KATHMANDU UNIVERSITY JOURNAL OF SCIENCE, ENGINEERING AND TECHNOLOGY
VOL. 8, No. I, FEBRUARY, 2012, pp 119-124

EFFECT OF DIFFERENT SUGARS ON SHOOT REGENERATION OF MAIZE (*ZEA MAYS* L.)

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Received 17 June, 2011; Revised 11 December, 2011

ABSTRACT
Effect of various concentrations of different sugars was investigated for induction of root and shoot from maize. The seedling development was induced with the application of fructose, maltose and sucrose at different concentrations (0.25%, 0.5%, 0.75%, 1.0%, 2.5%, 5%, and 10%) of each. Dissected embryos were transferred in ½ MS basal media fortified with various concentrations of different carbon sources. *In vitro* regenerated maize plantlets were healthy and attained a length of 12.2 cm at 1.0 % concentration in maltose within a week. Out of three sugars low concentration (0.25%- 2.5%) of maltose and sucrose exhibited the maximum shoot and root growth. All the concentrations of maltose and sucrose showed the good growth response of shoot and root.

INTRODUCTION
In vitro growth of plants is largely determined by the composition of the culture medium. Sugar in culture medium has been considered the sole carbon source for the growth of cells, buds, shoots, and even plantlets. Sugars enter the metabolism pathways and transformation of energy which are required for growth of cell. In plant tissue culture, sugar serves as a carbohydrate supply to provide an optimal culture condition for cell. The main components of most plant tissue culture media are mineral salts and sugar as carbon source and water [1]. Sugar is a very important component in medium and its addition is essential for *in vitro* growth and development of plants because photosynthesis is insufficient, due to the growth taking place in conditions unsuitable for photosynthesis or without photosynthesis [2]. The sugar concentration chosen is very dependant on the type and age of growth material; very young embryos require a relatively high sugar concentration. Generally the growth and development increases with increased sugar concentration, until an optimum is reached and then decreases at high concentrations. The most commonly used source of carbon is sucrose at a concentration of 2- 5%. Glucose and fructose are also known to support good growth of some tissues [3]. The exogenous carbohydrates support growth of the nutrient medium serve as energy and carbon sources and these carbohydrates affect the physiology and differentiation of tissues [4]. They also influence tissue growth, organ induction and differentiation [5-6]. Impact of different carbohydrates with other constituents of nutrient media are reported in several studies [7-9]. Among the sugars, sucrose is used as a principal carbon source for *in vitro* plant culture probably, because it is the most common carbohydrate in the phloem sap of many plants [10]. The objectives of this study were to investigate the influence of sugar concentration of the medium on growth and length of Maize seedlings. Maize plantlets were selected as model systems for this study.

Key words: Maize, Sucrose, Dextrose, Maltose, *in vitro* culture

MATERIALS AND METHODS

Plant Material and explants

Matured certified seeds of Maize were obtained from Seed Store, Kathmandu (Nepal). The seeds were washed under running tap water for 30 minutes and with Teepol for 5 minutes. After rinsing with sterile DDW, they were surface sterilized in 0.1% (w/v) $HgCl_2$ for 12 minutes and again rinsed thoroughly with sterile DDW. The maize grains were then dissected for embryo excision. Excised embryo was then transferred on 1/2MS [11] basal medium containing 0.5- 10% w/v different sugars concentration (sucrose, maltose, dextrose) and solidifies with 0.8% agar.

Medium and cultural conditions

Half MS basal media containing various concentrations of sugars (0.25% to 10%) were used for regeneration of shoots from the embryo of maize. The pH of medium was adjusted to 5.8 prior to the addition of agar 0.8% (w/v) agar and autoclaved at 15 lbs and 121^0C for 20 minutes. All the cultures were maintained at 25 ± 2^0C with 16 hrs photoperiod under 3000 lux fluorescent light intensity.

Hardening and acclimatization

Well rooted plantlets were carefully removed from culture vessels, washed under running tap water to remove the remnants of agar. After proper removal of agar dip the plantlet in the IAA solution (0.1%) for 1 minute and transferred to tray containing sand (Fig 5, 6). For 1 week, the potted plantlets were kept under transparent polythene membrane to ensure high humidity, and then they were kept in open in diffuse light for hardening. After 7 days, the surviving plants were transferred to pots containing garden soil and maintained in green house for acclimatization.

Statistical Analysis

Each experiment was repeated three times and each treatment had 10 replicates. The number of explants exhibiting regeneration was identified and the size of the shoot and root were determined. The data on size of shoot and root per explants were analyzed using standard deviation.

RESULTS AND DISCUSSION

A perusal of the data in Table 1-3 reveals that effect of different concentration of sugars on shoot regeneration in maize grain. Various types and concentrations of carbon sources were used to study their effect on shoot regeneration from excised embryo of maize. Variation in shoot response was observed in different sugars and author found that moderate concentration of maltose and sucrose enhance the root and shoot growth in comparison to dextrose. Among the different sugars, maltose and sucrose were found to be superior for plant regeneration when explant was embryo. Observation on shoot response of maize recorded that the root and shoot length decline when sugar concentration is increased. In dextrose, root and shoot length at 1.0% concentration were found to be maximum i.e., 8.5+0.5 cm and 10.2+2.1 cm, respectively (Table: 1, Fig. 3).

Fig. 1 Root & shoot regeneration

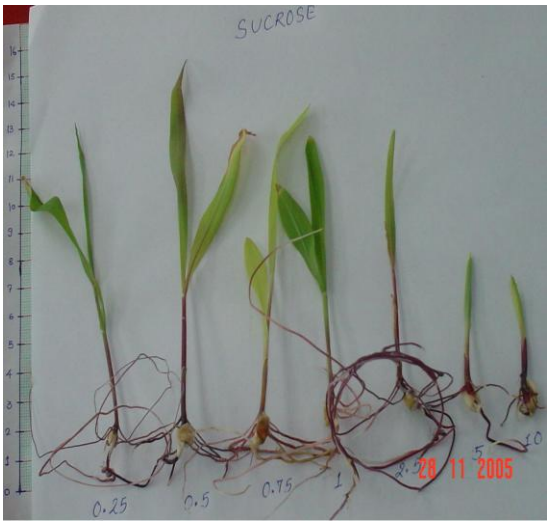

Fig. 2 Effect of sucrose on seedling

Fig. 3 Effect of dextrose on seedling

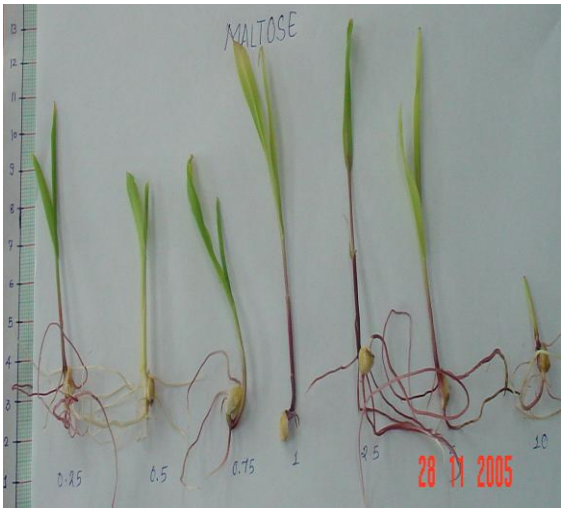

Fig. 4 Effect of maltose on seedling

Fig. 5 3days old seedling

Fig. 6 Acclimatization in sand

In maltose, highest shoot length was obtained at 1% (12.2+1.4 cm) and root length at 0.25% (9.0+1.3) (Table: 2, Fig. 4). In sucrose, Maximum root (11.4+0.3 cm) and shoot (12.0+0.6 cm) were observed at 0. 5% and 0.75%, respectively (Table: 3, Fig 1, 2). Observations showed that moderate concentrations of maltose enhances the shoot length and sucrose stimulates the root growth. The growth and multiplication of shoots *in vitro* are affected by many factors, one of which is the concentration and type of exogenous carbon source added to the medium. The carbon sources serves as energy and osmotic agents to support the growth of plant tissues [12]. In addition growth and root initiation are highly energy requiring processes that can occur at the expense of available metabolic substrates, which are mainly carbohydrates [13]. Sucrose have been proved to be better for shoot proliferation than other carbon sources in micropropagation of several plant species such as patchouli *Pogostemon cablin* Berth [14] *Centell asiatica* L. [15] and Peach root [16]. But in the present study best seedling growth was obtained on maltose supplemented medium. In *Solanum nigrum* L., among the different carbon sources fructose at 4% proved to be better choice for multiple shoot regeneration followed by sucrose, maltose and glucose [17]. Shoot length decreases at higher concentration of carbon sources may be due to the inhibition of organogenesis [18]. However, further research is needed to know the impact of carbon sources on development of shoots and the physiological changes during growth of Maize.

Table 1: Effect of different concentrations of Dextrose on shoot regeneration from Maize embryo.

Concentration of Dextrose	% Shoot regeneration	Shoot length (cm)	Root length (cm)
0.25%	100%	8.0± 1.1	4.5 ±1.0
0.5%	100%	9±0.8	3.5±1.4
0.75%	100%	8.5±1.8	2.0±0.4
1%	100%	10.2±2.1	8.5±0.5
2.5%	100%	10.0±1.3	5.5±0.4
5%	100%	6.4±1.6	4.0±0.3
10%	100%	4.5±2.0	1.5±0.2

Table 2: Effect of different concentrations of Maltose on shoot regeneration from Maize embryo.

Concentration of Maltose	% Shoot regeneration	Shoot length (cm)	Root length (cm)
0.25%	100%	8.2± 1.0	9.0 ±1.3
0.5%	100%	6.4±0.7	6.5±1.0
0.75%	100%	7.0±1.1	4.5±0.4
1%	100%	12.2±1.4	5.0±0.2
2.5%	100%	10.5±1.1	8.5±0.6
5%	100%	8.2±0.8	8.1±1.0
10%	100%	2.5±0.4	4.3±0.6

Table 3: Effect of different concentrations of Sucrose on shoot regeneration from Maize embryo.

Concentration of Sucrose	% Shoot regeneration	Shoot length (cm)	Root length (cm)
0.25%	100%	11.8± 0.7	11.1 ±1.4
0.5%	100%	12.0±0.6	10.3±1.2
0.75%	100%	11.2±1.2	11.2±0.3
1%	100%	10.1±1.8	8.0±0.7
2.5%	100%	8.5±0.4	10.9±0.8
5%	100%	5.0±0.6	4.5±0.3
10%	100%	4.4±0.3	1.3±0.2

ACKNOWLEDGEMENT
The author is grateful to the Department of Biotechnology, Kathmandu University for providing all necessary facilities for research work.

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