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In vitro tuberization in potato (*Solanum tuberosum* L.)

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Abstract

In vitro microtuber formation potentiality of potato was investigated to establish a rapid disease free seed production system in potato. MS medium supplemented with 4 mg/L of KIN showed best performance in respect of multiple shoot regeneration and microtuber formation. Simple MS medium was not able to produce any micro tuber under *in vitro* condition. Dark condition better responded to tuberization than light condition. Among the three different explants (nodal segment, sprout and shoot apex) nodal cutting showed the best performance on days to microtuber formation and average weight of microtuber. MS + 6% sucrose + 4 mg/L KIN combination of treatment was best for *in vitro* tuberization among the parameters under study.

Key words: potato, microtuber, kinetine (KIN), sucrose, nodal segment

Introduction

The potato (*Solanum tuberosum* L.) one of the important vegetable crop in Bangladesh is a staple food crop in many countries of the world as well. Potato is an annual herbaceous plant, which is vegetabively propagated by the tuber. Tuberization in potato is a highly complex developmental process, which may be modified in various ways. Tuberization can be induced under *in vitro* condition. Many researchers used different growth regulators for *in vitro* induction of microtuber in potato (Hossain and Sultana, 1998). The anti-gibberellin compound [Chloro Choline Chloride (CCC)] has strong effect on *in vitro* tuberization (Tovar et al., 1985; Dodds et al., 1988). *In vitro* tuber can produced through out the year. The potential value of tissue culture in potato production has been widely recognized. This technology has been used for disease free seed production in many countries (Wang and Hu, 1982). Seed production technique of potato can be designed with *in vitro* multiplication through either plantlet regeneration or microtuber production. But microtuber method has several merit over plantlet regeneration. Microtubers 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. Extensive physiological research has revealed that tuberization is controlled by several factors, such as hormonal combination, ratio of photo period, nutrient compositions etc. (Vreugdenhil & Struik, 1989, Coleman et al., 2001; Tugrul & Samanci, 2001). Although, researches have been carried out on micro tuber production in potato, very little attention has been paid on the *in vitro* tuberization with Kinetine (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 KIN and sucrose for successful microtuber production in potato.

Materials and methods

The experiment was conducted at the Department of Biotechnology, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh. The well establish potato cultivar viz. Diamant was collected from Tuber Crops Research Centre (TCRC), Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh. Disease free healthy tubers were wash with detergent in running water and then 2/3 times wash with distill water. The clean materials were treated with 300 ppm GA_3 for rapid sprouting. One week old sprouts were used as initial explant. The sprouts were surface sterilized by dipping in 0.5 $HgCl_2$ solution for 3-5 minute and then washed several times with autoclaved distilled water. These explants were cultured in MS solid medium (pH = 5.8) in test tube. The bud sprouted

Table 1. Effect of KIN on microtuber formation in potato

Media	No of explant	Days to shoot formation	No. of shoot per explant	Highest Shoot length (cm)	Days to microtuber formation	No. of microtuber per explant	Avg. wt. (mg) of microtuber
Simple MS	20	13.13	2.13	3.20	0	0	0
MS + 1 mg/L KIN	20	8.29	4.03	1.04	48.27	2.18	187
MS + 2 mg/L KIN	20	6.93	5.79	8.80	39.04	2.99	218
MS + 3mg/L KIN	20	6.00	8.11	7.63	34.92	4.01	309
MS + 4mg/L KIN	20	5.09	9.28	6.88	28.72	4.01	389

into plantlet having 4-5 nodal segments within one month. Shoot apex, sprout and nodal cutting were collected from regenerated plantlet and used as explants for *in vitro* tuberization in potato. MS media were prepared in two different sucrose concentrations *viz*. 3% and 6% respectively. Kinetine (KIN) was used as a induced hormone for microtuber production. MS media were supplemented with 1, 2, 3, 4 mg/L of Kinetine (KIN) for the present study. For each concentration of hormone and sucrose 20 replicates were prepared. Tuberization media were poured into well establish plantlet. Fifty percent of the cultures were incubated under 16 hour of photoperiod at 2500-3000 lux and rests of the materials were incubated under dark condition at 17± 3^0C. Microtubers were harvested after 45-55 days of incubation. Data were recorded on days to shoot formation, number of shoot per explant, shoot length, days to micro tuber formation, number of micro tuber per explant and average weight of microtuber.

Results and discussion

In vitro tuberization in potato was studied in Diamant variety. Various explants, sucrose and Kinetine (KIN) concentrations were examined in detail. The experimental finding is presented on different sub-headings.

***In vitro* shoot and microtuber formation**

MS media supplemented with different concentration of KIN were used for micro tuber production. The data are presented in Table 1. It was revealed that, simple MS media had the ability to produce shoot under *in vitro* condition. But days required for shoot regeneration was highest (13 days) in control condition. The minimum time (5.09) was required in MS + 4 mg/L KIN treatment, followed by MS + 3 mg/L and MS + 2 mg/L of KIN, respectively. The time variation between two treatments was very less. Shoot per explant was the highest (9.28) in MS + 4 mg/L KIN which was followed by 3, 2 and 1 mg/L KIN, respectively. The simple MS medium produced lowest number of shoot (2.13) per explant. Highest shoot length (8.80 cm) was observed in MS + 2 mg/L KIN which was followed by MS + 3 mg/L and MS + 4 mg/L KIN, respectively (Fig 1). It is interesting to note that, in higher concentration of KIN, number of shoot per explant was higher but the shoot length was lower. On the other hand, in 2 mg/L of KIN shoot length was higher but number of shoot per explant was lower. It reveals that, higher concentration of KIN has the ability to produce more plantlet per explant. As observed in Table 1, micro tuber was not produced in simple

Fig 1. Multiple shooting and more than two microtuber formation due to application of MS + 4 mg/L KIN.

Fig 2. Microtuber production under dark condition.

Table 2. Effect of photoperiod on *in vitro* microtuber production

Treatment	Media	Explant	Days to tuberization	No. of microtuber/ explant	Agv. wt. (mg) of microtuber	Colour of microtuber
Light condition	MS + 6% sucrose + 4mg/L KIN	Nodal segment	35.07`	1.93	113	Green
Dark condition	MS + 6% sucrose + 4mg/L KIN	Nodal segment	21.93	3.87	305	Brown

Table 3. Role of sucrose on *in vitro* tuberization

Media	Sucrose concentration	Total explant	Days to tuberization	No. of microtuber/ explant	Agv. wt. (mg) of microtuber
MS	3%	20	52.67	0.00	0.00
MS	6%	20	45.29	2.39	119
MS + 4mg/L KIN	3%	20	34.73	3.69	201
MS + 4mg/L KIN	6%	20	29.13	4.78	397

MS medium. Days required for microtuber formation was the highest (48.27 days) in MS + 1 mg/L of KIN and the lowest (28.72 days) in MS + 4 mg/L of KIN. It indicates that, gradually less period was required in higher concentration of KIN application. Number of microtuber per explant was statistically identical in the case of MS media with 3 and 4 mg/L of KIN application. Rest of the treatments also gave similar trend for this parameter. Average fresh weight of microtuber was the highest (389 mg) in MS + 4 mg/L KIN treatment and it was the lowest (187 mg) in MS + 1 mg/L of KIN treatment. Its reveals that, higher concentration of KIN had potentiality to produced heavier micro tuber than lower concentration. BAP and cytokinin were found to stimulate the tuberization process (Hussey and Stacey, 1984; Abbott and Belchel, 1984). The present finding is in conformity with the earlier findings (Kotkas & Peter, 1998; Al-Momani et al., 2000; Shibli et al., 2001).

Effect of photoperiod on microtuber production

MS medium supplemented with 4 mg/L KIN showed best response in tuberization. Hence, only this treatment was used to study the effect of photoperiod on microtuber production. The results were presented in Table 2. *In vitro* tuberization potentiality of Diamant variety was influenced by photoperiod. Tuber formation period of the culture was the lowest (21.93 days) under dark condition and it was the highest (35.07 days) in light condition. Number of tuber per explant (1.93) and average weight (305 mg) of microtuber were also higher in dark condition than light condition. Under light condition most of the culture produced green microtuber. It might be due to synthesis of the alkaloid solanin. It was also interesting to note that, green micro tuber produced sprouts (Fig 2 &3).

Similar phenomenon was reported by Wany and Hu (1982). On the other hand, brown coloured tuber was observed in dark condition and did not showed no sprout initiation. The experimental finding reveal that, dark condition is favorable for tuberization. Mares et al. (1981) observed that microtuberization at 16 or 24 hours photperiod was better than 8 hours. Wattimena (1983) indicated that the longer the photoperiod, better was the tuberization.

Fig 3. Biggest size of microtuber production under light condition due to application of MS + 6% sucrose + 4 mg/L KIN

Role of sucrose on *in vitro* tuberization

The effect of sucrose on microtuber production was presented in Table 3. The earlier finding revealed that MS media supplemented with 4 mg/L of KIN showed the best result on tuber production. Hence only this concentration was considered for the present study. Two different concentrations of sucrose were applied with and without hormone to study its effect on microtuber formation. Its showed that 3% sucrose without hormone was not able to produce any microtuber under *in vitro* condition,

Table 4. Response of explant on *in vitro* tuberization

Explant type	Media	No. of explant culture	No. of culture showing microtuber	Percent (%) of explant formed microtuber	Days to microtuber formation	No. of microtuber per explant	Avg. wt. (mg) of microtuber
Shoot Apex	MS + 4 mg/L KIN	50	43	86	33.89	3.67	378
Sprout	MS + 4 mg/L KIN	50	39	78	37.09	2.16	269
Nodal segment	MS + 4 mg/L KIN	50	47	94	30.91	5.12	309

where as 6% sucrose had little effect on tuberization. For all the parameter under study it showed the poorest performance. The treatment MS + 4 mg/L KIN + 6% sucrose showed the best result among all the treatments. It took only 29.13 days for microtuber formation; number of microtuber also higher (4.78) per explant and the average weight of microtuber also the highest (397 mg) in this treatment.

Gopal et al. (1998) Pelacho et al. (1994), Haque (1996), Amma and Maity (1998) have obtained maximum number of microtuber in different cultivars in the media supplemented with 4-5 mg/L KIN. The present finding is a proof of the previous workers. El-Sawy et al. (2007) reported that sucrose is an important factor for microtuber formation. The highest tuber formation was achieved when 12% sucrose was added to culture media.

Response of explant on *in vitro* microtuber production

The response of explant on microtuber formation were presented in Table 4. It was notice that, the culture obtained from nodal segment showed the highest percent (94%) for microtuber production and it was the lowest (78%) in the case of sprout explant. The explant nodal segment took only 30.91 days for microtuber formation and it was the highest (37.09 days) in sprout explant. The highest (5.12) number of microtuber per explant was observed in nodal cutting and it was the lowest in the case of sprout, where as shoot apex gave the intermediate (3.67) number of microtuber per explant. Average weight was the highest (370 mg) in for shoot apex explant and the lowest (269 mg) for sprout explant. Among all the parameter under study, explant nodal segment as responded well for microtuber production (Fig 4).

Leclere et al (1994) observed that, shoots produced microtubers rapidly and in higher number compared to nodal cutting. Myeong et al (1990) studied the influence of several factors affecting *in vitro* tuberization of shoot nodes in potato. It reveals that, more microtubers were produced on the nodes taken from middle and basal part of shoot than on those from upper part. Kanwal et al. (2006) revealed that node as an explant showed better result on microtuber production. The present finding is similar with the reports of the previous workers.

Fig 4. Different types of microtuber production under different treatments.

In vitro tuberization was studied on MS medium supplemented with different concentrations of KIN in the potato cultivar Diamant. Sprout, shoot apex and nodal cutting were used as explant on different concentration of sucrose for microtuber production. It concluded that, MS medium supplemented with 6% sucrose and 4 mg/L KIN response well for all the parameter studied. Dark condition took minimum time for tuberization than light condition. Maximum average weight of microtuber (397 mg) was obtained from the treatment MS + 6% Sucrose + 4 mg/L KIN.

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