INNSPUB

International Journal of Agronomy and Agricultural Research (IJAAR)
ISSN: 2223-7054 (Print) 2225-3610 (Online)
http://www.innspub.net
Vol. 7, No. 1, p. 118-124, 2015

RESEARCH PAPER OPEN ACCESS

Effect of sucrose on inducing *in vitro* microtuberization in potato without using any growth hormone

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Article published on July 17, 2015

Key words: Potato, Microtuberization, Sucrose concentration.

Abstract

The present *in vitro* experiment was conducted to investigate the effect of the various concentrations of sucrose on potato plantlets growth and microtuberization. It was observed that increasing sucrose level in the media influenced the plant growth negatively. 3% sucrose concentration in the medium showed comparatively early root/shoot emergence and highest mean root and shoot length (6.16 cm and 8.28 cm, respectively) with greater number of nodes (7.90). However, regarding microtubers (Mt) formation, treatment with 8% sucrose concentration has higher microtubers number with larger size (mean diameter 6.84mm). The mean weight of Mt was also highest (97.0mg) at 8 % sucrose concentration followed by T1 (70.00mg). It has been concluded on the basis of results that MS medium supplemented with 8% sucrose level and without any growth hormone is the best for *in vitro* microtuber formation in potato.

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Introduction

The potato (*Solanum tuberosum* L.) is an annual herbaceous plant and a staple food crop in many countries of the world. It is vegetatively propagated by tubers. In Pakistan several yield reducing factors in potato crop have been reported including viral diseases which causes about 83% yield losses (Abbas *et al.,* 2012). Among viruses PVX, PVY, PVS, PLRV, PVA and PVM are most significant in Pakistan (Gul *et al.,* 2013). Ahmad and Ahmad, (1995) reported that the seed borne viruses are spreading in potato growing areas of Pakistan through seed.

Shortage of good quality seed has been recognized as the single most important factor limiting potato production in the developing countries. All conventional potato seed production systems are characterized by low multiplication rate and progressive accumulation of degenerative viral diseases during clonal propagations. Tissue culture is possibly the first biotechnological approach used to eliminate viruses and to produce disease-free potato seed. *In vitro* microtuber production has solved the problem of *in vivo* plantlets transplantation, disease free potato seed stocks storage and easy to handle healthy germplasm exchange and conservation of valuable cultivars (Kefi *et al*., 2000; Rosu *et al*., 2004; Kanwal *et al*., 2006).

Microtubers are the first generation of potato seed produced by tissue culture from axially part of *in vitro* plantlets leaves. Since microtuberization is a complex physiological process regulated by many factors including sugar concentration in the culture medium (Ranall, 2007). Previous studies has mainly focused on the use of growth regulators for microtuberization in potato (Estrada *et al*. 1986; Vecchio *et al*. 1994). The present *in vitro* study was conducted to evaluate the effect of various sucrose concentrations for microtuber formation in potato variety desiree.

Materials and methods

The present *in vitro* experiment was conducted in the tissue culture laboratory at Hazara Agriculture Research Station, Abbottabad during the year 2014.

Media preparation

Basal Murashige and Skoog (1962) MS medium containing 1.0 mgl^{-1} Ca-pentothenate, 0.25 mgl^{-1} Gibberellic acid (GA3), 100mgl^{-1} Myoinositol and agar (8g/l) with pH 5.8 adjusted either with 0.1 N KOH or 0.1 N HCl before sterilization was used in this study. Sucrose was added to the medium at five different concentrations i.e 0% (T0), 3% (T1), 6% (T2), 8% (T3) and 11% (T4). Each treatment was replicated 20 times. Treatment with 0% sucrose concentration served as control. The media was poured into test tubes @ 10ml per test tube and autoclaved at 121°C under 15 psi pressure for 15 minutes.

Experimental material and methods

The explants of potato variety Desiree having at least 6 nodes per plantlet were obtained from the tissue culture laboratory at Hazara Agriculture Research Station, Abbottabad and each plantlet was cut into nodal segments. Each segment was inoculated aseptically on the media in the test tubes under sterilized conditions in laminar flow cabinet. The test tubes were incubated in growth chamber at 20°C under 16 h light and 8 h dark photoperiod for about 30 days. Then the plantlets were shifted to dark until microtuber formation. Data were analyzed by using computer software statistics 8.1 and least significance difference test (LSD) at 95% level of significance was used to assess significant difference between various treatments. The root/shoot emergence data was recorded after 5 days and one week after culturing while other growth parameters data was recorded after 30 days of inoculation.

Results

In the present study the effectiveness of different sucrose concentrations on growth and microtuber formation in the potato cv. Desiree was evaluated.

No of days to shoot and root emergence

The data in table 1 shows that all the 5 treatments i.e, T1(3%), T2 (6%), T3 (8%) T4 (11%) and T0 (0%) with different sucrose concentrations showed variation in no. of days to shoot and root emergence. In

treatment T1 all the cultured nodes showed shoot emergence at the fifth day of culturing (Fig. 1B) as compared to other treatments (Table 1) while in the control (T0) only 10 plantlets showed shoot emergence. Similar response of plantlets to various treatments was observed regarding root emergence (Table 1; Fig.1A).

Table 1. Average number of potato plantlets showing root and shoot emergence.

No. of days	Shoot emergence					Root emergence				
	T1	T2	T3	T4	T0	T1	T2	T3	T4	T0
On 5th day	20	15	16	18	10	20	10	8	9	6
On 8th day	20	20	20	20	20	20	20	20	20	20

Shoot length

There is significant difference (P≤0.05) between the shoot lengths of all the five treatments (Table 2). The highest mean shoot length (8.28 cm) was observed in treatment T1 having 3% sucrose level followed by T2 (6% sucrose) in which the mean shoot length was 6.40 cm (Fig. 2). The lowest shoot length (3.48 cm) was recorded in control (T0) (Table 2).

Table 2. Mean values for growth parameters of potato variety Desiree after 30 days of culturing.

Treatments	No. of leaves	No. of Roots	No. of nodes	Shoot length	Root length
3% sucrose level (T1)	9.75a	8.45a	7.90a	8.28a	6.16a
6% sucrose level (T2)	6.50b	4.10bc	5.45b	6.40b	4.86b
8% sucrose level (T3)	5.60c	4.40b	4.60c	5.02c	3.40c
11% sucrose level (T4)	4.85d	3.55c	3.85d	4.28c	3.00c
Control (T0) 0% sucrose level	4.05e	2.80d	3.05e	3.48d	2.40d

Means followed by different letters in a column indicate significant differences at P≤0.05.

Root length

Data regarding root length presented in Table 2 showed that all the treatments differed significantly (P≤0.05) in their root length except treatments T3 and T4 in which no significant difference was observed (3.40 and 3.00 cm respectively). The highest root length was recorded in the treatment T1 (6.16) whereas control showed the smallest mean root length (2.40 cm) (Table 2).

Number of leaves

The data pertaining to numbers of leaves revealed that plantlets of the treatment T1 showed significantly greater number of leaves (9.75) whereas the treatment T4 and T0 showed lowest leaves number (4.85 and 4.05) (Table 2).

Number of roots

Number of roots per plantlet were recorded after 30 days of culturing (Table 2). Highest mean number of roots (8.45) were recorded in treatment T1 while treatment T0 showed lowest mean no of roots (2.80) which differed significantly.

Number of nodes

The data revealed that all the treatments differed significantly in their number of nodes (Table 2). The highest mean number of nodes were recorded in the treatment T1 which was 7.90 nodes per plantlet whereas the treatment T0 showed lowest average no.

of nodes (3.05).

Number of microtubers

Microtubers (Mt) formed after 8 weeks of culture are in the range of 0-4 microtuber /plantlet (Table 3). No significant difference (P≥0.05) was observed among treatments (Table 3) regarding mean number of microtubers. A relatively higher number of microtubers (Mt) was observed in treatments with sucrose concentration 3 and 8% while in the control treatment (T0) no Mt were formed.

Table 3. Effect of sucrose concentrations on *in vitro* potato microtuberization.

Treatments	Fresh wt. of Microtuber(mg)	Diameter of Microtuber(mm)	No of microtubers	No. of eyes per microtuber
3 % sucrose level (T1)	70.00ab	5.86b	2.05a	3.00ab
6 % sucrose level (T2)	57.50b	5.35b	1.90a	2.65b
8 % sucrose level (T3)	97.00a	6.84a	2.10a	3.35a
11 % sucrose level (T4)	55.50b	5.56b	1.95a	2.80b
Control (T0)	0.00c	0.00c	0.00b	0.00c

Means followed by different letters in a column indicate significant differences at P≤0.05.

Weight of microtuber

The mean fresh weight of microtubers (Mt) were measured at the time of harvest which differed significantly among treatments (P≤0.05) (Table 3). The highest mean weight of Mt was observed in treatment T3 (97.0mg) followed by T1 (70.00mg), T2 (57.50mg) and T4(55.50mg).

Microtuber diameter

The diameter of microtubers (Mt) formed in treatment T3 (8 % sucrose) differed significantly (P≤0.05) from other treatments. The microtubers of T3 are comparatively larger in size with mean diameter of 6.84mm.

Number of eyes per microtuber

Table 3 showed that the microtubers formed in treatment T3 have more number of eyes (3.35) followed by treatment T1 in which mean no. of eyes was 3.00 which differed significantly (P≤0.05) from other treatments in which comparatively lesser no. of eyes was observed.

Discussion

Results revealed that treatment T1 having 3% sucrose have shoot/root emergence in all cultured nodes as compared to other treatments just after 5 days of culturing. This may be due to the fact that plants require an optimum level of carbon source for its normal development and when any plant is brought under stress by either increasing or decreasing sucrose concentration in the nutrient media, the plants shoot/root emergence will be delayed as reported by Gulsen and Domanoglu (1991) and Adjei, (2001) who found that 3% sucrose level in tissue culture medium can regenerate more shoots in Quince and pineapple *in vitro* regeneration, respectively.

The data presented in Table 2 showed that shoot length, no. of leaves, no. of nodes, and root number decreases as sucrose concentration increases. The possible reason for this is that the *in vitro* growth and multiplication of shoots are affected by the concentration of exogenous carbon source added to the medium. Gauchan (2012) reported variation in shoot response in maize plantlets on different sugar concentrations and found that moderate maltose and sucrose concentration enhance the root/shoot growth, whereas the root and shoot length decreases

with increasing sugar concentration. Plant growth and root initiation are energy requiring processes and occur at an optimum level of metabolic substrates, which are mainly carbohydrates (sucrose) as reported in previous studies (Calamar *et al.*, 2002; Tauquer *et al.*, 2007). Sucrose in tissue culture acts both as an energy source and as an osmoticum and affects plant growth by reducing water content of tissues at higher concentrations. Khan *et al.,* 2006 reported that sugar concentration regulates the osmolarity of the culture media and therefore plays an important role during morphogenesis in sugarcane.

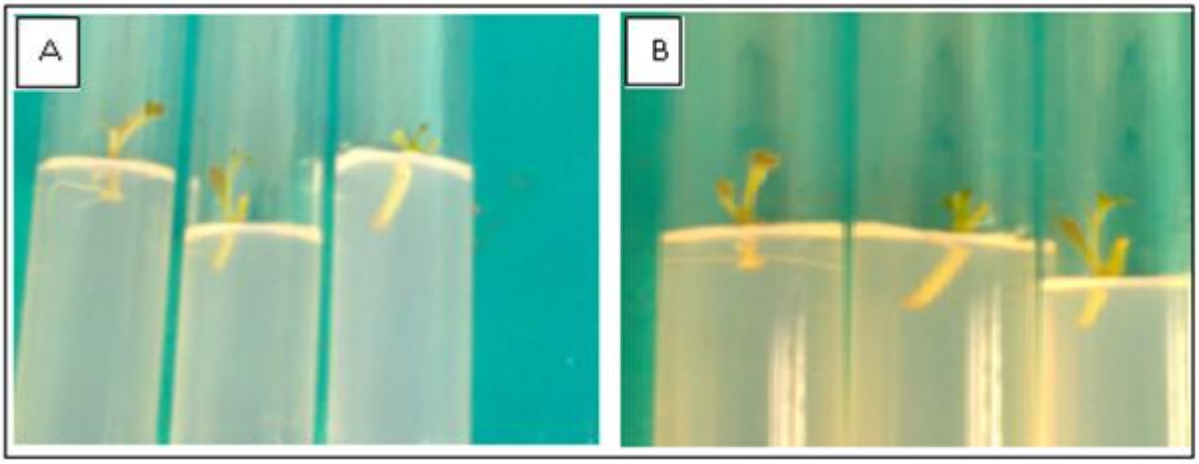

Fig. 1. Potato plantlets showing root (A) and shoot (B) emergence under *in vitro* conditions on 5th day of culturing.

Regarding microtuber formation results showed that as the sucrose concentration increases up to 8% (T3), the microtuber number, average fresh weight, size, and average no. of eyes per microtuber also increases however, further increase in sucrose concentration (11%) not only increases the time period for microtuberization but also decreases the number of microtubers per plantlet.

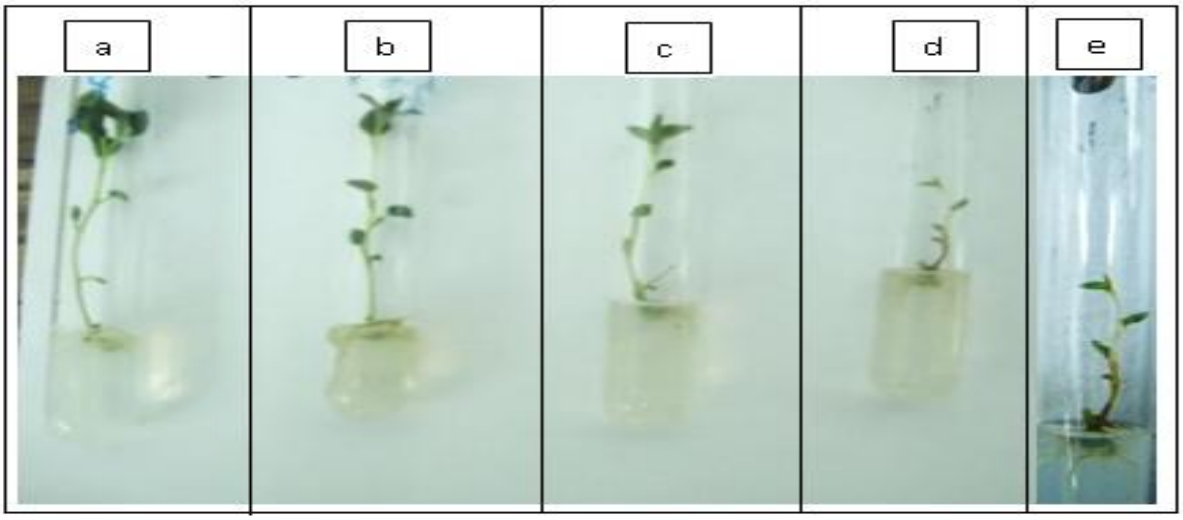

Fig. 2. Plantlets of potato cv. Desiree showing difference in growth rate after one month of culturing on MS media with different sucrose levels i.e (a): 3% (b): 6% (c): 8% (d): 11% (e): 0%).

From the results, it is quite obvious that very low (T0) or high (T4) sugar concentration is unsuitable for optimal microtuberization because low sucrose concentration is not enough to induce microtubers and high concentration may increase the osmotic properties of medium which disturb the pH and

balance of nutrients. In agreement to this Oparka and Wright (1988) and Khuri and Moorby (1995) demonstrated that the high sucrose level not only assimilated and converted to starch for the microtuber development but also increases the osmolarity of the media. Dodds *et al.*, 1992 reported that low and high sucrose concentrations influenced microtuberization negatively and as a result less microtubers produced. The present findings are in accordance with the earlier findings of Carlson (2004) and Miranda *et al.*, 2005 who reported best microtuber induction response on MS medium supplemented with 80g/L sucrose.

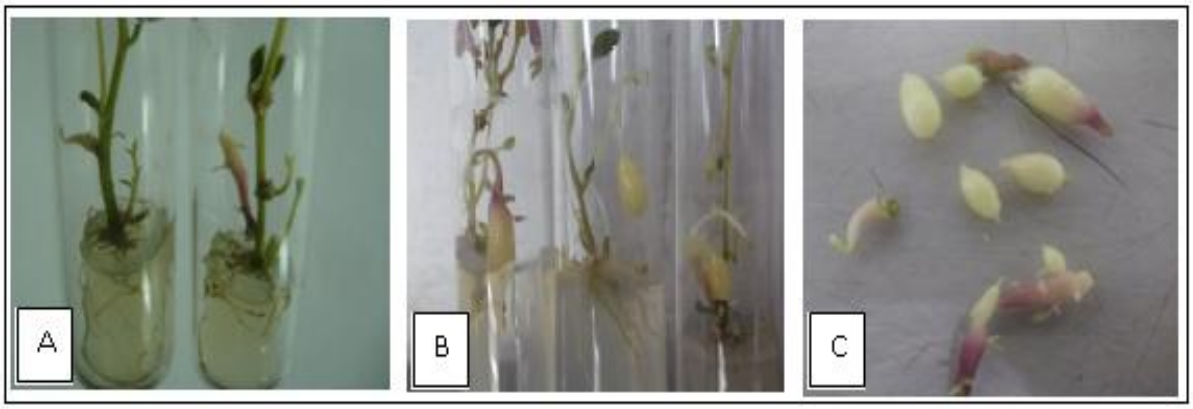

Fig. 3. Plantlets of potato variety Desiree showing *in vitro* microtubers development (A and B); (C) Microtubers after harvesting.

Conclusion

It is concluded on the basis of the results of this study that MS medium supplemented with 8% sucrose and without any growth hormone is the best for *in vitro* microtuber formation in potato which is a cost effective approach for developing countries like Pakistan. However, further research is needed to know the relationship between carbon source and the physiological changes during growth of *in vitro* growing potato plantlets for early *in vitro* microtuber formation**.**

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