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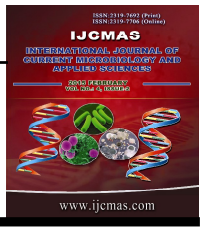

Original Research Article

The role of sucrose and light duration on *in vitro* tuberization for two cultivars of Potato *Solanum tuberosum L.*

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Keywords

Potato, Microtuber, Light duration, Sucrose

A B S T R A C T

Arnova and Reverra were evaluated using different concentration of sucrose (60 and 80 g/l) and different light durations period (5 and 10 days, 16 /8 hour light/ dark) before placed in darkness. Data of % tuberization, numbers, diameters and weights of microtubers were investigated. Results indicated that there was a significant interaction effect of cultivars, sucrose and light duration on all parameters. Average % tuberization varied from 60 to 93.33% irrespective of varieties, sucrose concentration and light duration. The highest tuber number, size and weight were at 80 gm sucrose under 10 days light duration in both cultivars.

Introduction

The traditional method of potato propagation is usually subjected to bacterial, fungal and virus infection and causes many diseases to transmit from one generation to another, a process called degeneration. Degeneration is a problem in potato tuber production, decreasing yield and quality (Shibli *et al.*, 2001). Over the last few years, attention has been paid to explore tissue culture to decrease degeneration through virus elimination (eliminate approximately 100% viruses) and germplasm conservation. Tissue culture technique is used to produce pre-basic, virus-free seed potatoes known as microtubers, which have tremendous advantages in terms storage and transportation because of their small size and weight (Kanwal *et al.*, 2006).

The possible ways of *in vitro* tuber induction based upon several factors, such as growth regulators, sucrose concentration, photoperiod and genotypes (Pruski *et al.*, 2002; Saha *et al.*, 2013; Seabrook *et al.*, 1993; Shibli *et al.*, 2001).

In general, increasing the sucrose concentration from 10 to 80 gm/l increased the percentage and earliness of microtuberization. According to Al-Taweel *et al.*, (2004) the highest percentage of tuberization (70%) with higher microtuber weight (58.8 mg) was at 6% sucrose. In the other study, Imani *et al.* (2010) reported that maximum number and size of potato microtubers were achieved when sucrose at 60 gm/l. While Iqbal *et al.* (2006) showed

that the maximum tuber induction was at 90gm/l sucrose. Differences among varieties number of microtubers have also been reported (Nistor *et al.,* 2010; Shojaei *et al.,* 2009). Many researchers suggested that tuberization is under the control of photo-period, Seabrook *et al.,* (1993) indicated that the microtuberization efficiency increased under long days (16/8 h day/night) compared with short days (8/16 h day/night), followed by short days or continuous darkness. Furthermore, Gopal (1996) observed faster rate of microtuberization under continuous darkness, while, Al-Taweel *et al.,* (2004) observed that exposure shoots to a short-day conditions (8/16h day/night) during the propagation period for one week increased the percentage of tuberization and microtubers weight with highest percentage of tuberization and higher mean weight compared to long day treatment of 16h light.

In Iraq, importation of certified potato tubers costs very high. Therefore, alternative methods to obtain potato tubers which can be practiced locally and maintain freedom of diseases have to find, one of these methods is i*n vitro* tubers. Microtubers enter the seed production chain to produce the certified potato seeds to be sold to the farmers. The main objective of the present study was to standardize the media microtubers induction for two local potato varieties under different light duration and sucrose concentration.

Material and Methods

This research was conducted at Genetic Engineering Department of Agricultural Research Directorate in Ministry of Science and Technology/Iraq. Potato tubers of Arnova and Reverra were brooked the dormancy and sprout. The sprouts were cleaned and sterilized by dipping in 2% sodium hypochlorite for 10 min (Al-taweel *et al.,* 2004). Shoot tips at 0.1- 0.3 mm with leaf primordial were existed and placed on MS medium (Murashige and Skoog, 1962) in such modification (Al-Salihi, 2002) with 8% agar. The cultures were incubated in growth room chamber at 25°C±2 under photoperiod of 16 h light and 8 h dark. Nodal cuttings (with length 1-2 cm in with one pair of shorten leaves, containing the axillary meristematic tissue) were collected from regenerated plantlets (after3 sub culture) and used as explants for *in vitro* tuberization of potato. The potato plantlet regenerated from meristems, were fragmented for obtaining nodal cuttings which planted in 20ml of liquid MS salt supplemented with 0.4, 100, 2, 2, 1 and 4 mg/l of Thiamine –HCL, Inositol, Glycin, Nicotinic Acid, Indole Acetic Acid, Kintein, respectively, with two different levels of sucrose (60 and 80 gm /l) and incubated under two light duration (5 days and 10 days, photoperiod 16 h light and 8 h dark) before placed in darkness. All the plantlets were placed in a growth room chamber at 18±2 °C with darkness until microtubers harvest.

After the tuberization period was completed (90 days) data were recorded for %tuberization, number of microtubers/plantlet, microtuber weight (gm), microtuber diameter (cm).

Experimental Design

Data for the experiments was analyzed as for the factorial completely randomized design (Steel and Torrie, 1982) in five replications, which the factor A include genotypes, factor B include two levels of sucrose concentrations and factor C include tow light duration. Each replication consisted of three nodal sections per culture vessel (15x5 cm). Analyses of variances were conducted LSD tests were used for mean separation ($P< 0.05$).

Result and Discussion

In the microtuberization experiment, when single nodes were cultured in media, axillary buds developed into stolon followed by stolon growth and branching then cessations of stolon longitudinal growth and tuber induction by swelling at the end of the stolon (sessile). The microtubers usually are approximately of the size of a pea seedpod and vary in color, number, size (diameter) and weight. The results in table 1 showed that there were no significant differences between varieties in term of % tuberization, tuber number and size, while Arnova significantly surpassed Reverra in weight of microtuber which reached 0.26 gm. Although there were no significant effect on %tuberization with increasing of sucrose levels, but sucrose at 80 gm/l excel in microtuber number, diameter and weight which reached 1.20 microtuber/ plant, 0.61 cm and 0.27 gm respectively. 10 days light duration significantly increased % tuberization (86.67%), microtuber diameter (0.66 cm) and their weight (0.29gm) compared with 5 days light duration.

Interaction between cultivars and sucrose (Table 2) revealed that in Reverra variety, adding 80 gm/l sucrose to the medium significantly increased % tuberization (80%), microtuber number (1.30), diameter (0.69 cm) and weight (0.24 gm) compared with 60 gm sucrose, while in Arnova cultivar increased sucrose from 60 to 80 gm/l did not effect on microtuber number and weight. Interaction between cultivars and light duration (Table 3) showed that in both cultivars incubated the tissue under 10 days light duration before darkness significantly increased all traits except tuber weight. Also it can easily be observed from the interaction between sucrose and light (Table 4) that 10 days of light duration significantly increased % tuberization, tuber number, diameter and weight compared with 5 days of light duration in both levels of sucrose.

The results given in table 5 illustrate the interaction between varieties, light duration and sucrose. Arnova had the highest % tuberization when using 60 gm/l sucrose in both light duration (5 and 10 days), while the highest % tuberization in Reverra variety was in using 80 gm/l under 10 days light duration. In Arnova variety, although there were no significant differences in % tuberization, microtuber number, diameter and weight between light duration treatments at 60 gm/l sucrose, but in 80 gm/l sucrose treatment increase light duration significantly increased these characters, % tuberization reached 60 and86.67%, microtuber number reached 0.8 and 1.4, the diameter of microtubers reached 0.33 and 0.77 cm and the weight of microtuber reached 0.18 and 0.42 gm under 5 and 10 days respectively, that mean light duration significantly affected on tuber initiation only in high concentration of carbon source.

In Reverra variety it look like more sensitive to sucrose concentration compared with light duration, there were no significant increase in tuber number (0.6 and 0.67) and weight (0.06 and 0.12 gm) under 5 and 10 days respectively at 60 gm/l sucrose and also at 80 gm/l sucrose the same effect was found, but from other hand there were significant differences in microtuber number, size and weight between 60 and 80 sucrose treatments under the same light duration. In general, the highest microtuber number, size and weight were at 80 gm/l sucrose under 10 days light duration in both cultivars.

Differential response between varieties dependence of microtuber production agreed well with the results by other workers who

reported that genotypes under the same cultural conditions showed a wide range of variations in their growth pattern (Gopal *et al.,* 1998; Srivastava *et al.,* 2012; Singh *et al.,* 2001), these processes are regulated by specific gene expression patterns (Bachem *et al.,* 2000; Gargantini *et al.,* 2009).

Modification of environmental factors, such as photoperiod are very important (Al-Taweel *et al.,* 2004; Gopal,1996; Rahman *et al.,* 2013; Seabrook *et al.,* 1993), darkness after light duration were reported to increase microtubers (Alisdair and Willmitzer, 2001) by enhanced tuberonic acid synthesis (chemically very similar to jasmonc acid) which plays important role in tuber formation during *in vitro* condition.

Raising sucrose concentrations are efficient to improve *in vitro* microtuber production as described by a number of investigators. The high sucrose concentrations are essential for microtuber induction, influencing this process through the osmotic effect (Motallebi- Azar *et al.,* 2013), and by serving as energy source. Sucrose converted to starch in microtubers developing, increased cell division and expansion of the stolon end are followed rapidly by a massive deposition of starch and storage protein as a result of coordinated expression of genes involved in starch and protein biosynthesis (Prat *et al.,* 1990; Visser *et al.,* 1994), accumulation of starch leads to increase in size and weight of microtubers.

Table.1 Effect of mean sucrose, light duration and cultivars on microtuberization

Treatments		%Tuberization	microtuberno.	microtuberdiameters (cm)	microtuberweight (gm)
Cultivars	Arnova	83.33	1.12	0.55	0.26
	Revera	73.33	0.97	0.52	0.16
L.S.D 0.05		n.s	n.s	n.s	0.08
Sucrose (gm/l)	60	80.00	0.88	0.47	0.16
	80	76.67	1.20	0.61	0.27
L.S.D 0.05		n.s	0.28	0.13	0.08
Light duration (days)	5	70.00	0.93	0.41	0.14
	10	86.67	1.15	0.66	0.29
LSD 0.05		12.17	n.s	0.13	0.08

Table.2 Effect of sucrose and varieties on microtuberization

varieties	sucrose (gm/l)	%tuberization	microtuberno.	microtuber diameters (cm)	microtuberweight (gm)
Arnova	60	93.33	1.13	0.95	0.22
	80	73.34	1.10	0.52	0.30
Revera	60	66.67	0.63	0.35	0.09
	80	80.00	1.30	0.69	0.24
LSD 0.05		17.22	0.40	0.19	0.12

Table.3 Effect of light duration and varieties on microtuberization

varieties	Light duration (days)	%Tuberization	microtuberno.	microtuber diameters (cm)	microtuberweight (gm)
Arnova	5	76.67	0.97	0.46	0.16
	10	90.00	1.27	0.64	0.36
Revera	5	63.34	0.90	0.36	0.11
	10	83.34	1.04	0.69	0.21
LSD 0.05		17.22	n.s	0.19	0.12

Table.4 Effect of light duration and sucrose on microtuberization.

sucrose (gm/l)	light duration (days)	%tuberization	microtuberno.	microtuber diameters (cm)	microtuberweight (gm)
60	5	76.67	0.87	0.37	0.10
	10	83.34	0.90	0.57	0.21
80	5	63.34	1.00	0.46	0.17
	10	90.00	1.40	0.75	0.36
LSD 0.05		17.22	0.40	0.19	0.12

Table.5 Effect of sucrose, light duration and cultivars onmicrotuberization

varieties	sucrose (gm/l)	light duration (days)	%tuberization	microtuber no.	microtuber diameters (cm)	microtuber weight (gm)
Arnova	60	5	93.33	1.13	0.60	0.14
		10	93.33	1.13	0.57	0.31
	80	5	60.00	0.80	0.33	0.18
		10	86.67	1.40	0.77	0.42
Reverra	60	5	60.00	0.60	0.13	0.06
		10	73.34	0.67	0.57	0.12
	80	5	66.67	1.20	0.59	0.17
		10	93.33	1.40	0.80	0.30
			24.35	0.56	0.27	0.17

Economical use of microtubers is possible if the *in vitro* tuberization rate is satisfactorily high and if the size of microtubers is sufficiently large (Dobranszki *et al.*, 1999). Garner and Blake (1989) indicated that use of 80 compared to 40 or 120 gm sucrose/l advanced the initiation of tuberization and gave more and larger. Nistor *et al.*, (2010)

graded microtubers into 3 size- groups, <0.5, 0.5-1 and > 1 cm. Microtubers larger than 0.2 cm can be further propagated, but only microtubers larger than 0.4 cm are suitable for long-term storage. It is necessary to increase the tuber size because increasing size resulted in a significant increase in the viability and sprouting ability of microtubers with reduced durations of dormancy and weight loss at the end of storage (Park *et al.*, 2009; Sharma *et al.*, 2012).

Microtuberization in potato is influenced by many factors including source, light and genotypes. Among these, sources have been suggested to play a prominent role. For germplasm conservation, microtubers should be induced in medium supplemented with high concentration of sucrose 80 gm/l under 10 days light duration before darkness.

References

Alisdair, R., Willmitzer, L. 2001. Molecular and biochemical triggers of potato tuber development. *Plant Physiol.*, 127: 1459–1465.

Al-Salihi. A. 2002. Radiosensitivity of potatoes (*Solanumtuberosum* L.) propagated *in vitro* to gamma radiation. Ph.D., Thesis. College of Agriculture, University of Baghdad.

Al-Taweel, K., Al-Maarri, K., Kheeti, M., Abdul-Kader, A. 2004.Effects of some factors influencing on in vitro tuberization of potato cv. «Draga». Damascus University, *J. Agric. Sci.*, 2(20): 265–280.

Bachem, C., van der Hoeven, R., Lucker, J., Oomen, R., Casarini, E., Jacobsen, E., Visser, R. 2000. Functional genomic analysis of potato tuber life-cycle. *Potato Res.*, 43: 297–312.

Dobranszki, J., Tabori, K., Ferenczy, A.1999. Light and genotype effects in vitro tuberization of potato plantlets. *Potato Res.*, 42: 483–488.

Gargantini, P., Giammaria, V., Grandellis, C., Feingold, S., Maldonado, S., Ulloa, R. 2009. Genomic and functional characterization of StCDPK1. *Plant Mol. Biol.*, 70:153–172.

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.

Gopal J. 1996. *In vitro* selection, genetic divergence and cross prediction in potato. Ph.D., Thesis, Punjab Agricultural University, Ludhiana (Pb), India.

Gopal, J., Minocha, J., Ghosal, S. 1998. Variability in response of potato genotypes to *in vitro* propagation. *Potato J.*, 25: 119–124

Imani, A., Gharemanzadeh, R., Azimi, J., and Janpoor, J. 2010. The effect of various concentration of 6-benzylaminopuine (BAP) and sucrose on *in vitro* potato(*Solanumtuberosum* L.,) microtuber induction. *Am. Eur. J. Agric. Environ. Sci.*, 8: 457 459.

Iqbal, H., Zubeda, CH., Aish, M., Rehana, A., Saqlan, S., Hamid, R. 2006. Effect ofChlorocholine chloride, Sucrose and BAP on *in vitro* tuberization Potato (*Solanum tuberosum* L. cv. Cardinal. *Pak. J. Bot.*, 38: 275–282.

Kanwal, A., ALI, A., and Shoaib, K. 2006.*In vitro* microtuberization of potato (*Solanumtuberosum* L.) cultivar Kuroda- a new variety in Pakistan. *Int. J. Agri. Biol.*, 8: 337–340.

Motallebi–Azar, A., Kazemiani, S., Yarmohamadi, F. 2013.Effect of sugar/osmotica levels on *in vitro* microtuberization of potato (*Solanum tuberosum* L.).*Russ. Agricult. Sci.*, 39: 112–116.

Murashige, T., Skoog, F. 1962. A revised for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant,* 15: 473 – 497.

Nistor, A., Campeanu, G., Atanasiu, N., Chiru, N., Karacsonyi, D. 2010. Influence of potato genotypes on "*in vitro*" production of microtubers. *Rom. Biotechnol. Lett.,* 15: 5317–5324.

Park, S., Jeon, J., Kim, H., Hong, S., Aswath, C., Joung, H. 2009. The effect of size and quality of potato microtubers on quality of seed potatoes in the cultivar 'Superior'. *Scientia Horticulturae,* 120: 127–129.

Prat, S., Frommer, W., Hofgenm, R., Keil, M., Kossmann, J., Koster-Topfer, M., Liu, X., Muller, B., Pena-Cortes, H., Rocha-Sosa, M., Sanchez- Serrano, J., Sonnewald, U., andWillmitzer, L. 1990. Gene expression during tuber development in potato plants. *FEBS Lett.,* 268: 334 –338.

Pruski, K., Astatkie, T., Nowak, J. 2002. Effects of media, jasmonic acid and photoperiod on microtuber production in two potato cultivars (*Solanumtuberosum* L.). *In vitro Cell. Dev. Biol. Plant,* 38: 203–209.

Rahman, M., Haider, S., Hossain, M., and Islam, R. 2013. Involvement of jasmonic acid and light periods on potato microtuber growth response. *Int. J. Biosci.,* 3: 87–94.

Saha, S., Ahmed, M., Islam, M.., Remme, R., Ali, M. 2013. Effect of different levels of sucrose on microtuberization and different substrates on minituber production resulted from potato meristem culture. *J. Agr. Vet. Sci.,* 6: 58–62.

Seabrook, J., Coleman, S., Levy, D. 1993. Effect of photoperiod on *in vitro* tuberization of potato (*Solanumtuberosum* L.). *Plant Cell Tissue Org. Cult.,* 34: 43–51.

Sharma, K., Venkatasalam, E., Kumar, V. 2012. Storability and sprouting behavior of micro–tubers of some Indian potato cultivars. *Potato J.,* 39: 31–38.

Shibli, R., Abu-Ein, A., and Ajlouni, M. 2001.*In vitro* multiplication of virus-free 'Spunta' potato. *Pak. J. Bot.,* 33: 35–41.

Shojaei, T., Sepahvand, N., Omidi, M., Abdi, H., Naraghi, S. 2009. The effect of plant growth regulators, cultivars and substrate combination on production of virus free potato minitubers. *Afr. J. Biotech.,* 8: 4864–4871.

Singh, D., Chaubey, I., Singh, S. 2001. Rapid seed multiplication in potatoes through micro-tubers. *J. Indian Potato Assoc.,* 28: 103–104.

Srivastava, A., Diengdoh, L., Bag, T., Singh, B. 2012. *In Vitro* micropropagation and micro-tuberization potential of selected potato varieties. *Indian J. Hill Farm.,* 25: 14–17

Steel, R., Torrie, J. 1982. Principles and Procedures of Statistics, 2nd Edition. McGraw-Hill International Book Company. Auckland, London.

Visser, R., Vreugdenhil, D., Hendriks, T., Jacobsen, E. 1994. Gene expression and carbohydrate content during stolon to tuber transition in potatoes (*Solanumtuberosum*). *Physiol. Plant.,* 90: 285–292.