Shoot multiplication of Pear cv. Othmany as affected by Sugar type and nutrient media *In vitro*

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Abstract

The effect of adding three types of sugars (sucrose, Glucose or mannitol) at concentration of 30 $g.L^{-1}$ each to MS [13] or WPM [11] media and the effect of nutrient Conc. of MS and WPM in (half strength, full strength, full and half strength and double strength of nutrient)contain 2.5 $mg.L^{-1}$ BA and 0.25 $mg.L^{-1}$ IBA, on shoot multiplication of the pear plants cv. Othmany were studied. The results after eight weeks of multiplication, MS medium achieved the best result in all the studying characters, No. of shoots (3.70), shoot length (2.63 cm) and the No. of leaves (27.33). However, the sucrose had a significant effect on No. of shoots and No. of leaves (5.39 and 40.28) respectively, but the longer shoot was achieved in adding Glucose to the medium, which differs significantly from Mannitol. The effect of concentration of Nutrient media on shoots multiplication, the results showed that the higher No. of Shoots and leaves were achieved in WPM (4.81 and 41.54) respectively that not differ significantly of MS. As soon as the best significantly length of shoots (3.46 cm) was achieved in MS in comparison with WPM.

Keywords: Tissue culture, Sucrose, Glucose, Mannitol, MS, WPM, Nutrient Concentration.

[1]*The research is derived from a master's thesis for the first researcher.

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Introduction

Pear trees (*Pyrus* spp. L.) are one of the most important fruit trees in the world. It came in the second stage after apples in terms of production [15]. In addition to the importance of its fruit as a nutrient, it is used to treat blood pressure in old age, atherosclerosis, kidney disease and the treatment of the symptoms caused by heart, kidney, and liver [17]. Othmany variety is a very good species, original home is from Syria and one of the most important varieties cultivated in Iraq, the fruit mature early, its trees are heavy load, the fruits are large in size, conical shape drawn from the neck of the fruit and wide at the top, yellow with red cheek from the sun-exposed side, sweety, and Juicy [14].

The needed for a good injury-free and Appropriate to Iraqi environmental grafts in Iraq in order to achieve the expansion of pear tree cultivation, as soon as, there is no research on the multiplication of the Othmany species, so it's despite its excellence in many of its vegetative and productive qualities. In response to the prevailing environmental conditions in Iraq and the high demand for its fruits, the study aimed to investigate the effect of Types of Sugars and the concentrations of nutrient media (MS and WPM) on shoots multiplication *In vitro*.

Shatnawi *et al*. [19] studied the effect of adding different concentrations (0, 15, 30, 45 and 60 $g.L^{-1}$) of (Socruse, Glucose, Fructose) each other to MS medium supplemented with 1 $mg.L^{-1}$ BA and 0.1 $mg.L^{-1}$ IBA on shoots multiplication of pear *Pyrus syriaca*, they found the best value of shoot multiplication and shoot length achieved in media supplemented with 15 $gm.L^{-1}$ sucrose was (6.8 shoot and 78.6 mm). Yaseen *et al.*[24] used different type of sugars (sorbitol, sucrose, Glucose or Mannitol) and its concentrations (0, 5, 15, 25, 35 and 45 $g.L^{-1}$) as a carbon source added to MS medium supplemented with 1.5 $mg.L^{-1}$ BAP and 0.4 $mg.L^{-1}$ NAA, as affected on apple rootstock M_9 and M_{26}. After four weeks, the results showed that the best multiplication of shoots was in media supplemented with 35 $g.L^{-1}$ of all types of sugars except the Mannitol. The sorbitol significantly increased the No. of shoots compared with sucrose and Glucose achieved (9.8, 7.9 and 3.4 shoots) respectively for M_9 rootstock in comparison with (4.7, 4.0 and 2.6 shoots) for M_{26} rootstock. Abu Raya *et al.* (2010) cultured single node of the almond plant on MS medium supplemented with 2 $mg.L^{-1}$ BA and 0.1 $mg.L^{-1}$ IBA and 0.1 $mg.L^{-1}$ GA_3 with adding different concentrations (0, 10, 20, 30 and 40 $g.L^{-1}$) of Glucose or fructose or sucrose. They noticed that the shoot multiplication increased in medium supplemented with 40 $g.L^{-1}$ sucrose or Glucose (60 and 55 shoots) respectively after 6 weeks.

On the other hand, Amiri [5] cultured Almond shoot tips *Prunus amygdalus* cv. Binazir in different conc. (0, 0.2, 1 and 2) of Fossard nutrient media (de Fossard, 1976) containing 0.75 $mg.L^{-1}$ BA and 0.75 $mg.L^{-1}$ NAA. After 8 weeks, The results observed the best shoot multiplication was (7.8 shoots) in medium with double conc. of nutrient. Another study conducted by Anirudh and Kanwar [6] on wild trees of Japanese pear *Pyrus pyrifolia* (Burn F.) cv. Nakai, cultured on MS Medium with full strength or half strength of nutrient media or MS with 400 $mg.L^{-1}$ Ammonium nitrate (NH_4NO_3) and WPM supplemented 1.5 $mg.L^{-1}$ BA interacted with 0.5 $mg.L^{-1}$ IBA on shoot multiplication. The best results were in shoot cultured on WPM achieved (11.20 shoot) in comparison with the full strength of MS medium achieved (10.21 shoots) after 8 weeks.

Materials and Methods

This study was conducted in the Laboratory of Tissue and Plant Cell culture/Department of Horticulture and Landscape Design/ College of Agriculture and Forestry/the University of Mosul. The stem cutting was collected from soft shoot newly grown 5-6 cm in length, the donor plants of Othmany cv. growing in the Lath House, transported to the laboratory, the old leaves were removed, except for 2-3 leaves near to the end of shoot tip, then the shoots were washed in tap water for 30 minutes and then transferred to the Laminar-flow cabinet for to sterilize the shoot surface, which was done by Immersing in $HgCl_2$ solution at the concentration of $1g.L^{-1}$ for 20 minutes[8]. The shoot washed with distilled and sterile water four times in a row with constant stirring and then transferred to sterile Petri dishes and attended the ends of the Apical shoots 1cm length. During the Initiation stage, the shoot tips were planted on solid MS medium supplemented with 1 mg BA (Benzyl

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Adenine). After four weeks, it was possible to obtain enough tissue mother farms to carry out the multiplication stage which contain two experiments, first experiment: study the effect of adding different types of sugars (sucrose, Glucose or mannitol) at a concentration of 30 $g.L^{-1}$ each to MS [13] or WPM [11] media, to multiplicate the shoots taken in 1 cm length, while in the second experiment: studied the effect of different concentrations of media (half the concentration of nutrient, full concentration of nutrient, one and a half concentration of nutrient, double the concentration of nutrient), With adding 2.5 $mg.L^{-1}$ BA + 0.25 $mg.L^{-1}$ IBA. The experiments were carried out at 10 replication per treatment and one shoot per replicate. After 8 weeks of multiplication, the parameter was taken Was: a number of shoots, shoots lengths (cm) and the number of leaves/shoots, although the shoots were re-cultured without cutting in the same medium, every 4 weeks after the beginning of each experiment above.

The experiments conducted as factorial with 10 replicate per treatment [3]. The data analyzed by using (SAS, 2001) and data tested statistically by using Duncan multiple tests at 0.05 [7].

Results and Discussion

I: Effect of sugar type and nutrient medium and interaction in shoots multiplication.

Shoots number:

The results are shown in Table (1) and Figure (1) that there is no significant difference between the two-type median effect in the number of shoots, with 3.70 shoot/shoot obtained in WPM media compared to 3.44 shoot/shoot in MS media. while the effect of the types of sugars, it is noted that sucrose had the best effect in stimulating the multiplication, and achieved the largest number of shoots in media supplemented with 5.39 shoots/shoot and differed significantly from medium that contained Glucose or mannitol, which were 3.29 and 2.04 shoots/shoot respectively, and did not differ significantly between them. The reason why sucrose is superior to Glucose may be due to the fact that sucrose is a binary sugar that, when sterilized with high temperature, turns into Glucose and fructose, that the shoot used the Glucose and then fructose [4]. thus, the effect of sucrose sugar is higher through the role of both Glucose and fructose compared to the role of Glucose alone.

Rather than, the superiority of sucrose over mannitol, it may be due to the combination of mannitol (as alcoholic), which did not succeed in providing the right conditions for multiplication. Used in the nutrient medium. Salman [18] stated that Roberts [16] had indicated that cellular differentiation could be strongly influenced by the concentration and quality of carbohydrates used in the nutrient medium.

These results are consistent with what Shatnawi *et al*. [19] when studying pear culture *Pyrus syriaca In vitro* that sucrose had the best effect in multiplying of shoot compared to Glucose.

It should be noted from the same table that the interaction of the nutrient medium and types of sugars had a significant effect in the multiplication of shoots, which achieved the largest number of shots from the shoots cultured medium with sucrose-equipped (5.38 and 5.40 shoots/shoot) for both MS and WPM medium respectively, so it did not differ significantly with medium contained Glucose, but differed significantly from media contained mannitol, which caused the lowest multiplication of shoot that achieved 2.38 and 1.70 shoots/shoot for MS and WPM medium, respectively.

The reason why sucrose overcome of Glucose and mannitol when added to the MS and WPM medium is due to the different roles played by each of them in their impact on the nutrient media and then on the response of the shoot to multiplication. Al- Kanani (1987) noticed that carbohydrates play two roles in tissue culture, provide the energy needed for the growth of the shoot and the minimum exudation pressure of the nutrient medium, which certainly varied according to the type of sugar and the nutrient medium and the common effect of their intervention reflected its impact on the eternal relations of the nutrient medium and the energy processed from it, then to the shoot Consequently, the effect of sugars has varied in causing multiplication from one medium to another.

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Table(1): Effect of sugar type and nutrient medium and their interaction supplemented with 2.5 mg.L^{-1} BA and 0.25 mg.L^{-1} IBA on shoots number after 8 weeks.

Type of sugar	WPM medium	MS medium	Effect of sugar
Sucrose	5.38 a	5.40 a	5.39 a
Glucose	2.57 ab	4.00 ab	3.29 b
Mannitol	2.38 b	1.70 b	2.04 b
Effect of Medium	3.44 a	3.70 a	

*Means in each column which have the same letter did not differ significantly at p> 0.05.

Shoots lengths (cm):

The data in the table (2) showed, there is no significant effect of the nutrient medium in shoot lengths, The effect of sugar type on shoot lengths, was best in the medium supplemented with Glucose, which reached 2.90 cm and did not differ significantly from the medium supplemented with sucrose that achieved 2.73 cm, but differed significantly from the medium supplemented with mannitol (1.56 cm). So, there was no significant difference in the shoot lengths between the media supplemented with sucrose and mannitol.

The reason for the superiority of Glucose over sucrose to increase shoot lengths may be due to the increase in the number of shoots formed in sucrose-equipped medium (Table 1), which reduced their chances of obtaining nutrients and thus reduced their lengths.

These results are consistent with what Abou Rayya *et al*,[1] found when they multiply the almond, that Glucose has a greater effect than sucrose in increasing shoot lengths. Moreover, the effect of the interaction between the nutrient medium and the type of sugar added to the medium in shoot lengths, it is noted that the addition of Glucose to the medium WPM achieved the highest means of shoot lengths (4.06 cm), which is significant difference to all other interactions except the interactions of sucrose with MS medium, which formed shoots amounted to an Its length is 3.22 cm.

The difference in the effect of sugars from one medium to another in increasing shoot lengths may be caused by the balance of these two factors, which in turn provided the best state for the growth and elongation of shoots.

Data in tables 1 and 2, was showed that the addition of mannitol, which is considered form alcoholic sugars to the nutrient medium, did not play any role in stimulating the multiplication of shoots and for all the characters studied, but rather discouraged the growth of the shoot, possibly due to the osmosis tension in the nutrient medium it contains. Vitova *et al*,. [23] noted that the osmosis resulting from the addition of high concentrations of mannitol (30-35 g.L^{-1}) restricts or reduces the benefit of the processing of mannitol energy or carbon sources for the shoots, which is lower than other types of sugars. Hilae and Te Chato [10], have indicated that the presence of the factors causing the osmosis stress with high concentrations leads to damage to the leaves.

Table(2): Effect of sugar type and nutrient medium and their interaction supplemented with 2.5 mg.L^{-1} BA and 0.25 mg.L^{-1} IBA on shoots length after 8 weeks.

Type of sugar	MS medium	WPM medium	Effect of sugar
Sucrose	3.22 a	2.24 b	2.73 ab
Glucose	1.73 b	4.06 a	2.90 a
Mannitol	1.53 b	1.58 b	1.56 b
Effect of Medium	2.16 a	2.63 a	

*Means in each column which have the same letter did not differ significantly at p> 0.05.

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Figure (1): Effect of sugars and nutrient media shoots multiplication after 8 weeks of cultivation

Results shown in table (3) show no significant differences between the two types of medium in the number of leaves formed on shoots, with 27.33 leaves/shoot for WPM versus 25.11 leaves/shoot for MS medium. As for as, the effect of the type of sugar added to the nutrient medium, it is noted that there is a significant difference between the types of sugars in number of leaves formed on the shoots, as it is the highest No. of leaves in media supplemented with sucrose and reached 40.28 leaves/shoot and significantly differ from medium supplemented with Glucose which achieved 25.94 leaves/shoot, which differed significantly from medium supplemented with Mannitol achieved (12.45 leaf/shoot).

Table(3): Effect of sugar type and nutrient medium and their interaction supplemented with 2.5 mg.L^{-1} BA and 0.25 mg.L^{-1} IBA on leaves number after 8 weeks.

Type of sugar	WPM medium	MS medium	Effect of sugar
Sucrose	42.25 a	38.30 a	40.28 a
Glucose	16.57 ab	35.30 a	25.94 b
Mannitol	16.50 b	8.40 b	12.45 c
Effect of Medium	25.11 a	27.33 a	

*Means in each column which have the same letter did not differ significantly at p> 0.05.

The reason why the different types of sugars have different in No. of leaves/shoot (Tables 1 and 2) maybe because these sugars provide energy to the shoot, which it's

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characterized by its ability to self-feed and need the energy of sources [4]. As soon as the effect of the interaction between the type of sugars and the concentration of nutrient media is clear from table (2 and 3) that MS and WPM medium supplemented with sucrose achieved a higher values of leaves number was 42.25 and 38.30 leaves/shoot, while the lowest number of leaves was in media supplemented with mannitol. The reason for the different number of leaves formed in media supplemented with different types of sugars is explained by van Huylenbroeck and Debergh [22] that some sugars added to the nutrient medium inhibit or decrease the manufacture of chlorophyll and photosynthesis, possibly due to their effect on osmotic pressure [2].

II: Effect of MS or WPM nutrient conc. on multiplication of shoots.

Shoots number:

Data in the table (4) and figure (2) showed, there is no significant difference between the two types of media in shoot number. With regard to the effect of concentration of nutrients, it is noted from the same table that the increase in concentration of nutrient in media reduced the ability of the shoot to multiply, with the largest number of shoots in the treatment of half concentration of nutrient and 4.98 shoots/shoot, which did not differ significantly from the treatment of full concentration or 1.5 concentration of nutrient, which caused less than doubling with 2.55 shoots/shoot segments.

The decrease in the number of shoots formed in the nutrient medium with the high concentration of their nutrients can be explained on the basis that the absorption of nutrients by the shoot is caused by the increased application of the negative osmosis of the cells of the plant segment on the application of the negative stress of the nutrient medium and when the concentration increases The nutrients in medium reflected the situation of nutrient medium negatively osmosis and became higher than the of shoot cells, which negatively affected the absorption of nutrients from the nutrient medium and thus the weakness of the growth and multiplication of the shoot [12]. Whereas the effect of the interaction between the two types of medium and the concentration of their nutrients, it is noted that the best rate of the number of shoots is in WPM medium with the full concentration of its nutrients, that giving 6.00 shoots/shoot, which is not significantly different from the other interaction except the interacted of MS and WPM with half the concentration of nutrients, which consisted of 2.00 and 3.10 shoots/ shoot respectively.

The difference in the effect of MS nutrient concentration from WPM may be due to the high concentration of nitrogen for the medium MS or the high concentration of its total nutrients [20] or possibly due to the different quality of the composition of nutrients for the media and their concentrations such as nitrogen and calcium added like $Ca(NO_3)$ to WPM medium, which is more plant-ready than NH_4NO_3 and $CaCl_2$ in MS medium [21] or may be due to the difference in the osmosis tension in the nutrient media resulting from the different concentrations of its constituent mineral nutrients [12]. These results are agreed with Banno *et al*. [9] and Anirudh and Kanwar [6] when they breed pear trees in different media, noting that the WPM medium was best at stimulating the multiplication of branches from MS medium with the full concentration of nutrients or half concentration.

Table(4): Effect of MS or WPM nutrient conc. and their interaction supplemented with 2.5 mg.L^{-1} BA and 0.25 mg.L^{-1} IBA on shoots number after 8 weeks.

Nutrient Conc.	Media		Effect of Conc.
	Medium MS	Medium WPM	
0.5	5.40 ab	4.56 ab	4.98 a
1	3.90 a-c	6.00 a	4.95 a
1.5	3.80 a-c	5.56 ab	4.68 a
2	2.00 c	3.10 bc	2.55 b
Effect of Media	3.78 a	4.81 a	

*Means in each column which have the same letter did not differ significantly at p> 0.05.

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Shoots length (cm):

From the results of table (5), it is clear that there is a significant effect of the nutrient medium in shoot lengths in MS medium achieved 3.46 cm compared to 2.42 cm in the WPM medium. On the other hand, there was no significant difference in the lengths of shoots developing in all the concentrations of the studied medium nutrients. The interaction between the concentration of the medium nutrient and the type of nutrient medium, it is noted that the highest length of shoots obtained from shoot cultured in MS medium with a full concentration of nutrient, which reached 4.22 cm and did not differ significantly from other treatments except the WPM medium with half concentration of its nutrients and the full concentration which achieved lowest length of shoots1.99 and 2.11 cm respectively.

The reason for the low length of shoots growth in WPM medium with full concentration of its nutrients may be due to the high number of Shoots formed in this medium (Table 5), which has led to competition between the shoots to nutrient, especially if taken into account the inappropriateness of the osmosis relationships of other media to The shoot multiplication despite the high amount of nutrient (Table 5).

These results are agreed with Anirudh and Kanwar [6] when they attempt to reproduce the Japanese pear *Pyrus pyrifolia* (Burm F.) Nakai by tissue culture as we showed that the full-concentration of MS achieved the best lengths of shoot compared to the MS medium with half concentration or WPM media conc.

Table(5): Effect of MS or WPM nutrient conc. and interaction between them in shoots length (cm) after 8 weeks.

Nutrient Conc.	Media		Effect of Conc.
	Medium MS	Medium WPM	
0.5	2.51 ab	1.99 b	2.25 a
1	4.22 a	2.11 b	3.17 a
1.5	3.00 ab	2.66 ab	2.83 a
2	4.10 a	2.93 ab	3.52 a
Effect of Medium	3.46 a	2.42 b	

*Means in each column which have the same letter did not differ significantly at p> 0.05.

Leaves number:

Table (6) showed there is no significant difference in the number of leaves formed on the shoots. the highest number of leaves developing on WPM media that achieved 41.54 leaf /shoots, while the No. of leaves on MS medium was 34.08 leaves/shoots.

As for the effect of concentration of nutrient media, the same table showed that the highest number of leaves was from in the shoots cultured in the full concentration of nutrients (46.97 leaves/shoots), which was not significantly different from the treatment of 0.5 and 1.5 concentrations, but it differs significantly to the treatment of double concentration of nutrients achieved the lowest number of leaves and 26.97 leaves/shoots.

The interaction between the two types of medium and the concentration of their nutrient, it is noted from the same table that the largest number of leaves are in the shoots cultured on the WPM medium with a full concentration of nutrients and reached 53.33 leaves/shoots, which did not differ significantly from the treatment of 1.5 concentration of WPM and half and the full concentration of MS media but significantly differs from other treatment.

The reason why the number of leaves overcomes developed from shoots cultured in full conc. of WPM, may be due to the effect of this treatment in giving the largest number of total shoots (Table 4) and the largest number of shoots longer than 0.5 cm (Table 5).

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MS Medium

WPM Medium

Figure (2): Effect of types and concentrations of Nutrient media in multiplication of shoot tip after 8 weeks of culture.

(0.5)= Half strength of nutrient. (1)=Full strength of nutrients.

(1.5)= Full and half strength of nutrient. (2)= double strength of nutrients.

Table(6): Effect of MS or WPM nutrient conc. and interaction between them in Leaves' number after 8 weeks.

Nutrient Conc.	Media		Effect of Conc.
	Medium MS	Medium WPM	
0.5	42.80 ab	35.56 bc	39.18 ab
1	40.60 ab	53.33 a	46.97 a
1.5	31.60 bc	44.67 ab	38.14 ab
2	21.33 c	32.60 bc	26.97 b
Effect of Medium	34.08 a	41.54 a	

*Means in each column which have the same letter did not differ significantly at p> 0.05.

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