

Effect of different levels of sucrose on microtuberization and different substrates on minituber production resulted from potato meristem culture

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Abstract: The present research work was undertaken to choose the most effective sugar concentration in *in vitro* microtuberization from cultured shoot tips and to select the best substrates for minituber production from *ex vitro* transferred plantlets. Percentage of survivability of microtuber producing plants increased with the increase of sucrose concentration. Best survival rate and average diameter of tuber per plant was found at 10% sucrose. Minituber production on direct field showed best performance. It was observed that after 30 days, maximum shoot length per plant (188.87 mm) was found on the direct field whereas the lowest shoot length per plant (66.81 mm) was on coconut dust. Lowest weight of tuber per plant was also recorded on the substrates containing coconut dust. The highest weight of tuber was found in direct field. In case of, average diameter of tuber per plant, 25% soil + 75% coconut dust showed minimum result and on the other hand, in direct field, large size of tubers were found.

Key words: Microtuberization, Minituber, *In vitro*, *Ex vitro*, Potato

I. Introduction

Microtubers ("in vitro" developed tubers) are miniature seed potatoes and they represent an intermediary phase between "in vitro" plantlets and minitubers. Microtubers are the first generation of potato seed from tissue culture, being used to solve the problems of transplanting the plantlets from "in vitro" to "in vivo" conditions. The microtubers offer a lot of advantages to storage, transport and mechanization due to their little size and reduced weight. They can be planted directly in the soil and they can be produced in any period of the year. Microtuber production "in vitro" is very important for producing and storage of valuable seed potato stocks.

Potato microtubers obtained by "in vitro" culture from single-node cuttings are convenient for handling, storage and exchange of a healthy germplasm, representing an important component, along with plantlets and minitubers, for seed potato production programs (Roşu R., 2004). Microtuber production represents in the same time an efficient method for obtaining a healthy material, by which the process of production is reduced with 3-4 years. The sucrose is the most critical stimulus for tuber induction (Wang, 1982 and Abbott, 1986), as results from the review of Struik (1999), regarding induction, initiation and growth of potato tubers. The high sucrose concentrations are essential for "in vitro" microtuber induction, influencing this process through the osmotic effect and by serving as energy source (Simko I. 1994, Perl A. 1991, Struik PC.1999)

Minitubers are generally produced in the greenhouse in many countries around the world. The source of material is either the plantlets developed inside the growth chamber of tissue culture laboratory or the already produced mini or micro tubers in the greenhouse and in the laboratory, respectively. Greenhouse should be facilitated with temperature, light, humidity and air circulation for the production of minitubers.

In *in vivo* condition average tuber weight and tuber number were determined as different depending on the production technique (Struik, 1999). Khuri *et al.*, (1995) found that the tuber numbers of various potato cultivars were between 6.2 to 7.9 in plantlets grown in polyethylene bags. Present research work has been undertaken to fulfill the following specific objectives to choose the most effective sugar concentration in *in vitro* microtuberization from cultured shoot tips and to select the best substrates for minituber production from *ex vitro* transferred plantlets.

II. Materials and Methods

2.1 Experimental site:

The experiment was carried out at the Plant Tissue Culture Laboratory of Agrotechnology Discipline, Khulna University, Khulna-9208, Bangladesh, during April 2010 to January 2011.

2.2 Type of explants:

In vitro grown potato explants were used as source materials for microtuberization and minituber production.

2.3 Constituents of media for microtuberization of potato:

For microtuber formation, MS medium supplemented with BAP-5.0 mg l⁻¹, Chloro Choline Chloride (CCC)-500 mg l⁻¹ and different concentrations of sucrose-5%, 6%, 7%, 8%, 9%, 10% is used.

2.4 Substrates used in pots for minituber formation:

Following substrates were employed for minituber production:

- | | |
|----------------------------|-----------|
| 1. Soil | 100% |
| 2. Sand | 100% |
| 3. Coconut dust | 100% |
| 4. Soil : Sand | 50% : 50% |
| 5. Soil : Coconut dust | 25% : 75% |
| 6. Soil : Coconut dust | 50% : 50% |
| 7. Soil : Coconut dust | 75% :25% |
| 8. Directly into the field | |

2.5 Experimental design

All the experiments were laid out in a Completely Randomized Design (CRD) with five replications for each treatment. In that case, a plastic pot containing different substrates with a single healthy plant was considered as a replication.

2.6 Procedure for microtuberization

After taking all the precautions to ensure aseptic condition, *in vitro* grown healthy plantlets were selected for further inoculation. All inoculation and aseptic manipulations were carried out under a laminar airflow cabinet. plantlets were removed carefully and cut the shoot tips measuring 2 cm in length. Then they were placed onto nutrient media containing test tubes for microtuberization.

After inoculation of the explants, the microtuber induction cultures were incubated in the dark at 24°C and relative humidity 65%, maintaining a temperature of 18° C. Microtuber was first observed at 35 days after inoculation.

The experiment was laid out in a Completely Randomized Design (CRD) with ten replications. A test tube (15 cm length) containing 15 ml medium with a single shoot tip of potato was considered as a replication.

2.6 Procedure for minituber production

For minituber production of potato, *in vitro* cultured plants were transplanted into plastic pots (4 inch size) with different substrates (soil, sand, coconut dust and their different combination) and some were directly into the field. Soil, sand and coconut dust were autoclaved at 121° C for 15 minutes before transplantation. After proper mixing of different substrates according to each treatment, they were kept in plastic pots. Few minutes later after watering, plants were transferred into pots. Transferred plants were placed into growth chamber with 85% humidity for 24 hours. After that, they were kept in net house.

2.7 Parameters studied and data collection

To study the effect of different sugar concentrations on *in vitro* microtuberization,

- survivability of plant,
- percentage of plant producing microtuber,
- no. of microtuber per plant and
- average diameter of tuber per plant were measured.

After 20 days interval, data were collected.

For experiment of *ex vitro* minituber production, data were collected by measuring

- percentage of survivability of plants up to 30 days,
- leaf color,
- shoot length per plant,
- no. of tuber per plant,
- average diameter of tuber per plant and
- average weight of tuber per plant.

Leaf color of every plant was observed visually.

2.8 Analysis of data

Recorded data for all of the experiments were analyzed for ANOVA (Analysis of Variance) with the help of computer using MSTAT-C program and the means were compared according to Duncan's Multiple Range Test (DMRT) and Least significance Differences (LSD).

III. Results and Discussion

The results of the experiments (Table 1) revealed that the percentage of plants producing microtuber increased with the increase of sucrose concentrations. Percentage of survivability also increased with the increase of sucrose concentration. The highest survival rate (80%) was found at 10% sucrose. The highest number of microtuber was 2.17 observed on 10% sucrose where as the lowest number was 1.33 at 6% sucrose. In case of average diameter of tuber plant⁻¹, it was observed that 10% sucrose showed best result. Average diameter of tubers varied from 3.12 mm to 5.11 mm irrespective of sucrose concentrations.

Table 1. Effect of different sucrose concentration in presence of BAP (5 mgL⁻¹) and CCC (500 mg L⁻¹) on *in vitro* microtuberization from cultured shoot tips.

Sucrose concentration (%)	Survivability (%)	Plants producing microtuber (%)	No. of microtuber plant ⁻¹	Average diameter of tuber plant ⁻¹ (mm)
5	50	40	2	3.12
6	50	40	1.33	3.91
7	50	50	1.5	4.44
8	60	60	2	4.50
9	70	60	2	4.62
10	80	70	2.17	5.11
Level of significance	NS	NS	0.01	0.01
LSD	-	-	1.363	2.285

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 (Pruski *et al.*, 2003). For optimal plantlet growth, sucrose level sustainability are necessary and if it is rapidly hydrolyzed into glucose and fructose making the long term maintenance of desirable sucrose level is difficult. The present investigation was tended to find out the effect of sugar level on microtuberization and found that microtuberization increased with the increasing sugar level and the optimum concentration was 10% sucrose which was dissimilar to Pruski *et al.*, (2003).

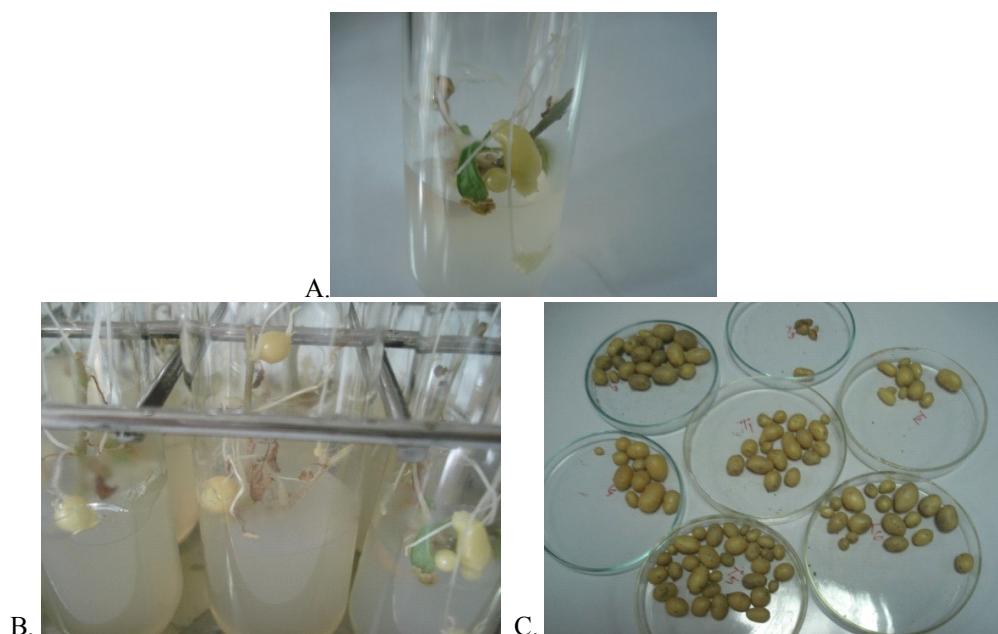


Plate 1. Effect of sucrose on *In vitro* microtuberization. A. Microtuber formation at 5% sucrose. B. Microtuber formation at 10% sucrose. C. Harvested Microtubers from *in vitro* grown plantlets.

In vitro grown potato plantlets were carefully removed, washed and planted into different substrates to investigate *ex vitro* performance of plants and performance of minituber production. Survival rate of plants after 30 days was found similar for all substrates whereas, leaf colors of plants vary from substrates to substrates.

Minituber production on direct field showed best performance. It was observed that after 30 days, maximum shoot length plant⁻¹ (188.87 mm) was found on the direct field whereas the lowest shoot length plant⁻¹ (66.81 mm) was on coconut dust. Average weight of tuber plant⁻¹ varied from 0.77 gm to 6.17 gm irrespective of different substrates. Lowest weight of tuber plant⁻¹ was recorded on the substrates containing coconut dust; although soil, sand, coconut dust, 50% soil + 50% sand, 25% soil+ 75% coconut dust, 50% soil + 50% coconut dust and 75% soil + 25% coconut dust were found statistically similar effective for weight of tuber. The highest weight of tuber was found in direct field. In case of, Average diameter of tuber plant⁻¹, 25% soil+ 75% coconut dust showed minimum result and on the other hand, in direct field, large size of tubers were found (Table 2).

Table 2. Ex vitro minituber production using different substrates.

Substrates	Survivability (%)	Leaf color	Shoot length(mm)	No. of tuber plant ⁻¹	Average diameter of tuber plant ⁻¹ (mm)	Average weight of tuber plant ⁻¹ (gm)
Soil	100	Green	131.76b	3.4bc	15.20	2.47b
Sand	100	Yellowish	69.32c	2.0bc	15.09	1.23b
Coconut dust	100	Yellowish	66.81c	1.0c	12.94	0.77b
50% soil + 50% sand	100	Light green	95.85bc	2.75bc	14.85	1.5b
25% soil + 75% coconut dust	100	Yellowish	92.14bc	4.6bc	12.49	1.36b
50% soil + 50% coconut dust	100	Light green	117.4b	4.5bc	12.53	1.63b
75% soil + 25% coconut dust	100	Green	135.34b	5.6b	14.37	2.6b
Direct on field	100	Dark green	188.87a	9.4a	15.5	6.17a
Level of significance	NS		0.01	0.01	NS	0.01

Data in a column followed by same letters are not significantly different according to DMRT.

In *in vivo* condition average tuber weight and tuber number were determined as different depending on the production technique (Leifert and Struik, 1992; 1999). Keller *et al.*, (2006) found that the tuber numbers of various potato cultivars were between 6.2 to 7.9 in plantlets grown in polyethylene bags. In the study of minituber production, result showed that plants which were directly transplanted into field represented best result than other substrates.





Plate. 2. Minituber formation from different substrates in *ex vitro* condition.

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