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Acceleration of Microtuber Induction in Potato by Cytokinine and Photoperiod Adjustment

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ABSTRACT: Protocol was developed for acceleration of microtuber induction in potato. It was observed that cytokinine (Kin), and both dark and light period had great influence on *in vitro* tuberization. Regarding concentration of Kin, 8 mg/l was found excellent for microtuber induction and formation under both dark and light conditions. Under continuous dark condition, higher percentage of explants (shoot) induced microtuber was observed than the short and long period of light conditions. For increasing the number and weight of microtuber product/explant, use of long photoperiod (16 h) is recommended.

KEY WORDS: Microtuber, photoperiod, cytokinine.

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INTRODUCTION

Potato (*Solanum tuberosum* L) is most productive, common, and multiuse horticultural vegetable crop. It conquered about 50% among root crop production in the world[1]. In vegetatively propagated crops, once systematically infected with a viral disease, the pathogen can be passed from one vegetative generation to next. Especially in potato, contamination by a pathogen can severely reduce the total yield of the crop[2]. Since many tropical countries are not able to produce high quality seed tubers due to a lack of vector—free production area, the importation of certified seed and the resultant high costs can become a major constraint to potato production[3].

Microtuber is an alternative end product of micropropagation, produced by allowing *in vitro* plantlets to grow under the tuber inducing conditions. In recent years, interest has developed in many countries in the induction of potato tubers under *in vitro* condition for diseases free microtuber production, and for germplasm conservation and distribution. Use of micro propagated plantlets for production of tuber seeds in field shows many difficulties (laborious and high failure rates) in many tropical countries. *In vitro* tubers have been used to remove this problem. Microtuber could be harvested *in vitro* conditions, stored, shipped and planted conveniently. Besides these, in general microtuber—derived plants produced higher yield than cutting derived plants both under screen—house and field conditions (data not shown). There are reports on the induction of microtubers in potatoes[4~12]. Although *in vitro* production of potato tubers or microtuberization was achieved more than 40 years ago, the application of microtubers in reliable model research system has been slow to develop. Several factors such as the use of growth regulators in microtuber induction and growth media, the mixotropic nature of the *in vitro* system, and cultivar—specific responses have led to interpretive difficulties[13]. *In vitro* tuberization is a complex physiological process regulated by many factors. Cytokinine and photoperiods are two major factors of this process. Cytokinine such as Kinetine and BAP have shown to stimulate tuber initiation *in vitro* under high sucrose level and total darkness[2,6,8,12,14,15]. The effect of photoperiod on *in vitro* tuberization was also noticed by

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other workers[6,7,9,16].

So, for acceleration of microtuber induction further efforts could be given through cytokinine and photoperiod adjustment. In this paper, we have reported on using Kin, and both dark and light for high induction and production of microtuber.

MATERIALS AND METHODS

Diseases free (cheeked through ELISA test) meristem derived plantlets of cultivars Diamant and Cardinal were used as primary source of explants for *in vitro* tuberization (See inside back cover, Figure A). Nodal segments of 2～8 cm length with 5～6 leaves of plantlets were inoculated for microtuber induction (See inside back cover, Figure B). The experiment was conducted in two phases during the years 2001～2003, in the Department of Botany, University of Rajshahi, Rajshahi — 6205, Bangladesh. In the 1st phase, the explants were cultured on MS[17] medium supplemented with 30% sucrose as source of carbon and different concentrations of BAP (6—benzylaminopurine) and Kin (6—Furfuryl aminopurine) for cytokinine adjustment. The culture was incubated under continuous dark condition. On the basis of results of 1st phase experiment, the 2nd phase of experiment was started for further acceleration of microtuber development. Here also different concentrations of Kinetine were tested under two photoperiods (8 h and 16 h light) including continuous dark as control.

The nature of microtuber formation was studied through weeks needed for microtuber formation, percentage of explants induced tuber, number of microtuber formation/shoot explant and total weight of microtuber/shoot (mg).

RESULTS

Results of cytokinine (BAP and Kin) effects on *in vitro* tuberization under continuous dark condition (1st phase of experiment) showed that the use of plant hormone (cytokinine) was essential for acceleration of microtuber induction and production (Table 1). In both cultivars, the performance of microtuber formation was very poor in MS_0 (control) medium. Kin was more effective than BAP for *in vitro* tuberization. In cv. Diamant for shoot induced microtubers (%), 43.5 were noticed in BAP, whereas it was 70.6 in Kin. For no. of microtuber/shoot, 2.1 and 2.6 were observed in BAP and Kin, respectively. In case of weight of microtuber/shoot (mg), 104.0 and 172.3 were weighted in BAP and Kin, respectively. In cv. Cardinal, similar trends were observed. Here also Kin showed more responsive than BAP. For traits, shoot induced microtubers (%), no. of microtuber/shoot and weight of microtuber/shoot, the following mean data were recorded, 40.0, 2.0 and 98.6, and 46.3, 2.4 and 13.5 in BAP and Kin, respectively. Regarding weeks to microtuber induction completion, no significant difference was observed in respect to types of cytokinine and kinds of cultivar tested. It varied from 6 to 12 weeks. However, difference was observed in the use of different concentration of BAP and Kin. Similar difference was also noticed for the other characters. With few exceptions, use of 8 mg/l Kin was found most effective for the studied traits. Considering all treatments, cv. Diamant showed better response than cv. Cardinal.

The results on testing role of photoperiod (2nd phase of the experiments) under five concentrations of Kin (2, 4, 6, 8 and 10 mg/l) on *in vitro* tuberization in potato are presented in Table 2. Here continuous dark was considered as control treatment. From the result, it was observed that use of continuous dark is more effective than use of light for high percentage of explant (shoot) induced microtuber. From the mean percentage of photoperiod effect, it was found that maximum (70.6%) explants were able to induce microtuber when no light (control) was used. On the other hand, only 44.0% and 39.4% explants (shoot) were able to induce microtuber under 8 h and 16 h of light, respectively. However, for producing high number with high weight of microtuber/explant, 16 h of light was found more effective than the control (continuous dark). Here no. 3.51 microtuber/shoot and 182.3 mg microtuber/shoot were noticed for 16 h light treatment. On the other hand, only 2.7 microtuber/shoot and 169.36 mg microtuber/shoot were noticed for continuous dark treatment. Regarding use of 8 h light treat-

ment, moderate effect was observed. Here 44%, no. 1.77 and 135 mg were reported for the traits of shoot induced microtubers, microtuber no. /shoot and weight microtuber/shoot, respectively.

Regarding the color of microtuber grown under dark and light conditions, we observed that microtubers grown in dark condition was whitish (See inside back cover, Figure C and E), whereas microtuber grown in light condition was greenish (See inside back cover, Figure D and F).

Table 1 Effect of cytokinine (BAP and Kin) on *in vitro* tuberization of potato (cvs. Diamant and Cardinal) in MS semi solid medium containing 30 g/l of sucrose under continuous dark condition. Data were recorded after 12 weeks of subculture

Cultivars	Growth regulators	Concentrations of BAP and Kin (mg/l)	Weeks to microtuber induction	Shoot induced microtuber (%) ($\bar{x}$ ±SE)	No. of microtuber/shoot ($\bar{x}$ ±SE)	Weight of microtuber/shoot (mg) ($\bar{x}$ ±SE)
Diamant	BAP	2	8—9	30±1.73	2.1±0.11	90.8±2.88
		4	8—9	32±0.57	2.5±0.23	98.3±1.15
		6	7—8	45±1.15	2.7±0.10	102.4±1.73
		8	7—8	47±1.00	2.3±0.50	122.0±1.15
		10	8—9	50±1.15	1.8±0.11	108.6±1.00
		12	9—10	57±1.15	1.3±0.23	101.9±1.73
	Mean	—	—	43.5	2.1	104.0
	KIN	2	8—9	45±1.15	2.5±0.17	129.6±0.90
		4	7—8	58±0.57	2.6±0.28	112.5±0.28
		6	7—8	71±1.73	2.9±0.23	184.5±1.60
		8	6—7	87±1.00	3.1±0.50	218.6±1.15
		10	8—9	92±1.15	2.4±0.23	201.6±1.73
		12	8—9	41±0.57	2.2±0.28	187.3±1.00
	Mean	—	—	70.6	2.6	172.3
	MS_0 control	—	<12	0.50±0.14	0.4±0.11	60.0±1.73
Cardinal	BAP	2	9—10	20±1.73	1.8±0.11	84.0±1.60
		4	8—9	30±2.88	2.1±0.11	91.0±1.73
		6	8—9	50±2.30	2.4±0.23	98.5±1.00
		8	7—8	55±1.52	2.6±0.26	115.8±1.15
		10	7—8	45±1.15	1.5±0.35	102.3±1.47
		12	8—9	40±1.73	1.7±0.11	100.5±1.32
	Mean	—	—	40	2.0	98.6
	KIN	2	8—9	30±1.00	2.1±0.23	97.0±1.47
		4	7—8	35±1.73	2.4±0.26	101.5±1.15
		6	7—8	45±1.15	2.7±0.17	128.6±1.73
		8	6—7	65±2.30	2.8±0.17	185.5±1.15
		10	8—9	57±1.73	2.5±0.23	167.9±1.15
		12	9—10	51±1.15	1.9±0.20	108.3±1.60
	Mean	—	—	46.3	2.4	131.5
	MS_0 control	—	<12	0.40±0.14	0.4±0.13	45.0±1.00

DISCUSSION

Results reveal that the acceleration of microtuber formation varied with types and concentrations of plant hormones, dark and light period and genotypes. There are reports that *in vitro* tubers can be sessile on the nodes of the stem[18,19] or can be axillary or terminally formed on new growing shoots[6,12,20]. In our experiment, it was clearly observed that phytohormones, especially cytokinine, are very useful for the acceleration of *in vitro* tuberization in potato. Requirement of cytokinine for *in vitro* tuberization has also been reported by several workers[3,7,21~24]. Here, we observed that among the cytokinine, Kin is most effective for microtuber induction and production. This is supported by other workers[3,6,25]. Higher percentage of explant was induced to produce microtuber in dark condition than in light condition. Use of continuous darkness in production of microtubers was also reported earlier[2,3,6,8,12,14,26~28].

Table 2　Effect of different concentrations of Kin and of photoperiods on *in vitro* tuberization in potato after 12 weeks of subculture

Treatment		Weeks to microtuber induction	Shoot induced microtubers (%) ($\bar{x}$ ±SE)	Microtuber number/shoot ($\bar{x}$ ±SE)	Weight of microtuber/Shoot (mg) ($\bar{x}$ ±SE)
Photoperiods	Kinetin (mg/l)				
Continuous dark (control)	2.0	8—9	45±1.15	2.5±0.28	129.6±0.97
	4.0	7—8	58±1.52	2.6±0.23	112.5±1.15
	6.0	7—8	71±1.73	2.9±0.05	184.5±1.73
	8.0	6—7	87±1.15	3.1±0.11	218.6±1.43
	10.0	8—9	92±1.15	2.4±0.17	201.6±1.15
8 hours light	2.0	9—10	32±1.00	1.03±0.05	167.8±1.15
	4.0	8—9	40±0.57	1.01±0.05	98.6±1.52
	6.0	8—9	43±1.15	1.90±0.11	103.8±0.81
	8.0	7—8	57±1.73	2.91±0.11	124.6±1.15
	10.0	8—9	48±1.52	2.10±0.28	180.5±1.73
16 hours light	2.0	10—12	27±1.15	3.49±0.05	118.3±1.52
	4.0	9—10	36±1.73	3.59±0.28	141.6±1.73
	6.0	8—9	40±1.00	3.50±0.17	202.6±2.15
	8.0	7—8	51±1.15	3.59±0.11	214.3±2.45
	10.0	9—10	43±0.57	3.38±0.28	235.0±4.04
Mean of photoperiod effect					
0			70.6	2.70	169.36
8h			44.0	1.77	135.00
16h			39.4	3.51	182.30
Mean of Kinetin effect					
2.0			34.66	2.34	138.56
4.0			44.66	2.40	117.56
6.0			51.33	2.76	163.63
8.0			65.00	3.20	185.83
10.0			61.00	2.62	205.70

The 2nd phase of experiment shows that photoperiod has distinct effect on induction and production of microtuber. This is supported by the reports of other workers[6,7,9,12,16]. Here, we observed that long photoperiod (16 h) is better than short photoperiod (8 h) for increasing the production of microtuber. Kefi et al[7] reported use of 16 h photoperiod for *in vitro* tuberization of potato in MS medium containing different additives. Under 16 h photoperiod, inhibition to the production of microtubers was reported by Pruski[9]. We also noticed that use of light is better than no light use for significant production of microtubers (number of microtubers and weight/shoot). Our results are supported by the work of Pruski[9]. He observed much better production of microtubers (number of tubers and weight) on solid agar than on liquid medium, and under the 8 h photoperiod compared to no light. He further reported that tuber-bulking rates were lower in the darkness than under the 8 h photoperiod.

Regarding the color of microtuber grown under dark and light conditions, our results are also supported by Pruski[9]. He reported that microtubers derived from 8 h photoperiod were greenish and seemed less juvenile than the tubers from 0 h light. Such microtubers performed better in the field or the greenhouse than microtubers produced in darkness.

In conclusion, it may be recommended that for acceleration of microtuber induction and production, use of plant hormone (cytokinine) is most needed. In

our findings, Kin (8 mg/l) is effective. Regarding use of photoperiod, it depends upon the genotype used and objective of microtuber productions. For the production of high percentage of shoot induced microtuber, continuous dark period is recommended, whereas for production of high no. with high weight of microtuber/shoot, use of long period of light is recommended.

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