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Role of sucrose, glucose and maltose on conventional potato micropropagation

Rahman, M.H.*, Islam, R., Hossain, M. and Islam, M.S.

Plant Breeding and Gene Engineering Lab, Department of Botany, University of Rajshahi, Rajshahi-6205, Bangladesh.

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Five potato cultivars including one indigenous potato cultivar (Shilbilaty, as check cv) were subjected with sucrose (a conventional carbon source), glucose and maltose in MS media and evaluate their efficiency to *in vitro* growth profile. It was found that sucrose, glucose and maltose containing media were statistically indistinguishable in their action to different growth parameters. Sucrose-media were well capable in performing optimum growth but in some cases glucose and maltose also performed well specially an increasing the nodal number per plantlet (a fundamental of multiplication rate). Highest number of nodal countings was noted in maltose media (cv. Shepody) compare to that of sucrose nutrients (cv. Shilbilaty). The fresh mass of plantlet of all cultivars were also maximum in maltose medium except to this was All Blue. Leaf proliferation were noted highest in maltose media (cv. Diamant) followed by glucose (cv. Shilbilaty). The sucrose media results shortened internode than that of maltose and glucose counterpart only exception to this was in Atlanta. In this experiment sucrose gave inferior values than maltose and glucose. Maltose media improved growth status particularly in Shepody, Atlanta and Diamant .

Key words: potato micropropagation, growth, sugar type

Introduction

Sucrose has been established as prime component for potato micropropagation (Khuri and Moorby, 1995). There have been several reports comparing the effects on micropropagation of a range of sucrose concentrations and established that 3% sucrose was the optimum level for *in vitro* potato micropropagation (Wang and Hu, 1982; Khuri and Moorby, 1995; Danielle *et al.*, 2003; Pruski *et al.*, 2003). Surose is frequently used as a carbon source in plant tissue culture media. Its hydrolysis into glucose and fructose has been demonstrated in a wide variety of plant cell and tissue cultures (Georage, 1993;

*Corresponding author: M H Rahman; e-mail: hasanur7@yahoo.com

Nuutila *et al.*, 1997). Akita and Takayama, (1994a) also reported that in their potato microtuber jar fermentar, all the remaining sugars were glucose and fructose after 10 weeks of in vitro growth *i.e.* total sucrose degraded into glucose and fructose. Such a hydrolysis of sucrose makes its utilization as the superior carbon source in potato micropropagation very insufficient (Yu *et al.,* 2000). For optimal plantlet growth, sucrose level sustainability are necessary and if it is rapidly hydrolysed into glucose and fructose making the long term maintenance of desirable sucrose level is difficult. Chandra *et al*. (1988) compared sucrose, glucose fructose, mannose and mannitol at concentration ranging from 4 to 12% for potato microtuberization but they did not indicate the role of those sugars on micropropagation, a preliminary step for potato microtuberization. To facilitate the sucrose hydrolysis and their after effect on *in vitro* micropropagation growth traits with other monosaccharide (glucose) and disaccharide (maltose), the culture were grown on liquid media. Liquid media have been successfully used for potato by Tovar *et al*. (1985) and Rosell *et al*. (1987). The hydrolytic nature of sucrose also causing fluctuations of pH in the media and limiting to uptake nutrients by the plantlets and also possible formation of more complex molecules in the media (Leifert *et al*., 1992). Furthermore, autoclaving is also a contributory factor of sucrose hydrolysis and a large amount of it breaks down during the growth of the plantlets (Kanabus *et al*., 1986). The effect of substituting sucrose with glucose and maltose on potato micropropagation nature were not much investigated. Lower level of nitrate supply were provided in MS micropropagation media with a view of developing more vigorous plantlets as reported by Zarrabeita *et al.* (1997) and also an agreement with our previous experiment (unpublished data).

The culture was initiated by nodal microcuttings, a breaking point of potato multiplication systems in the commercial production of high quality potato seed. Since the quality of plantlets produced from nodal cuttings has been a priority, over the years, the effects of liquid media on the growth of micropropagated potato shoots have been studied in relation to the availability of nutrients and water in the culture medium. Nitrogen and sugar assimilation in the new grown shoots and their water content have been evaluated for various cultivars in various regeneration systems (Pruski, 2007). No growth regulators were added to operate this experiment as previously established by several authors (Abbot and Belcher, 1986; Garner and Blake, 1989; Ahsan *et al.,* 2003; Alsadon *et al*., 2004).

The present investigation was tended to find out the effectiveness of different sugar types in distinguish with conventional sucrose media on basic growth traits of five important potato CV's. The information may attribute the significant physiological behavior of potato plants, efficient field utilization of planting materials, *in vitro* microtuber yield and other carbohydrate relevant study.

Materials and methods

In vitro stock plants of meristematic origin in five potato (*Solanum tuberosum* L.) cultivars including Shilbilaty (a indegenous cv, regrded as check cv), Shepody, Atlanta, All Blue and Diamant were propagated using single nodes of every 3-4 weeks as the regular micropropagation methods. In this study nodal explants of same age and size of different cultivars were used and cultured in different sugar media with due regard to find out their sugar inducing *in vitro* response. A single nodal segments were separated (1 cm long with single leaf) and cultured in MS liquid media (having ¼ strength of KNO_3 and NH_4NO_3 without changing other components of the MS basal media) containing 3%, sucrose, glucose and maltose. The pH of all the media were adjusted to 6.0. The sucrose containing media were autoclaved for 20 min and the glucose and maltose solution were filter sterilized through 0.22 m membrane filters (type GS, Millipore Corporation, USA). The cultivation was performed at 25±1°C in a well set growth room conditions for 3 weeks under fluorescent lightening (Philips) of 800 lux (16/8, D/N cycle) at the top of the culture vessels.

Determination of growth regime

The plantlets grown *in vitro* for 3 weeks were harvested from all replicate vessels of a treatment in each cultivar and evaluated their micropropagation status. The parameters considered were a) plantlet height (cm), b) number nodes/plantlet, c) internode length (cm), d) number leaves /plantlet and e) fresh mass of plantlet (mg). Each treatment consisted of 10 culture vessels and each vessel was a replicate containing 5 segments of each cultivar. All the culture vessels were arranged randomly in growth chamber. At the end of 3-week period 4 culture vessels from each treatment were chosen randomly and the plantlets were pooled out and measured as per growth parameters described. The experiment was repeated twice with almost similar results. The data on different parameters were evaluated using ANOVA and mean differences were separated by DMRT at 5 % level of significance.

Results

The plantlets grown in each treatment was compared after 3-week period. All the tested cultivars showed variable response to different sugar sources. The plantlet height changed over 3 weeks time courses revealed that significant differences existed among the cultivars in response to sucrose, glucose and maltose media (Fig. 1). The plant height of all cultivars was maximum in maltose-media as compared to that of sucrose and glucose-media (Table 2).

Exceptional to this was Shilbilaty (sucrose-media) and All Blue (Gulcose-media). The multiplication rate *i.e.* number of nodal points per plantlet was found optimum in maltose-media and in this case Shepody (6.50 nodes/plantlet) and Diamant (6.0 nodes/plantlet) were comparable. Whereas all Blue and Atlanta yielded more nodal points in glucose-media than sucrose and maltose supplemented media (Table 2). It was observed that nodal points increased with the decrease of internode length. It was interestingly noted that among the cultivars, Diamant proliferated highest number of leaves (7.04 leaves/ plantlet) in maltose-media followed by Shilbilaty (6.75 leaves/plantlet) in glucose-media. Sucrose media showed inferior to increasing leaf number for all the tested cultivars. It was also noticed that the maltose grown plantlets resulted highest fresh mass than sucrose and glucose grown plantlets. Only exceptional results were given by All Blue (163.25 mg). The 3% sucrose media resulted inferior in most of the cases than the media with 3% glucose and maltose. From this experiment, it is demonstrated that the response of different potato cultivars significantly varied at different sugar types. The results also demonstrated that the check cultivar (Shilbilaty) performed superior than other cultivar studied in all the traits except in fresh mass of plantlet and in this regard Shilbilaty was comparable to Shepody. The average values of three sugar media on cultivar performences were noted best in Shilbilaty including plant height (8.76cm), nodal number per plantlet (5.25), internode length (1.95 cm) and leaves per plantlet (6.08). Hence the results indicted that Shilbilaty (no report has not yet been noticed so far) is the promising cultivar for *in vitro* micropropagation scheme.

Glucose Maltose Sucrose

Plate 1 Photographs showing five potato cultivars growing in three sugar containing MS media, 21 days after culture.

Discussion

The experiment support our intention that it is very important to study the sugar effects at low nitrate MS media with due regard to optimum plantlet growth and their subsequent effect may be of good information in the refinement of tissue culture media. Sucrose is a prime carbon source of potato micropropagtion and influence of developing vigor plantlets but it was not fully

explain the performances of other disaccharides or monosaccharide as far as optimum potato micropropagation is concerned. There is a general agreement in the literature that sucrose is required in the medium for potato micropropgation (Pruski *et al*, 2003; Yu *et al.,* 2000). Here the data indicated that not only the sucrose, glucose and maltose could be the positive alternative to produce optimum microshoots. No distinguisable differences were observed among the three sugar types on response to micropropagation traits of five potato cultivars and appeared interchangeable. In *Albizzia* root explants however it has been found that maltose rather than sucrose favors the regeneration and development of healthy and vigorous plantlets (El Maataouri *et al.*, 1998). Hossain *et al* (2005) reported that maltose containing MS media having 0.2 mg/l BA and 1.5 mg/l Kin resulted the higher shoot number per explant in Indian Pennywort (*Centella asiatica* L.).

Cultivar responded to sugar types were varied as far as plant height and plantlet fresh mass is concerned. Sometimes it was very difficult to make a statistically valid comparison among three sets of treatments (two disaccharides and one monosaccharide). It was also noted that media with 3% fructose had deleterious effect to *in vitro* plantlet growth (data not mentioned).In some anther culture and somatic embryogenesis system maltose has also been shown to be more preferable to sucrose in the medium. It was suggested that maltose in the micropropagation media remained largely intact *i.e*. not hydrolysed (sucrose immediately hydrolysed). This is a sharp contrast to the known fate of sucrose in the micropropagation media *i.e*. rapid and complete hydrolysis (Yu *et al.,* 2000; Yoon and Leung, 2004). Beside this, commercial sugar is impure sucrose and there may be present some other substances which may not suitable for plant tissue culture (Hossain *et al.,* 2005). Further research is going to clarify the carry over effect of glucose and maltose onto potato microtuberization and subsequent seed potato production chain.

Table 1. F value of different growth traits of five potato cultivars and three sugar treatments.

Source variation	df	F Value				
		Plant height (cm)	Number of nodes/plantlet	Internode length (cm)	Number of leaves/ plantlet	Plantlet fresh mass (mg)
Cultivar (C)	4	12.40**	3.91*	5.27*	0.39ns	8.68*
Treatment(T)	2	2.09ns	1.54ns	2.45ns	1.87ns	1.41ns
C × T	8	2.07ns	4.20*	1.52ns	1.24ns	1.63ns
Error	45					

**, *, ns = highly significant, significant and non-significant respectively at 5% level .

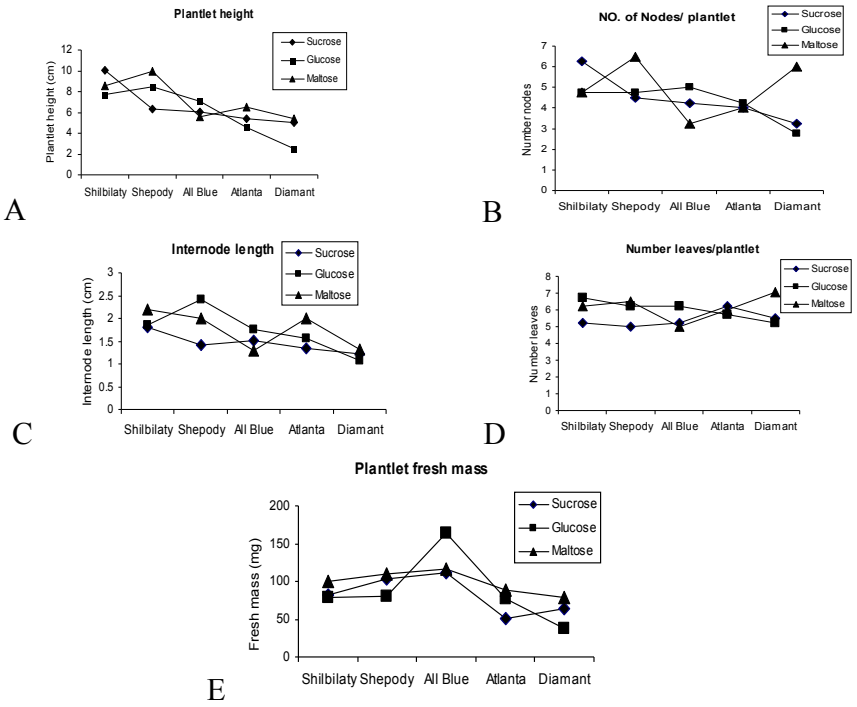

Fig. 1. Mean performances of five potato cultivars on different *in vitro* growth traits (A - E) in sucrose, glucose, and maltose containing MS media. Data 21 days after culture.

Table 2. Mean value of sugars on influence to different growth traits in five potato cultivars. Data after 21-day period culture.

Sugar type	Growth characteristics				
	Plant height (cm)	Number of nodes/plantlet	Internode length (cm)	Number of leaves/ plantlet	Plantlet fresh mass (mg)
Sucrose	6.56a	4.45a	1.46a	5.45a	82.30a
Glucose	6.03a	4.30a	1.73a	6.05a	87.40a
Maltose	7.23a	4.90a	1.76a	6.15a	98.80a

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