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In Vitro Studies on Microtuber Induction in Potato

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ABSTRACT

This research was planned to study the effect of various concentrations of BAP and sucrose on *in vitro* potato (*Solanum tuberosum* L.) microtuber induction. Explants from *in vitro* grown plants were cultured on Murashige and Skoog (1962) medium supplemented with iron (150 gL^{-1}), concentrations of sugar (0, 3, 6 and 9%) and 6-benzylaminopurine (0, 0.2, 1 and 5.0 µM). The sucrose 6% and BAP 1 µM showed maximum number of microtubers. Sucrose and BAP had also significant ($p<0.05$) effect on shoot and root length. MS medium supplemented with sucrose and BAP significantly induced the microtubers in Santa, Cardinal, Diamant and Desiree.

Key Words: Microtuber; Virus; Potato

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). It plays a remarkable role in human diet as a supplement to wheat and rice. Potato is composed of 80% water, 2-3% protein and 18% carbohydrates (Thompson & Kelly, 1957). It is the number one vegetable crop in Pakistan and produced 1.72 million tonnes per annum from an area of 105.6 thousand hectares (Anonymous, 2001).

In Pakistan, basic problem for potato production is the availability of good quality seed. Imported potato seed is used to minimize the threat of potato seed born and viral diseases like potato leaf roll virus (PLRV), potato virus Y (PVY) and potato virus X (PVX). The viruses are the major cause of degeneration of seed and results in severe yield losses (Hooker, 1986; Lazar & Georgescu, 1987). Production and supply of seed potatoes in Pakistan is dependent on import of basic virus free seed from Holland. Imported seed is mostly cultivated as a spring crop which serves as a source of seed supply for the autumn crop in the plains and summer crop in the hills. In the same season aphid (*Myzus persicae*) attack is very high, which is the main insect vector for virus transmission (Rich, 1993).

Tissue culture techniques are used worldwide to produce pre-basic, virus-free seed potatoes known as microtubers. The microtubers are sown in a protected environment to produce minitubers (basic seed). The basic seed enters the seed production chain to produce the certified seed to be sold to the farmers. The main objective of the present study was to standardize the media for potato plant growth and microtuber induction.

MATERIALS AND METHODS

Four varieties Santa, Cardinal, Diamant and Desiree were collected from different sources. Potato tubers were washed with tap water and kept in craft paper bags. These bags were stored under dark conditions for one and a half month at constant temperature of 25°C for etiolated shoot sprouts (Ahmad & Khan, 1993). Etiolated shoots were washed in tap water and surface sterilized in 70% alcohol for 1-2 minutes and then for 15 minutes in 10% sodium hypochlorite with the addition of one or two drops of Tween-20 (Ahmad *et al.*, 1993). Finally explants were washed with double distilled autoclaved water and cultured on MS medium to achieve secondary explants which were sub-cultured on MS media supplemented with iron (150 gL^{-1}), Sucrose (0, 3, 6 and 9%) and 6-benzyl aminopurine (0, 0.2, 1 and 5 µM). Medium pH was adjusted to 5.7. After inoculation of shoot tips and nodes on media, the test tubes were placed at a temperature of 27±2°C under 16 h photoperiod.

RESULTS AND DISCUSSION

The treatment of 1 µM BAP induced maximum number of microtubers (0.53 per plant) followed by 5 µM BAP (0.36). BAP 0.2 µM and 0 µM produced 0.28 and 0.19 number of microtubers per plant, respectively (Table 1). It is also clear from Table I that 6% sucrose level yielded the highest number of microtubers (0.69) per plant; 9% sucrose level was also superior for the number of microtubers (0.49) per plant than 3% sucrose level, which induced 0.18 microtubers per plant. While control did not produce any microtuber. It is obvious from the results that BAP concentrations and sucrose level have significant impact for microtuber induction. Our findings are in accordance with Hoque *et al*. (1996), Khuri and Mooby (1996), Pelacho *et al*. (1999), Teisson and Alvard

Table I. Comparison of means and interaction of BAP (Benzyl Aminopurine) concentration and sucrose levels (percentage) in the media for number of microtubers per explant

Sucrose Level	BAP 0 µM	BAP 0.2 µM	BAP 1 µM	BAP 5 µM	Means of sucrose
0 %	0.00	0.00	0.00	0.00	0.00 d
3 %	0.00	0.15	0.35	0.20	0.18 c
6 %	0.40	0.60	1.0	0.75	0.69 a
9 %	0.35	0.35	0.75	0.50	0.49 b
Means of BAP	0.19 c	0.28 bc	0.53 a	0.36 b	

Table II. Comparison of means and interaction of BAP (Benzyl Aminopurine) concentration and sucrose levels (percentage) in the media for weight of microtuber

Sucrose Level	BAP 0 µM	BAP 0.2 µM	BAP 1 µM	BAP 5 µM	Means of sucrose
0 %	0.00	0.00	0.00	0.00	0.00 d
3 %	0.00	0.04	0.08	0.04	0.04 c
6 %	0.079	0.14	0.17	0.17	0.14 a
9 %	0.069	0.07	0.16	0.08	0.10 b
Means of BAP	0.04 c	0.06 bc	0.10 a	0.07 ab	

(1999), Sarkar and Niak (1997), Dimitrova and Ruseva (1988), and Yu *et al*. (2000).

The comparison of means of microtuber weight for different concentrations of BAP used is shown in Table II. It is clear that the highest microtuber weight (0.10 g) was in the medium supplemented with 1 µM BAP followed by medium with 5µM BAP producing 0.07 g microtuber (Table II). Microtuber weight at 3 and 9% sucrose levels was 0.04 and 0.10 g, respectively. The highest microtuber weight (0.14 g) was yielded by 6% sucrose level. Results revealed that means for microtuber weight was higher at 1 µM BAP concentration and 6% sucrose level. The results are in accordance with Hussey and Stacey (1984), Estrada *et al*. (1986), Rosell *et al*. (1987) and Lillo (1989).

The interaction of varieties, BAP and sucrose levels indicates that maximum shoot length (7.40 and 7.30 cm) was recorded in 'Desiree' and 'Cardinal' varieties cultured on 1 µM BAP with 9 and 6% sucrose levels, respectively which was non-significant ($P<0.05$) with 'Santa' (7.10 cm) at 5 µM BAP and 6% sucrose level. No shoot initiation was observed at 0% sucrose level irrespective of varieties and BAP concentration. These results indicate that the variety 'Desiree' produced the highest shoot length at 1 µM BAP concentration and was statistically similar with 'Cardinal' and 'Diamant'. Our results are in accordance with the results of Hoque *et al*. (1996). They recommended 1 mgL^{-1} BAP for higher shoot length in cultivars 'Cardinal' and 'Diamant'. Similar findings were reported by Liu *et al*. (1999) who observed that sucrose level was very important for shoot length. Results showed that after optimum (6%) sucrose level, shoot length decreased in the same manners as were reported by Wareing and Jenning (1980).

The overall interaction of the three factors studied for the response of root length (cm) is presented in Table IV, which showed that the maximum root length (3.46 cm) was examined on cultivar 'Santa' cultured on 5 µM BAP at 6% sucrose level and it is non significant with 'Cardinal' and 'Diamant' cultured on 1 µM BAP with 6% sucrose level and also non significant ($P<0.05$) with 'Desiree' cultured on 1 µM and 5 µM BAP with 9% sucrose level. Sucrose at 0% yielded no root length for all varieties and BAP concentrations. 'Desiree' was the best for root length than others on low BAP levels but higher sucrose levels. The highest root length (3.46 cm) was generated by 'Santa' at higher BAP concentration but at 6% sucrose. Similarly, 1 µM BAP concentration at 6% sucrose level was the best for 'Cardinal' and 'Diamant' regarding root length.

In conclusion, the results of present study suggested that standardization of culturing media significantly improve the potato plant growth and microtuber induction. The increase in BAP concentration

Table III. Shoot length (cm) of the varieties as affected by the amount of BAP and Sucrose in the culturing media

Sucrose/ Levels	Desiree				Diamant				Cardinal				Santa			
	BAP0 µM	BAP0.2 µM	BAP1 µM	BAP5 µM	BAP0 µM	BAP0.2 µM	BAP1 µM	BAP5 µM	BAP0 µM	BAP0.2 µM	BAP1 µM	BAP5 µM	BAP0 µM	BAP0.2 µM	BAP1 µM	BAP5 µM
0 %	0.00p	0.00p	0.00p	0.00p	0.00p	0.00p	0.00p	0.00p	0.00p	0.00p	0.00p	0.00p	0.00p	0.00p	0.00p	0.00p
3 %	4.10lmn	4.52jklmn	5.20defg	5.08fghijk	3.70no	3.80mno	6.34bcd	4.60ijklmn	3.62no	3.70no	5.90def	3.70no	3.10o	3.74mno	4.12klmn	5.58defgh
6 %	5.20efghij	5.50defghi	6.30bcd	5.64defgh	5.08fghijk	6.12cde	6.90abc	5.96cdef	4.12klmn	6.12cde	7.30a	6.06cdef	4.04lmno	5.72defg	5.54defghi	7.10ab
9 %	5.60defgh	6.38bcd	7.40a	6.10cde	4.14 klmn	5.50defghi	6.24bcd	4.32jklmn	3.92lmno	4.32jklmn	5.52defghi	4.84ghijkl	3.70no	4.70hijklm	4.48jklmn	5.92def

Table IV. Root length (cm) of the varieties as affected by the amount of BAP and Sucrose in the culturing media

Sucrose/ Levels	Desiree				Diamant				Cardinal				Santa			
	BAP0 µM	BAP0.2 µM	BAP1 µM	BAP5 µM	BAP0 µM	BAP0.2 µM	BAP1 µM	BAP5 µM	BAP0 µM	BAP0.2 µM	BAP1 µM	BAP5 µM	BAP0 µM	BAP0.2 µM	BAP1 µM	BAP5 µM
0 %	0.00s	0.00s	0.00s	0.00s	0.00s	0.00s	0.00s	0.00s	0.00s	0.00s	0.00s	0.00s	0.00s	0.00s	0.00s	0.00s
3 %	2.10mnopqr	2.52fghijklm	2.68defghi	2.50fghijklmn	2.12mnopqr	2.30hijklmno	2.80bcdefg	2.18lmnopq	1.72r	2.08nopqr	2.92bcdef	1.80qr	1.82pqr	2.10mnopqr	1.86pqr	3.12abc
6 %	2.62efghijk	2.72bcdefgh	2.90bcdef	2.70cdefghi	2.64efghij	2.72bcdefgh	3.14ab	2.82bcdefg	2.28ijklmno	2.88bcdef	3.10abcd	2.90bcdef	2.42ghijklmno	2.84bcdefg	2.90bcdef	3.46a
9 %	2.90 bcdef	3.10abcd	3.12abc	3.00bcde	2.20klmnopq	2.56fghijkl	2.90bcdef	2.32hijklmno	2.22jklmnop	2.50fghijklmn	3.00bcde	2.10mnopqr	2.02opqr	2.52fghijklmn	2.44ghijklmno	2.92bcdef

significantly induced maximum number of microtubers with highest weight. The MS medium supplemented with 6% sucrose level yielded maximum number of microtubers with highest microtuber weight in all the studied varieties.

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