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NOTE

THE EFFECTS OF DIFFERENT KINDS AND CONCENTRATIONS OF CARBON SOURCE ON *IN VITRO* SHOOT MULTIPLICATION OF PERSIAN WALNUT

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ABSTRACT

This experiment was carried out to study the effects of different carbohydrate sources (fructose, glucose and sucrose) at different concentrations (20, 30 and 40 g l^{-1}) on shoot multiplication of Persian walnut (*Juglans regia* L.). *In vitro* propagated shoot tips of *J. regia* L. cv. 'Serr' were cultured on DKW medium containing 1.0 mg l^{-1} 6-benzylaminopurine (BA) and 0.01 mg l^{-1} indole-3-butyric acid (IBA) solidified with 2.2 g l^{-1} Phytagel. No significant differences were observed among the different kinds of carbohydrates for shoot fresh weight and main shoot length. Maximum callus fresh weight at basal ends of shoots was obtained with glucose and this was significantly greater than fructose or sucrose. Explants cultured on media containing glucose produced maximum number of axillary shoots. Different concentrations of carbohydrates did not have any significant effect on shoot fresh weight and main shoot length. There was a significant difference among the three different concentrations

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of carbohydrates for callus fresh weight and axillary shoot numbers. The best microshoots were obtained using 20 g l^{-1} sucrose.

Key words: Carbon sources, *In vitro* shoot multiplication, Persian walnut.

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اثرهای انواع مختلف کربوهیدرات با غلظت های متفاوت بر افزونگری شاخساره درکشت درون شیشه ای گردوی ایرانی

یوسف علی سعادت و مایکل هنرتی

به ترتیب دانشجوی سابق دکترای علوم باغبانی (اکنون استادیار پژوهشی مرکز تحقیقات منابع طبیعی و امور دام استان فارس) و استاد باغبانی دانشگاه کالج دوبلین، دوبلین، ایرلند.

چکیده

این پژوهش به منظورمطالعه اثرهای انواع مختلف کربوهیدرات (سوکروز، فروکتوز و گلوکز) با غلظت های متفاوت (۲۰، ۳۰ و ٤۰ گرم در لیتر) بر افزونگری شاخساره درگردوی ایرانی (*Juglans regia* L.) انجام گردید. نوک شاخساره های تولید شده درشرایط درون شیشه ای از رقم 'Serr' بعنوان ریزنمونه روی محیط کشتDKW دارای ۱ میلی گرم درلیتر بنزیل آدنین (BA) و ۰/۱ میلی گرم درلیتر ایندول بوتیریک اسید (IBA) که با ۲/۲ گرم در لیترفیتاجل نیمه جامد شده بود کشت شدند. تفاوت معنی داری بین کربوهیدرات های مختلف برای وزن تر شاخساره وطول ساقه اصلی مشاهده نگردید. حداکثر وزن تر پینه با استفاده از گلوکز درته شاخساره ها تشکیل شد که به طور معنی داری بیشتر از سوکروز و فروکتوز بود. ریزنمونه های کشت شده روی محیط کشت های دارای گلوکز حداکثر شاخساره های جانبی را تولید نمودند که به طور معنی داری بیشتر

از تیمارهای دارای فروکتوز بودند اما با تیمارهای دارای سوکروز تفاوت معنی داری نداشتند. غلظت های مختلف کربوهیدرات تاثیر معنی داری بر وزن تر شاخساره و طول ساقه اصلی نداشتند، اما بر وزن تر پینه و تولید شاخساره های جانبی تاثیر معنی دار نشان دادند. با استفاده از ۲۰ گرم در لیتر سوکروز بهترین شاخساره ها ایجاد شدند.

INTRODUCTION

Normally for the culture of plant cells, tissues or organs, it is necessary to incorporate a carbon source into the medium. Most researchers have used 30 g l^{-1} sucrose in walnut culture media (5, 8, 9, 10, 11). However, when *in vitro* proliferation of *Juglans regia* L. was studied in relation to the carbon source by Gruselle *et al.* (4), the best result was obtained with 30 g l^{-1} fructose. The addition of 100 mg l^{-1} ammonium dihydrogen phosphate ($NH_4H_2PO_4$) to the basal medium containing the original G6 (3) macroelements and 30 g l^{-1} fructose greatly improved the shoot proliferation of walnut. Marques Silva and Dias (6) reported that in *in vitro* cultures of Persian walnut media containing fructose and glucose produced more and longer shoots than medium with sucrose. No factorial comparisons of different carbon sources and their concentrations have been reported for walnut shoot multiplication. This experiment was designed to study the effects of different carbon sources at different concentrations on *in vitro* growth of *J. regia* L.

MATERIALS AND METHODS

In vitro propagated shoot tips of *Juglans regia* L. cv. 'Serr' with two to three leaves 0.5 cm in length were cultured on DKW medium (7) containing 1.0 mg l^{-1} BA and 0.01 mg l^{-1} IBA solidified with 2.2 g l^{-1} Phytagel. The pH of media was adjusted to 5.8 ± 0.05 before the addition of gelling agent and autoclaving. The cultures were grown at 27±1 °C, in a 16-hr photoperiod under cool white Philips fluorescent lamps producing a Photosynthetically active radiation (P.A.R) of 75µmol m^{-2} s^{-1} Electrical conductivity of media was measured using a portable electroconductivity

meter after removing the shoots from vessels at the end of the experiment. Data of callus fresh weight, shoot fresh weight, main shoot length, axillary shoot numbers and bud number on shoots were collected after five weeks.

The experiment was laid out as a factorial experiment with three different carbohydrates (fructose, glucose and sucrose) as factor A, and three different concentrations (20, 30 and 40 g l^{-1}) of carbohydrate as factor B. Treatments were arranged in a completely randomized design with five replications. A Magenta GA_7 vessel with polypropylene closures containing 50 ml of medium and five explants was used as a replicate. Statistical analysis was performed using the programme GLM on SAS (1). Mean comparisons were performed by Tukey's Studentized Range (HSD) test.

RESULTS

No significant differences were observed between different kinds of carbohydrate for shoot fresh weight and main shoot length (Table 1). Maximum callus fresh weight at the basal ends of shoots was obtained with glucose and this was significantly greater than fructose or sucrose. Explants cultured on media containing glucose produced the most axillary shoot numbers and this was significantly different from fructose-containing media, but not significantly different from media with sucrose. There was a significant difference for bud number on shoots between fructose and sucrose, but neither of these was significantly different from glucose (Table 1).

Different concentrations of carbohydrates did not have any significant difference on shoot fresh weight and main shoot length (Table 1). There was a significant difference among the three concentrations of carbohydrates for callus fresh weight and axillary shoot numbers. Bud number on shoots of explants cultured on media containing 20 g l^{-1} and 30 g l^{-1} carbohydrate did not show significant differences, but both of them were significantly different from treatments containing 40 g l^{-1} carbohydrate. Although bud number on shoots in media containing 40 g l^{-1} sugar was maximum, the shoot morphology was not good and chlorosis of leaves was observed. At 20 g l^{-1} carbohydrate, the number of axillary shoots

was the least with larger leaves, but the appearance of the microshoots was the best.

Table 1. *In vitro* growth of *Juglans regia* L. explants on different kinds and concentrations of carbohydrates†.

Carbohydrate source	Callus fresh weight (g)	Shoot fresh weight (g)	Main shoot length (cm)	Axillary shoot numbers	Bud number on shoots	Electrical conductivity (m S cm^{-1})
Fructose	1.38[a]	1.16[a]	5.10[a]	2.13[a]	21.25[a]	1.37[a]
Glucose	1.98[b]	0.99[a]	4.00[a]	3.35[b]	24.97[ab]	0.64[b]
Sucrose	1.27[a]	1.09[a]	4.30[a]	2.95[ab]	26.65[b]	1.72[a]
Carbohydrate concentration						
20 g l^{-1}	0.87[a]	1.02[a]	4.18[a]	1.41[a]	19.76[a]	2.58[a]
30 g l^{-1}	1.40[b]	1.12[a]	4.95[a]	2.73[b]	23.83[a]	0.94[b]
40 g l^{-1}	2.31[c]	1.09[a]	4.21[a]	4.13[c]	28.72[b]	0.35[c]
Interaction	ns	ns	ns	*	*	*

† Each value is the mean of 75 explants, and values followed by the same letter are not significantly different (P< 0.05).

* Significant (P<0.05).

ns Not significant (P<0.05).

Electrical conductivity of media was measured at the end of experiment and a significant difference was observed between glucose and other kinds of carbohydrates (Table 1). Electrical conductivity of media containing 40 g l^{-1} carbohydrate was the least and showed a significant difference from media containing 30 g l^{-1} or 20 g l^{-1} carbohydrate. Electrical conductivity of media containing 30 g l^{-1} carbohydrate was also significantly different from media containing 20 g l^{-1} The interactions of different carbohydrates and their concentrations for shoot fresh weight, callus fresh weight, and main shoot length were not significant, but were significant for axillary shoot numbers, bud number on shoots, and electrical conductivity (Table 1). A significant interaction indicated that different sources of carbohydrates affected axillary shoot numbers, bud number on

shoots and electrical conductivity differently at each level of sugar concentration.

DISCUSSION

In this study no significant difference was obtained for shoot fresh weight and main shoot length by using different types of carbohydrate (fructose, glucose and sucrose). Maximum and significantly greater callus fresh weight and axillary shoot numbers per explant were obtained on media containing glucose rather than on other carbohydrates (Table 1). This is in contrast with the results of Gruselle *et al.* (4) who reported that the best results were obtained using fructose at 30 g l^{-1} This difference might be due to using different basal nutrient media (DKW vs G6) or explants originating from different cultivars of *J. regia* L. In contrast to the results of Yu and Reed (12) for hazelnut, general appearance and growth habit of cultures were the best on sucrose and the worst on glucose. This difference in response might be due to different requirements of the plant species. In general, it seems that sucrose is a good source of carbohydrate for *in vitro* culture of Persian walnut and confirms the idea of George (2) that sucrose is the best carbohydrate for plant tissue culture.

The number of axillary shoots per explant and callus fresh weight of explants cultured on media containing 40 g l^{-1} carbohydrate was significantly greater than on media containing 30 g l^{-1} carbohydrate and both of them were significantly greater than on media containing 20 g l^{-1} carbohydrate (Table 1). This confirms the results reported by Gruselle (4) that 30 g l^{-1} carbohydrate was better than 15 g l^{-1} carbohydrate, which caused vitrification of explants. Despite no significant difference for main shoot length of explants, cultures on media containing fructose were longer than those on media containing sucrose, and this is in agreement with the reports of Marques Silva and Dias (6) for Persian walnut and Yu and Reed (12) for hazelnut. The electrical conductivity of the media at the end of the experiment and the callus fresh weight of explants were significantly different among the three different concentrations of carbohydrates in this research (Table 1). As the concentration of the carbohydrates in the media was increased, the amount of callus fresh weight increased, but the

electrical conductivity of the media decreased. In addition, chlorosis and deficiency symptoms were obvious in cultures on media containing 30, or 40 g l^{-1} carbohydrates. In addition, there were no significant differences for shoot fresh weight of explants among the different concentrations of carbohydrate (Table 1). It can be concluded that the chlorosis and deficiency symptoms of shoots on media containing 30 or 40 g l^{-1} carbohydrates, were due to the formation of callus, and the media nutrients being used for production of callus or accumulated in callus. The low electrical conductivity of media containing 30 or 40 g l^{-1} carbohydrates also supports this hypothesis. Moreover, the shoots produced on these media were not suitable for rooting. The general appearance and growth habit of cultures on media containing 20.0 g l^{-1} carbohydrates was best, with well developed leaves without chlorosis, or deficiency symptoms, and the electrical conductivity of media was higher than with other concentrations of carbohydrates. Therefore, it can be concluded that for commercial work it is preferable to use low concentrations of sugar for better quality shoots, which are optimum for rooting.

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