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Effect of Different Carbon Sources on *in vitro* Regeneration of Indian Pennywort (*Centella asiatica* L.)

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Abstract: An investigation was undertaken at Plant Biotechnology Laboratory, Biotechnology Discipline, Khulna University, Khulna to study the performance of different carbon sources and growth regulators on *in vitro* plantlet formation of Thankuni (*Centella asiatica* L.) from nodal segment. Among the carbon sources, sucrose showed the best results on shoot formation in presence of BA or Kn. In these cases the sucrose along with BA obtained 100% and sucrose along with Kinetin obtained 90% shoot formation, respectively. Maximum shoot length was obtained (2.67±0.059 cm) in sucrose containing BA medium. Other carbon sources showed less performance than sucrose both on BA and Kn containing media. Interestingly maltose showed the highest number of shoot/explant (4.0±0.0017) in presence of BA. In combination of BA+Kn, the percentage of shoot formation was higher (90%) in presence of sucrose, but the number of shoot/explant was maximum (3±0.035) in presence of maltose. In root formation, sucrose was found superior than other carbon sources used in present experiment. In this case IBA obtained the highest percentage of root (80%) with maximum length (1.5±0.019 cm), whereas, gur and maltose did not produce any root.

Key words: Carbon source, shoot, root, auxin, cytokinin

INTRODUCTION

Indian pennywort (*Centella asiatica* L.) is a member of big family Apiaceae under genus *Centella.* In Bengali it is known as Thankuni, thankuri, brahmabuti, brahmokuti. Thankuni is one of the most frequently found medicinal plants in Bangladesh. Traditional and herbal medicine practitioners have been using this plant in treatment of different types of diseases for centuries after centuries[1].

The plant Thankuni is considered as a useful alternative tonic in diseases of the skin, nerves and blood. Leaf juice is astringent and given to children for treatment diarrhoea. Poultice of leaves are used to relieve distention due to flatulence in herpes and retention of urine. Decoction of leaves dissolves vesicle calculi and acts as diuretic. Leaves and roots are also regarded as tonic and stomachache and given to sick and convalescing patients; also used as remedies for diarrhoea, dysentery and rheumatic affections. The plant has special influence on the genitourinary tract; it has also emmenague action[2].

Mass propagation of plant species through *in vitro* culture is one of the best and most successful examples of commercial application of plant tissue culture technology. *In vitro* propagation may provide a practical solution for cloning of Thankuni plants. It has been estimated that more than 10^6 plantlets can be produced per year from the culture of a single node of *C. asiatica*[3]. Therefore, the present study was undertaken to determine the effects of different carbon sources such as glucose, maltose, gur, sucrose and commercial sugar on the number and length of the shoots, to find out the suitable carbon source for *in vitro* rooting of Thankuni micro shoots, to identify the best medium to initiate organogenesis and regeneration of shoots and roots and to produce maximum number of plantlets from each explant *in vitro.*

MATERIALS AND METHODS

This experiment was conducted at Plant Biotechnology Laboratory, Biotechnology Discipline, Khulna University, Khulna during January to June 2002. In this experiment nodal segments of Thankuni (*Centella asiatica* L.) plants were used as explant. The plant material was collected from the fields of Khulna University Campus, Khulna, Bangladesh.

They were washed under continuous flashing of running tap water for 30 min and then treated with a

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Table 1: Effects of different carbon sources in the presence of BA and Kn in MS medium on shoot regeneration

Cytokinin	Carbon sources used (3 %)	Percentage of shoot formation	Avg. length of the shoot/culture (cm)	No. of shoot/ explant±SE
	Sugar	80	2.64±0.053	3.0±0.013
	Gur	-	-	-
BA	Glucose	90	1.76±0.027	3.0±0.0016
1.5 mg L^{-1}	Sucrose	100	2.67±0.059	2.0±0.023
	Maltose	70	1.94±0.031	4.0±0.0017
	Sugar	70	1.81±0.025	1.0±0.012
	Gur	-	-	-
Kn	Glucose	80	1.47±0.014	1.0±0.0032
1.5 mg L^{-1}	Sucrose	90	2.01±0.036	1.0±0.014
	Maltose	50	1.79±0.017	1.0±0.0043

Table 2: Effects of carbon sources presence of different combination of BA+Kn in MS medium on shoot regeneration

Cytokinins	Carbon sources used (3%)	Percentage of shoot formation	Avg. length of the shoot/culture (cm)	No. of shoot/ explant ± SE
	Sugar	70	1.42±0.013	2.0±0.019
	Gur	-	-	-
BA+Kn	Glucose	80	1.08±0.02	1.0±0.0051
1.5+0.2 mg L^{-1}	Sucrose	90	1.36±0.029	2.0±0.027
	Maltose	50	1.48±0.025	3.0±0.035
	Sugar	70	1.15±0.023	1.0±0.0036
	Gur	-	-	-
BA+Kn	Glucose	90	1.0±0.018	2.0±0.024
0.2+1.5 mg L^{-1}	Sucrose	90	1.8±0.046	2.0±0.039
	Maltose	50	1.31±0.033	3.0±0.056

Table 3: Effects of different carbon sources in the presence of auxins like IBA and NAA in MS medium on root regeneration

Auxins	Carbon sources used (3%)	Percentage of root formation	Avg. length of the root/culture (cm)
	Sugar	50	1.33±0.009
	Gur	-	-
IBA	Glucose	30	0.8±0.02
0.2 mg L^{-1}	Sucrose	80	1.50±0.019
	Maltose	-	-
	Sugar	40	1.19±0.02
	Gur	-	-
NAA	Glucose	30	1.00±0.021
0.2 mg L^{-1}	Sucrose	70	1.20±0.056
	Maltose	-	-

solution of the Savlon (5% v/v) for 10 min and finally surface sterilized with $HgCl_2$ (0.1% w/v) for 10 min. Lastly, the material was washed three times with autoclaved distilled water to remove any trace of $HgCl_2$. After surface sterilization, the explants were again carefully cut into pieces about 2-3 cm in size by a sharp surgical blade bearing one bud in each explant and placed in a petridish and then the explants were inoculated at the natural orientation in the test tube containing 15 mL of MS medium supplemented with different concentrations of a single hormone or combination of hormones.

RESULTS AND DISCUSSION

Explant from field grown mature plants of *Centella asiatica* were cultured on MS media supplemented with cytokinins (viz., BA and Kn) at different concentrations and their combinations and auxins like IBA and NAA for proliferation of auxiliary shoots and roots containing different carbon sources like commercial sugar, gur, glucose, sucrose, maltose etc. for morphogenic responses like shoot induction, root induction, growth rate etc.

Effect of different carbon sources in medium containing BA and Kn: Five treatments containing 1.5 mg L^{-1} BA were employed for regeneration of shoots. Data collections were performed after 7 days till 28 days of inoculation. Hundred percent shoot induction was observed on medium containing sucrose as carbon source. The average length of the shoot/culture was the best for sucrose (2.67±0.059 cm) but number of shoot/explant (2.01±0.023), which is lower than maltose (4.0±0.0017). The performance of sugar and glucose was fair in case of number of shoot/explant (Table 1). Media containing 1.5 mg L^{-1} Kn, 90% shoot induction was observed on medium containing sucrose as carbon source. The average length of the shoot/culture (2.01±0.036 cm) and number of shoot/explant (1.0±0.014) was the best for sucrose (Table 1). In case of gur shoot induction, average length of the shoot/culture and number of shoot/explant was nil.

Effect of different carbon sources on media containing BA+Kn at two different combinations: Five treatments containing 1.5 mg L^{-1} BA+0.20 mg L^{-1} Kn, 90% shoot induction was observed in medium containing sucrose as carbon source rather than any other carbon sources tested. The average length of the shoot/culture (1.48±0.025 cm) and the average number of shoot/explant (3.0±0.035) was the best in case for maltose (Table 2).

Similar treatments containing 0.20 mg L^{-1} BA+1.50 mg L^{-1} Kn, 90% shoot induction was observed on medium containing sucrose and glucose as carbon source rather than any other carbon sources tested. The average length of the shoot/culture was the best in case of sucrose (1.8±0.046 cm) and average number of shoot/explant was (3.0±0.056), the best in case for maltose (Table 2). In case of gur shoot induction, average length of the shoot/culture and number of shoot/explant was nil.

Effect of different carbon sources in medium containing IBA and NAA: Five treatments containing 0.2 mg L^{-1} IBA were employed for regeneration of roots. Data collection was performed after 5 weeks of inoculation. Eighty percent root induction was observed on medium containing sucrose as carbon source. The average length of the root/culture was the best for sucrose (1.50±0.019 cm). The performance of sugar and glucose was 1.33±0.009 and 0.8±0.02 cm, respectively (Table 3). Media containing 0.2 mg L^{-1} NAA, 70% root induction was observed in medium containing sucrose as carbon source rather than any other like commercial sugar, gur, glucose and maltose. The average length of the root/culture (1.2±0.056 cm) was the best. The performance of sugar and glucose was (1.19±0.02 cm) and (1.0±0.021 cm), respectively (Table 3). In case of gur and maltose root induction, average length of the root/culture was nil.

Sugar and gur is impure and may be some other chemicals were present that might have a bad effect on explant. During autoclave sucrose is broken down into glucose and sucrose, which are the ultimate carbon source. Glucose is then utilized first, followed by fructose. Interestingly, in presence of maltose the number shoot/explant was the best.

The usual carbon source for plant tissue culture is sucrose. Glucose and fructose are also been used. Sucrose has a long-term effect. Sucrose is broken down during autoclave and converted into glucose and fructose[4]. Glucose is then utilized first, followed by fructose. In this experiment, glucose was utilized first for both sucrose and glucose containing media. Medium containing sucrose utilized fructose later, medium contain glucose had no alternative sucrose for later use. Commercial sugar is impure sucrose, which was also broken down into glucose and fructose. In the results, it can be seen that the performance of commercial sugar is less than sucrose and glucose. The probable cause for this is that commercial sugar is impure and there may present some other toxic substances, which may not suitable for plant tissue culture. Maltose has been used as carbon source, but it is generally much inferior to sucrose and glucose. In this study multiple shoots were found for both in BA and Kn containing media. BA and Kn are cytokinins which have effective response on multiple shoot formation[5-8]. In this study roots were found both on IBA and NAA containing media. Both are synthetic auxin and are suitable for root induction[9-11]. The best root induction and highest survival rate when medium is supplemented with sucrose[12]. *In vitro* propagation of Thankuni showed better result for micropropagation. This plant was easily regenerated from nodal explants through shoot multiplication in cytokinins and root formation in auxins with minor problem of contamination. In this regeneration system high frequency of shoot formation and multiple shoot formation were observed which was a worthy feature of this investigation.

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