Evaluation of different levels of Sucrose, BAP and Photoperiod regimes for *In-vitro* Microtuber Production of Potato (*Solanum tuberosum* L.)

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Abstract
Single-node stem cuttings of potato cv. "Desiree" were cultured on a multiplication medium containing MS basal elements supplimented with 0.5 mg l^{-1} BAP, 0.01 mg l^{-1} NAA and 0.4 mg l^{-1} GA_3 for maintaining stock plantlets. The cultures were exposed to 25 ± 2^{0}C temperature and 16/8 hrs photoperiod with 75% relative humidity in a growth chamber. Six week-old plantlets from mother stock were treated with 10 ml MS liquid medium supplemented with four different levels of sucrose (100, 120, 140, 160 g l^{-1}) and four different levels of BAP (0, 2, 4, 6 mg l^{-1}) in all possible combinations to promote microtuberization (Experiment 1). The cultures were maintained at 15±2^{0}C temperature and 0/24 hrs photoperiod (total darkness). The treatment combination of 140 g l^{-1} sucrose and 6 mg l^{-1} BAP gave the highest microtuber fresh weight (277.8 ± 179.4 mg), highest microtuber dry weight (58.4 ± 33.4 mg), highest starch assimilation capacity of explant (605.8 ± 353.3 mg), highest cross sectional tuber area (66.7 ± 29.1 mm^2), maximum number of microtubers per plantlet (3.32 ± 0.12) and the highest number of eyes per microtuber (4.79 ± 0.06). The second experiment was designed to evaluate the effect of two different sucrose levels (120, 140 g l^{-1}) and three photoperiod regimes (0/24, 8/16, 16/8 hrs light/dark) on microtuberization of potato. As in experiment 1 stock plantlets were maintained at same culture conditions and 10 ml of prepared two sucrose solutions were poured into six-weeks- old plantlets in all possible combinations to promote microtuberization. Growth regulators were not used in this MS liquid medium. The cultures were maintained at 15 ± 2 ^{0}C temperature. According to the results, most suitable treatment combination was 140 g l^{-1} sucrose and 8/16 hrs photoperiod, which produced microtubers with highest fresh weight (469.1 ± 443.3 mg), highest dry weight (114.7 ± 113.8 mg), highest starch assimilation capacity per microtuber (469.1 ± 443.3 mg), highest cross sectional tuber area (97.1 ± 63. 5 mm^2) and the highest number of eyes per microtuber (16.1 ± 0.3). In conclusion, these results could be employed to adopt most optimum sucrose, BAP levels and photoperiod regime for effective microtuber production of potato (cv. Desiree) based on the MS basal solution.
Key words: Microtuberization, Microtubers, Sucrose, BAP, Photoperiod

Introduction

The propagation of potato by *in-vitro* culture of axillary buds is commonly used in the production of disease-free seed tubers and germplasm exchange and conservation (Ranalli *et al.*, 1994). The *in-vitro* propagated plantlets produce microtubers (approx. 2-10 mm in diameter) when incubated under suitable conditions (Wang and Hu, 1982; Estrada *et al.*, 1986). The cost of producing microtuber is relatively higher than that of *in-vitro* plantlets. Thus, the use of microtubers for the preservation of genetic resources is limited. Furthermore, microtubers are used as mother stock to fulfill the shortages of planting material and for culture renewal programs as conservation material because of the easiness of their maintenance and handling when compared to *in-vitro* plantlets. In Sri Lanka potato occupies approximately 6400 ha with a total annual production of over 75,000 t. Over 78,000 large and small scale farmers are engaged in potato cultivation. Out of the total cost of production, over 40-50% accounts for seed materials. Therefore, the Department of Agriculture has emphasized the need to provide farmers with seeds at low cost. The national potato seed production program was initiated in late 1996 at the Agriculture Research Station (ARS) at Sitha Eliya. At present ARS supplies advanced generation seeds to the farmers, which can be multiplied in farmer fields. Considering the above facts, experiments have been conducted to determine factors controlling the tuberization process in view of developing rapid and cost effective methods for large scale production of microtubers. *In-vitro* microtuberization process is controlled by physical, hormonal (chemical), nutritional and physiological factors. Among these, the need for comparatively high sucrose concentration as well as role of the hormones in the medium for microtuber formation have been revealed (Dodds, 1988). It would thus be of great advantage to investigate the combine effect of sucrose and cytokinin (BAP) with photoperiod on *in-vitro* microtuber production.

Materials and methods

Maintenance of explants (Mother Stock)

Single nodal stem cuttings of cv.Desiree were cultured *in-vitro*, at the rate of one segment per culture tube. Each tube contained 10 ml of MS (Murashige and Skoog, 1962) basal medium containing 30 g l^{-1} sucrose, 0.5 mg l^{-1} BAP, 0.01 mg l^{-1} NAA and 0.4mgl^{-1} GA_3. The culture tubes were sealed with polythene and incubated under 25±2^{0}C, 75% relative humidity and 16/8 hrs light/dark regime with 2500-3000 lux of light intensity.

Experiment 1. Effect of different levels of sucrose and BAP on mirotuberization

Six-week-old *in-vitro* plantlets from mother stock were treated with 10ml of MS liquid tuberization media containing using four different levels of sucrose (100, 120, 140, 160 g l^{-1}) and four levels of BAP (0, 2, 4, 6 mg l^{-1}) (Table 1). All the plantlets were placed in a cooling cabinet in 0/24 light/dark regime at 15±2 ^{0}C temperature with 75% relative humidity.

Table 1. Treatment combinations of sucrose and BAP levels tested for *in-vitro* microtuber production of potato

	BAP conc. (mg l^{-1})			
Sucrose conc.(g l-1)				
	0	2	4	6
100	T_1	T_2	T_3	T_4
120	T_5	T_6	T_7	T_8
140	T_9	T_{10}	T_{11}	T_{12}
160	T_{13}	T_{14}	T_{15}	T_{16}

Experiment 2. Effect of different levels of sucrose and photoperiod regimes on microtuberization

Six-week-old *in-vitro* plantlets from mother stock were treated with 10 ml of MS liquid media containing two levels of sucrose (100, 120 g l^{-1}) and incubated at 15±2 ^{0}C under three different photoperiodic regimes (0/24, 8/16, 16/8 hrs light/dark regimes) as indicated in table 2.

Table 2. Treatment combinations of sucrose levels and photoperiod regimes tested for *In-vitro* microtuber production of potato

	Photoperiod regime (light/dark) hrs		
Sucrose conc .(g l^{-1})			
	0/24	8/16	16/8
120	S_1	S_2	S_3
140	S_4	S_5	S_6

Experimental Design

The two experiments were conducted in factorial completely randomized design with seven replicates for experiment 1 and eight replicates for experiment 2.

Data recording and statistical analysis

At the onset of foliage senescence (browning of leaves and / or stems) plantlets were taken out of the culture tubes and microtubers were harvested. Data were recorded on six parameters, namely microtuber fresh weight (MFW), microtuber dry weight (MDW), starch assimilation capacity of single explant (SAC), cross sectional tuber area (CSTA), number of microtubers per explant (NT) and number of eyes per microtuber (NE).

Results

All treatments employed in experiments 1 and 2 induced the formation of microtubers (Tables 3 and 4). The combination of 140 g l^{-1} sucrose and 6 mg l^{-1} BAP (T_{12}) was the most effective treatment in experiment 1 (Table 3) and the combination of 140 g l^{-1} sucrose and 8/16 hrs light/dark regime (S5) was the best treatment in experiment 2 (Table 4).

Microtuber Fresh Weight (MFW)

The results indicated that 140 g l^{-1} sucrose and 6 mg l^{-1} BAP (T_{12}) was the most effective treatment (277.8 mg) and 140 g l^{-1} sucrose without BAP (T_9) was the least effective treatment (103.8 mg) in experiment 1 (Table 3). The effect of BAP was shown to be significant ($p>0.014$).

The results of experiment 2 indicated that 140 g l^{-1} sucrose and 8/16 hrs light/dark regime (S_5) was the most effective treatment (469.1mg) whereas 140 g l^{-1} sucrose and 0/24 hrs light/dark regime (S_4) was the least effective treatment (Table 4). The main effect of photoperiod was significant ($p>0.044$).

Microtuber Dry Weight (MDW)

The effect of various treatments on this parameter was similar to that of microtuber fresh weight. In Experiment 01, 140 g l^{-1} sucrose and 6 mg l^{-1} BAP (T_{12}) gave rise to the highest (58.4 mg) MDW whereas T_9 gave rise to the lowest MDW (Table 3).

In experiment 2, the highest MDW was recorded in 140 g l^{-1} sucrose and 8/16 hrs light/dark regime (S_5) (114.7 mg) and the lowest was observed in treatment S_4 (25.3 mg) (Table 4). The main effect of photoperiod was significant ($p>0.001$) regarding this parameter.

Table 3. Effect of BAP and sucrose levels on yield parameters of potato microtubers

Treatments	MFW	MDW	SAC	CSTA	NT	NE
T1	184.7	32.0	221.7	46.42	1.35	4.11
T2	244.5	48.4	378.7	60.02	1.64	3.68
T3	268.2	55.0	352.7	60.14	1.46	4.38
T4	224.8	44.2	332.2	51.40	1.86	3.38
T5	176.1	70.8	317.8	45.87	1.76	3.08
T6	232.5	51.7	271.7	54.47	1.24	3.13
T7	214.1	44.5	335.2	52.18	1.95	3.38
T8	255.8	46.3	326.0	58.02	1.35	4.53
T9	103.8	18.8	301.5	37.05	2.67	3.53
T10	243.2	49.4	313.5	54.97	1.24	3.76
T11	238.2	52.0	551.1	56.65	2.47	4.66
T12	277.8	58.4	606.6	66.72	3.32	4.79
T13	135.4	31.8	329.1	39.20	2.58	3.24
T14	255.0	51.8	385.8	56.94	1.79	3.96
T15	168.8	33.0	337.8	46.56	2.00	4.08
T16	151.8	30.7	296.0	40.75	1.86	3.80
CV%	24.19	32.08	24.33	38.66	24.25	10.04
LSD ($\alpha = 0.05$)	60.70	-	10.19	10.61	0.81	0.11

MFW = Microtuber Fresh Weight; MDW= Microtuber Dry Weight; SAC = Starch Assimilation Capacity; CSTA =Cross Sectional Tuber Area; NT = Number of Tubers (microtubers) per plantlet; NE = Number of Eyes per microtuber

Starch Assimilation Capacity (SAC)

This parameter indicated the total capacity of the explant to assimilate starch into tubers (i.e. the total fresh weight of the tubers produced by a single explant).

Microtubers produced with 140 g l^{-1} sucrose and 6 mg l^{-1} BAP (T_{12}) combination recorded the highest SAC (605.8 mg) and 100 g l^{-1} sucrose and 0 mg l^{-1} BAP (T_1) recorded the lowest (221.7 mg). Main effect of sucrose was significant ($p>0.040$) for experiment 1.

Regarding experiment 2, 140 g l^{-1} sucrose and 8/16 hrs light/dark regime (S_5) recorded the highest (469.1 mg) SAC and 140 g l^{-1} sucrose and 0/24 hrs light/dark regime (S_4) recorded the lowest (137.4 mg). Main effect of photoperiod was highly significant ($p>0.001$) for the parameter.

Cross Sectional Tuber Area (CSTA)

Highest CSTA (66.7 mm^2) was observed in the 140 g l^{-1} sucrose and 6 mg l^{-1} BAP (T_{12}) treatment and the lowest (37.05 mm^2) was in 140 g l^{-1} sucrose without BAP (T_9) (Table 3). Main effect of BAP was significant ($p> 0.036$) for experiment 1.

In experiment 2, 140 g l^{-1} sucrose and 8/16 hrs light/dark regime (S_5) treatment was very effective (97.05 mm^{-2}) and 140 g l^{-1} sucrose and 0/24 hrs light/dark regime (S_4) (34.6 mm^2) was the least effective (Table 4). Main effect of photoperiod was highly significant ($p>0.0002$) for this parameter.

Number of Tubers per Plantlet (NT)

In experiment 1, combination of 140 g l^{-1} sucrose and 6 mg l^{-1} BAP (T_{12}) was the most effective (3.32 tubers/plantlet) and 140 g l^{-1} sucrose and 2 mg l^{-1} BAP (T_{10}) was the least effective (1.24 tubers/plantlet). Main effects of sucrose ($p>0.0029$) and BAP ($p>0.0470$) were significant (Table 3).

In experiment 2, no significant difference in NT was observed among the treatments tested (Table 4).

Number of Eyes per microtuber (NE)

Microtubers produced with 140 g l^{-1} sucrose and 6 mg l^{-1} BAP (T_{12}) had the highest number of microtubers (4.79 eyes/microtuber) and 120 g l^{-1} sucrose and 0 mg l^{-1} BAP (T_5) recorded the

lowest (3.08 eyes/microtuber).The interaction effect of BAP and sucrose was highly significant ($p>0.0051$) for this parameter (Table 3).

In experiment 2, 140 g l^{-1} sucrose and 8/16 hrs light/dark regime (S_5) recorded the highest number of eyes/ microtuber (16.08) and 140 g l^{-1} sucrose and 0/24 hrs light/dark regime (S_4) gave rise to the lowest (2.7). The interaction effect of sucrose and photoperiodic regime was highly significant ($p> 0.0017$) for this parameter (Table 4).

Table 4. Effect of sucrose levels and photoperiod on yield parameters of potato microtubers

Treatments	MFW	MDW	SAC	CSTA	NT	NE
S_1	171.5	29.8	171.5	47.9	1	4.1
S_2	411.5	84.8	411.5	95.8	1	11.3
S_3	437.7	89.1	437.7	82.1	1	7.9
S_4	137.4	25.3	137.4	34.6	1	2.7
S_5	469.1	114.7	469.1	97.1	1	16.1
S_6	268.1	55.5	268.1	58.6	1	7.7
CV%	26.65	37.40	26.65	29.49	-	18.72
LSD ($\alpha = 0.05$)	15.02	36.80	15.02	24.84	-	0.27

MFW = Microtuber Fresh Weight; MDW= Microtuber Dry Weight; SAC = Starch Assimilation Capacity; CSTA =Cross Sectional Tuber Area; NT = Number of Tubers (microtubers) per plantlet; NE = Number of Eyes per microtuber

Discussion

The findings of the present study on the effect of sucrose and BAP on microtuberization are in agreement with those of Oparka and Wright (1988), Khuri and Moorby (1996), Vreugdenhil and Struik (1989), Bradford and Yang (1980), who reported enhanced microtuber induction when *in-vitro* plantlets were treated with higher levels of sucrose and BAP. It was observed that shifting *in-vitro* potato plantlets grown under long days in a medium with low levels of sucrose to short days and medium with high sucrose concentration bring about the better formation of microtubers.

The high sucrose level increased the osmotic potential of the culture media. It has also been shown that starch synthesis is regulated by the osmolarity of the media (Oparka and Wright, 1988). Thus the high osmotic potential would enhance the starch accumulation process occurring at the stolon tip. High sucrose concentration may help to trigger rapid starch biosynthesis and induction of microtuber formation at the stolon tip.

The microtuber fresh weigh, dry weight, starch assimilation capacity, cross sectional tuber area and number of microtubers per explant increased with the combination of relatively high sucrose and BAP concentrations. A probable reasons would be that, the BAP could disturb the balance of endogenous levels of growth regulators with a simultaneous increase of osmotic potential of the medium.

In this experiment fresh weight and dry weight of the microtubers were reduced with 16% (160 gl^{-1}) of sucrose than the 14% (140 gl^{-1}) of sucrose. That may be due to the higher osmotic potential in the tuberization medium than the accepted level (Chandra *et al.*,1992; Yu *et al.*, 2000). At 14% sucrose level, there was no significant increase in the fresh weight of microtubers compared to the other levels of sucrose.

Starch assimilation capacity of the explant was a better parameter than fresh weight of the microtubers for assessing microtuberization capacity, when plantlets produce larger tubers as well as smaller tubers under the same conditions, the average fresh weight was significantly reduced. However, the starch assimilation capacity of the explant accounts for the total capability of converting the nutrients into starch.

The promotion of microtuberization on cultured shoots by cytokinin has been demonstrated by many workers (Wang and Hu 1982; Hussey and Stacey 1984 and Ortiz- Montiel 1987). The present study also indicated an increase in microtuber number, fresh weight, dry weight, starch assimilation capacity and cross sectional tuber area upon supplementing MS medium with comparatively high level of BAP (6 ml l^{-1}). The above result was obtained under total darkness (0/24 hrs) and at confirms the findings of Hussey and Stacey (1984). Also, the findings of Garner and Blake (1989) and Ranalli *et al.*, (1997) agree with the results obtained in experiment 2 under total darkness, who reported that microtubers could be induced even without the use of growth regulators.

The findings on the effects of photoperiod and sucrose on microtuberization agree with those of Wang and Hu (1982), Hussey and Stacey (1984), Ortiz-Montel and Lozoya-sal-dana (1987) and Garner and Blake (1989) who also found better microtuber induction when *in-vitro* plantlets were incubated under short day conditions with higher sucrose levels in the medium. Further, it is a well established fact that under *in-vitro* conditions tuberization in whole plantlets is hastened during short days and at low temperatures.

Short photoperiods may not be the only inductive stimulus for tuberization. However, short photoperiods may act as a permissive agent (Pelocho and Mingo-Castle, 1991) for tuberization in some potato cultivars. It appears that the effect of light on *in-vitro* potato cuttings was more of a photoperiodic nature rather than a quantitative effect (Pelacho and Mingo-Castle, 1991).

The number of eyes per microtuber was higher in 8/16 hrs light/dark photoperiod. The faster rate of microtuberization and early senescence of the plantlets (Gopal, 1996) may have resulted in fewer eyes being produced in microtubers cultured in the dark than those cultured in light.

Conclusions

- MS medium supplemented with 140 g l^{-1} sucrose and 6 mg l^{-1} BAP resulted in the highest microtuber fresh weight, dry weight, starch assimilation capacity per plantlet, cross sectional tuber area and highest number of microtuber per plantlet under the culture conditions of 15 $\pm 2^0$C and 0/24 hrs light/dark regime.
- The MS medium supplemented with 140 g l^{-1} sucrose and 8/16 hrs light/dark regime resulted in the highest microtuber fresh weight, dry weight, starch assimilation capacity per plantlet, cross sectional tuber area and highest number of eyes per microtuber at 15 $\pm 2^0$C .
- *In-vitro* microtuberization can be induced with or without BAP. But, physical conditions of 15 $\pm$ 2°C and 8/16 hrs light/dark regime with 140 g l^{-1} sucrose and without BAP in the medium could be selected for low cost of production, as it gave the largest as well as heaviest microtubers

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