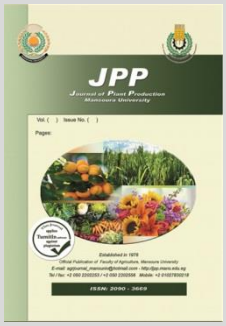
Journal of Plant Production

Journal homepage: www.jpp.mans.edu.eg
Available online at: www. jpp.journals.ekb.eg

Effect of Carbon Source in Woody Plant Medium with Different Salt Strengths on Oak (*Quercus aegilops* L.) Micropropagation

Laylan H. Fadhaladeen[1*] and Rafail S. Toma[2]

[1]Department of Forestry, College of Agric. Engine. Sci., UoD, Iraqi Kurdistan Region *
[2]Department of Horticulture, College of Agricultural Engineering Sciences, UoD, Iraqi Kurdistan Region
rshtoma@uod.ac



ABSTRACT

This study aimed to test the influence of different carbon sources in woody plant medium with various salt strengths on *in vitro* propagation of oak. The results showed that, sucrose was better than the other sugars by giving the highest shoots number, length of shoots and leaves number per explant (2.05, 1.30 and 17.67 respectively). The highest shoots number per explant was achieved when 45 gl^{-1} of glucose and 30 gl^{-1} of sucrose were added by giving 3.44 and 3.33 shoots/ explant respectively followed by 45 gl^{-1} sucrose and 30 gl^{-1} of glucose by giving 2.67 and 2.11 shoots/ explant respectively. On the other hand, full Woody Plant Medium salt strength gave the best multiplication results while the quarter Woody Plant Medium salts strength gave the lowest values. The highest roots number per explant was achieved when full salts strength was used which gave 5.40 roots followed by half salts strength with 2.60 roots then quarter salts strength by 0.30 root which was the lowest parameter. Furthermore, the highest rooting percentage was acquired in both full and half salts strength which was 100% and the lowest rooting rate was 30% in quarter salts strength. Sucrose was the best carbon source by giving the best root system and giving the highest rooting percentage (100%) followed by glucose (60%). The produced oak plantlets were successfully hardened and acclimatized to be transferred to the permanent field in the greenhouse with a success reached 85%.

Keywords: Oak, Micropropagation, sugars, carbon source, Woody plant medium, salt strength

INTRODUCTION

Quercus species are widely distributed in the Mediterranean forest. This genus comprises nearly 500 species (Manos *et al.*, 1999) which belongs to *Fagaceae* family and Fagalis genera. Four of these species are present naturally in Kurdistan region of Iraq (*Q. aegilops. Q. infectoria*, *Q. libani* and *Q. machranthera*). These four species are occupy about 70% of Kurdistans forest (Shahbaz, 2010).

These trees have ecological and economic importance including their use as food and refuge for many organisms including insects, birds, and mammal (Purohit *et al*., 2002a); they play a vital function for soil and water conservation, and helps to recuperate degraded environments. Oaks also are source of fodder, fire wood; charcoal (Shahbaz, 2010)

Productive shoot multiplication of forest trees, like oaks, is quite difficult because of the long time period acquired for achieving physiological maturity however, natural regeneration is affected by highly unstable annual seed production (Diaz Pontones and Reyes-Jaramillo, 2009). Due to these problems, researchers were encouraged to find asexual propagation (vegetative) methods for such an important tree propagation that would allow achieving progeny from plus-trees. Conventional propagation ways, like cutting, shoots-rooting and grafting are quite difficult furthermore, require high quantities of maternal materials. An alternative method to these is *in vitro* propagation that allows the mass production of true-to-type and identical plantlets in a short time periods. These techniques are very effective because most plant cells have totipotency potentiality and each single cell possesses the material of genetic information necessary to produce an entire plant. *In vitro* propagation, therefore, can be used to propagate a huge number of plants which are genetically identical to the donor plant, as well as to each another (Preesman, 2005).

Carbohydrates are essential in whole living plant cells as an energy source and as carbon-skeletons for the processes of biosynthesis. In plant tissues cultures, a subsequence carbohydrate supply from the culture medium is very essential, because the photosynthetic activity of tissues grown under *in vitro* conditions is reduced due to low light intensity, high relative humidity and limited gas exchange (Kozai, 1991). Carbohydrates are also essential in tissue culture as osmotic material agents (Thorpe, 1982). The most commonly universal used sugar is sucrose for micropropagation approaches being so utilizable generally by tissue culture techniques. The refined sugar is totally pure for almost all practical protocols (Aloni**,** 1980). The choosing of sucrose as the best suitable source of energy for culture media follows several comparisons among possible options. Some of the pioneer works of this type on the nutrition carbohydrate of plant tissues was applied by Gautheret (1945) when normal carrot tissues were used. Sucrose was noticed to be the first ranked option source of energy following by glucose, maltose or raffinose. Fructose sugar was less used and mannose and

* **Corresponding author.**
E-mail address: laylan.fadladeen@uod.ac
DOI: 10.21608/jpp.2019.59754

lactose were the least effectives. Sucrose has been noticed to be the best option carbohydrate; glucose is found to enhance and support growth equally well, and in a few species of plants it may act better in *in vitro* growth and development than the act of sucrose, or encourage organogenesis where sucrose cannot; (George *et al*., 1993).

The ideal concentration of sucrose to enhance *in vitro* morphogenesis or growth process varies between various genotypes, sometimes even between plants which are closely are relating to each other. Investigations by Molnar (1988) have declared that the ideal concentration of sucrose might depend on the other amendments applied to the culture medium. It was found that the culture dry weight of the proliferated explant increase about 2.5 times when compared with those cultured on medium containing 20 gl^{-1} sucrose. However, sucrose concentration influence directly on the kind of morphogenesis. So (87 mM) is suitable for organogenesis and more than this level is better for somatic embryogenesis derived from immature sunflower embryos (Jeannin *et al*., 1995). When some non-digestive sugars as (maltose, melibiose, cellobiose, sorbitol and mannose) were added to low sucrose level, this lead to the production of a fluffy fibrous roots, this mean that sucrose has a nutrition instead of osmotic (Rier and Beslow, 1967). Furthermore, the rate of respiration increase when the concentration of carbon source increase specially glucose and sucrose. On the other hand, the inorganic nutrient uptake could depend on the level of carbohydrates where the high nutrient rate in the medium cannot be observable unless the carbohydrates level will increase too (Gamborg *et al*., 1974).

Rooting stage is recognized by preparation of proliferated shoots for rooting and then transfer successfully to soil. In the rooting stage, the common growth regulators used are auxins which are responsible for the formation of adventitious roots. In general, the low auxin concentration is better for root formation while for callus induction the high auxin concentration is better.

From the review of literature of *in vitro* culture of oak species, the most common auxins used in the rooting step are IBA and NAA. Evidence has been produced that reduction of the salts' strength in the medium achieves better rooting of plantlets. For rooting stage of micropropagation of *Q. robur* L., Chalupa (1984) used half-strength medium salts with two kinds of auxins (IBA and NAA) in various concentrations (0.1-0.5 mgl^{-1}) where high rooting percentages (70-95%) were achieved at 0.3 mgl^{-1} IBA and 0.1 mgl^{-1} NAA. The aim of this study was to examine which hormone concentration and medium is the most suitable for micropropagation of *Quercus aeqilops*. Furthermore, testing different kinds of carbon sources including various sugars at different levels.

MATERIALS AND METHODS

For initiation stage, alternative nodes were taken from microshoots that obtained from oak embryo culture grown on WPM (Lloyd and McCown, 1980) including 0.5 mgl^{-1} GA_3. They were cultivated on WPM containing 3 mgl^{-1} BA for eight weeks. For avoiding contact with phenols, explant transferred to its opposite side then to fresh medium after the first and second day of culturing on initiation medium. Each 3 weeks, they were transferred to the same but fresh medium. After 8 weeks culturing on initial medium, the micro-shoots were cut off and cultivated on proliferative medium.

For proliferative stage, Woody Plant Medium was used, supplemented with 3 mgl^{-1} BA as well as four kinds of carbon sources including sucrose, fructose, glucose and sorbitol at 15, 30, 45 and 60 $g.l^{-1}$. For avoiding contact with phenols, explant transferred to its opposite side then to fresh medium after the first and second day of culturing on multiplication medium; Each 3 weeks, they were transferred to the same but fresh medium. After 8 weeks culturing on multiplication medium, the multiplication parameters were recorded as shoot number mean per explant and their length mean and the microshoots were transferred to the rooting stage.

At rooting stage, the microshoots were cultured on Woody Plant Medium full, ½ and ¼ salt strengths supplemented with 1 mgl^{-1} NAA. For avoiding contact with phenols, explant transferred to its opposite side then to fresh medium after the first and second day of culturing on rooting medium; Each 3 weeks, they were transferred to the same but fresh medium.

Additionally, different carbon sources including sucrose, glucose, fructose and sorbitol were tested at 15, 30, 45 and 60 $g.l^{-1}$ respectively. For avoiding contact with phenols, explant transferred to its opposite side then to fresh medium after the first and second day of culturing on rooting medium; Each 3 weeks, they were transferred to the same but fresh medium. After 8 weeks culturing on multiplication medium, the multiplication parameters were recorded as shoot number mean per explant and their length mean and the microshoots were transferred to the rooting stage. 8 weeks later the root parameters were registered as the average number of roots per micro-shoot, the root length mean and rooting percentage.

The whole tests were coordinated depending on Complete Randomized Design (CRD) with 5 replicate for every treatment. The condition of cultures incubation were 25± 2° C temperature, 16 hours light exposure with 1000 lux (Toma and Rasheed, 2012).

RESULTS AND DISCUSSION

Results

Effect of carbon sources on multiplication stage of oak

The current results of the study indicate the ability of *in vitro* propagation of this hard-to-propagate forest tree. The parameters showed significant statistical differences as treated with various experimental tested factors. Table (1) illustrates the effect of sucrose, glucose, fructose and sorbitol in different concentrations (15, 30, 45 and 60) gl^{-1} on shoot multiplication of oak after eight weeks in culture.

In general, sucrose was better than the other carbon sources by giving the highest means of shoot number, length of shoots and leaves number per explant which were 2.30, 1.30 and 17.67 respectively. However, fructose and sorbitol gave the lowest parameters where fructose gave 1.33 shoot and 10.84 leaves per explant and sorbitol gave the lowest shoot length which was 1.12 cm. According to the data, the greatest shoot numbers per explant was achieved when 45 gl^{-1} of glucose and 30 gl^{-1} of sucrose were used by giving 3.44 and 3.33 shoots respectively followed by 45 gl^{-1} sucrose and 30 gl^{-1} of glucose by giving 2.67 shoot and 2.11 shoots respectively. While the less shoot numbers per explant was acquired when 45 gl^{-1} of fructose and 60 gl^{-1} of glucose and fructose were used which gave 1.0 shoot. Moreover, the

longest shoot was acquired when 45 gl^{-1} of glucose was used which was 1.67 cm followed by 60 gl^{-1} fructose with 1.50 cm. while the shortest length of shoot was acquired when 15 gl^{-1} sorbitol was used which gave 0.94 cm. Furthermore, the greatest leaves number per explant was obtained when 45 gl^{-1} of glucose and 30 gl^{-1} of sucrose was used by giving 25.56 and 25.22 leaves respectively followed by 45 gl^{-1} of sucrose by giving 23.11 leaf. While the lowest number of leaf per explants was obtained when 15 gl^{-1} of sorbitol was used by giving 6.33 leaf as shown in figure (1). Similar results were reported by chalupa (1984), Manzanera and pardos (1990), Sanchez (1996), Puddephat (1999), Purohit (2002) Vieites *et al*. (2004) and Liao and Chuang (2014).

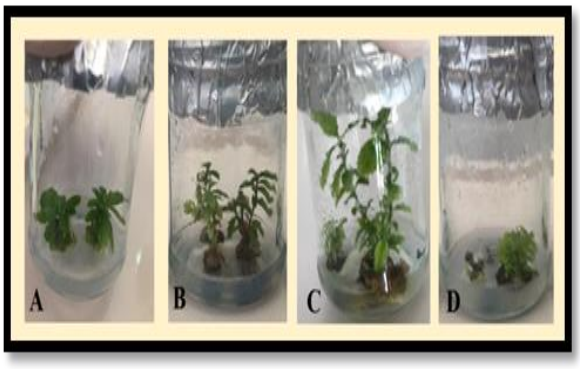


Figure 1. Effect of carbon source on multiplication stage of oak
A: 15 $g.l^{-1}$ fructose. B: 45 $g.l^{-1}$ glucose. C: 30 $g.l^{-1}$ sucrose. D: $g.l^{-1}$sorbitol.

Table 1. Effect of different sugar sources on shoot multiplication of oak *Quercus aegiops* L. after eight weeks in culture

Sugars (g/l)		Shoot number/ explant	Sugar means	Shoot length mean (cm)	Sugars means	Number of leaves/ explant	Means of sugars
Sucrose	15	1.44 c	2.30 a	0.97	1.30 a	9.11 b	17.67 a
	30	3.33 a		1.47 a		25.22 a	
	45	2.67 b		1.48 a		23.11 a	
	60	1.78 c		1.27 a		13.22 b	
Glucose	15	0.78 d	1.83 a	1.00 a	1.23 a	6.67 b	12.73 b
	30	2.11 b		0.84 a		11.67 b	
	45	3.44 a		1.76 a		25.56 a	
	60	1.00 d		1.31 a		7.00 b	
Fructose	15	1.56 c	1.33 a	1.32 a	1.30 a	14.67 b	10.84 b
	30	1.78 c		1.09 a		12.78 b	
	45	1.00 d		1.29 a		6.89 b	
	60	1.00 d		1.50 a		9.00 b	
Sorbitol	15	1.44 c	1.50 a	0.94 a	1.12 a	6.33 b	12.78 b
	30	1.67 c		0.97 a		10.89 b	
	45	1.56 c		1.18 a		10.56 b	
	60	1.33 c		1.38 a		10.56 b	

Effect of Woody Plant Medium salt strength on rooting stage of *Q. aegilops* L.

Table (2) shows the effect of Woody Plant Medium full, half and Quarter salts strength on rooting stage of oak after eight weeks in culture. Generally, full Woody Plant Medium salt strength recorded the best results while the quarter Woody Plant Medium salts strength gave the lowest parameters as shown in figure (2). From the table, it is clear that the highest number of root per explant was achieved when full salts strength was used which gave 5.40 roots followed by half salts strength with 2.60 roots then quarter salts strength by 0.30 root which was the lowest parameter. Moreover, this treatment produced some basal callus as shown in figure (2) which caused an easily break and cut of roots during the preparation of plantlets for acclimatization stage as well as it is not desirable as it contrary influence the survival of plantlets in the field (Purohit *et al*. (2002) so the half Woody Plant Medium salt strength was the best treatment in giving the best root system.

Furthermore, the highest rooting percentage was acquired in both full and half salts strength which was 100% and the lowest rooting rate was 30% in quarter salts strength. Similar results were reported by Sanchez (1996), Purohit *et al*. (2002), Vengadesan and Pijut (2009), Linan *et al*. (2011) and Pandey and Tamta (2014).

Figure 2. Effect of woody plant medium salt strength on rooting stage of oak.
A: ¼ strength B: ½ strength C: full strength

Table 2. Effect of Woody Plant Medium salts strength on rooting of oak *Quercus aegiops* L. after eight weeks in culture

woody plant medium salts strength	Rooting Percentage (%)	Number of Roots/ Explant	Mean Length of Roots (cm)
Full (1/1)	100 a	5.40 a	2.60 a
Half (1/2)	100 a	2.60 b	1.30 b
Quarter (1/4)	30 b	0.30 c	1.27 b

Effect of various carbon sources on rooting stage of *Q. aegil*ops L.:

Table (3) illustrates the effect of various concentration of sucrose, glucose, fructose and sorbitol on rooting stage of oak. In general, sucrose was better than other carbon sources in giving better root system as well as for giving the greatest rooting percentage followed by glucose. According to the table, the highest mean of rooting rate with 87.5% as well as the highest mean number or roots with 4.28 root and the longest mean of root with 2.78 followed by glucose with 27.50%, 1.35 cm. while fructose gave the lowest parameters with 0 %, 0, 0 cm and 0 roots.

It is clear that the highest rooting rate was achieved when 30, 45 and 60 gl^{-1} were used which gave 100% followed by 60 gl^{-1} glucose, 45 gl^{-1} glucose, 15 gl^{-1} sucrose, 60 gl^{-1} sorbitol and 45 gl^{-1} sorbitol with 60, 50, 50, 30 and 20% respectively. While the rest treatments were 0%. Furthermore, the longest root was acquired when 60 gl^{-1} of glucose was used which gave 4.30 cm followed by 45 and 60 gl^{-1} sucrose with 3.23 cm. Moreover, the greatest number of root was acquired when 45 and 60 gl^{-1} sucrose were used with 5.60 and 5.50 root respectively followed by 5.40 roots by using 30 gl^{-1} sucrose. While the lowest number of root was recorded when 15 and 30 gl^{-1} of glucose, fructose and sorbitol as well as 45 and 60 gl^{-1} of fructose were used which gave 0 root Similar results were reported by sanches (1996), Purohit *et al.* (2002), Vengadesan and Pijut (2009), Linan *et al.* (2011) and Pandey and Tamta (2014). It is very important to mention that in two of fructose treatments (15 and 30 gl^{-1}) the shoot system is develop instead of producing roots. The produced oak plantlets were successfully acclimatized and transferred to the open field with 85% of success.

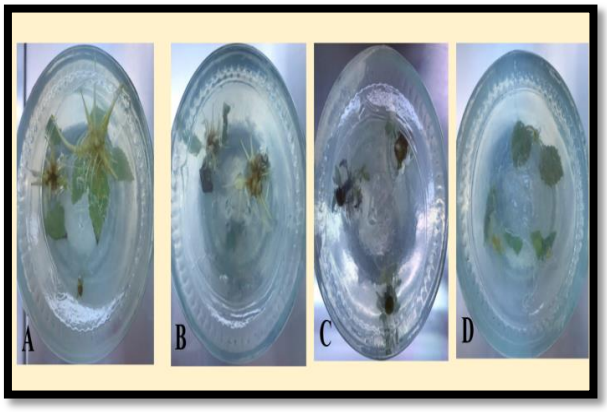


Figure 3. Effect carbon source on rooting stage of oak.
A: Sucrose B: Glucose C: Fructose D: Sorbitol

Table 3. Effect of different sugar sources on rooting stage of oak *Quercus aegiops* L. after eight weeks in culture

Carbon sources		Rooting percentage (%)	Means of sugars	Root length means (cm)	Means of sugars	Root number/ explant	Means of sugars
Sucrose	15	50	87.50 a	2.08 c	2.78	0.60 c	4.28 a
	30	100		2.56 c		5.40 a	
	45	100		3.23 b		5.60 a	
	60	100		3.23 b		5.50 a	
Glucose	15	0	27.50 b	0	1.35	0 d	0.55 b
	30	0		0		0 d	
	45	50		1.09 d		1.20 b	
	60	60		4.30 a		1.00 b	
Fructose	15	0	0 d	0 e	0	0 d	0 c
	30	0		0 e		0 d	
	45	0		0 e		0 d	
	60	0		0 e		0 d	
Sorbitol	15	0	12.50 c	0 e	0	0 d	0.71 b
	30	0		0 e		0 d	
	45	20		0.20 e		1.00 b	
	60	30		0.30 e		1.83 b	

Discussion

In spite of the fact that carbon sources are of major significance for organogenesis *in vitro*, the metabolism of carbon *in vitro* is yet not plainly comprehended (Kozai, 1991). It is completely firmed that carbohydrates requirement contingent on the culture stage and might show distinction depending on the species. The multiplication of *Q. aegilops* L. is highly affected by carbon source (Fig. 1). Regarding to the former scores on oak shoot multiplication (Romano *et al*., 1992), it is reported that adding of 30 gl^{-1} sucrose extremely promoted shoot multiplication and elongation. In this study, fructose and sorbitol didn't catalyze shoot multiplication (Fig. 1). These carbohydrates didn't have a constant baleful influence on the tissue, at least though 2 multiplication cycles, since cultures continued growth after transferring to rooting medium. To be confirmed concerning the physiological influince of fructose at 15, 30, 45 and 60 gl^{-1} were contrasted to equimolar concentrations of sucrose and glucose. The reduce of the root numbers and rooting rate noticed for the above carbon source combinations could be assignable to inhibitory influence fructose as carbon source (Oka and Ohyama 1986; Chauvin and Salesses 1988; Hew and Mah 1989; Welander *et al*. 1989; Borkowska and Szczerba 1991; Moncousin *et al.* 1992) are perhaps because of the various in the plant types sensitivity to the degeneration products produced as a results of autoclaving as hydroxymethylfurfural and furfural (Hsiao and Bornman 1989; Uosukainen and Vasara 1992; Druart and de Wulf 1993). The opposite results acquired using sorbitol illustrate that this kind of sugar doesn't strongly hydrolize by *Q. aegilops* L. cultures (Fig. 1), while in other species as *Prunus seracus*, sorbitol is well hydrolized (Borkowska and Szczerba 1991). However, in Malos as well as in closest plant species sorbitol is a great dissolved sugar. Sorbitol overwhelmingly added to the medium as an osmotic potential adjustment in plants, so it is not utilized and hydrolize.

The results acquired could be refer in this situation to osmotic influence. Furthermore, this carbon source may not be hydrolized by *Q. aegilops* L. tissues, where it is not existing as a carbon source in oak species. (Zimmermann

& Ziegler 1975). The results approaching in this investigation backup the supposition that the influence of greater glucose and sucrose concentrations on *Q. aegilops* L. multiplication as well as rooting may not be only osmotic (figs. 1 and 3). If it was correct, so for all carbon sources researches the content of water of cultures will follow up the same way when contrast as equimolar concentrations instead of percentage concentration and carbon source reliance. This denoted that for *Q. aegilops* L. tissue culture, carbon sources may be compared depends on the available carbon number, independent of molarity.

Most of the tests on the induction of roots used 20-30 gl^{-1} sucrose in the form of carbon source (Nemeth, 1986) and it commonly agreed that a reducing in carbohydrates progress rooting (Kozai, 1991). Several researches found that a favorable response to increase in carbohydrates. Manzanera and pardos (1990) and Romano *et al*. (1995) working with *Q. suber* L., deduced that the rooting rate increased by increasing the concentration of sucrose. The results of this study also observed that the number of root and root length is increased with increasing the concentration of carbon source (table 3). The concentration of carbon source adjust the osmotic pressure of the medium (Thompson and Thorpe, 1987) and at a great osmotic pressure the medium is known to decrease the plant height and slow growth (Short *et al*., 1987).

Although the plant growth regulators especially auxins have a critical role in lateral root initiation. But carbon /nitrogen ratio has been also reported as having an effect on root initiation. It was established that the lateral root formation and their development are greatly affected by the increasing of the carbon source and decreasing the nitrogen in the medium. However, these data also illustrate that taking-up of sucrose metabolism cause to create a more greatly root shoots (MacGregor *et al*., 2008). On the other hand, a high equipping of nitrogen inhibited the development of lateral root when the plantlet were grown on a low carbon concentration media (Zhang and Forde, 1998). But in the present investigation the full salt strength gave the highest number of root may be due to that the Valonia oak is a woody plant and need a high concentration of nitrogen to get the enough energy to develop and produce roots. Comparatively a few studies have been conducted on the influence of carbon /nitrogen ratio on *in vitro* root initiation (Welander, 1976, Welander, 1978, Hyndman *et al*. 1982 and Gabryszewska, 2010).

REFERENCES

Aloni, R. (1980). Role of auxin and sucrose in the differentiation of seive and tracheary elements in plant tissue cultures. Planta 150, 255-263.

Borkowska, B. and Szczerba, J. (1991). Influence of different carbon sources on invertase activity and growth of sour cherry *(Prunus cerasus* L.) shoot cultures. J. Exp. Bot. 42:911-915.

Chalupa, V. (1984). In vitro propagation of oak (*Quercus robur* L.) and linden (*Tilia cordata* Mill.). Biol. Plant. 26: 374-377.

Chauvin, J. E. and Salesses, G. (1988). Effet du fructose sur la micropropagation du chfitaignier *Castanea* sp. C. R. Acad, Sc. Pads, Serie III 306:207-212.

Diaz-Pontones, D. and Reyes-Jaramillo, I. (2009). Production and acclimatization of Quercus hintonii Warburg (Fagaceae). Botan. J. 27: 131-143.

Druart, P. and De Wulf, O. (1993). Activated charcoal catalyses sucrose hydrolysis during autoclaving. Plant Celt Tiss. Org. Cult. 21: 97-99.

Gabryszewska, E. (2010). The effects of glucose and growth regulators on the organogenesis of *Paeonia lactiflora* Pall. *in vitro*. J Fruit Ornam Plant Res. 18(2):309–320.

Gamborg, O.L.; Constabel, F. and Shyluk, J. P. (1974). Organogenesis in callus from shoot apices of Pisum sativum. Physiol. Plant. 30, 125-128.

Gautheret, R. J. (1945) Une voie nouvelle en biologie végétale: la culture des tissus. Gallimard, Paris.

George, E.F. (1993). Plant propagation by tissue culture I: The Technology, Exegenetics Ltd., Edington, UK.

Hew, C. S. and Mah, T.C. (1989). Sugar uptake and invertase activity in *Dendrobium* tissues. New Phytol. 111: 167-171.

Hsiao, K. C. and Bomman, C. H. (1989). Cyanide-initiated oxygen consumption in autoclaved culture medium containing sugars. Plant Cell Rep. 8:90-92.

Hyndman, S. E.; Hasegawa, P. M.; Bressan, R. A. (1982). The role of sucrose and nitrogen in adventitious root formation on cultured rose shoots. Plant Cell Tissue Organ Cult.1:229–238. http://dx.doi.org/10.1007/ BF02318919.

Jeannin, G.; Bronner, R. and Hahne, G. (1995). Somatic embryogenesis and organogenesis induced on the immature zygotic embryo of sunflower (Helianthus annus L.) cultivated in vitro: Role of Sugar. Plant Cell Rep. 15, 200-204.

Kozai, T. (1991). Micropropagation under photoautotrophic conditions. In: Debergh PC, Zimmerman RH (Eds) Micropropagation: Technology and Application (pp 447-469). Kluwer Academic Publishers, Dordrecht.

Liao, Y. and Chuang, M. (2014). Micropropagation of *Quercus aliena* Blume var. from explants of mature trees. Tiwan J. For Sci. 92 (2): 117-131.

Linan, J.; Cantos, M.; Troncoso, J.; Garcia, J. L.; Fernandes, A. and Troncoso, A. (2011). Some propagation methods for cloning hplm oak (Quercus ilex L.) plants. Cent. Eur. J. Biol. 6(3): 395-364.

Lloyd, G. and McCown. Commercially-feasible micropropagation of mountain laurel, Kalmia latifolia, by use of shoot-tip culture. B., Int. Plant Prop. Soc. Proc. 30, 421 (1980).

MacGregor, D. R.; Deak, K. I.; Ingram, P.A. and Malamy, J. E. (2008). Root system architecture in *Arabidopsis* grown in culture is regulated by sucrose uptake in the aerial tissues. Plant Cell. 20:2643–2660. http:// dx.doi.org/ 10.1105/ tpc. 107. 055475.

Manos, P.S., Doyle, J.J. and Nixon, K.C. (1999). "Phylogeny, biogeography, and processes of molecular differentiation in *Quercus* subgenus *Quercus* (Fagaceae)". *Mol. Phylogenet. Evol.*, 12: 333-349.

Manzanera, J.A. and Pardos, J.A. (1990). Micropropagation of juvenile and adult *Quercus suber* L. *Plant Cell Tiss. Org. Cult.*, 21: 1-8.

Mareno, G.; Bertazza, G.; Magnanini, E. and Altan, A. D. (1993). Comparative effects ofsorbitoI and sucrose as main carbon energy sources in micropropagation of apricot. Plant Cell Tiss. Org. Cult 34:235-244.

Molnar, S. J. (1988). Nutrient modifications for improved growth of Brassica nigra cell suspension cultures. Plant Cell Tissue Organ Cult. 15, 257-267.

Moncousin, C.; Ribaux, M.; O'Rourke, J. and Gavillet, S. (1992). Effects of type of carbohydrate during proliferation and rooting of microcuttings of *Malus* Jork 9. Agronomic 12:775-781.

Nemeth, G. (1986). Induction of roofing. In: Bajaj YPS (Ed). Biotechnology in Agriculture and Forestry, Trees I (pp 49-64). Springer-Verlag, Berlin

Oka, S. and Ohyama, K. (1986). Mulberry *(Morus atba* L.). In: Bajaj YPS (Ed) Biotechnology in Agriculture and Forestry, Trees I (pp384-392). Springer-Verlag, Berlin

Pandey, A. and Tamta, S. (2014). *In vitro* propagation of the important tasar oak (*Quercus serrata* thumb.) by casein hydrolysate promoted high frequency shoot proliferation. J. of Sustain. Forest. 33: 590-603.

Preesman (2005). Roskam Young plants. (www.roskam-youngplants.com), (www.preesman.com).

Pua, E. C. and Chong, C. (1985). Regulation of *in vitro* shoot and root regeneration in 'Macspur' apple by sorbitol (d-glucitol) and related carbon sources. J. Amer. Soc. Hort. Sci. 110:705-709.

Puddephat, I. J.; Alderson, P. G. and Wright, N. A. (1999). *In vitro* root induction in axillary microshoots of *Quercus robur* L. Ann. appl. Biol. 134: 233-239.

Purohit, V. K.; Tamta, s.; Chandra, S.;Vyas, T.; Palni, L. M. K. and Nandi S. K. (2002). *In vitro* multiplication of *Q. leucotrichophora* and *Q. glauca*: Important Himalayan oaks. Plant Cell. Tiss. And Org. Cult. 69: 121-133.

Purohit, V.K.; Palni, L.M.S.; Nandi, S.K. and Rikhari, H.C. (2002a). *In vitro* regeneration of *Quercus fl oribunda* Lindl. through cotyledonary nodes: an important tree of Central Himalaya. Current Sci., 833: 312-316.

Rier, J. P. and Beslow, D. T. (1967). Sucrose concentration and the differentiation of xylem in callus. Bot. Gaz. 128, 73-77.

Romano, A. and Martins- Loucao, M. A. (1992). Micropropagation of mature cork –oak (*Quercus suber* L.): Establishment problems. *SCIENTIA* gerondensis, 18: 17-27.

Sanchez, M.; San-Jose, M. C.; Ballester, A. and Vieitez, A. M. (1996). Requirements for in vitro rooting of *Quercus robur* and *Quercus rubra* shoots derived from mature trees. Tree physio. 16: 673-680.

Shahbaz, S. E. (2010). Tree and shrubs afield guide to the trees and shrubs of Kurdistan Region of Iraq. First edition, University of Duhok publication. No. 2232/12/2009.

Short, K.; Warburton, J. and Robert, A. (1987). *In vitro* hardening of cultured cauliflower and chrysanthemum plantlets to humidity, Acta Hort. 212:329-33

Thompson, M. and Thorpe, T. (1987). Metabolic and non-metabolic roles of carbohydrates. In: Bonga J.M. and Durzan, D.J. Cell and Tissue Culture in Forestry (pp 89-112) Martinus Nijhoff Publishers, Dordrecht.

Thorpe, T. (1982). Carbohydrate utilization and metabolism. In: Bonga, J. M. & Durzan D. J. (Eds) Tissue Culture in Forestry (pp 325-368). Martinus Nijhoff Publishers, London.

Toma, R. S. and Rasheed K. A. (2012). In Vitro Propagation through Seed Culture and Regeneration of Asparagus Densiflorus L. through Callus Cultures Derived from Hypocotyls. Int. J. Pure Appl. Sci. Technol., 9(2) (2012), pp. 94-102

Uosukainen, M. and Vasara, T. (1992). Effects of autoclaving on micropropagation medium. In: COST 87 Session, Dijon, France, 1992.

Vengadesan, G. and Pijut, P.M. (2009). *In vitro* propagation of northern red oak (*Quercus rubra* L.). In Vitro Cell. Dev. Biol-Plant, 45: 474-482.

Vieites, A. M. (2004). Somatic embryogenesis in mature *Quercus robur* tree. Plant cell. Tissue and organ culture. 76: 283-287.

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

Welander, T. A. (1976). Effects of nitrogen, sucrose, IAA, and kinetin on explants of *Beta vulgaris* grown *in vitro*. Physiol Plant. 36:7–10. http:// dx.doi.org/ 10.1111/ j.1399-3054.tb05018.

Welander, T. A. (1978). Influence of nitrogen and sucrose in the medium and irradiance of the stock plants on root formation in *Pelargonium* petioles grown in vitro. Physiol Plant. 43:136–141. http://dx.doi. org/10.1111/ j. 1399-3054.1978.tb01581.

Zhang, H. and Forde, B. (1998) An *Arabidopsis* MADS box gene that controls nutrient-induced changes in root architecture. Science. 279:407–409. http://dx.doi.org/ 10.1126/ science.279.5349.407.

Zimmermann, M. H. and Ziegler, H. (1975). List of sugars and sugar alcohols in sieve-tube exudates. In: Pirson A & Zimmermann MH (Eds) Encyclopedia of Plant Physiology, New Series Vol I (pp 480-503). Springer Berlin, Heidelberg.

تأثير المصدر الكاربوني في وسط النباتات الخشبية بقوى مختلفة من الأملاح في الإكثار الدقيق للبلوط (*Quercus aegilops* L.)

ليلان حسين فضل الدين[1] و روفائيل شليمون توما[2]

[1]قسم الغابات، كلية علوم الهندسة الزراعية، جامعة دهوك، اقليم كوردستان العراق

[2] قسم البستنة، كلية علوم الهندسة الزراعية، جامعة دهوك، اقليم كوردستان العراق

استهدفت الدراسة دراسة تأثير مستويات مختلفة من السكريات المضافة الى وسط زراعة النباتات الخشبية بقوى مختلفة من الاملاح في الاكثار الدقيق خارج الجسم الحي لنبات البلوط؟ أظهرت النتائج بأن استخدام السكروز كان الأفضل مقارنة ببقية السكريات المضافة في مرحلة التضاعف الخضري، وذلك بإعطاء أكبر عدد من الفروع وأطوالها وزيادة عدد الأوراق المتكونة للجزء النباتي (٢.٠٥ و ١,٣ و١٧,٦٧ على التوالي). تم الحصول على اكبر عدد من الفروع من خلال اضافة ٤٥ غم/ لتر من الجلوكوز و٣٠ غم/ لتر من السكروز وصل الى ٣,٤٤ و ٣,٣٣ فرع/ جزء نباتي على التوالي وتلتها اضافة ٤٥ غم/ لتر من السكروز و٣٠ غم/ لتر من الجلوكوز وذلك بتكوين ٢.٦٧ و ٢,١١ فرع/ جزء نباتي على التوالي. من جانب آخر، القوة الكاملة من أملاح بيئة زراعة النباتات الخشبية أعطت أفضل النتائج في مرحلة التضاعف الخضري بينما أدى استخدام ربع قوة الأملاح الى الحصول على أقل النتائج. تم الحصول على اكبر عدد من الجذور باستخدام القوة الكاملة من أملاح الوسط الزراعي وذلك بتكوين ٥,٤٠ جذر/ جزء نباتي تلاه استخدام نصف قوة الاملاح الذي أنتج ٢,٦٠ جذر/ جزء نباتي وأخيراً اتخدام ربع قوة الأملاح الذي أعطى أقل المقادير. أعلى نسبة تجذير (١٠٠%) تم تسجيلها مع كل من القوة الكاملة ونصف القوة من الأملاح بينما أقل نسبة تجذير (٣٠%) تم الحصول عليها عند استخدام ربع قوة الأملاح. من جانب آخر، كان السكروز أفضل مصدر كاربوني في وسط الزراعة في مرحلة تكوين الجذور من خلال رفع نسبة التجذير الى ١٠٠% تلاها الجلوكوز الذي أعطى فقط ٦٠%. نبيتات البلوط المنتجة، تم تقسيتها ونقلها تدريجياً من ظروف المختبر الى الحقل الدائم في البيت الزجاجي وبنسبة نجاح وصلت الى ٨٥%.