South Western Journal of
Horticulture, Biology and Environment
P-Issn: 2067- 9874, E-Issn: 2068-7958

Vol. 4, No 1, 2013
pp. 66-81

THE STUDY OF CARBON SOURCES EFFICIENCY ON *IN VITRO* POTATO MICROTUBERIZATION

Alireza MOTALLEBIAZAR[1,*] and Samaneh KAZEMIANI[1]

1. Department of Horticultural Sciences, Faculty of Agriculture,
University of Tabriz, Tabriz 51666, Iran
*Corresponding Author: motallebiazar@gmail.com

Abstract. *One of the most critical stages of produced virus free potato plants is rapid and efficient in vitro microtuberization. Carbon sources and their concentration is important factor on potato microtuberization. This study was conducted to develop a protocol for rapid microtuber formation for potato micropropagation and germplasm conservation by different carbon sources and their concentrations. In order to evaluate the effects of five concentrations of sucrose (80,100,120,140 and 160 g/l) and five concentrations of mannitol (0.0, 10, 20, 30 and 40 g/l with 80 g/l sucrose) a factorial experiment based on completely randomized design with five replications was carried out in tissue culture laboratory, Faculty of Agriculture, University of Tabriz. Single node explants from in vitro potato shoots were cultured on microtuberization media and they incubated on darkness condition at 20 ±1°C in growth chamber. Analysis of variance revealed significantly differences among treatments for induction and formation of microtuber, length, diameter, weight tubers and rate of microtuber induction. Sucrose was the more effective on microtuberization process than mannitol. When mannitol used in medium, it led to a decrease microtuberization traits. The use of high concentrations of sucrose than 80 g/l was useful on in vitro microtuberization. Increasing of sucrose concentrations were improved efficiently in vitro microtuber production without negative side effects. The present study describes an efficient method for in vitro microtuberization of potato cultivars which could be considered for large scale multiplication and propagation of this important vegetable crop. This finding helps to rapid micropropagation of commercial*

potato cultivars by using high concentrations of sucrose in microtuberization medium.

KEYWORDS: *rapid microtuberization, mannitol, sucrose, shoot proliferation, Solanum tuberosum, virus free potato plants*

INTRODUCTION

Microtubers generally originate as aerial structures on the stem, occasionally a few microtubers may be formed in the medium (Motallebi-Azar *et al*, 2011; Ali *et al*, 2009). Microtubers are convenient for handling, storage and transport of germplasm (Thieme 1992; Gorden and William 1993; Hussein *et al*, 2006). Microtubers also provide more flexible planting options and reduce the risk of disease infection in the field (Yu *et al*, 2000). Induction and growth microtubers were influenced by some factors such as genotype (Ahloowalia 1994), type of explants (Khuri and Moorby 1996), light quality (Pelacho and Mingo-Castel 1991), photoperiod (Hussey and Stacey 1984), temperature (Akita and Takayama 1994), carbon source (Garner and Blake 1989) and growth regulators (Ortiz-Montiel and Lozoya-Saldana 1987; Tan *et al*, 2010; Shirani *et al*, 2010). Several reports have evaluated the effects of carbon source on microtuberization (Dodd's *et al*, 1992; Garner and Blake 1989; Hussey and Stacey 1984). Dodd's *et al*. (1992) showed that optimal concentration of sucrose ranged from 60-80 g/l. Higher or lower sucrose concentrations that 80 g/l leads to slower microtuberization, fewer and smaller microtubers (Yu *et al*, 2000). Sucrose may be playing a dual role in microtubers development. A part from being a suitable carbon source that is easily assimilated by the microplants and converted to starch in developing microtubers. Sucrose, at a concentration of 80 g/l, also provides a favorable osmolarity for microtubers development (Khuri and Moorby 1995; Yu *et al*, 2000). The substitution of the culture medium carbon source by somatically active solutes has shown that sugars act as a carbon source and as osmotic regulators. In this case, the most frequently used solutes are the two-alcohol sugars mannitol and sorbitol

(George 1993). Important and unexploited data is the probable non-uptake and or non-metabolization of mannitol, a fact, which makes it suitable for use in osmotic studies. The treatment with a combination of sucrose and mannitol reinforces this hypothesis. As in change some of the assessed traits, probably due to the osmotic potential in the culture medium when compared to the potential of the medium containing only sucrose. This hypothesis could confirm by adding a treatment with sucrose as carbon source but with and osmotic contribution (Paiva Neto and Campos Otoni 2003; Ehsanpour *et al*, 2005).

In this study, plant materials were cultured on various levels of sucrose for microtuber induction and then investigated the effects of different concentrations of mannitol on medium including 80 g /l sucrose for microtubers formation and growth.

MATERIALS AND METHODS

In vitro shoot multiplication

In vitro potato shoots (*Solanum tuberosum* cv. Agria) were routinely subcultured every 3-4 weeks by placing 5 single node cuttings on each jar containing 30 mL of semi-solid MS medium supplemented by 30 g/l sucrose and 8 g/l agar. The culture was maintained at 22±2°C under photoperiod 16 h light/ 8 h dark (Motallebi-Azar *et al*, 2011).

In vitro microtuberization

Single node cuttings were cut aseptically from *in vitro* shoots and then cultured on different culture media consisting of two types of carbon source at five concentrations. Five treatments including 80, 100, 120, 140 and 160 g/l sucrose and five other treatments were 0.0, 10, 20, 30 and 40 g/l mannitol mixture of one level of sucrose, 80 g/l. Five single node cuttings were cultured in each Petri dish (8 cm). Petri dishes were sealed with parafilm and incubated under continuous dark at 20±1°C in a culture room. After 30 days, data were recorded for microtuber induction percentage (enlarged cells in the node location, size less than 3 mm), microtuber formation percentage (larger than 3 mm), microtuber fresh weight (mg), length and diameter of microtuber (mm), induction rate (n: number of induced microtuber, t: days):

$$\frac{(n_1 \times t_1) + ... + (n_n \times t_n)}{t_n}$$

The experiment was conducted in a factorial experiment based on completely randomized design with five replications. Data obtained from this study were analyzed using SPSS software Ver.16. The means of treatments were compared using Duncan's multiple range tests at 5% probably level.

RESULTS AND DISCUSSION

In all culture media, microtuber induction from axillary bud was initiated 5-6 days after culture (Figure 1).

Figure 1. Microtuber induction 5-6 days after culture.

In some treatments, induced microtubers were growth and its size varied from 3-11 mm during one month (Figure 2). However, due to lack of suitable conditions, microtuber induction were occurred on some explants, but didn't developed to microtuber (larger than 3 mm). This buds were growth and shoot elongated (0.5-5 cm) (Figure 3). In most cases microtubers were globular and sometimes ellipsoid (Figure 2). Skin color of microtubers was usually cream, sometimes with purple spots (Figure 4).

Figure 2. Size and shape of microtuber
(left: globular and 9 mm, right: ellipsoid and 3 mm)

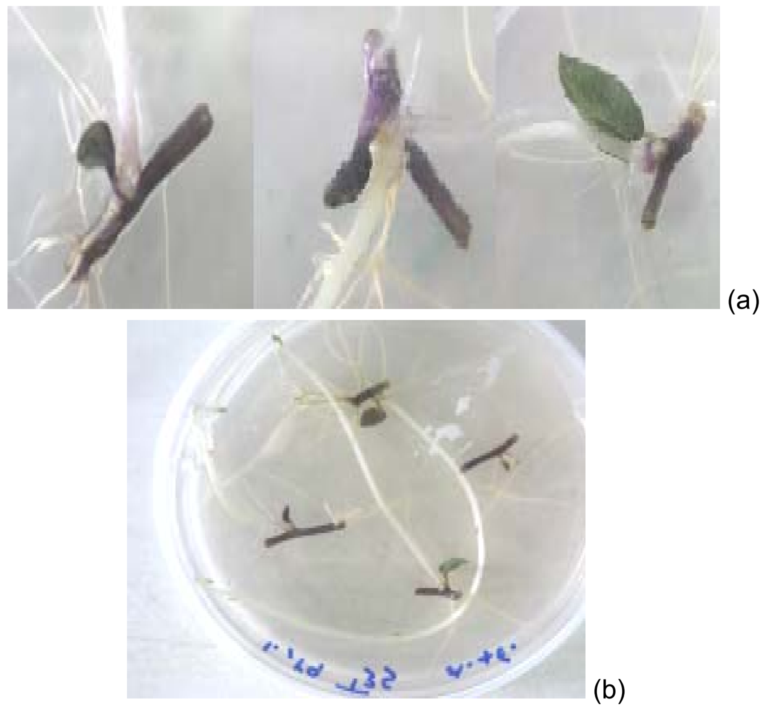

Figure 3. (a) Lake of growth of induction microtuber,
growth of single node and shoot formation;
(b) Shoot growth and root formation

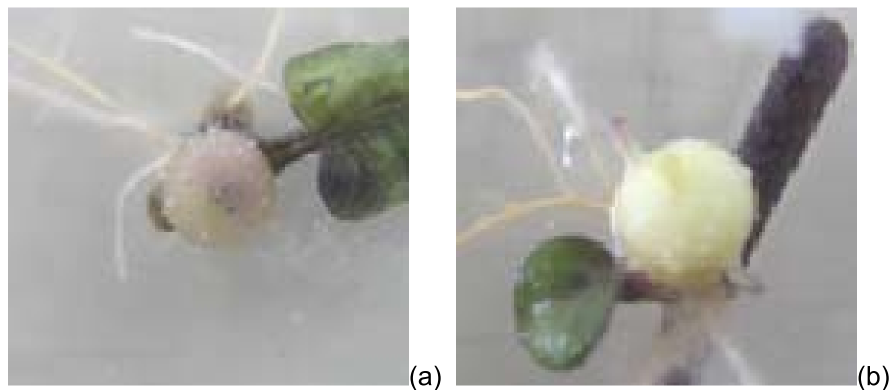

Figure 4. Color of microtuber (a) cream color with purple veins (b) cream color

Number and length of root per explant were 9-36 and between 0.5-8 cm (Figure 3). Harvested microtubers were transferred to pots containing peat, perlite and vermicompost (1:1:1) and they germinated on mist conditions during 4-5 days (Figure 5). Microtubers also can be cultured in MS medium free hormones, there germinated during the 5-7 days and *in vitro* plants produced desirability (Figure 6).

Figure 5. Microtubers cultured in MS medium without hormones, who germinated during the 5-7 days

Figure 6. Microtubers after harvest were transferred to pots containing peat: perlite: vermicompost (1:1:1) and germinated on mist conditions during 4-5 days.

Analysis of variance showed that microtuber induction percentage was significantly affected by different treatments ($p<0.01$). Microtuber induction percentages in treatments containing only sucrose were significantly higher than culture media with mannitol. Minimum microtuber induction percentage was observed in treatments containing different concentrations of mannitol and this indicated that adding mannitol to the medium significantly decreased in induction of microtuber percentage. However, non significant differences were observed among different concentrations of mannitol. When the medium was containing only sucrose, in the high levels of sucrose was increased microtuber induction percentage, but maximum was observed in concentration of 120 g/l medium without hormones (Figure 7).

Analysis of variance showed that the microtuber formation percentage was affected by carbon source ($p<0.05$) (Table 1). The microtuber formation in culture media containing different concentrations of sucrose was higher than the culture media containing mannitol and so, adding of mannitol had been a significantly decrease in the microtuber formation. However,

significant differences weren’t observed among different concentrations of mannitol. In addition, although different concentrations of sucrose were high microtuber formation percentage, however, non significant differences observed among the concentrations of sucrose (Figure 7).

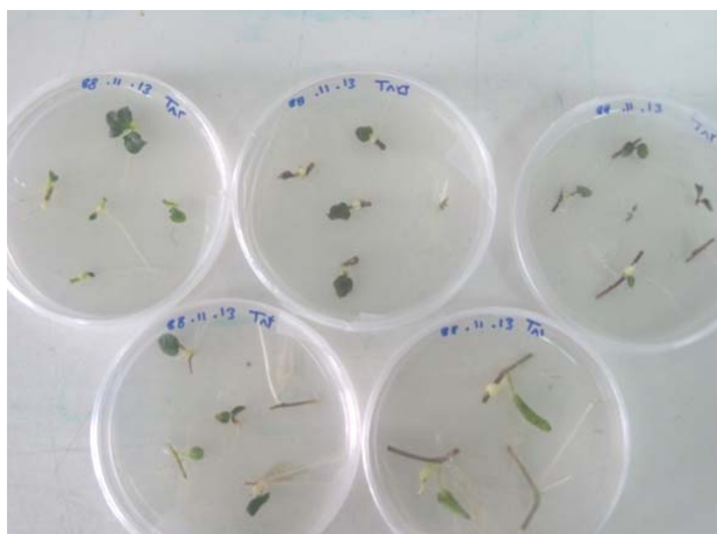

Figure 7. Different to start microtuberization to from explants to other explants in each Petri dish

Table 1. Analysis of variance table

Microtuber Source	DF	Mean square					
		Microtuber induction	Microtuber formation	Microtuber lenght	Microtuber diameter	Rate of weight	Microtuber induction
Treat	8	0.321**	0.185*	0.173**	0.050**	0.001**	2.157**
Error	36	0.094	0.062	0.034	0.015	0.001	0.472

Levels of significance are: *: p<0.05 and **: p<0.01

In each Petri dish, initiation microtuberization was occurred in different times to from explants to other explants. In some explants, initiation microtuberization were take placed of 5-6 days after culturing, but in a small

number of explants microtuberization began after 14-20 days and therefore in this explants, the size of microtubers were small at the recorded time (Figure 8).

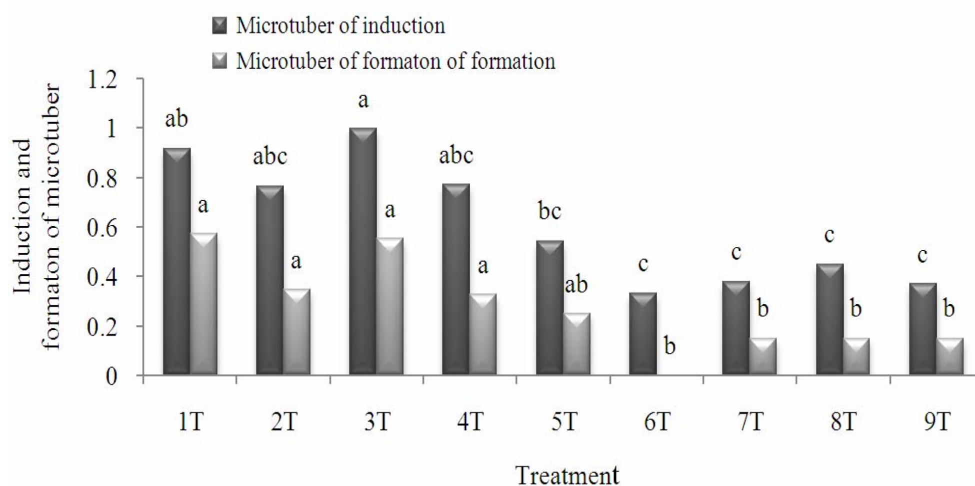

Figure 8. Effect of different concentrations of sucrose and mannitol on induction and formation of microtuber (T1: 160 g/l sucrose, T2: 140 g/l sucrose, T3:120 g/l sucrose, T4:100 g/l sucrose, T5:80 g/l sucrose, T6:10 g/l mannitol, T7:20 g/l mannitol, T8:30 g/l mannitol, T9:40 g/l mannitol)

Analysis of variance for the induction rate showed that culture media containing different concentrations of sucrose had more quickly induction rate than the culture media containing mannitol ($p<0.01$)(Table 1). In other words, sucrose in microtuber induction medium was caused earlier microtuberization from explant. The induction rate was increased by higher concentrations than 80 g/l sucrose and in 160 g/l sucrose was maximum (Figure 9). In culture media containing mannitol, induction rate were less than culture media containing sucrose.

Increasing mannitol concentrations were decreased significantly induction rate and the minimum rate of induction was observed at 40 g/l mannitol (Figure 9).

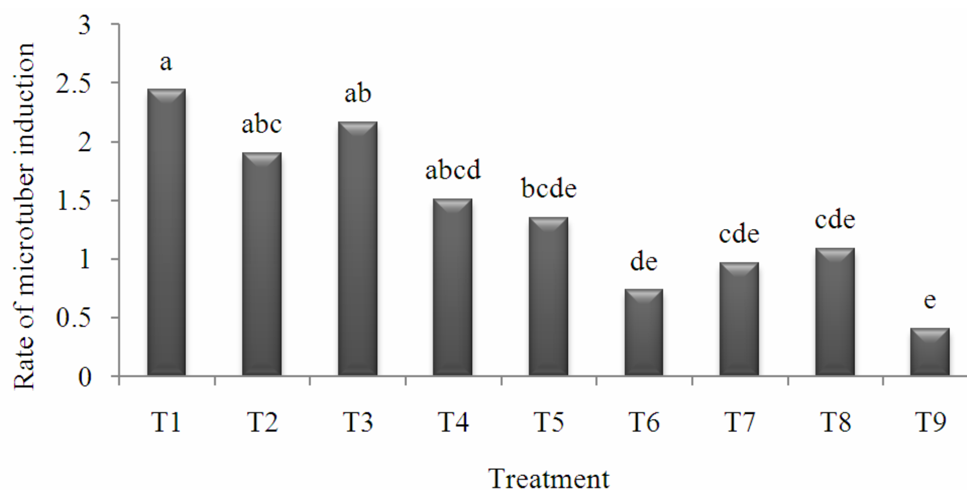

Figure 9. Effect of different concentrations of sucrose and mannitol on rate of microtuber induction (30 days). (T1: 160 g/l sucrose, T2: 140 g/l sucrose, T3:120 g/l sucrose, T4:100 g/l sucrose, T5:80 g/l sucrose, T6:10 g/l mannitol, T7:20 g/l mannitol, T8:30 g/l mannitol, T9:40 g/l mannitol)

Microtubers length were significantly affected by carbon source significantly ($p<0.01$). So that the significant difference was observed between the culture medium containing different concentrations of sucrose. Higher microtuber length was observed with increasing sucrose concentrations up to 120 g/l and with further increases sucrose decreased microtuber length, but non significant difference were observed in sucrose concentrations up to 120 g/l. Analysis of variance revealed significant differences among different concentrations of mannitol for length of microtuber. Its increasing of microtuber length was observed on increasing the concentrations of mannitol up to 30 g/l and then its length decreased by 40 g/l (Figure 10). Microtuber diameter was significantly affected by sucrose concentrations. But, significantly different wasn't in sucrose concentrations up to 120 g/l. Diameter was added increasing of mannitol to 30 g/l and then were observed decreasing in diameter (Figure 10).

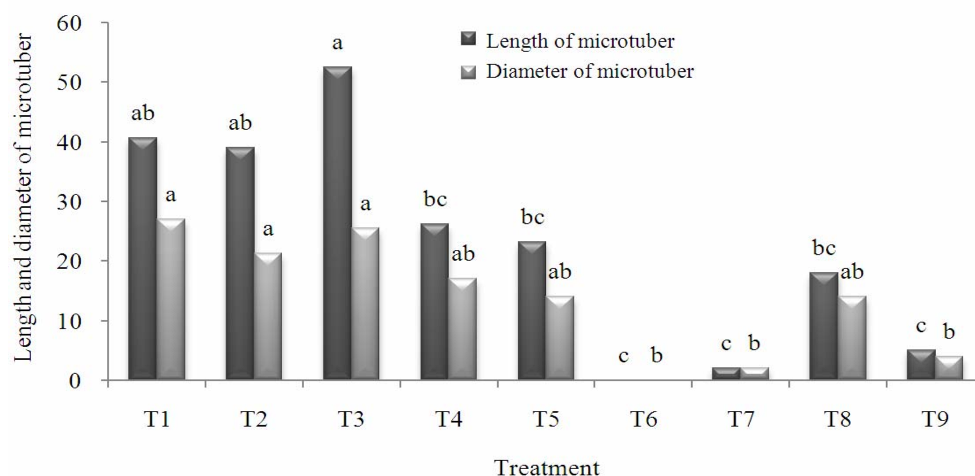

Figure 10. Effect of different concentrations of sucrose and mannitol on length and diameter of microtuber (T1: 160 g/l sucrose, T2: 140 g/l sucrose, T3:120 g/l sucrose, T4:100 g/l sucrose, T5:80 g/l sucrose, T6:10 g/l mannitol, T7:20 g/l mannitol, T8:30 g/l mannitol, T9:40 g/l mannitol)

Microtubers weight also was significantly affected by carbon source ($p<0.01$). In more than 100 g/l sucrose was added on microtuber weight. But among them did not exist significant differences. Adding mannitol due to small microtuber production, its weight was low, it is non significant and it canceled. But microtuber weight was equal in the concentration of 30 g/l mannitol approximately with 100 g/l sucrose, perhaps osmotic pressure and nutrient source is appropriate (Figure 11).

In all culture media, microtuber induction from axillary bud was initiated 5-6 days after culture. In some treatments, induced microtubers were growth and its size varied from 3-11 mm during one month. However, due to lack of suitable conditions, microtuber induction were occurred on some explants, but didn’t developed to microtuber (larger than 3 mm). This buds were growth and shoot elongated (0.5-5 cm) (Figure 3). In most cases microtubers were globular and sometimes ellipsoid (Figure 2). Skin color of microtubers was usually cream, sometimes with purple spots (Figure 4).

Number and length of root per explant were 9 - 36 and between 0.5-8 cm (Figure 3). Harvested microtubers were transferred to pots containing peat, perlite and vermicompost (1:1:1) and they germinated on mist conditions during 4-5 days (Figure 5). Microtubers also can be cultured in MS medium free hormones, there germinated during the 5-7 days and *in vitro* plants produced desirability (Figure 6).

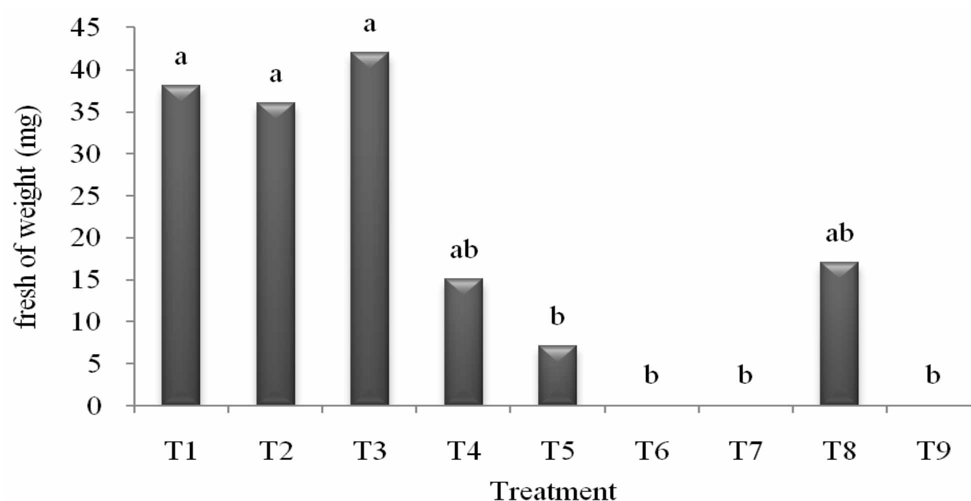

Figure 11. Effect of different concentrations of sucrose and mannitol on weight of microtuber (T1: 160 g/l sucrose, T2: 140 g/l sucrose, T3:120 g/l sucrose, T4:100 g/l sucrose, T5:80 g/l sucrose, T6:10 g/l mannitol, T7:20 g/l mannitol, T8:30 g/l mannitol, T9:40 g/l mannitol)

Analysis of variance showed that microtuber induction percentage was significantly affected by different treatments ($p<0.01$). Microtuber induction percentages in treatments containing only sucrose were significantly higher than culture media with mannitol. Minimum microtuber induction percentage was observed in treatments containing different concentrations of mannitol and this indicated that adding mannitol to the medium significantly decreased in induction of microtuber percentage. However, non significant

differences were observed among different concentrations of mannitol. When the medium was containing only sucrose, in the high levels of sucrose was increased microtuber induction percentage, but maximum was observed in concentration of 120 g/l medium without hormones (Figure 7). In most reports the maximum sucrose was used for microtuberization 90 g/l, that this has been with using hormone (Seabrook 2005; Hussein *et al*, 2006; Khuri and Moorby 1996; Karami 2008).

Analysis of variance showed that the microtuber formation percentage was affected by carbon source ($p<0.05$) (Table 1). The microtuber formation in culture media containing different concentrations of sucrose was higher than the culture media containing mannitol and so, adding of mannitol had been a significantly decrease in the microtuber formation. However, significant differences weren't observed among different concentrations of mannitol. In addition, although different concentrations of sucrose were high microtuber formation percentage, however, non significant differences observed among the concentrations of sucrose (Figure 7). The suitable effects of sucrose in microtuberization also were reported by Yu *et al*. (2000) they showed that replacing 80 g/l glucose and fructose instead of 80 g/l sucrose was produced small microtubers, because osmolarity of medium was being unfavorable. So the fact that since the report wasn't by adding mannitol to the culture medium of microtuberization, there can be concluded that only the role of mannitol in the medium of microtuberization plays osmolarity and because lack of nutritional and energy roles cannot be effective in the growth and formation microtuber.

In each Petri dish, initiation microtuberization was occurred in different times to from explants to other explants. In some explants, initiation microtuberization were take placed of 5-6 days after culturing, but in a small number of explants microtuberization began after 14-20 days and therefore in this explants, the size of microtubers were small at the recorded time (Figure 8). Analysis of variance for the induction rate showed that culture media containing different concentrations of sucrose had more quickly induction rate than the culture media containing mannitol ($p<0.01$)(Table 1). In other words, sucrose in microtuber induction medium was caused earlier microtuberization from explant. The induction rate was increased by higher

concentrations than 80 g/l sucrose and in 160 g/l sucrose was maximum (Figure 9). Khuri and Moorby (1995) showed that sucrose concentrations increased was increasing osmotic pressure, led to be increasing microtuberization. In culture media containing mannitol, induction rate were less than culture media containing sucrose. Increasing mannitol concentrations were decreased significantly induction rate and the minimum rat of induction was observed at 40 g/l mannitol (Figure 9). These results confirms findings Hussein *et al*. (2006) and Khuri and Moorby (1995) that sucrose is the most critical stimulating for the formation of microtuber and sucrose is essential as a osmotic, source of energy and in higher concentrations as a signal for the formation of microtuber.

Microtubers length were significantly affected by carbon source significantly ($p<0.01$). So that the significant difference was observed between the culture medium containing different concentrations of sucrose. Higher microtuber length was observed with increasing sucrose concentrations up to 120 g/l and with further increases sucrose decreased microtuber length, but non significant difference were observed in sucrose concentrations up to 120 g/l. Analysis of variance revealed significant differences among different concentrations of mannitol for length of microtuber. Its increasing of microtuber length was observed on increasing the concentrations of mannitol up to 30 g/l and then its length decreased by 40 g/l (Figure 10). Microtuber diameter was significantly affected by sucrose concentrations. But, significantly different wasn’t in sucrose concentrations up to 120 g/l. Diameter was added increasing of mannitol to 30 g/l and then were observed decreasing in diameter (Figure 10).

Microtubers weight also was significantly affected by carbon source ($p<0.01$). In more than 100 g/l sucrose was added on microtuber weight. But among them did not exist significant differences. Adding mannitol due to small microtuber production, its weight was low, it is non significant and it canceled. But microtuber weight was equal in the concentration of 30 g/l mannitol approximately with 100 g/l sucrose, perhaps osmotic pressure and nutrient source is appropriate (Figure 11).

CONCLUSION

Microtuberization on *in vitro* condition are controlled by different factors including: growth regulators, carbon source, temperature and light. Using different concentrations of sucrose and mannitol showed that high levels of sucrose had positive effects on microtuber induction, formation, as well as the weight and size of microtuber. The use of different levels of mannitol had negative effect on microtuberization characterizations. Therefore, the using of 160 g/l sucrose was useful on *in vitro* microtuberization. Increasing of sucrose concentrations were improved efficiently *in vitro* microtuber production without negative side effects.

REFERENCES

Ahloowalia, B.S. (1994): Minitubers for seed potato production. Farm Food 4: 4-6.

Ali, C., Reda, D.M., Rachid, R., Houria, B. (2009): cadmium induced changes in metabolic function of mitochondrial isolated from potato tissue (*Solanum tuberosum* L.). Am. J. Biochem. Biotechnol., 5: 35-39.

Akita, M., Takayama, S. (1994): Induction and development of potato. Tubers in a jar fermentor. Plant Cell Tiss. Organ Cult., 36: 177-182.

Dodd’s, J.H., Silva-Rodriguez, D., Tovar, P. (1992): Micropropagation of potato (*Solanum tuberosum* L.). In: Biotechnology in Agriculture and Forestry Vol.19: high-tech and micropropagation ш, Y.S.P. Bajaj (Ed.). Springer Berlin Heidelberg, New York, pp: 91-106.

Ehsanpour, A.A., Razavizadeh, R. (2005): Effect of UV-C on drought tolerance of alfalfa (*Medicago sativa*) callus. Am. J. Biochem. Biotechnol., 1: 107-110.

Garner, N., Blake, J. (1989): The induction and development of potato microtubers *in vitro* on media free of growth regulating substances. Ann. Bot., 63: 663-674.

Hussein, I., Chaudhry, Z., Muhammad, A., Asghar, R., Naqvi, S.M.S. (2006) Effect of chlorocholine chloride, sucrose and BAP on *in vitro* tuberization in potato (*Solanum tuberosum* L. cv. Cardinal). Pakestan J. Bot., 38: 275-282.

Hussey, G., Stacey, N.J. (1984): Factors affecting the formation of *in vitro* tubers of potato (*Solanum tuberosum* L.). Ann. Bot. 53: 565-578.

Karami, O. (2008): Induction of embryogenic callus and plant regeneration in carnation (*Dianthus caryophyllus* L.). OnLine J. Biol. Sci., 8: 68-72.

Khuri, S., Moorby, J. (1995): Investigations into the role of sucrose in potato cv. Estima microtuber production *in vitro*. Ann. Bot., 75: 295-303.

Khuri, S., Moorby, J. (1996): Nodal segments or microtubers as explants for *in vitro* microtuber production of potato. Plant Cell Tiss. Organ. Cult., 45: 215-222.

Motallebi-Azar, A., Kazemiani, S., Kiumarsie, F., Mohaddes, N. (2011): Shoot proliferation from node explants of potato (*Solanum tuberosum* cv. Agria). II. effect of different concentrations of NH4NO3, hydrolyzed casein and BAP. Romanian Biotech. Let., 16: 6199-6204.

Ortiz-Montiel, G., Lozoya-Saldafia, H. (1987): Potato minitubers: Technology validation in Mexico. Amer. Potato J., 64: 535- 544.

Paiva Neto, V.B., Campos Otoni, W. (2003): Carbon sources and their osmotic potential in plant tissue culture: Does it matter? Scientia Hortic., 97: 193-202.

Pelacho, A.M., Mingo-Castel, A.M. (1991): Effects of photoperiod on kinetin-induced tuberization of isolated potato stolons cultured *in vitro*. Amer. Potato J., 86: 533-541.

Seabrook, J.E.A. (2005): Light effects on the growth and morphogenesis of potato (*Solanum tuberosum* L.) *in vitro*: A Review. Amer. J. Potato Res., 82: 353-367.

Shirani, S., Sariah, M., Zakaria, W., Maziah, M. (2010): Scalp induction rate responses to cytokinins on proliferating shoot-tips of banana cultivars (*Musa* spp.). Am. J. Agric. Biol. Sci., 5:128-134.

Tan, S.H., Musa, R., Ariff, A., Maziah, M. (2010): Effect of plant growth regulators on callus, cell suspension and cell line selection for flavonoid production from pegaga (*centella asiatica* l. urban). Am. J. Biochem. Biotechnol., 6: 284-299.

Thieme, R. (1992): An *in vitro* potato cultivar collection: Microtuberization and storage of microtubers. Plant Genetic Res. Newslet., 88/89: 17-19.

Yu, W.C., Joyce, P.J., Cameron, D.C., McCown, B.H. (2000): Sucrose utilization during potato microtuber growth in bioreactors. Plant Cell Rep., 19: 407-413.