World Journal of Agricultural Sciences 2 (2): 156-161, 2006
ISSN 1817-3047

Influence of Different Carbon Sources on Wild Pear *(Pyrus syriaca)* Growth and Sugar Uptake

[1]*Mohamad A. Shatnawi,* [2]*Rida A. Shibli,* [3]*Hussein Migdadi,*
[2]*Ahed Obeidat,* [2]*Khalil Ereifej and* [2]*Abdalleh M-Abu-Ein*

[1]Faculty of Agriculture Technology, Al-Balqa Applied University, Al-Salt 19117, Jordan
[2]Faculty of Agriculture, Jordan University of Science and Technology, P.O. Box 3030, Irbid, Jordan
[3]National Center for Agricultural Research and Technology Transfer (INCARD), Baqa 19381, Jordan

Abstract: *In vitro* proliferation and rooting of *Pyrus syriaca* were tested on Murashige and Skoog (MS) medium enriched with varying concentrations (0.0, 1.5, 3.0, 4.5, or 6.0%) of sucrose, glucose or fructose as main carbon sources. The morphological characteristic, content of sucrose, fructose or glucose in shoot, root and in medium were studied. Increasing the sucrose concentration did not have any effect on dry weight, while the supplement of glucose caused a significant increase in dry weight. Fructose did not cause any significant differences. Rooting occurred with the medium supplemented with sucrose at various concentrations and low fructose concentrations 1.5-3%. No rooting occurred on medium supplemented with glucose. Examination of the change in total carbon source content showed that accumulation of sucrose, fructose and glucose appeared to be varied. Growth of *Pyrus syriaca* and accumulation of carbon sources under *in vitro* conditions are largely influenced by the composition of the carbon source in the culture medium.

Key words: Fructose · sucrose · glucose · micropropagation · *Pyrus syriaca*

INTRODUCTION

Pyrus syriaca (wild pear) grows wild in Jordan, it is considered to be highly suitable by the growers in the region. It is grown in marginal and dry areas in a wide range of soil types and considered to be tolerant to many biotic and abiotic stresses. *P. syriaca is* becoming an endangered species due to deforestation and continuous removal by people. However, micropropagation techniques have been used for the rapid and large-scale propagation of *P. syriaca* [1]. The main characteristic of this technique is the production of a large number of uniform mother stock plants. Thus plants produced are usually of a superior quality and better health compared with conventional propagation methods [1, 2].

Sucrose has been considered the most common carbon source used in plant tissue cultures due to its efficient uptake across the plasma membrane [3-6]. Previous studies have shown that successful *in vitro* cultures have an inadequate photosynthetic ability and to acquire sugar as a carbon source for hetro or mixtrophic growth [7]. Ebrahim *et al.* [8] reported the presence of sucrose in the medium increases the intracellular sucrose concentration. Increasing the concentration of sugar in the medium not only increases the availability of carbon sources but also stimulates growth as a result of more negative water potential in the medium [9, 10]. On the other hand a study by Cunha and Fernandes-Ferreira [11] on *Linum usitatissimum* showed that medium supplemented with monosaccharides (glucose or fructose) at concentration of 4% gave consistently highly embryogenic cultures with higher somatic embryo frequencies and higher growth rate compared with medium supplemented with either sucrose or maltose.

Soluble sugars, mainly glucose, fructose and sucrose are preferred as the substances responsible for osmotic adjustment in tissue water osmotic stress [12]. In addition, a study by De Reik *et al.* [10] on *Rosa multiflora* indicated that hydrolysis of sucrose in the culture medium occurred in both multiplication and root induction stage. Sucrose before being utilized in metabolic activity has to split up into its constituent monosaccharides by the enzyme invertase [4]. The disaccharide sucrose is widely used as energy source and/or osmoticum in plant cell and tissue cultures, it also improves root system quality in

Corresponding Author: Dr. Mohamad A. Shatnawi, Faculty of Agriculture Technology, Al-Balqa Applied University, Al-Salt 19117, Jordan

Carica papaya [13]. However, sucrose is not always the best carbohydrate to achieve best multiplication and regeneration. Fructose and raffinose increased the number of embryos produced in *Spinacia oleracea* as reported by Komai *et al.* [14].

Different plant species showed differences in their preference to the various forms of sugar [9]. Replacing a sugar with another could initiate embryogenesis [15]. As little information is available on the effect of different components of carbon sources on the *in vitro* micropropagation of *Pyrus syriaca,* we addressed this question in the present study. Morphogenic responses of *P. syriaca* and quantitative analysis of the carbohydrate in the media and the cultures were studied as a function of plant growth *in vitro*.

MATERIAL AND METHODS

One year buds of mature dormant wood of wild pear were collected from a single healthy wild pear (*Pyrus syriac*a) in Jarash (North Jordan). Wood was cut into 10-15 cm long, the basal ends were immersed in tap water and kept at room temperature at 24±°C 2/day, 18 ±2°C night for 3 weeks until buds broke and new growth was initiated [1]. Bud scales were excised and buds were surface sterilized in 5% chlorox (5.25 sodium hypochlorite) plus 0.1 Tween-20 (surfactant) for 10 min and then rinsed three times with sterile distilled water (for 5 min each time) under laminar air flow cabinet. Shoot tip (5 mm) were excised aseptically and inoculated on ½ strength MS medium [16] containing 0.1 g L^{-1} polyvinylpyrrolidine (PVP). The medium was supplemented with 0.5 mg L^{-1} benzyl adenine (BA) and 0.1 mg L^{-1} napthalenean acetic acid (NAA), 30 g L^{-1} sucrose and 8.0 g L^{-1} Difco Bacto agar. Medium pH was adjusted at 5.8 prior sterilization. Cultures were kept in the dark for at least 2 d, then moved to the growth chamber (24±2°C), under 16 h light (photosynthetic flux 40-50 μmol m^{-2} S^{-1}) /8 h dark photoperiod. Shoot tips were transferred to fresh medium every 3 d, for three times to minimize phenolic exudation and browning. Two weeks later microshoots were transferred to fresh media before starting experimentation. Subculturing was performed every four weeks to establish a massive mother stock culture before initiating experiments.

Microshoots were subcultured on 50 mL media (in 250 mL Erlenmeyer flask) supplemented with sucrose, fructose or glucose at 0.0, 1.5, 3.0, 4.5 or 6.0%) using shoot proliferation medium containing 1.0 mg L^{-1} BA and 0.1 mg L^{-1} IBA [1]. As similar experiment was also done by subculturing microshoot to rooting medium containing 0.2 mg L^{-1} IBA [1]. Each treatment consisted of 20 replicates arranged in a completely randomized design and the experiments were repeated five times. Growth parameters were taken after eight weeks on shoot number, shoot length, fresh weight and dry weight. Medium sample shoots and roots samples were used for analysis carbohydrate.

Dry Samples of 2.0 g were placed in test tube in 10 mL of 80% ethanol and vortex for 30 min, then kept on water bath shaker at 75 rpm at 30°C for 30 min. Samples were then centrifuged at 3000 rpm for 8 min. The supernatant was collected and the extraction was repeated twice. Supernatant was mixed with 2 mL lead acetate and 2 mL of oxalic acid and vortex to precipitate protein and fat. Then the whole mixture was centrifuge for 8 min at 3000 rpm. The supernatant was then taken to a 50 mL flask and the total volume was made to 50 mL by adding deionized distilled water, then 0.1 mL sample was taken and mixed with 0.2 mL phenol and 5 mL H_2SO_4. The sample was then kept for 20 min at 30°C with shaking. Sugar was then analyzed using spectrophotometer-phenol sulfuric acid method [17]. Collected data were statistically analyzed using analysis of variance and means were separated according to the least significant differences (LSD) at 0.05 level of probability using MSTATC.

RESULTS AND DISCUSSION

Growth of *in vitro* culture is strongly influenced by the different carbon sources (Table 1). Moreover, fructose showed no effect on number of shoots of *P. Syriaca.* Previous study by Shibli *et al.* [1] shows that sucrose has been used as carbon sources (at low concentration 3%) in the nutrient media for both *in vitro* propagation and *in vitro* rooting of *Pyrus syriaca.* Increased sucrose, or fructose concentrations were inhibitory to root formation (Table 2). While at higher sucrose concentration there were decrease in shoots dry matter (Table 1). Maximum shoot dry weight was recorded at 3% sucrose (Table 2). However, the decrease in accumulation of dry matter of cultures grown under higher supply carbon sources might be due to decrease in water potential of the medium [9, 18]. Moreover, the hydrolysis of sucrose in the culture medium associated with sucrose hydrolosing enzymes involved in a cycle of sucrose adsorption and/or uptake and subsequent secretion of glucose and fructose to the cytosol caused a decrease and accumulation of dry weight at high concentration [10].

Table 1: Growth responses of *in vitro* grown wild pear (*Pyrus syriaca*) to different concentrations of sucrose, fructose or glucose. Medium supplemented with 1.0 mg L^{-1} BA and 0.1 mg L^{-1} IBA

Carbon source	Shoot number	Shoot length (mm)	Fresh weight (g)	Dry weight (g)
Sucrose (%)				
0.0	0.0d	0.0c	0.0c	0.0c
1.5	6.8a	78.6a	0.703a	0.136a
3.0	4.6ab	67.0a	0.774a	0.139a
4.5	2.6bc	53.0b	0.39b	0.108ab
6.0	1.8cd	39.4b	0.294b	0.076b
Fructose (%)				
0.0	1.0a	19.8a	0.154c	0.012e
1.5	1.0a	31.2a	0.519a	0.089a
3.0	1.0a	21.6a	0.436ab	0.067b
4.5	1.0a	23.2ab	0.349b	0.064c
6.0	1.0a	19.8a	0.125c	0.044d
Glucose (%)				
0.0	1.2ab	36.6a	0.245b	0.06a
1.5	1.2ab	32.4ab	0.255b	0.079a
3.0	1.4a	28.0ab	0.355a	0.031a
4.5	1.0b	24.0b	0.205b	0.063a
6.0	1.2ab	23.0b	0.191b	0.016a

Means with columns for each sugar source having different letters are significantly different according to LSD test at $p \leq 0.05$, n= 5, Approximately 20 shoots were tested for each replicate

Root formation requires carbon sources; high concentration of sucrose decreases the number of roots significantly (Table 2). No root formations were recorded with the supplementation of glucose. Lower fructose concentrations (1.5-3%) were more favourable for root initiation than higher concentration (0.45-0.6%) (Table 2). In this study no root formations were recorded with the supplemented of glucose in the medium. These results clearly demonstrate that sucrose is a superior carbon source for *in vitro* growth than glucose and fructose. However, to achieve optimal proliferation rate, sucrose concentration should be added at the optimal level. Sucrose hydrolysis into glucose and fructose has been reported in a wide variety of plant cells and tissue culture [1, 6]; this may cause sucrose to be optimal for *in vitro* rooting of wild pear. Shoot regeneration of *Lilium longiflorum* was enhanced when sucrose concentration was used at 3 or 4% after 60 days of culture. On the other hand, several coniferous species showed that sucrose represents the best carbon source which support somatic embryo maturation [19-21]. The supplements of fructose in proliferation medium showed that sucrose, glucose and fructose in shoots increased significantly with the increasing of fructose (Table 5). The same patterns were seen in the medium. While in the rooting medium fructose showed significant difference at 3% compared with 4.5 or 1.5%. This may be due to adventitious shoot formation; applied fructose is used as a source of energy, building and also maybe in accordance with a possible regulatory role of glucose.

There are great differences in the ability to accumulate sucrose, glucose or fructose and use it as the source of carbon and energy. Maximum concentrations of sucrose were reported in both proliferation and rooting in medium was with the supplement of 6% sucrose (Table 3).

Table 2: Growth responses of *in vitro* grown wild pear (*Pyrus syriaca*) to different concentration of sucrose, fructose or glucose. Medium supplemented with 0.2 mg L^{-1} IBA Results

Carbon source	No. of shoot	Shoot length (mm)	No. of root	Root length (mm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)
Sucrose (%)								
0.0	0.0b	0.0d	0.0b	0.0c	0.0d	0.0e	0.0a	0.0c
1.5	1.2a	52.4a	3.2a	9.4bc	0.443a	0.102a	0.041a	0.008ab
3.0	1.0a	32.4b	2.2ab	30.2ab	0.271b	0.044c	0.025a	0.009a
4.5	1.0a	38.4b	1.2ab	34.0a	0.313b	0.086b	0.033a	0.006ab
6.0	1.0a	23.2c	1.1ab	28.0ab	0.124c	0.039d	0.02a	0.004bc
Fructose (%)								
0.0	1.0a	19.0b	0.0b	0.0b	0.204bc	0.01d	0.0c	0.0c
1.5	1.6a	21.2b	0.6ab	3.4ab	0.142c	0.026c	0.006b	0.002b
3.0	1.0a	31.4a	2.0a	8.2a	0.266ab	0.052c	0.015a	0.003a
4.5	1.4a	24.4ab	0.0b	0.0b	0.533a	0.067a	0.0c	0.0c
6.0	1.4a	19.8b	0.0b	0.0b	0.232bc	0.052b	0.0c	0.0c
Glucose (%)								
0.0	1.0	22.0a	0.0	0.0	0.15ab	0.013d	0.0	0.0
1.5	1.0	18.0a	0.0	0.0	0.198ab	0.018c	0.0	0.0
3.0	1.0	21.6a	0.0	0.0	0.217a	0.02b	0.0	0.0
4.5	1.0	20.8a	0.0	0.0	0.148ab	0.046a	0.0	0.0
6.0	1.0	15.6a	0.0	0.0	0.133b	0.012e	0.0	0.0

Means with columns for each sugar source having different letters are significantly different according to LSD test at p = 0.05, n= 5
Approximately 20 shoots were tested for each replicate

Table 3: Sucrose, fructose or glucose contents (%) of shoots, roots and medium, 8 weeks after *in vitro* grow of wild pear on proliferation or rooting medium. Medium supplemented with sucrose as a carbon sources

Sucrose concentration (%)	Sucrose % in shoot	Glucose % in shoot	Fructose % in shoot	Sucrose % in root	Glucose % in root	Fructose % in root	Sucrose % in medium	Glucose % in medium	Fructose % in medium
Proliferation medium									
0.0	0.0e	0.0e	0.0e	-	-	-	0.0e	0.0d	0.0d
15	11.2d	17.9d	6.8d	-	-	-	5.0d	6.10c	4.0c
3.0	17.3b	26.4a	11.7c	-	-	-	6.7c	7.10b	6.0b
4.5	15.9c	24.5b	12.6b	-	-	-	7.4b	7.80a	6.8a
6.0	18.5a	24.0c	16.4a	-	-	-	8.1a	8.40a	7.3a
Rooting medium									
0.0	0.0e	0.0e	0.0d	0.0e	0.0e	0.0e	0.0d	0.0d	0.0d
1.5	8.9d	14.4d	4.6c	13.2c	10.0d	19.6d	6.2c	9.9c	5.8c
3.0	9.7c	15.6c	5.7b	18.9d	17.4c	32.8c	8.6ba	10.3c	6.5b
4.5	12.5b	16.7b	12.7a	19.8b	18.3b	35.2b	10.2a	12.4a	8.8a
6.0	26.0a	24.5a	12.2a	21.0a	19.0a	36.1a	10.0a	11.9b	8.7a

Table 4: Sucrose, fructose and glucose content (%) in shoot, root and medium, 8 weeks after *in vitro* grow of wild pear on proliferation or rooting medium. Medium supplemented with fructose as a carbon source

Fructose concentration (%)	Sucrose % in shoot	Glucose % in shoot	Fructose % in shoot	Sucrose % in root	Glucose % in root	Fructose % in root	Sucrose % in medium	Glucose % in medium	Fructose % in medium
Proliferation medium									
0.0	2.4e	3.1e	4.5e	-	-	-	0.05cd	0.25e	0.11e
1.5	13.0d	18.2d	11.0d	-	-	-	1.29c	2.50c	1.01c
3.0	14.9c	20.8d	12.7c	-	-	-	2.34bc	4.60b	2.40b
4.5	17.6b	24.5b	14.9b	-	-	-	3.50b	6.88a	3.60a
6.0	23.9a	33.4a	20.7a	-	-	-	9.72a	1.24d	0.65d
Rooting medium									
0.0	12.0c	16.0c	12.6bc	0.0c	0.0c	0.0c	0.04e	0.24e	0.13d
1.5	14.0b	20.0b	13.9b	20.0b	22.0b	19.0b	1.35d	2.70d	1.41c
3.0	12.0c	17.0c	10.7c	23.0a	25.0a	21.0a	2.35c	4.98c	2.62b
4.5	17.0b	23.0b	14.0b	0.0c	0.0c	0.0c	3.98b	7.75b	4.04a
6.0	20.0a	28.0a	17.0a	0.0c	0.0c	0.0c	4.86a	9.37a	4.92a

Table 5: Sucrose, fructose and glucose content (%) in shoot, root and medium, 8 weeks after *in vitro* grow of wild pear (*P. syriaca*) on proliferation or rooting, medium supplemented with different concentration of glucose as a carbon sources

Glucose Concentration (%)	Sucrose % in shoot	Glucose % in shoot	Fructose % in shoot	Sucrose % in root	Glucose % in root	Fructose % in root	Sucrose % in medium	Glucose % in medium	Fructose % in medium
Proliferation medium									
0.0	8.0a	11.8a	24.0a	-	-	-	0.18d	0.38d	0.91a
1.5	7.1b	9.2b	19.0c	-	-	-	1.69c	1.98c	4.62d
3.0	6.3c	6.0d	19.0c	-	-	-	2.35b	2.58b	6.98c
4.5	7.0b	9.4b	20.0b	-	-	-	3.3a	3.47a	9.56a
6.0	5.6d	7.0c	16.0d	-	-	-	2.7b	3.17a	8.57b
Rooting medium									
0.0	5.3e	7.0c	14.oe	-	-	-	0.34c	0.4c	0.52e
1.5	7.0b	11.0a	21.0b	-	-	-	0.50c	0.91b	2.73d
3.0	6.0d	7.0c	16.0d	-	-	-	1.99b	2.54a	8.38b
4.5	8.0a	9.bd	23.0a	-	-	-	2.69a	2.81a	8.49a
6.0	6.0c	9.0b	20.0c	-	-	-	2.93a	2.79a	8.06c

Proliferation medium 1.0 mg L^{-1} BA and 0.1 mg L^{-1} IBA. Rooting medium supplemented with 0.2 mg L^{-1} IBA. Means with columns for each sugar source having different letters are significantly different according to LSD test at p = 0.05, n= 5. Approximately 20 shoots were tested for each replicate
-No rooting occurred and no results presented her

When 6% sucrose was supplemented to the proliferation medium, there were significant differences in the sucrose, glucose and fructose content of the shoot. The highest sucrose in shoot was with 6% (Table 3). Reik *et al.* [10] indicated that sucrose molecules are hydrolyzed by cell wall or plasmalemma located invertasese, this may be the reason for accumulation of high sucrose. Moreover, sucrose hydrolyzing enzyme was involved in a cycle of sucrose adsorption and/or uptake and subsequent secretion of glucose and fructose to cytosol [10]. High sucrose showed low uptake of sucrose in shoot, which is significant to low concentration (Table 3).

Uptake of sucrose, glucose and fructose did not seem to follow the same pattern when the medium was supplemented with fructose. In both rooting and proliferation there were significant differences in sucrose concentration in shoots with the supplemented of 6% fructose 1.5% (Table 4). Strobel *et al.* [22] showed that sucrose concentration had to be raised from the standard 3 to 6% to obtain maximum growth rate of *Gallium verum* cell suspension cultures growing in MS medium. After four weeks of growth, the fructose added in the medium affected the sucrose, fructose and glucose content in the shoot root or the medium (Table 4). Increased fructose in the proliferation medium increased significantly the sucrose, glucose and the fructose in shoot (Table 5). This is similar to a previous study by Blanc *et al.* [12] on somatic embryo of *Hevea brasiliensis*. With the supplement of fructose to the rooting medium, highest sucrose was recorded with 6% fructose, which was significant to 4.5%. On the other hand when the medium was supplemented with 4.5-6.0% fructose no significant differences were recorded in the medium (Table 5). The supplemented of glucose to the medium caused no root formation in both mediums (rooting and shooting). Moreover, there were significant differences in sucrose, glucose and fructose in the medium supplemented with different glucose concentration.

In conclusion, these results showed that carbon source and uptakes by the plant under this investigation differ. There are great differences in the content of carbon source in shoots, roots or medium. The medium supplemented with high sugar source is not necessary to achieve high number of new shoots. Moreover, there were fundamental changes in the medium after being supplemented with sucrose, fructose and glucose after the incubation periods.

REFERENCES

1. Shibli, R.A., M.M. Ajlouni, A. Jaradat, S. Aljanabi and M. Shatnawi, 1997. Micropropagation in wild pear (*Pyrus syriaca*). Sci. Hort., 68: 237-242.
2. Zhang, L.X.U., X. Sun, H. Zhang, T. Tang and K. Tang, 2003. Factors influence shoot regeneration from cotyledons of tetraploid *Isatis indigotica* Fort. *In vitro*. Cell. Dev. Biol-Plant, 39: 459-462.
3. Leifer, C., K.P. Murphy and P.J. Lumsden, 1995. Mineral and carbohydrate nutrition of plant cell and tissue culture. Crit. Revi. Pla. Sci., 14: 83-109.
4. Shimon-Kerner, N., D. Mills and J.C. Merchuk, 2000. Sugar utilization and invertase activity in hairy-root and cell-suspension cultures of *Symphytum officinale*. Plant Cell Tiss. Org. Cult., 62: 89-94.
5. Sima, B.D. and Y. Desjardins, 2001. Sucrose supply enhances phosphoenolpyrute carboxylase phosphorylation level in *in vitro Solanum tubersum*. Plant Cell Tiss. Org. Cule., 67: 235-242.
6. Yu, C.U., P.J. Joyce, D.C. Cameron and B.H. McCown, 2000. Sucrose utilization during potato microtuber growth in bioreactors. Plant Cell Rep., 19: 407-413.
7. Grout, B.W.W. and M.J. Ashton, 1978. Trasnplanting of cauliflower plants regenerated from meristem culture. Carbon dioxide fixation and the development of photosynthetic ability. Hort. Res., 17: 65-71.
8. Ebrahim, M.K.H., O. Zingheim, R. Veith, E.E. Abo Kassem and M.E. Komor, 1999. Sugar uptake and storage by sugarcane suspension cell at different temperatures and high sugar concentrations., J. Plant Physiol., 154: 610-616.
9. Lipavska, H. and D. Verugdenhil, 1996. Uptake of mannitol from the media by *in vitro* grown plant. Plant Cell Tiss. Org. Cult., 45: 103-107.
10. Riek, J.D., A. Piqueras and P.C. Debergh, 1997. Sucrose uptake and metabolism in a double layer system for micropropagation of Rosa multiflora. Plant Cell Tiss. Org. Cul., 47: 269-278.
11. Cunha, A. and M. Fernandes-Ferreira, 1999. Influence of medium parameters on somatic embryogenesis from hypocotyls explant and flx (*Linum usitatissium* L.). J. Plant Physiol., 155: 591-597.
12. Blanc, G., N. Micvhaux-Ferriere, C. Teisson, L. Lardet and M.P. Carron, 1999. Effects of carbohydrate addition on the induction of somatic embryogenesis in *Hevaea barsiliensis*. Plant Cell Tiss. Org. Cule., 59: 103-112.
13. Monmarson, S., N. Michaux-Ferriere and C. Teisson, 1994. Cal embryogenes haute issus de plantes de semis chez *Carica papaya* L.: obtention et germination of embryons somatiques. Fruits, 49: 279-288.
14. Komai, F., I. Okuse, K. Saga and K. Harda, 1996. Improvement on the efficiency of somatic embryogenesis from *spinash* root tissue by applying various sugar. J. Japanese Soc. Hort. Sci., 65: 67-72.
15. Scott, P. and R.L. Lyne, 1994. The effect of different carbohydrate sources upon the initiation of embryogeneisi from barley microspors. Plant Cell Tiss. Org. Cult., 36: 129-133.

16. Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassay with tobacco tissue culture. Plant Physiol., 14: 437-497.
17. Association of Official Analytical Chemists (AAOC), 2000. Official method AOAC for sugar using spectrophotometer-phenol sulfuric acid method. Washington D C.
18. Shibli, R.A., M.A. Smith and L.A. Spomer, 1992. Osmotic adjustment and growth responses of three (*Chrysanthemum morifolium* Ramat) cultivars to osmotic stress induced *in vitro*. J. Plant Nutri., 15: 1373-1381.
19. Tremblay, L. and F.M. Tremblay, 1995. Maturation of black spruce somatic embryos: sucrose hydrolysis and resulting osmotic pressure of the medium. Plant Cell Tiss. Org. Cult., 42: 39-46.
20. Iraqi, D. and F.M. Tremblay, 2001. Analysis of carbohydrate metabolism enzymes and cellular contents of sugars and proteins during spruce somatic embryogenesis suggests a regulatory role of exogenous sucrose in embryo development. J. Exp. Bot., 52: 231-241.
21. Iraqi, D., Q. Le, M.S. Lamhamedi, M. Francine and F.M. Tremblay, 2005. Sucrose utilization during somatic embryo development in black spruce: involvement of apoplastic invertase in the tissue and of extracellular invertase in the medium. J. Plant Physiol., 162: 115-125.
22. Strobel, J., M. Hieke, M. Gebaure, E. Wind and D. Groger, 1990. The influence of organic and inorganic chemical factors on cell growth and anthraquinone formation in suspension culture of *Gallium verum. Biochem.* Physiol. Pflanzen, 186: 117-124.