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EFFECT OF CHLOROCHOLINE CHLORIDE, SUCROSE AND BAP ON *IN VITRO* TUBERIZATION IN POTATO (*SOLANUM TUBEROSUM* L. CV.CARDINAL)

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Abstract

Protocols for *in vitro* tuberization of potato (*Solanum tuberosum* L.) variety Cardinal were studied. Virus free cultures were multiplied on Murashige & Skoog (1962) medium containing 1.0 mg/l GA_3. When the number of shoots was maximum the cultures were shifted on tuber induction medium. *In vitro* tuberization media consisted of MS along with different concentrations of CCC, BAP and Sucrose. It was observed that 200 mg/l of CCC or 90g/l sucrose in the mediums resulted in maximum tuber induction (16.5 and 15.6 tubers/flask respectively) followed by BAP @ 4.0mg/l producing 9.0 tubers/flask. It was also observed that complete darkness was essential for tuber induction.

Introduction

Microtubers could be used as an alternate source for basic seed produced through tissue culture. Potato is among one of the most important cash crops and is widely cultivated in the world. In Pakistan, unavailability of healthy seed to the growers has affected the per hectare yield of potato. The infestation of seed results in considerable reduction in yield, due to several viral, fungal and bacterial diseases (Malik, 1995). A lot of foreign exchange has been spent every year so far, for the import of healthy seed. Good quality disease free basic seed produced through tissue culture could play a major role for increase in per hectare production of potato crop.

Previously, it was a routine practice to transport *in vitro* plantlets and cutting to Northern areas of Pakistan for pre-basic and basic seed production. *In vitro* plantlets of potato have high rate of mortality during transportation, transplantation and acclimatization. So there is a need to provide alternate source material which could have less risk during acclimatization and establishment. Microtubers can be stored for longer period, handled and transported easily than plantlets, thus these could be an ideal propagating material (Rosell *et al.,* 1987; Tabori *et al.,* 1999). Microtubers are also used as explant source in genetic transformation studies (Gordon & William 1993).

There are reports on the induction of microtubers on MS medium using different plant growth regulators by Gopal *et al.,* (1998), Dobranszki & Mandi (1993), Dobranszki (1996), Dobranszki *et al.,* (1999) and Nowak *et al*., (1999). In the present study different factors were studied which affect the microtuberization in potato cv. Cardinal which has red skin and mostly accepted by potato growers and consumers in Pakistan. Microtubers were produced on MS medium with different concentrations of BAP, CCC and sucrose.

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Materials and Methods

The process of microtubers production consisted of the following three phases viz., thermotherapy, *In vitro* multiplication and *In vitro* tuberization.

Thermotherapy and meristems excision

Thermotherapy is an important step for eradication of different viruses especially in case of potato. The planting materials (field tubers) were subjected to thermotherapy. The tubers were sown in sterilized peat moss mixture (sand, clay and peat moss in 1:1:2 ratio) and incubated at 34^{o}C - 37^{o}C for a period of three weeks in thermotherapy chamber along with continuous florescent light. Hoagland solution was sprayed after every two-day interval to enhance the vegetative growth. After three weeks of treatment the shoot apices were taken and then meristems were excised in aseptic condition under a stereo microscope.

In vitro multiplication

In vitro multiplication was carried out by nodal fragments on MS (1962) liquid and solid media containing Biotin @1 mg/l, Ca Pantothenic acid @1 mg/l and different concentrations of GA_3 ranging from 0.0-2.0 mg/l. Five nodes (2-3 cm) were cultured per 250 ml flask, and were kept under 16 hours photoperiod at 25 ± 2^{o}C. Shoot growth and multiplication rate was examined for each treatment after every 2nd week. Shoot multiplication reached to the maximum and shoot growth up to 6-8 cm after fifteen days. The cultures were shifted on tuber induction medium and incubated for 15 days with photoperiod of 16 hrs and then were shifted to complete darkness for a period of two months.

In vitro tuberization

For tuber induction, MS medium was supplemented with BAP ranging from 0.0-4.0 mg/l, sucrose from 30-120 g/l and CCC from 0.0 to 250 mg/l (Table 2). The results were recorded after every 15 days until tubers matured. For each treatment 20 replicates were used and data was analyzed by M.STAT programme.

To study the affect of light, 50% of the cultures were kept under complete darkness while remaining were left under16 hrs photoperiod with a light intensity of 2000 lux at 25±2^{o}C.

Results and Discussion

Thermotherapy and meristem culture

Thermotherapy at a temperature of 34-37^{o}C for a period of 3 weeks followed by meristem culture was found to be appropriate to produce virus free plants of potato. The plants regenerated from meristem were multiplied for the production of disease free microtubers. ELISA showed that PLRV, PVX, PVV, PVA, PVM and PVS were absent in the *in vitro* plants produced. Quak (1977), Mellor & Smith (1977) have reported that thermotheropy and meristem culture proved to be a highly successful method for inactivation or elimination of viruses from perennial crop plants specifically potato to obtain virus free stock material.

Table 1. Number of shoots on MS media containing different concentrations of GA_3.

GA_3 mg/l	Average number of shoots/ flask (Liquid)	Average number of shoots/ flask (Solid)
0.0	5	5
0.5	10	8
1.0	16	12
2.0	12	10

In vitro Multiplication

The virus free cultures were multiplied on MS medium containing GA_3 by nodal fragmentation method. Maximum number of shoots was observed on MS liquid medium containing GA_3 @ 1.0 mg/l (Table 1) producing 16 shoots/flask from 5 nodes. On the other hand, same media composition in solid state produced 12 shoots/flask. It was noted that higher concentration of GA_3 (2.0 mg/l) has no effect on shoot numbers both in liquid as well as in solid state, but showed elongation in shoot size.

In vitro tuberization

Effect of light: It was observed that complete obscurity was an essential factor in tuber induction. Cultures kept under 16 hrs., photoperiod were not able to produce microtubers. During incubation under light, GA_3 is synthesized which inhibits tuber induction while darkness enhanced tuberonic acid synthesis, which plays important role in tuber formation. Tuberonic acid is a glucoside of 12- hydroxil-Jasmonic acid involved in tuber induction as reported by Alisdair & Willmitzer (2001), Jackson (1999) and Simko *et al.,* (1996). Dobranszki *et al.,* (1999) and Donnelly *et al.,* (2003) have also demonstrated that microtuberization efficiency has been increased by short day's exposure or continuous darkness during culture condition. Similarly Zaida & Elizabeth (1991) have reported that shoots grown under 16 hrs photoperiod when placed under darkness induced tuber formation.

Effect of BAP: Tuber formation started after 3 weeks when medium contained BAP at higher concentrations (3.0 to 5.0 mg/l) under dark condition. These tubers matured after 5 weeks of initiation and were harvested when the tuber size was more than 1.0 cm in diameter. Maximum number of tubers was obtained (9/flask) when MS medium was supplemented with BAP @ 4.0 mg/l (Table 2, Ti-3), and variance was 1.0 indicating that all the cultures performed uniformly. Le (1999) reported that media containing BAP alone has significant effect on microtuber diameter and fresh weight as compared to media used in combination with CCC and Kn. The number of tubers decreased with the decrease in concentration of BAP and only shoot proliferation was observed. When BAP was used in combination with increased concentration of sucrose @ 90 g/l (Table 2, Ti-4) no significant increase in tuber number and size was observed. Husey & Stacey (1984), Rosell *et al.,* (1987), Donnelly *et al.,* (2003) reported that growth regulators (BAP, 2iP, Kn and NAA) could induce microtubers in potato *in vitro* but in some cases the effect of growth regulators was found to be genotype dependent.

Table 2. *In vitro* tuberization on MS media containing different concentrations of CCC, sucrose and BAP in dark condition.

MS + Chlorocholine Choride mg/l (Ti-1)	Mean No. of Tubers	Variance
0.0	0.0	0.0
100.0	2.2	.70
150.0	3.60	.300
200.0	16.50	3.50
250.0	11.0	2.0
MS + Sucrose gm/l (Ti-2)		
30	0.0	0.0
60	4.8	.70
90	15.60	3.30
120	9.0	1.0
MS + BAP mg/l (Ti-3)		
0.0	0.0	0.0
1.0	0.0	0.0
2.0	.60	0.30
3.0	3.60	0.30
4.0	9.0	1.0
5.0	6.0	0.8
MS and 90 g/l sucrose + BAP mg/l (Ti-4)		
0.0	15.5	3.3
1.0	4	1.2
2.0	5.6	1.0
3.0	7.8	1.5
4.0	10	2.3
5.0	8.4	1
MS and 90 g/l sucrose + CCC mg/l(Ti-5)		
0.0	15.4	3
100	3	1
150	6	1.5
200	16	2
250	13	1

Effect of CCC: When medium contained CCC @ 200 mg/l, the number of tubers (Fig. 2) was maximum i.e., 16/flask (Table 2, Ti-1). With the increase in numbers, the size of the tubers decreased (less than 1 cm) indicating that total storage of starch remained same. As the concentration of CCC was decreased below optimum the number of the tubers decreased indicating that CCC played a vital role in tuber induction. With the increase in CCC concentration, tuber formation and shoot growth was reduced indicating growth-retarding effect. Presence of CCC in the medium reduces GA_3 biosynthesis and increases tuberonic acid synthesis, which enhances the tuber formation. Stecco & Tizio (1982) and Vecchio *et al.*, (1994) reported that the growth retardant usually suppress GA_3 synthesis and may stimulate microtuberization.

Fig.1. *In vitro* tuber induction on media containing BAP.

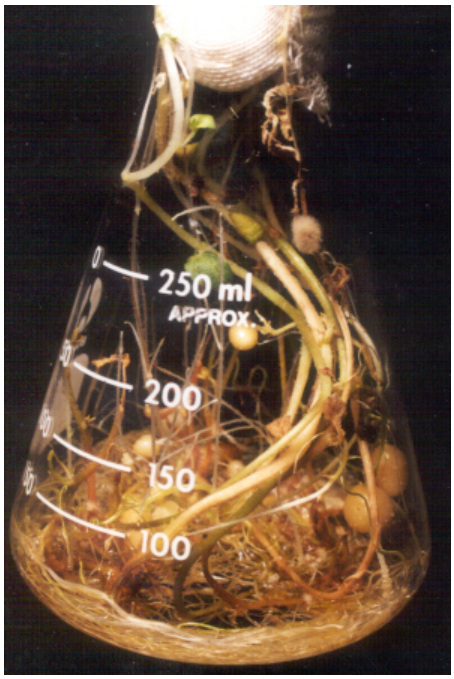

Fig. 2. *In vitro* tuber induction on media containing CCC.

Fig. 3. *In vitro* tuber induction on media containing Sucrose.

When the concentration of CCC was increased upto 250 mg/l, the number of tubers decreased again indicating that upto certain level CCC may enhance tuber formation as also reported by Zaida & Elizabeth (1991), Hussey & Stacey 1984, Rosell *et al.*, (1987). Earlier Tovar *et al.*, (1985) have also demonstrated that CCC is a key compound for *In vitro* tuberization, but the optimum dose for tuberization was higher than the optimized dose of our study. When CCC was used with higher concentration of sucrose, no considerable change on the tuber formation was observed (Table 2, Ti-5) which indicates that presence of CCC dominated the tuber induction behavior whether used alone or in combination.

Effect of sucrose: In MS media containing different concentration of sucrose, the maximum number of tubers 15.6/flask was produced when 90 g/l sucrose was added (Fig. 3, Table 2, Ti-2). It was observed that low level of sucrose @ 30 g/l produced the healthy shoots and no tuber formation occurred indicating that low level of sucrose was responsible for vegetative growth of shoots in potato. At higher levels of sucrose, the osmolarity of the medium increased and plants underwent stress. Due to this stress, plants behavior shifted towards maturity which leads to tuber formation. This may be the reason why maximum number of tubers were harvested when 90 g/l sucrose was used. The maximum size of these tubers was 1.5 cm during the whole study. Sucrose seems to be the most critical stimulus for tuber formation. It may be essential as an osmoticum, as an energy source and at higher concentration may have a role as a signal for microtuber formation as reported by Wang & Hu, (1982) and Khuri & Moorby (1995).

When comparison was taken into consideration between CCC, BAP and sucrose, it became quite evident from the results (Table 2) that CCC is at par with sucrose for *in vitro* tuberization in Potato. However CCC is classified as a carcinogenic agent so it can be conveniently replaced by sources for the same purpose. Similarly growth hormones e.g. BAP may induce variations when applied *in vitro* at such a high concentrations as used for tuberization. This study therefore, indicates that sucrose may serve as a cheap, safe and superior agent for microtuberization, as compared to CCC and plant growth regulators.

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