of concentrations (0-120 g l^{-1}). For each sugar added, the highest number of buds and shoots was formed at concentration of 30 g l^{-1}. Lower and higher concentration of sugars minimized number of buds and shoots particularly at concentrations of 0 and 120 g l^{-1}. The highest number of buds (5.0) and shoots (2.4) occurred on sucrose-containing medium, whereas sorbitol produced the lowest number of buds and shoots. In the present study, it seems that sorbitol was not the recommended sugar for strawberry proliferation media over the whole concentration range. Fructose tended to produce cultures with less compact and large shoots compared with the other sugars. Tissues grown on medium supplemented with 90 and 120 g l^{-1} concentration showed red and white pigmentations with no signs of buds possibly due to osmotic stress.
Keywords: Tissue culture, carbon source, sucrose, strawberry, *Fragaria x ananassa*.

INTRODUCTION

It is well known that a carbohydrate supply is required for plant cell, tissue and organ culture in order to satisfy energy demand (Paiva *et al*., 2003) and they act also as osmotic agents. In plant tissue culture a continuous supply of carbohydrates is essential, since the photosynthetic activity of *in vitro* plant tissues is reduced due to low light intensity, high relative humidity and limited gas exchange (Kozai, 1991).

Sucrose has been used as a carbohydrate source in most plant tissue culture. It is assumed that sucrose is the best carbohydrate in culture media because it is the main form of carbohydrate translocated from source to sink in most of the species (Bogunia and Przywara, 1999). Despite the widespread and successful use of sucrose in plant tissue culture, other sugars have also been reported as being suitable as carbon sources for *in vitro* culture of many species. It was found that different patterns of morphogenesis were attributed to the type of sugar and its concentration used in culture. Sorbitol has been shown to be an effective carbon source for apple tissue *in vitro* (Welander *et al*., 1989). Alkhateeb (2001) has found that in culture of date palm cv. Khanezi the shoots are capable of utilizing fructose, glucose or maltose as the sole carbon source for vegetative growth, as well as for root formation. In tissue culture of *Fagus sylvotica*, glucose found to be the best carbon source for both axillary branching and adventitious shoot regeneration (Cuenca and Vieitez, 2000). Fructose at 3% greatly improves the proliferation of walnut (Gruselle *et al*., 1995). In tomato, the effect of sugars was significantly different with maltose giving the highest number of shoots followed by glucose (El-Bakry, 2002). Whereas, in other

study, Gubiš *et al*. (2005) found that among sugar types sucrose at 3% induced the highest number of shoots in tomato.

The main objective of the present study was to determine the influence of different carbon sources, concentrations and their interaction on bud and shoot formation *in vitro* culture of strawberry plant.

MATERIALS AND METHODS

The Chandler strawberry (Fragaria x ananssa) shoot cultures used in this study originated from vegetative meristem tips and were cultured on Murashige and Skoog (1962) supplemented with BAP 0.2 mg l^{-1} and IBA 0.2 mg l^{-1} according to Alkhateeb (1997).

Stock cultures were maintained by monthly transfer of 5 mm shoot tips to jars with 40 m1 of Murashige and Skoog medium supplemented with 1 mg l-1 BAP and 30 g l-1 sucrose, pH adjusted to 5.7. Cultures were maintained at 25± 2°C under fluorescent light with 16/8 h (day/night) light regime.

Shoot tips were proliferated on 40 ml of the medium as described for stock cultures, with the following carbon sources: sucrose, glucose, fructose, maltose and sorbitol, each at different concentrations of 0, 30, 60, 90 and 120 g l^{-1}. Each treatment had 10 replicates in a factorial experiment laid out in a complete randomized design with two factors, namely type of sugar and its concentrations. Culture was incubated at 25±2°C in 16 hours photoperiod with three 40W fluorescent lamp. After 4 weeks from the onset of the experiment, several growth parameters were recorded. Number of buds (before producing true leaves) and shoots (plantlets that have two-three small leaves) per culture were determined. Shoot height was measured the length shoot. After obtaining the fresh weight of culture, it was kept in a forced air oven 70 °C for 72 hours for dry weight determination.

The data were statistically analyzed according to the technique of analysis of variance (ANOVA) for the factorial experiment in a completely randomized design according to Gomez and Gomez (1984). The treatment means were compared using least significant difference (LSD) at 5 % level of probability. All statistical analysis was performed using the facility of computer and SAS software package (SAS, 2001).

RESULTS

Results collected in Table 1 and Fig. 1(A) showed that both type of carbon and concentration and their interaction had a significant effect on number of buds. Sucrose produced more buds, as compared with the other sugars, whereas sorbital produced the lowest. Concentration of 30 g l^{-1} produced the highest significant number of buds, compared with the other concentrations. Sucrose of 30 g l^{-1} gave the best result in number of buds (15.4 buds) of strawberry cultured *in vitro*.

Data in Table 1 and Fig. 1(B) indicated that both type and concentration of sugars and their interaction had a significant effect on shoots number. Sucrose and glucose produced more number of shoots than the other tested sugars. An increase in shoots number was observed at 30 g l^{-1}

and was significantly different from the others. On the other side, the lowest number of shoots was found at concentration of 120 g l^{-1}. However, sorbitol at 120 g l^{-1} did not produce any shoots. Concerning the effect of the interaction between types and concentrations of sugar on number of shoots, data in Fig. 1(B) clear that sucrose at 30 g l^{-1} produced the highest values (5.6 shoots), compared with others.

Sugar types, sugar concentrations and their interaction had a significant effect on shoot length. The shoot length was significantly decreased as sugar concentration increased (Table 1). The concentration of 30 g l^{-1} produced the longest shoots, followed by 60 and 90 g l^{-1}. Glucose and sucrose produced the longest shoots (1.06 and 1.04 cm), while sorbitol produced the lowest value (0.24 cm). Glucose at 30 g l^{-1} was superior with no significant different with sucrose.

Fresh weight of culture was significantly affected by sugar type and concentrations as well as their interaction (Table 1 and Fig. 1D). Maltose and fructose produced the highest amount of fresh weight. The highest amount of fresh weight was observed at concentration of 30 g l^{-1} (6.90), followed by 60 g l^{-1} (5.18), whereas the lowest was recorded with concentration 120 and 0 g l^{-1} sugar. Regarding the dry weight of culture, data in Table (1) and Fig. 1(E) show that sugar types and concentrations had a significant effect. Fructose, maltose and sucrose produced the highest amount of dry weight, whereas sorbitol produced the lowest. Concentration of 60, 90 and 30 g l^{-1} significantly improved dry weight. The interaction between types and concentrations of sugar was significant. Fructose produced the highest amount of dry weight at concentration of 60 g l^{-1} (0.651 g), followed by maltose at concentration of 90 g l^{-1} (0.515 g).

Table 1: Number of buds and shoots, shoot length, fresh weight and dry weight as affected by sugar types and its concentration cultured *in vitro* of strawberry.

Characters / Treatments	No. of buds/ culture	No. of shoots/ culture	Shoot length (cm)	Fresh weight (g)	Dry weight (g)
A. Sugar type:					
Sucrose	5.0	2.4	1.04	3.55	0.328
Glucose	2.2	1.9	1.06	3.48	0.256
Fructose	2.2	0.9	0.70	4.88	0.360
Maltose	1.2	1.1	0.85	4.99	0.342
Sorbitol	0.3	0.3	0.27	1.74	0.105
LSD 5%	1.0	0.5	0.24	0.91	0.063
B. Sugar concentrations (g l^{-1}):					
0	0.38	0.4	0.36	0.95	0.029
30	6.40	3.0	1.31	6.90	0.337
60	2.38	1.4	0.97	5.18	0.395
90	1.16	1.1	0.83	3.56	0.367
120	0.64	0.7	0.46	2.05	0.264
LSD 5%	1.0	0.5	0.24	0.91	0.063

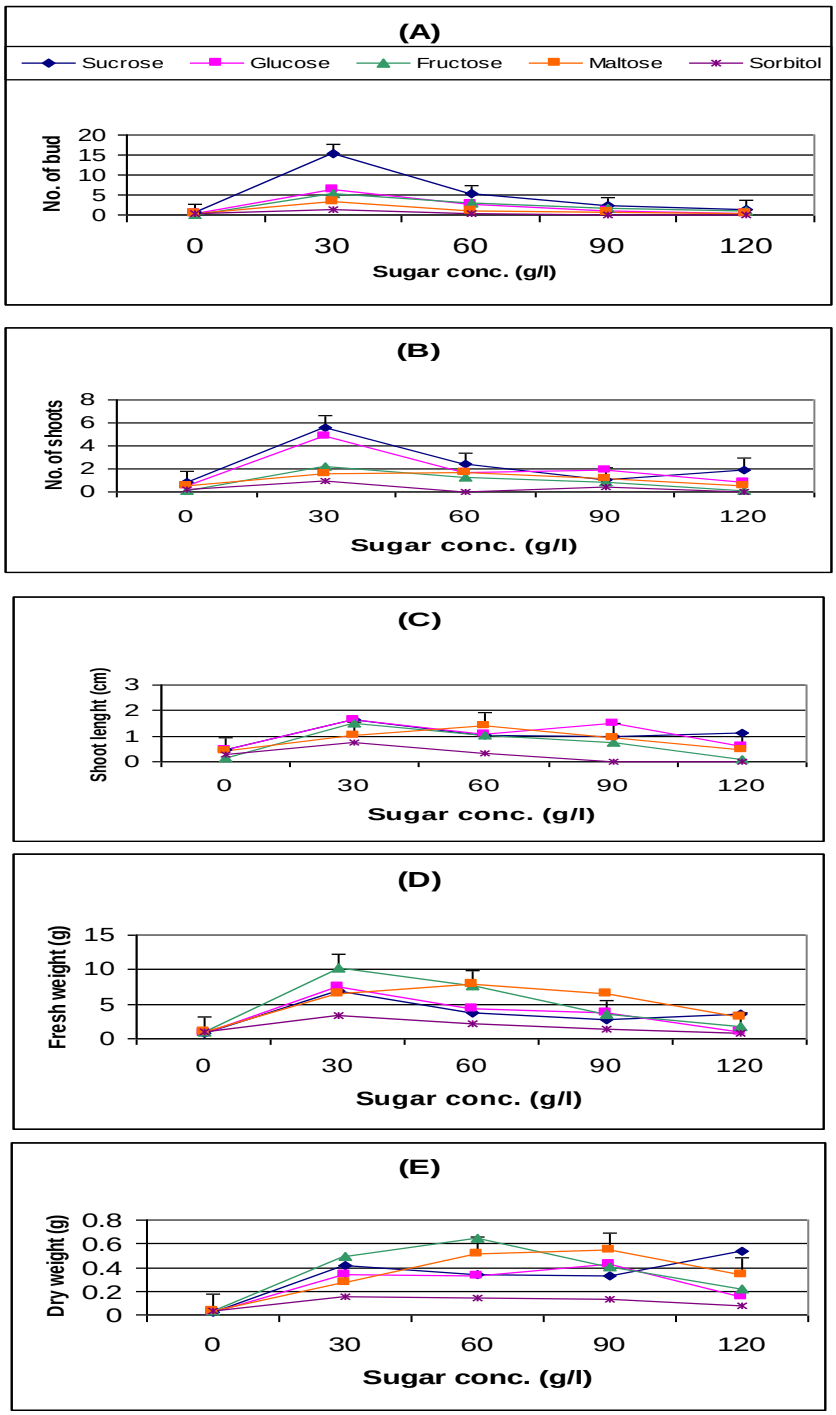

Fig. 1: Number of buds (A) and shoots (B), shoot length (C), fresh weight (D) and dry weight (E) of strawberry as affected by the interaction between sugar types and its concentration cultured *in vitro*.

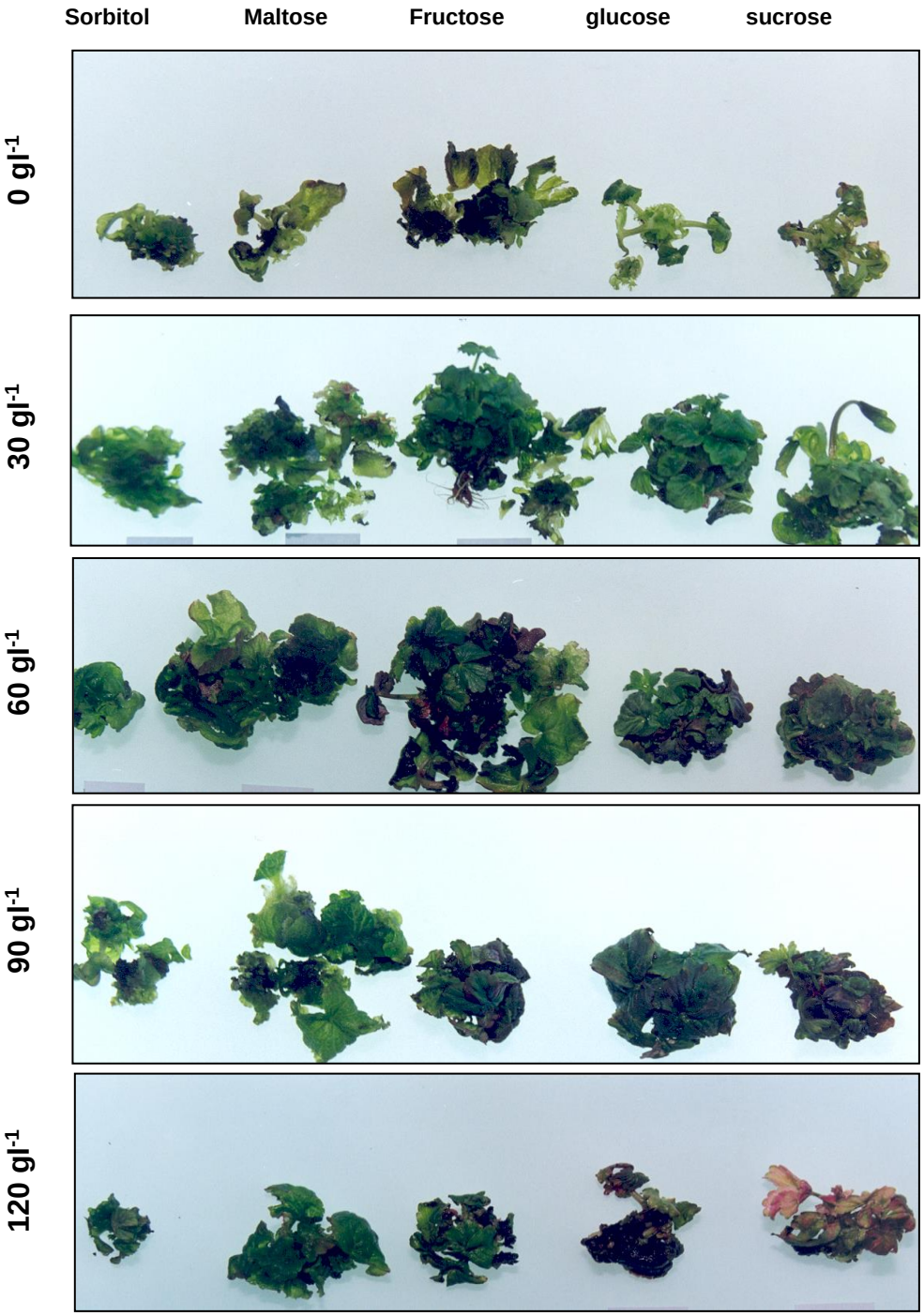

Fig. 2. Effect of sugar types and its concentrations on shoot number of strawberry cultured *in vitro*.

DISCUSSION

There was a clear effect for sugar types and concentrations on the shoot multiplication rate of strawberry. For each sugar added, the highest number of buds and shoots/ culture was formed at concentration of 30 g l^{-1}. Lower and higher concentration of sugars minimized the number of shoots produced especially with concentration of 90 and 120 g l^{-1}, respectively. The highest shoot fresh weight occurred with 30 g l^{-1} fructose and glucose. Fructose tended to produce cultures with less compact and large shoots that from a practical point of view, were easier to separate them during transfers. Furthermore, in rooting stage, it is expected that it may be much easier to produce healthy and good root system in such shoots. The lowest number of shoots as well as fresh weight of culture occurred when sorbitol was used even though it is reported that sorbitol is the major sugar of translocation in Rosaceae family (Bieleski, 1982) to which strawberry plant is a member. In the present study, it seems that sorbitol was not the recommended sugar for strawberry proliferation media over the whole concentration range. It is clear that the negative results obtained with sorbitol showed that it is not efficiently metabolized by strawberry plants, since glucose, fructose and maltose were supplemented at concentration equal molar to sorbitol and they gave positive results. These results were supported by the finding of Alkhateeb (1997) which indicate that sucrose is the major sugar transported in phloem of strawberry grown *in vivo.* In addition, low levels of glucose, fructose and galactose were detected.

Media devoid of sugar did not produce shoots, indicating the importance of sugar in the organ initiation process *in vitro*. Similarly, in *Begonia rex*, Mangat *et al*. (1990) found that there was a heavy accumulation of starch in tissue cultured on sucrose medium, whereas there was no starch deposition or organogenesis in tissue culture on mannitol and carbohydrate free medium. Also, they found during shoot primordium development there was a decrease in the starch content of the cultured tissue indicating the utilization of the polyglucon in the organogenesis process. Glucose has been shown to control gene expression both positively and negatively (Lee, 1987, German *et al*., 1990, Carlson *et al*., 1993). Therefore, increasing the sugar within the plant may increase the activities of some genes, subsequently increasing the production of energy or/and catalyzing other factors needed for organ initiation.

Higher concentration of sugar irrespective of the type caused red and white pigmentation (abnormal growth), compact and solid growth with no sign of bud formation (Fig. 2). Similar symptoms were quite evident in other studies dealing with carbon sources and concentration. These symptoms as well as the reduction of growth is probably not due to the extra sugar being toxic to the tissues, but to the high osmotic potential of a medium containing such a higher concentration of sugar (approximately – 1.5 MPa). The tissue may suffer from osmotic stress, and respond to this by retarded growth. This assumption was supported by Alkhateeb (1997), who found that the rate of uptake of glucose from the strawberry plantlet cultured in glucose medium varied with concentration and time during the culture period. There was a

rapid and heavy accumulation of carbohydrate (approximately 10 fold increase) in the plantlets that were cultured on medium containing 90 and 110 g l^{-1} glucose. This heavy accumulation of carbohydrates may cause the osmotic stress or and toxic effect and consequently resulting in the deterioration of the growth.

Stress-induced somatic embryogenesis has already been reported in carrot (Harada *et al*., 1990) and *Arabidopsis thaliana* (Miho *et al*., 2003) in which it was shown that somatic embryos could be induced by osmotic stress (sucrose, mannitol). Therefore, a short exposure to high concentration of sucrose 90 g l^{-1} and above may benefit or increase organ formation through inducing stress.

It seems that the superiority effect of sucrose over glucose or fructose due to the degradation of sucrose into glucose and fructose during media autoclaving (Pierik, 1989). This combination of glucose and fructose may acts better than being alone. The other explanation is the osmotic potential of medium being more negative with glucose or fructose than that of sucrose. Therefore, it seems that the differentiation of strawberry needs less osmotic potential of medium in order to grow better. Finally, it is possible that sucrose expend energy to break down into monosaccharide. This is in accordance with the findings that sucrose is degraded in to smaller units before uptake into cells in culture (Fowler, 1982). It is possible that the differentiation of strawberry plant needs less energy. Therefore, sucrose at concentration of less than 30 g l^{-1} may enhance the organ initiation better.

In conclusion, carbohydrates seem to play an important, but still unknown, regulatory role in the control of organ initiation in strawberry plant. Therefore, experiments that look at the specific roles that carbohydrates have inside the tissue of strawberry plants during the process of organ initiation are needed. Such experiments should examine the demand for energy or/and the activation of some genes during this process.

REFERENCES

Alkhateeb, A. A. (1997). The effect of carbohydrates on *in vitro* flowering of strawberry, *Fragaria x Ananassa* cv. Elsanta. Ph.D. Thesis, School of Plant Sciences, Reading University, England.

Alkhateeb, A. A. (2001). Influence of different carbon sources and concentrations on *in vitro* root formation of date palm (*Phoenix dactylifera* L.) cv Khanezi. Zagazig J. Agric. Res., 28: 597-608.

Bieleski, R. L. (1982). Sugar alcohols. In: Encyclopedia of Plant Physiology, New Series, Vol. 13A. Plant carbohydrates I (F.A. Loewus and W. Tanner, Eds), Springer-Verlag, Berlin, 159-192.

Bogunia, H. and Przywara, L. (1999). Role of carbohydrates in plant tissue culture. Wiadomosci Botaniczne, 43: 25-36.

Carlson, S.G. Fawcett. T.W. Barlett, J.D. Bernier, M. and Holbrook, N.J. (1993). Regulation of the C/EBP-related gene gad 153 by glucose deprivation. Mol. Cell Biol., 13: 4736-4744.

Cuenca, B. and Vieitez, A. M. (2000). Influence of carbon source on shoot multiplication and adventitious bud regeneration in *in vitro* peach cultures. Plant Growth Regulation, 32: 1-12.

El-Bakry, A. A. (2002). Effect of genotype, growth regulators, carbon source, and pH on shoot induction and plant regeneration in tomato. *In Vitro* Cellular & Developmental Biology Plant, 38: 501-507.

Fowler, M. W. (1982). Substrate utilization by plant-cell culture. J. Chem. Tech. and BioTech., 32: 338-346.

German, M. S., Moss, L. G. and Rutter, W. J. (1990). Regulation of insulin gene expression by glucose and calcium in transfected primary islet culture. J. Biol. Chemist., 265: 22063-22066.

Gomez, K. A. and Gomez, A. A. (1984). Statistical Procedures for Agricultural Research. 2nd Ed. John Wally & Sons .

Gruselle, R., Nicaise, C. and Boxus, P. (1995). Regulation of *in vitro* shoot multiplication in Persian walnut by different carbon sources and ammonium phosphate. Bulletin des Recherches Agronomiques de Gembloux, 30: 47-53.

Gubiš, J., Lajchová, Z., and Klčová, L. (2005). The effect of carbon source on plant regeneration in tomato. HortScience, 32: 6-8.

Harada, H., Kiyosue, T., Kamada, H. and Kobayashi, T. (1990). Stress-induced carrot somatic embyrogensis and its application to synthetic seed. In The Impact of Biotechnology in Agriculture (Sangwan, R.S. and Sangwan-Norreel, B.S., eds). The Netherlands: Kluwer Academ. Publishers pp. 129-157.

Kozai, T. (1991). Micropropagation under photoautotrophic conditions. In: Micropropagation: Technology and Application. (PP 447-469). Ed: Debergh, P.C., and Zimmermann, R.H. Kluwer Academic Publishers, Dordercht.

Lee, A.S. (1987). Coordinated regulation of a set of genes by glucose and calcium ionophores in mammalian cells. Trends Biochemical Sci., 12: 20-23.

Mangat, B.S., Pelekis, M.K. and Cassells, A.C. (1990). Changes in the starch content during organogenesis in *in vitro* cultured *Begonia rex* stem explants. Physiologia Plantarum, 79: 267-274.

Miho, I. I., Umehara, M., Satoh, S. and Kamada, H. (2003). Stress-induced somatic embryogenesis in vegetative tissues of *Arabidopsis thaliana*. Plant J., 34: 107-114.

Murashige, T., Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant., 15: 473-497.

Paiva, N., De, V. B., Otoni and W.C. (2003). Carbon sources and their osmotic potential in plant tissue culture: does it matter? Scientia Horticulturae, 97: 193-202.

Pierik, Ir. R. L. M. (1989). *In Vitro* Culture of Higher Plants. Mortinus Nijhoff Publishers, Bordrecht, Netherlands.

SAS Institute (2001). SAS for Windows, SAS User's Guide: Statistics. Version 8.0 e. SAS Inst., Inc., Cary, North Carolina.

Welander, M., Welander, N. T., Brackman, A. S., (1989). Regulation of *in vitro* shoot multiplication of *Syringa, Alnus* and *Malus* by different carbon sources. J. Hort. Sci., 64: 361-366.

تأثير مصادر و تراكيز مختلفه من الكربون على تكوين ونمو البراعم في نبات الفراوله المنزرع نسيجيا

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هدفت هذه الدراسة إلى معرفة تأثير مصادر كربون (السكروز و الجلكوز و الفركتوز والمالتوز والسوربيتول) بتراكيز مختلفة (صفر (مقارنه), ٣٠, ٦٠, ٩٠ و١٢٠ جم/ل) والتفاعل بينهم على تكوين ونمو البراعم في الفراولة خلال الزراعة النسيجية. وأشارت النتائج إلى أن تركيز ٣٠ جم/ل قد أعطى أفضل النتائج من ناحية تكوين البراعم والنموات القابله للتجذير بغض النظر عن مصدر الكربون. كما أن السكروز أعطى أعلى النتائج من ناحية تكوين البراعم (٥) والنموات القابله للتجذير (٢,٤) و خاصة تركيز ٣٠ جم/ل، بينما السوربيتول لم يعطي نتائج إيجابيه في جميع التركيزات المستخدمة. لذلك لا ينصح باستخدامه في إكثار الفراوله. وقد أعطى سكر الفركتوز نموات أقل تزاحما وأكبر في الحجم مما يعني السهوله في عملية فصل النموات أثناء مراحل الإكثار المختلفه. كما أثبتت الدراسة أن للفراولة القدرة على استخدام المصادر المختلفة من الكربون و بكفاءة أقل نوعا ما من السكروز, و يشذ عن هذه القاعدة سكر السوربيتول و الذي لم يعطي نتائج إيجابيه في جميع التركيزات المستخدمه. أوضحت نتائج الدراسة أن التراكيز العالية من الكربون (٩٠ جم/ل) وأعلى أدت إلى تدهور النمو و قلة البراعم المنتجة.