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Potato germplasm innovation through micro tuberization technique

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Abstract

In vitro micro tuber formation potentiality of potato was investigated to establish a rapid disease free seed production system as well as potato germplasm innovation. Micro tubers were induced to meristem derived *in vitro* grown shoots in culture bottles increasing the concentration of BA (6-Benzyl adenine), KIN (Kinetin) and sucrose in MS (Murashige and Skoog 1962) medium. KIN showed better performance than BA for micro tuber induction and among the three concentrations (6-10 mg/l) of KIN, 8 mg/l KIN was the most effective and more preferred concentration for micro tuber induction. It was observed that increase the level of KIN also increased the percentage of tuberization, number and weight of micro tuber/shoot. By increasing the concentration of sucrose increased the presentence (%) of *in vitro* tuberization. The media containing 60 g/l was found to be the best among three concentrations (40-70 g/l) of sucrose tested followed by 70 g/l sucrose for micro tuber induction for all four potato cultivars.

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Introduction

Potato (*Solanum tuberosum L.*) is the most important non-cereal food crop of the world. In monetary terms it ranks fourth in the world after wheat, rice and maize (Anonymous, 1999). It produces the largest quantity of carbohydrates per day per unit area among the food crops (Zaag & Horton, 1983). In Bangladesh potato substantially supplement of food requirements of the country after two cereals of rice and wheat (Sarker & Mostafa 2002). It is enriched with starch, protein, iron, magnesium, potassium and vitamin B&C, fat and fiber (Thompson and Kelly 1957). It provides roughly half of the world's annual production of all root of the diet of half a billion consumers in the developing countries (Ghislain et al 1999). To make Bangladesh self sufficient respect to vegetables, plant biotechnologists are trying to improve potato variety through integrated biotechnological approaches and *In vitro* tuberization is considered as one of the important tools. Tissue culture techniques are used worldwide to produce pre-basic, virus-free seed potatoes known as micro tuber. The micro tubers are sown in a protected environment to produce minitubers (basic seed). Seed production technique of potato can be designed with *in vitro* multiplication through either plantlet regeneration or micro tuber production. But Micro tuber method has several merits over plantlet regeneration. Micro tubers are very convenient and easy to transport, it can be easily stored for long time. Hence, it is necessary to establish a protocol for *in vitro* production of micro tuber for rapid multiplication. Although, researches have been carried out on micro tuber production in potato, very little attention has been paid on the *in vitro* tuberization with BA (6-Benzyl adenine), Kinetin (KIN), sucrose concentration and different types of explants to establish suitable regeneration protocol. Therefore, the experiment was designed to find out the best concentration for BA (6-Benzyl adenine), KIN (Kinetin) and sucrose for successful micro tuber production in potato.

The main objective of the present study was to standardize the media for potato plant growth and micro tuber induction. The use of micro tuber technology in seed tuber production, breeding programs, germplasm conservation, and research appears to have enormous potential.

Materials and methods

Plant materials

Disease free tissue cultured meristem derived plantlets of four potato (*Solanum tuberosum*) varieties-Lady rosety, Asterix, Shepody and Indurkani were used as primary sources of explant for *in vitro* tuberization which were obtained from the Potato germplasm bank of Plant Breeding and Gene Engineering Lab, Department of Botany, Rajshahi University.

Plant growth regulators

The plant growth regulators and additives were used for this experiment in different concentration was Benzyl adenine (BA), 6-Furfuryl amino purine or kinetin (KIN).

Nutrient basal salts

MS (Murashige and Skoog 1962) formulation was used in the present study. All chemical compounds including macro and micro nutrient, organic acid and inorganic acids, sugar, agar, KOH, 70% ethyl alcohol etc.

Others materials

The plant material and nutrient media were contained within some type of culture vessel; the whole constituting a culture system. The culture vessel such as bottle (12×5mm), conical flask (250ml, 1000ml), measuring cylinder glass rods, beaker, pipette pumps, rubber bands, filter paper, aluminum foils, marker pen, spirit lamp, forceps, needle, scalpels blade, firebox electronic balance, autoclave, pH meter, magnetic stirrer, Laminar airflow machine etc. were also used in the present experiment.

Methods

MS (Murashige and Skoog 1962) medium was used for node culture and further tuberization. Sucrose was added at the rate of 40-70 g/l. Plant growth regulators were added separately to different media according to requirements. Agar was added at the rate of 6.5 g/l for the preparation of semi-solid MS medium. Stock solution of PGR (plant growth regulators), the nutrient media were used. To prepare the stock solution of any of these plant growth regulators, 10 mg of solid growth regulators were placed in to a clean test tube and then dissolved in specific solvent. The volume of the solution was then made up to 100 by adding distilled water. It was stored in a refrigerator in 4°C-6°C temperature for ready to use at any time. Data were collected were percentage of *in vitro* tuberization, Number of micro tubers/shoot, weight of micro tubers/shoot. All the counted data imputed in mean value with standard deviation (M±SD) and it was tested with DMRT technique.

Table 1. Effect of various concentrations of BA (6-benzyl adenine) and control (MS media without hormonal treatment) on % of in vitro tuberization, No. of micro tubers/shoot, Weight of micro tuber/shoot (mg) of potato cultivars.

Treatment		% of *in vitro* tuberization	No. of micro tubers/shoot	Weight of micro tubers/shoot (mg)
Cultivar	BA(gm/l)	M±SD	M±SD	M±SD
Lady rosety	0	19.6±2.07i	0.58±0.16g	49±4.18g
	6	44.6±1.14e	3.3±0.97a	101.3±1.81f
	8	48±1.58c	2.22±0.14de	104±0.92e
	10	53±0.70a	1.74±0.27f	104.38±5.14e
Asterix	0	9.4±2.40j	0.3±0.15g	42±1.58h
	6	38±1.58g	2.84±0.11bc	101±1.92f
	8	45.6±1.14de	3.22±0.13ab	106±1.53d
	10	50±1.58b	1.9±0.15ef	111±1.58b
Shepody	0	8.2±1.48jk	0.2±0.07g	36±2.92i
	6	38.2±1.30g	2.58±0.13cd	103±1.32e
	8	46.4±1.14cd	3.32±0.13a	108±1.15c
	10	51.8±0.83a	1.86±0.18ef	114±1.12a
Indurkani	0	7.6±1.14k	0.44±0.08g	32±1.48j
	6	32.6±1.14h	2±0.15ef	101±1.24f
	8	41.2±1.31f	3.4±0.15a	103±2.12e
	10	46.8±1.31cd	1.7±0.12f	112±1.58b

Note: Same letter have no different, All the characters tested with DMRT.

Result and discussion

The present investigation was carried out to establish a protocol for the production of *in vitro* micro tuber at large scale. The highest 53% of explants showed *in vitro* tuberization and it was obtained in medium containing 10 mg/l BA in lady rosety and the lowest 32.6% *in vitro* tuberization was observed in 6 mg/l BA in indurkani. Highest number of micro tubers (3.4) was recorded in 8 mg/l BA in indurkani and the lowest (1.7) numbers of micro tubers was found in 10 mg/l BA in indurkani. The highest weight (114 mg) of micro tuber per shoot was recorded in 10 mg/l BA in shepody and the lowest weight (101 mg) of micro tuber per shoot was observed in 6 mg/l BA in lady rosety, asterix and indurkani. All the parameter is clearly different with the control and that is the lowest result (Table 1). Table 1 Effect of various concentrations of BA (6-benzyl adenine) and control (MS media without hormonal treatment) on % of *in vitro* tuberization, No. of micro tubers/shoot, Weight of micro tuber/shoot (mg) of potato cultivars.

Four cultivars of potato viz. Lady rosety, Asterix, Shepody and Indurkani and three concentrations of

KIN (6, 8, 10 mg/l) were used. Data were recorded after 12 weeks of culture and are shown in Table 2. For highest percentage (74%) of *in vitro* tuberization was observed in 8 mg/l KIN containing medium in Asterix variety and Lowest 40% was recorded in 10 mg/l KIN in Indurkani. Highest number (3.9) of micro tuber per shoot was found in 8 mg/l KIN containing medium in Asterix and lowest (2.5) number of micro tubers were found in 6 mg/l KIN in Indurkani. Highest micro tuber weight (202 mg) was found in 10 mg/l KIN containing medium in Indurkani and lowest weight (116 mg) was recorded in medium containing 6 mg/l KIN Indurkani. From this experiment it was concluded that 8 mg/l KIN fortified medium was found to be the most effective for *in vitro* tuberization in potato and control was clearly different to the treatment and was lowest value (Table 2).

Table 2. Effect of various concentrations of KIN (Kinetin) and control (MS media without hormonal treatment) on % of in vitro tuberization, No. of micro tubers/shoot, Weight of micro tuber/shoot (mg) of potato cultivars.

Treatment		% of in vitro tuberization	No. of micro tubers/shoot	Weight of micro tubers/shoot (mg)
Cultivar	KIN (mg/l)	M±SD	M±SD	M±SD
Lady rosety	0	5±1.58k	0.7±0.16j	21±1.58k
	6.0	61±1.58e	2.85±0.02e	185.3±0.16e
	8.0	68±1.58c	3.62±0.02c	200.5±0.16b
	10.0	53±1.58h	3.4±0.16d	190.4±0.16c
Asterix	0	4±1.58ks	0.7±0.16j	15±1.58l
	6.0	66±1.58d	2.76±0.11ef	189.2±0.16d
	8.0	74±1.58a	3.95±0.02a	200.3±0.16b
	10.0	70±1.58b	3.82±0.02b	202.4±0.16a
Shepody	0	5±1.58k	0.4±0.16k	12±0.71m
	6.0	58±1.58f	2.62±0.02g	140.5±0.16i
	8.0	61±1.58e	2.73±0.02fg	182.3±0.16f
	10.0	49±1.58i	2.66±0.02fg	168.5±0.16h
Indurkani	0	3±1.58s	0.3±0.16k	11±1.58n
	6.0	56±1.58g	2.5±0.16h	116.1±1.58j
	8.0	58±1.58f	2.65±0.02fg	170.6±1.58g
	10.0	40±1.58j	2.26±0.02i	140.46±1.65i

Note: Same letter have no different, All the characters tested with DMRT.

Table 3. Effect of sucrose on *in vitro* tuberization of potato cultivars.

Treatment		% of in vitro tuberization	No. of micro tubers/shoot	Weight of micro tubers/shoot (mg)
Cultivar	Sucrose (g/l)	M±SD	M±SD	M±SD
Lady rosety	0	8±1.58h	0.6±0.15f	12±1.58l
	40	66±4.18f	3±1.58cd	125.24±0.82h
	60	77±1.58a	3.5±0.15ab	205.12±1.61d
	70	70±1.58cde	1.7±0.15e	210.96±0.08a
Asterix	0	5±1.58i	0.4±0.15f	13±1.58k
	40	69±1.58e	3.1±0.15bcd	130.5±0.15g
	60	71±1.58cd	3.7±0.15ab	208.3±0.15c
	70	73±1.58bc	3±1.58cd	211±1.58a
Shepody	0	9.5±1.58h	0.3±0.15f	7±1.58m
	40	65.5±1.58f	3.3±0.15abc	120.5±0.15i
	60	76.5±1.58a	3.8±0.15a	205.3±0.15d
	70	70.5±1.58cde	3.4±0.15ab	209.5±0.15b
Indurkani	0	5±1.58i	0.4±0.15f	3±1.58n
	40	49±1.58g	2.58±0.19d	93.2±0.15j
	60	72±1.58bc	3.9±0.15a	155.2±0.15e
	70	65±1.58f	2.7±0.15cd	133.6±0.15f

Note: Same letter have no different, All the characters tested with DMRT.

Four cultivars of potato viz. Lady rosety, Asterix, Shepody and Indurkani and three concentrations of sucrose (40, 60, 70 g/l) and control were used (Table 3). It was observed that percentage of *in vitro* tuberization ranged from 49-76%. The highest 76% shoots induced micro tuber was observed in medium containing 60 g/l sucrose in shepody and lowest 49% *in vitro* tuberization was recorded in medium containing 40 g/l sucrose in indurkani. Number of micro tubers/shoot ranged from 1.7-3.9. The highest number (3.9) was found in medium containing 60 g/l sucrose in indurkani and lowest (1.7) number of micro tuber was observed in 70 g/l sucrose in lady rosety. The weight of micro tuber/shoot ranged from 93-211mg. The highest micro tuber weight (211 mg) was observed in medium containing 70 g/l sucrose in asterix and lowest micro tuber weight (93 mg) was observed in 40 g/l sucrose containing medium in indurkani. The media containing 60 g/l and 70 g/l sucrose were found to be the better among three sucrose concentrations and also in control (Table 3).

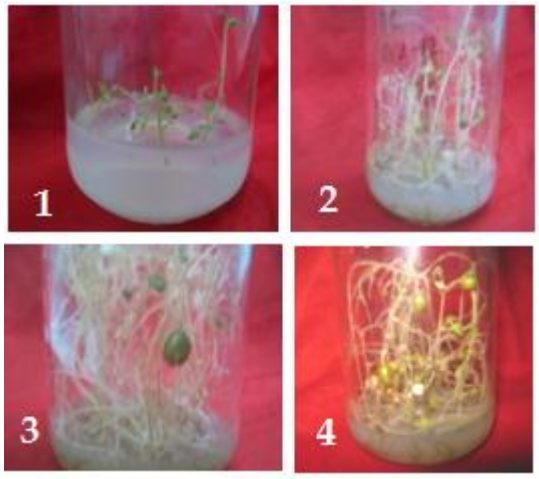

Fig. 1-4. Different stages of micro tuberization

In this experiment results reveal that the acceleration of microtuber formation varied concentration, types of phytohormone and genotypes. There are reports that *in vitro* tubers can be sessile on the nodes of the stem (Catchpole and Hillman, 1969, Mes and Menge, 1954) or can be axillaries or terminally formed on new growing shoots (Hussey and Stacey, 1984, Wang and Hu, 1982, Stallknecht, 1972). In the present experiment, it was clearly observed that phytohormonses, especially cytokinins are very useful for the acceleration of *in vitro* tuberization in potato. Requirement of cytokinin for *in vitro* tuberization has also been reported by several workers (Wattimena *et al.,* 1983, Kefi et al., 2001; Mingo-Castel *et at*., 1976, Wang and Hu, 1985). In this investigation it was observed that KIN is most effective for micro tuber induction and production. This was supported by other workers (Wattimena *et al.* 1983, Hussey and Stacey, 1984, Ziv. and Shemesh, 1996). In conclusion, it may be recommended for acceleration of micro tuber induction and production use of KIN is most needed and in this finding, KIN 8 mg/l was most effective. Micro tuberization was earliest at 6 mg/l and 8 mg/l BA. Either higher or lower concentration of this level delayed tuberization. Sucrose concentration of the medium was most important organic nutrient variable effecting rate and percentage of *in vitro* tuberization of potato.

Fig. 5. Different types of micro tuber production under different treatments

The results obtained in the present investigation (Table 3) showed that there was no significant difference between 40, 60 and 70% sucrose but on average, 60% sucrose produced more micro tubers than 40, 70, and in control. Many research workers also found that the optimum sucrose concentration for *in vitro* tuberization was between 60% and 70% (Catchpole and Hillman, 1969; Lawrence and Barker, 1963; Obata-

Sasamoto and Suzuki, 1979, Palmer and Smith, 1970, Stalknecht and Farnsworth, 1979, Wang and Hu, 1982). Increasing the sucrose concentration from 40% to 70% increased the earliness and percentage of *in vitro* tuberization (Alam *et al*. 2003a). According to Lo *et al*., (1972) this kind of response was a response to sucrose as an energy source and not as an osmotic modifier since the sucrose effect could not be replaced by mannitol. The result further showed that the frequency of tuberization increased with the increased concentration of sucrose and 60-70 g/l was found optimum. The concentration of sucrose above this limit resulted decrease of tuberization. This might be due to effect of high osmoticum (Ishak *et al*. 1992).

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