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Impact of carbon sources on callus induction and regeneration ability in banana cv. *Sabri*

Md. Jamal Hossain[1], Md. Abdul Bari[1], Nure Anzuman Ara[1], Md. Al Amin[1], Motiur Rahman[2], Kazi Md. Faisal Haque[2]

[1]*Biotechnology Laboratory, Institute of Biological Sciences, University of Rajshahi, Rajshahi-6205, Bangladesh*

[2]*Dept. of Genetic Engineering and Biotechnology, University of Rajshahi, Rajshahi-6205, Bangladesh*

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Abstract

The purpose of this study was to investigate the effect of three different carbon sources; sucrose, glucose and sorbitol alone and their combinations on *in vitro* callus induction and regeneration in banana cv. *Sabri*. Male flowers were used as experimental material in this investigation. The male flowers were cut into small pieces and cultured on MS medium supplemented with 2 mg/l 2, 4-dichloro phenoxy acetic acid (2, 4-D) + 1 mg/l α-naphthalene acetic acid (NAA) + 1 mg/l Indole-3-acetic acid (IAA) + 1 mg/l biotin+1 mg/l glutamine containing different percentages of sugars like sucrose, glucose and sorbitol singly and in their combinations. Glucose showed the highest performance rate for callus induction and 3 % concentration proved the optimal dose. Sucrose and sorbitol behave different in embryo formation and they produced the highest and lowest number of embryos, respectively in regeneration medium. In respect of overall performance the highest percentages shoot and root formation was obtained in the media containing 3% and 2% sucrose, respectively. These findings will be beneficial for the further experiment of other cultivars of banana.

* **Corresponding Author:** Md. Jamal Hossain ✉ jam_gene@yahoo.com

Introduction

Plant morphogenesis can be influenced both by physical and chemical factors. Among factors affecting plant regeneration, the genotype and the composition of the culture medium including carbohydrates and growth regulators play the vital roles in morphogenesis. Sugars are necessary in living cells as a source of energy, carbon skeletons for biosynthetic processes and materials for cell wall synthesis (Kutschera and Heiderich, 2002). Continuous supply of carbohydrates to plants cultured *in vitro* is essential because photosynthetic activity of *in vitro* growing tissues is usually reduced without steady supply of carbon in culture medium. These compounds are also necessary in media as agents ensuring proper osmolarity (Bogunia and Przywara, 2000). Moreover, sugars are signal molecules repressing or activating plant genes involved in many essential processes, including photosynthesis, glycoxylate metabolism, respiration, starch and sucrose synthesis and degradation, nitrogen metabolism, defense responses, cell cycle regulation, pigmentation and senescence (Jang *et al.,* 1997; Xiao *et al.,* 2000). For all these reasons, sugars have a great potential effect on the physiology, growth and differentiation of cells and may influence to shoot proliferation potential of explants as well as rhizogenesis (Gibson, 2000; Jain and Babbar, 2003; Nowak *et al.,* 2004).

Sucrose is often assumed to be the best choice of carbon source in cell and tissue culture media because it is the main sugar translocated in the phloem of many plants (Peterson *et al.,* 1999). However, there are a number of plants that can grow on carbohydrates other than sucrose and sucrose may not be the most suitable carbohydrate for plant regeneration. Regeneration *via* organogenesis or somatic embryogenesis was stimulated in a number of plants on the media containing Glucose, fructose, maltose, mannose or Sorbitol (Borkowska and Szczerba, 1995; Bach and Pawowska, 1993; Fuentes *et al.,* 2000; Tang, 2000; Blanc *et al.,* 2002; Sairam *et al.,* 2003).

Banana shoot culture has been well established and received commercial application but callus and cell suspension culture in banana is very difficult (Strosse *et al.,* 2004). According to them, initiation and maintenance of cell cultures is rather labour intensive and time consuming. However, since 1 ml of settled cells of a highly regenerable cell suspension can yield more than 100,000 plants. Cell cultures are most suitable for mass clonal propagation. Moreover, embryogenic cell suspensions are highly preferred as target material for protoplast culture and genetic engineering since the risk of chimerism is circumvented because of the unicellular origin of regenerated plants. We have also reported first the callus and cell suspension culture of brinjal in Bangladesh (Hossain *et al.,* 2007). Our present attempt is objected to perform detail study on the effect of carbon sources on callus induction and regeneration in banana.

Materials and Methods

Male flowers of *Musa* spp. cv. *Sabri* were used as experimental materials in this investigation. The initial plant material consisted of male inflorescences of banana were collected from field grown plants. Male flowers were collected from 10th to 18th hands of the inflorescences after 6-10 weeks of flower opening. Under a sterile condition, the outer overlapping bracts were removed and the remaining part (2-3cm in length) containing male flowers were isolated and surface sterilized by 70% ethyl alcohol for 1 minute. The male flowers were cut into small pieces and they were transferred in petridishes (9 cm in diameter) containing MS (Murashing and Skoog, 1962) medium which was supplemented with 2 mgl^{-1}2,4-D +1mgl^{-1}NAA+1mgl^{-1} IAA +1mgl^{-1} Biotin+1mgl^{-1}glutamine and different percentages (0%, 1%, 2%, 3%, 4%) (w/v) of different sugars: sucrose, glucose, sorbitol and their combinations which were autoclaved in 121°C temperature for 20 minutes and agarifide with 8 gml^{-1} agar. The pH of the medium was adjusted to 5.8. The media were autoclaved and plant growth regulators were sterilized using microbiological filter (pore size 0.2 µm). The explants (10 per petridish) were cultured in the continuous dark at 25±2 °C.

For embryo formation, the calli derived from male flowers, were subcultured on the agarifide MS medium supplemented with different carbon sources.

After 80-110 days the embryos were appeared in the solid MS medium. The embryos showed various polarities like unipolar and bipolar. The number of embryos per callus was calculated under microscope from the beginning of embryogenesis. For shoot and root formation, the bipolar embryos were transferred in MS medium containing different carbon sources and 0.5mgl^{-1}BAP+2mgl^{-1}IAA. The data of the formation of callus, roots and shoots were taken and the results of these experiments are expressed as either mean value or a percentage.

Results and discussion

Callus induction

Three types of carbon sources *viz.* sucrose, glucose and sorbitol were used singly or in combination with MS medium in order to study their respective performance for callus induction and regeneration. Flowers when cultured in MS medium without any sugar, the callus initiation and their subsequent growth were very low. Effect of different strength of carbon sources for callus induction and time periods are shown in Table 1. among the three carbon sources, the highest rate of callusing was obtained in MS medium containing 3% glucose. But replacement of glucose by sucrose or sorbitol caused to slow down the rate of callus induction. Moreover, when sucrose was used singly as a carbon source both callus induction and callus growth were remarkably ceased down.

The role of different carbon sources were also studied by some other workers and better performance of glucose was established over other sources in callus induction. Alina *et al.* (2006) studied the effect of carbon source on callus induction and regeneration ability in *Pharbitis nil.* addition of glucose instead of sucrose to the medium stimulated the induction of callus on flower buds and cotyledonary explants, but inhibited its growth on fragments of hypocotyls. Addition of fructose or glucose to the medium stimulated the organogenesis of shoots, whereas root organogenesis was inhibited on all explants used.

Sorbitol strongly inhibited both induction of callus and organogenesis on all explants used. Peterson *et al.*, 1999 also studied the effect of carbon sources on callus induction on leaves of *Miscanthus* when glucose exhibited most effective performance, but the differences between media containing sucrose, glucose, fructose and maltose were not observed in the case of inflorescences (Peterson *et al.,* 1999). It may be due to the fact, that the leaf is a place of synthesis of glucose, which is not transported by phloem, can be readily taken up by diffusion across the isolated tissues surfaces and metabolized by tissues cultured *in vitro*.

It is known that maltose also stimulates callus (probably when it is hydrolyzed into glucose) and increases regeneration better than sucrose or glucose, for example in *Oryza sativa* (Kumria *et al.,* 2001), *Lycopersicon esculentum* (El-Bakry, 2002) and *Glicyne max* (Sairam *et al.,* 2003). Using Sorbitol (sugar alcohol) resulted in very little callus induction on both leaf explants and immature inflorescences of *Miscanthus* (Petersen *et al.,* 1999) and on internodal explants of *Annona muricata* (Lemos and Baker, 1998). Similar results were observed in this study. It seems that sorbitol was not metabolized by the *Musa* spp. tissues. It might have brought about carbon starvation, without killing the explants.

Embryogenesis

Data presented in table 1 revealed that sucrose and sorbitol produced the highest and lowest number of embryos respectively whereas glucose slightly stimulated the embryogenesis. The results also indicated that glucose or sorbitol with sucrose in the ration of 1:1 markedly stimulated the embryogenesis in banana *in vitro* culture. As indicated previously, most of the vegetative growth characteristics measured under this study was enhanced with the increase of carbon sources in the medium up to 3%.

Table 1: Effect of different carbon sources alone or in their combinations on callus induction and embryogenesis in banana cv *Sabri*.

Sources of carbon	% of carbon sources	% of immature male flowers responded	Callus colour	Days taken for calli formation	% of calli induced somatic embryos	Days taken for embryogenesis
Sucrose	0%	48	W	40-50	45	110-130
	1%	53	W	35-45	56	110-130
	2%	69	W	30-40	62	100-120
	3%	61	LG	30-40	44	110-120
	4%	38	LG	30-40	41	110-120
Glucose	0%	53	W	40-55	38	120-140
	1%	60	W	40-55	43	120-140
	2%	68	LG	30-40	54	120-140
	3%	71	LG	30-40	47	120-140
	4%	64	LG	30-40	37	120-140
Sorbitol	0%	42	W	40-55	36	110-130
	1%	53	W	40-55	42	110-130
	2%	66	LG	30-40	49	110-120
	3%	44	LG	30-40	38	110-120
	4%	33	LG	30-40	34	110-120
½ Sucrose + ½ Glucose	0%	50	W	40-50	27	120-140
	1%	55	W	40-50	36	120-140
	2%	70	W	30-40	42	120-140
	3%	62	LG	30-40	42	120-140
	4%	49	LG	30-40	33	120-140
½ Sucrose + ½ Sorbitol	0%	39	W	40-50	38	120-140
	1%	48	W	40-50	45	120-140
	2%	61	W	30-40	50	120-140
	3%	53	LG	30-40	45	120-140
	4%	44	LG	30-40	39	120-140

Traore and Guiltinan (2009) reported that explants cultured continuously on maltose or sorbitol-containing media failed to produce embryos in cacao genotypes. During embryo maturation and conversion, they found no significant differences among glucose, fructose, maltose or Sucrose for embryo weight, total shoot and root production but the plantlets produced on glucose had shoots with normal leaves.

In the present study, the importance of sugar for embryos formation was clearly demonstrated as indicating by the reduction of embryo formation in the medium without any supplemented carbon source. Results shown in the table 2 indicated that there was insufficient sugar available in low concentrations in the present experiments led to a reduction in vegetative growth.

Table 2: Effect of different carbon sources alone and their combinations on embryo multiplication and organogenesis in banana cv. *Sabri.*

Sources of carbon	% of carbon sources	No. of globular embryos after multiplication	% of shoot formation	% of root formation	Shoot length. (cm)	Root length (cm)
Sucrose	0%	30	40	50	3.00±0.29	2.00±0.98
	1%	36	56	61	4.33±0.45	3.19±0.87
	2%	47	71	76	4.88±0.65	4.19±0.44
	3%	72	77	89	4.55±0.42	3.78±0.66
	4%	42	67	69	4.66±0.41	3.29±0.77
Glucose	0%	30	40	50	3.00±0.49	2.00±0.92
	1%	38	48	57	4.49±0.40	3.45±0.91
	2%	48	53	66	4.59±0.41	3.66±0.65
	3%	51	62	72	5.20±0.69	3.88±0.67
	4%	37	59	58	4.80±0.45	3.69±0.55
Sorbitol	0%	30	40	50	3.00±0.29	2.00±0.53
	1%	37	43	54	3.43±0.45	2.65±0.45
	2%	41	47	57	3.55±0.42	2.76±0.61
	3%	44	55	61	3.88±0.65	3.00±0.29
	4%	38	52	56	3.66±0.41	2.69±0.66
½ Sucrose + ½ Glucose	0%	30	40	50	3.00±0.49	2.00±0.45
	1%	39	44	59	4.59±0.40	3.65±0.88
	2%	47	58	78	4.69±0.41	3.76±0.35
	3%	59	71	64	4.99±0.69	3.85±0.67
	4%	36	64	72	4.80±0.45	3.61±0.46
½ Sucrose + ½ Sorbitol	0%	30	40	50	3.00±0.49	2.00±0.49
	1%	36	47	54	3.59±0.40	2.75±0.63
	2%	39	54	63	3.69±0.41	2.79±0.49
	3%	48	61	71	3.99±0.69	3.210±0.81
	4%	30	52	58	3.80±0.45	3.00±0.67

Regeneration

Mature somatic embryos capable of being converted into complete plantlets were separated by gentle shaking with double autoclaved water and they were used for shoot and root formation. The embryos were transferred in MS media containing 2 mgl^{-1}2,4-D +1mgl^{-1} NAA+1$mg1^{-1}$IAA+1mgl^{-1} biotin+1mgl^{-1} glutamine and different percentage of different sugars like sucrose, glucose, sorbitol singly and in their combinations. The highest percentage of shoot formation was 77 (table 2) in the media containing 3% sucrose. Glucose and sorbitol did not show any effective response on shoot formation. But their concentration with sucrose by the ratio of 1:1 influenced shoot formation. The shoot length in case of 3% glucose was highest (5.20 cm) than that recorded in control (3.00 cm) and combination of sucrose and glucose. However, the longevity of cultures was found shortened in control showing only 40% culture survived at 90 days (data are not shown).

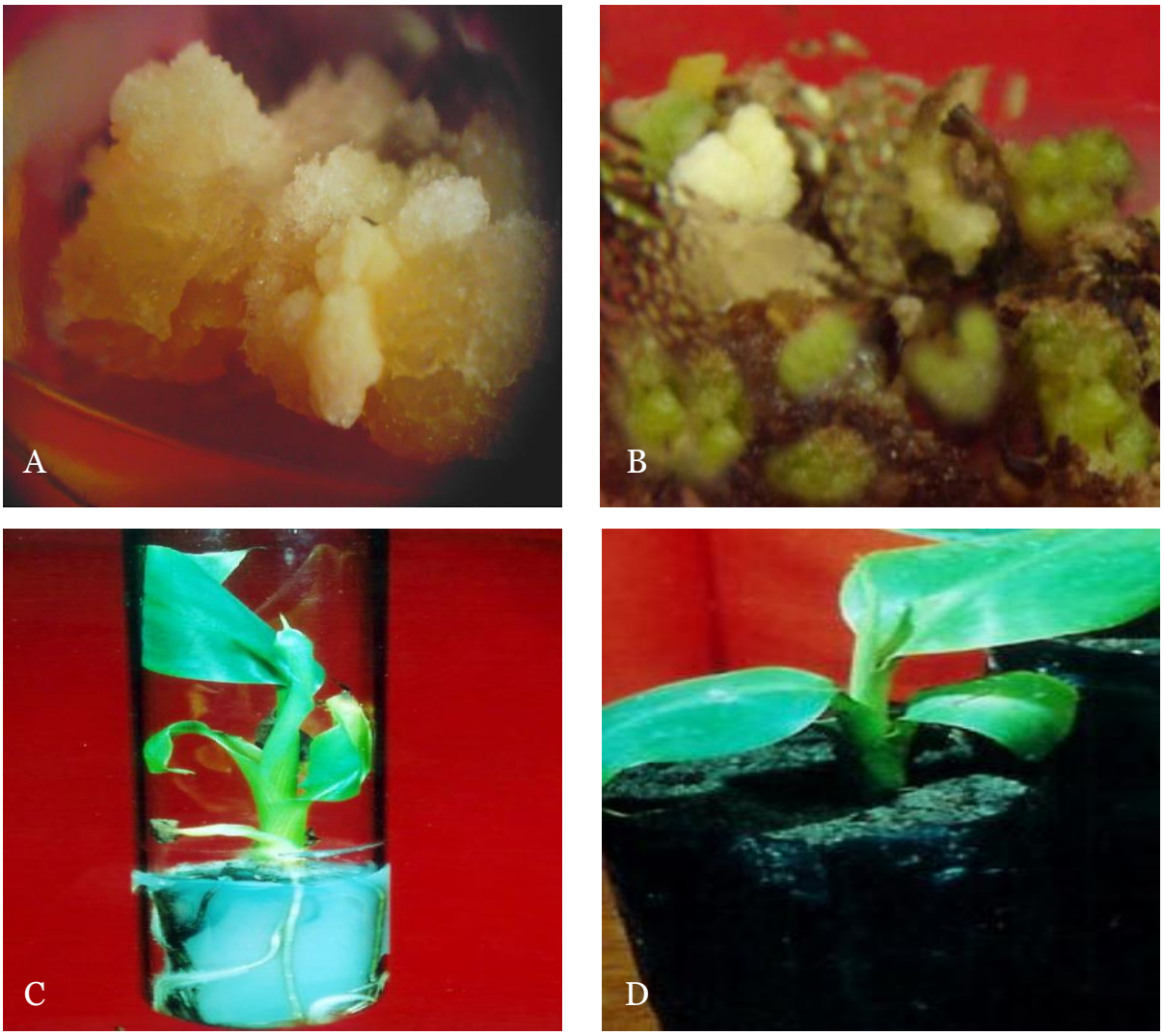

Fig.1. Effect of different carbon sources on plantlet regeneration through callus culture. Plate A (highest performance for callus induction on 3% glucose), B (highest performance for callus regeneration on 3 % sucrose), C & D rooted plant and potted plant respectively.

In addition to other characteristics rooting of proliferated shoot had great impact on tissue culturing of *Musa* spp. among the various treatments prolific rooting (89%) was observed in media supplemented with 3% sucrose. The higher (4%) and lower (1%) concentrations of sucrose, glucose or sorbitol did not promote proper rooting in *Musa* culture. Number of roots, root length and days required for root initiation (15-20) were also appreciable in case of 2% glucose. Similar results have also been reported by Priyakumari *et al.* (2002). The behavior of shoot proliferation may differ to root proliferation and same sugar type may not prove productive for both purposes.

Lipavska & Konradova (2004) also stated that the recommended concentration vary in different cultures, ranging from 1-6%; however, they were of the view that in many cases a detailed search for optimum sugar concentration has not been performed.

Transplantation of plantlets to pots

When the regenerated plantlets induced sufficient root (3-5 cm in length) they were considered ready to transfer in soil. The plantlets grown inside the culture vessels were brought out the controlled environment of growth chamber. They were then kept in the room temperature for 4-7 days to bring them in contact with normal temperature.

The plantlets were then removed carefully from the culture vessels. The roots of the plantlets were gently washed under running tap water to remove agar attached to the root zone. Immediately after that, they were transferred to small pots containing the following mixtures: Garden soil : Compost : Sand : : 1:1:1.

This study points to the fact that selection of carbon source played an important role in callus induction and regeneration in banana. The concentrations and combinations of carbon sources were similarly important for callus induction and regeneration in banana.

Conclusion

It can be concluded that among the different carbon sources used, glucose performed well followed by sucrose and sorbitol in terms of callus induction. Since sucrose and sorbitol are the better carbohydrate choices for embryo formation in callus regeneration of banana. However, further research is highly required to explore the effect of different variety of carbon sources on *in vitro* plant regeneration of other cultivars of banana.

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