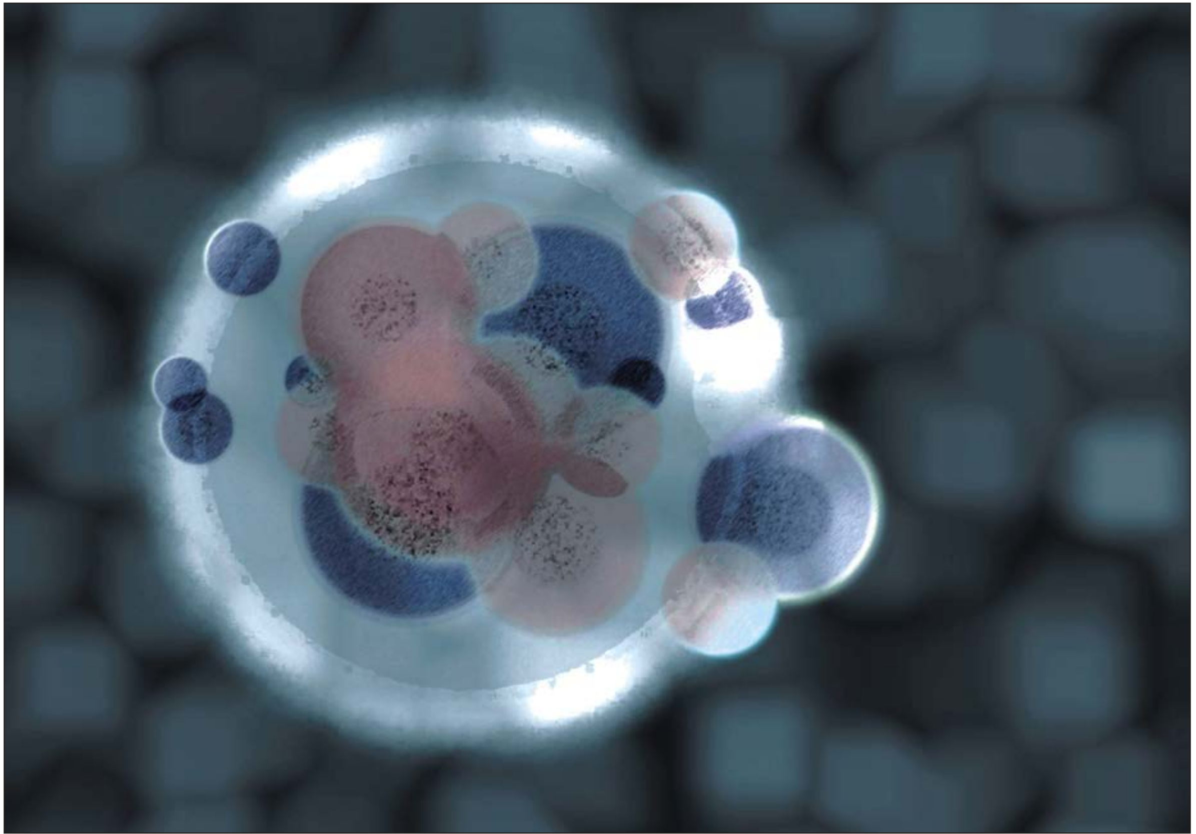

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Aseptic Multiplication and Maintenance of Bitter Gourd (*Momordica charantea* Linn.) as Affected by Sucrose, Agar and pH

[1]R.S. Sultana, [1]M.A. Bari Miah [2]M.M. Rahman, [1]M.H. Rahman and [1]M.U. Mollah
[1]Institute of Biological Sciences, Rajshahi University, Rajshahi-6205, Bangladesh
[2]Department of Botany, Rajshahi University, Rajshahi-6205, Bangladesh

Abstract: Effects of different concentrations of sucrose, agar and different levels of pH on *in vitro* axillary shoot multiplication of *Momordica charantea* Linn. were studied in the present investigation. The nodal segments from the field grown plant were used as testing plant material. For maximum number of shoot induction and multiplication in MS medium containing 2.0 mg L^{-1} BAP+0.2 mg L^{-1} NAA with 30 g L^{-1} of sucrose, 7 g L^{-1} of agar and 5.5-6.0 levels of pH proved more effective. When the medium having 30 g L^{-1} sucrose, the highest percentage of explant responded to shoot proliferation and that was 100%. This sucrose concentration also showed the optimum result for total number of shoot per culture and average length of shoots and that were 5.1±0.8 and 5.6±0.4 cm, respectively. The highest response of shoot proliferation from the nodal explant was observed on MS medium having 7 g L^{-1} of agar and the frequency was 100%. Among different levels of pH, the highest percentage of explant showing proliferation was observed on the media adjusted to pH 5.5-6.0 levels. It was proved by the present investigation that *in vitro* growth and shoot multiplication was affected by sucrose, agar and pH on the shoot induction medium.

Key words: Aseptic, multiplication, maintenance, bitter gourd

INTRODUCTION

Momordica charantea Linn. is a slender and creeping or climbing annual vine weak herb. It is a tropical vegetable and locally known as karalla, belonging to family Cucurbitaceae. It grows in tropical areas, including parts of the Amazon, East Africa, Asia and the Caribbean and is cultivated throughout South America as food yielding and medicine producing plant. In Bangladesh, it is cultivated as a vegetable crop all over the country[1]. *Momordica charantea* has a non-nitrogenous neutral principle charantin and on hydrolysis gives glucose and a sterol. The fruit pulp has soluble pectin but no free pectic acid. Galactouronic acid is also obtained from the pulp. Fruits contain glycosides, saponins, alkaloids, reducing sugars, resins, phenolic constituents, fixed oil and free acids[2]. It has been used extensively in folk medicine as a remedy for diabetes. In Ayurveda, the fruit is considered as tonic, stomachic, stimulant, emetic, antibilous, laxative and alterative. The fruit is useful in gout, rheumatism and sub acute cases of the spleen and liver diseases. It is supposed to purify blood and dissipate melancholia and gross humours. It has also been shown to have hypoglycaemic properties (anti-diabetic) in animal as well as human[3].

Plant tissue culture offers a potential tool for rapid clonal propagation of most herbaceous fruit crops. In plant breeding, these techniques would allow the rapid multiplication of a particularly desirable genotype, a potential for cost reduction of F_1 hybrids where seed costs are high due to hand labor and specifically in the propagation of triploid hybrid plants. Plantlets formation has already been reported in *Cucurbita pepo*[4], watermelon[5-7], cucumber[8,9], squash[10,11], teasel gourd[12]. The attempt of *in vitro* propagation of *Momordica charantea* Linn. was demonstrated by Islam *et al*.[13] and Sultana and Bari[14]. There have not been many studies on micropropagation of *Momordica charantea* Linn. in Bangladesh or in neighboring countries. For micropropagation of *Momordica charantea* requires developing protocol, which will be able to produce multiple shoots in a shorter time and also technically feasible. *In vitro* growth and shoot multiplication must be affected by sucrose, agar and pH on the shoot proliferation medium.

Sucrose is the most preferred carbon source in plant tissue culture. Plant cells and tissues in the culture medium lack of autotrophic ability and there fore need external carbon for energy. Even tissues, which are initially green or acquire green pigments under special

Corresponding Author: R.S. Sultana, Institute of Biological Sciences, Rajshahi University, Rajshahi-6205, Bangladesh

conditions during the culture period are not autotrophs for carbon. The addition of an external carbon source to the medium enhances proliferation of cells and regeneration of green shoots[15]. It is usually desirable for tissue explants to be in contact with but not submerged in the culture medium, solid or semisolid media often are used. Tissues cultured on such media maintain good contact with the media but also have good aeration. Agar is the most commonly used gelling agent in plant tissue culture work. It has also some effects on growth and development of the culture depending on its concentration and brand[16]. Plant cell and tissues require optimum pH for growth and development in cultures. While preparing a medium, the pH can be adjusted to the requirement of an experiment. The pH affects uptake of ions and for most of the culture media is adjusted before sterilization is considered optimal. Higher pH is likely to give a hard medium while a low level of pH results in unsatisfactory solidification of the agar.

Therefore, the present study was under taken to find out the effects of sucrose and agar and pH on *in vitro* shoot formation and multiplication of *Momordica charantea* over the cultural period. The objective of the present study was to determine the optimum cultural conditions for production of genetically stable multiple shoots from the explants.

MATERIALS AND METHODS

Shoot apices (4-6 cm) were collected from pot-grown seedling in out site condition. The shoots segments were defoliated after bringing to lab and treated with 1% Savlon for about 10 min. The material was then washed thoroughly under tap water and finally surface sterilized with 0.1% $HgCl_2$ for 5 min followed by washing thrice in autoclaved distilled water. The explants consisting of nodal segments were prepared from the surface sterilized material. They were then implanted on agar-gelled MS medium[17] for shoot proliferation. For shoot bud initiation and multiplication, the MS+2.0 mg L^{-1} BAP+0.2 mg L^{-1} NAA medium was supplemented with various concentrations of sucrose (10-60 g L^{-1}) and agar (4-10 g L^{-1}) and adjusted different levels of pH (3.5-7.0). All media were steam sterilized for 20 min at 121°C under 1.1 kg cm^{-2} pressure. The cultures were grown at 26±1°C under 16 h photoperiod with a photon flux density of about 70 μ mol $m^{-2}s^{-1}$. The experiment was conducted at Biotechnology Laboratory, Institute of Biological Sciences, University of Rajshahi, Bangladesh

RESULTS AND DISCUSSION

Effects of sucrose on shoot proliferation: Sucrose is an important factor for *in vitro* shoot proliferation. In this experiment, different concentrations of sucrose in MS medium were used for multiple shoot regeneration and development. Nodal segments were taken from *in vitro* cultures that grew on a particular medium combination for the present investigation. Nodal segments were cultured on MS medium having 2.0 mg L^{-1} BAP+0.2 mg L^{-1} NAA at eleven different concentrations (10-60 g L^{-1}) of sucrose. After 6 weeks of culture frequency of explant showing proliferation, number of total shoots per culture, number of usable shoots per culture, average length of shoots per culture were as shown in Table 1.

Among the different concentrations of sucrose in MS medium, the media having 30 g L^{-1} sucrose showed the highest percentage of explant responded to the shoot proliferation and that was 100% where the optimum result for number of total shoots per culture, number of usable shoots per culture and average length of shoots per culture were 8.80±0.25, 6.00±0.26 and 3.95±0.30 cm, respectively (Fig 1A).

Table 1: Effects of different concentrations of sucrose on growth and development of axillary shoots from nodal explants in MS medium containing 2.0 mg L^{-1} BAP+0.2 mg L^{-1} NAA. There were 15 explants in each treatment and data ($\bar{x}$±SE) were recorded after 6 weeks of culture

Different concentrations of sucrose (g L^{-1})	% of explant showed proliferation	No. of total shoots per culture	No. of usable shoots per culture	Average length of shoots per culture (cm)
10	46.7	2.90±0.16	2.44±0.42	2.15±0.13
15	66.7	3.00±0.16	2.90±0.33	2.36±0.18
20	80.0	5.26±0.24	5.00±0.28	4.00±0.20
25	86.7	6.50±0.27	5.10±0.30	3.85±0.43
30	100.0	8.80±0.25	6.00±0.26	3.95±0.30
35	86.7	4.16±0.20	3.00±0.37	3.00±0.36
40	73.3	3.20±0.24	2.95±0.36	2.50±0.29
45	46.7	4.38±0.30	4.00±0.36	3.25±0.40
50	40.0	2.80±0.28	2.70±0.40	2.10±0.24
55	33.3	2.62±0.16	2.54±0.46	2.00±0.15
60	33.3	2.09±0.20	1.84±0.42	1.90±0.19

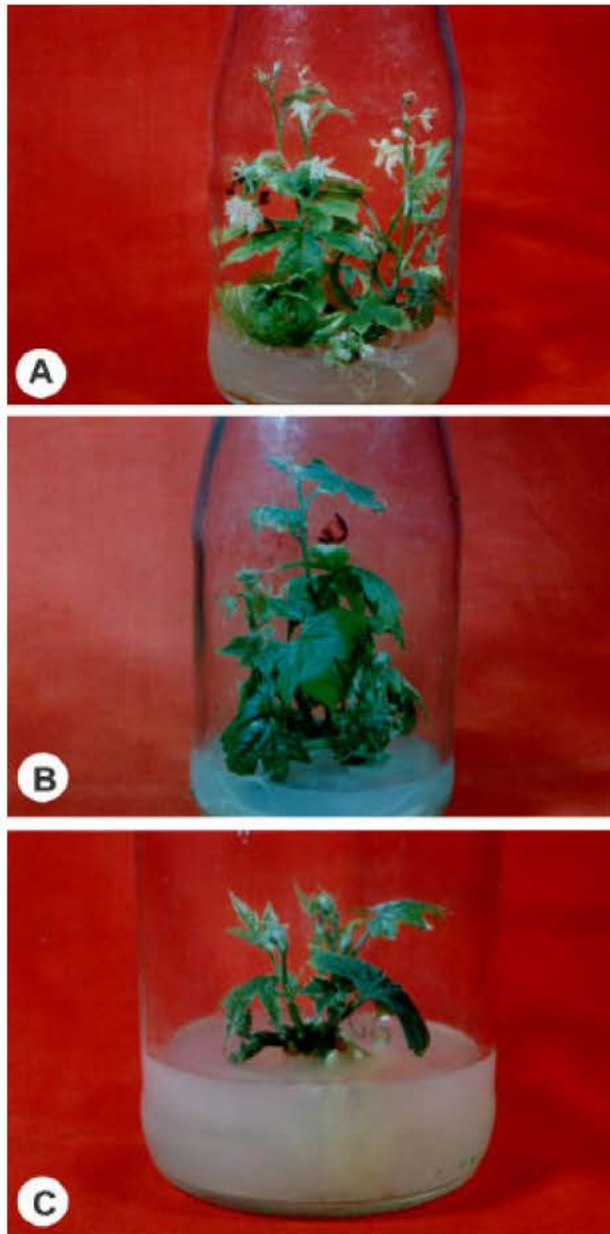

Fig. 1A-C: *In vitro* propagation from nodal segments of *Momordica charantea* Linn. as affected by sucrose, agar and pH, (1A) Shoot multiplication on MS+2.0 mg L^{-1} BAP+0.2 mg L^{-1} NAA with 30 g L^{-1} sucrose after 6 weeks of culture, (1B) Development of multiple shoot on MS+2.0 mg L^{-1} BAP+0.2 mg L^{-1} NAA with 7 g L^{-1} agar after 6 weeks of culture, (1C) A clamp of multiple shoots on MS+2.0 mg L^{-1} BAP+0.2 mg L^{-1} NAA adjusted with 5.5-6.0 pH level after 6 weeks of culture

From the present investigation it was observed that different concentrations of sucrose affected *in vitro* growth of *Momordica charantea* shoots variously. The *in vitro* grown shoots despite being green, do not rely on photosynthesis and grow as heterotrophs[18]. Inhibition of chlorophyll synthesis and shoot growth on sucrose deficient medium has also been reported by Amin and Jaiswal[19]. At 40 and 50 g L^{-1} sucrose concentrations, the shoot size was bigger but its number decreased and root growth was inhibited. The present findings also indicate that the sucrose not only acts as a carbon cum energy source in the medium but also acts as an osmoticum[20-21] and different concentrations of it act as one of the controlling factors for the induction and growth of shoots.

Effects of agar on shoot proliferation: Nodal explants were cultured on MS medium having 2.0 mg L^{-1} BAP+0.2 mg L^{-1} NAA at seven different concentrations (4-10 g L^{-1}) of agar to standardize the optimum agar concentration for maximum growth and development of the axillary shoots after 6-7 weeks (Table 2). The highest proliferation response the explant was observed on medium having 7 g L^{-1} of agar and the frequency was 100%. Number of total shoots per culture, number of usable shoots culture and average length of shoots per culture were also highest on the media that contained 7 g L^{-1} agar and they were 7.72±0.42, 6.30±0.35 and 4.10±0.44 cm, respectively (Fig. 1B).

They early spouting rapid growth shoots as were noticed on medium with lowest agar concentrations (2-4 g L^{-1}) could be distributed to the easy availability of nutrient elements like Ca, Mg, K and Mn in the soft-gel medium[22]. *In vitro* growth abnormalities like fascinated shoots and vitrified leaves have also been observed in guava cultures grown with lower concentrations of agar[19]. These growth anomalies are apprehended to be the changes in the metric potential of media water under lower gelling agent concentrations. Whereas, the highest proliferation response of the explants was observed on medium having 7 g L^{-1} agar (BDH) and the frequency was 100%. Contrary to this, reduced growth and less number of shoots on media gelled with 10 g L^{-1} agar could be due to restricted diffusion of macro nutrients[23] or reduced availability of organic matters and water[21,22,24]. It is evident from the results of present investigation and those of other[19,22] that concentrations of agar in the media can affect the culture growth in many ways. Therefore, the level of agar in the medium should be such that it minimizes the water loss and allows the good diffusion of nutrient elements.

Table 2: Effects of different concentrations of agar on growth and development of axillary shoots from nodal explants in MS medium containing 2.0 mg L^{-1} BAP+0.2 mg L^{-1} NAA. There were 15 explants in each treatment and data ($\bar{x}$±SE) were recorded after 6 weeks of culture

Different concentrations of agar (g L^{-1})	% of explant showed proliferation	No. of total shoots per culture	No. of usable shoots per culture	Average length of shoots per culture (cm)
4	40.0	2.25±0.13	2.05±0.19	2.20±0.34
5	66.7	3.60±0.14	3.10±0.46	2.80±0.52
6	80.0	5.75±0.34	5.11±0.26	3.65±0.21
7	100.0	7.72±0.42	6.30±0.35	4.10±0.44
8	73.3	4.00±0.13	3.80±0.21	3.30±0.62
9	60.0	3.00±0.17	2.82±0.36	2.63±0.53
10	46.7	2.65±0.51	2.20±0.19	2.50±0.18

Table 3: Effects of different levels of pH on growth and development of axillary shoots from nodal explants in MS medium containing 2.0 mg L^{-1} BAP+0.2 mg L^{-1} NAA. There were 15 explants in each treatment and data ($\bar{x}$±SE) were recorded after 6 weeks of culture

Different levels of pH	% of explant showed proliferation	No. of total shoots per culture	No. of usable shoots per culture	Average length of shoots per culture (cm)
3.5-4.0	40.0	3.21±0.23	2.20±0.46	2.35±0.37
4.0-4.5	46.7	4.33±0.16	2.55±0.23	2.80±0.31
4.5-5.0	60.0	4.75±0.26	5.11±0.20	3.00±0.29
5.0-5.5	73.3	5.90±0.24	3.59±0.24	3.55±0.36
5.5-6.0	100.0	6.52±0.19	4.61±0.23	4.06±0.43
6.0-6.5	66.7	5.00±0.15	3.20±0.24	3.25±0.26
6.5-7.0	53.3	4.62±0.22	3.00±0.16	2.85±0.17

Effects of pH on shoot proliferation: The pH of the culture media is an another important factor for the *in vitro* proliferation and healthy culture growth. In the present study, nodal segments were cultured on MS medium containing 2.0 g L^{-1} BAP+0.2 g L^{-1} NAA with adjusted to seven different levels of pH (Table 3). Among these pH levels, the highest percentage of explant showing proliferation was observed on the media adjusted to pH 5.5-6.0 and that was 100%. Number of total shoots per culture, number of usable shoot culture and average length of shoots per culture were 6.52±0.19, 4.61±0.23 and 4.06±0.43 cm, respectively (Fig. 1C).

From the present investigation, it was revealed that both lower (4.5) and higher (6.5) pH levels hindered multiple shoot proliferation. Comparatively less acidic pH 6.5 gave harder get, which might have adverse effects on regeneration proliferation of shoots. *In vitro* proliferation of *Cucurbita pepo*[4] watermelon[5-7], cucumber[8,9], squash[10,11], teasel gourd[12] shoots was increased significantly when the pH culture media was adjusted at 5.7±0.1 before autoclaving.

Plant regeneration through tissue culture technique would be a noble alternative process for improving the quality and faster production of *Momordica charantea*. *In vitro* culture techniques permit the shoot induction and multiplication under aseptic condition with reduced space requirements because of the small size of explant. It has been demonstrated that sucrose, agar and pH on the medium play an important role on *in vitro* growth and development of shoots. Therefore, the present report showed that in MS medium containing sufficient growth regulators combination, sucrose 30 g L^{-1}, agar 7 g L^{-1} and pH 5.5-6.0 level proved more effective on shoot multiplication.

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