Advances in Crop Science and Technology

Fufa and Diro, Adv Crop Sci Tech 2013, 1:4
http://dx.doi.org/10.4172/2329-8863.1000114

Research Open Access

The Effects of Sucrose on *in vitro* Tuberization of Potato Cultivars

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Abstract

Two potato varieties namely, 'Hunde' and 'Ararsa' were tested for *in vitro* Tuberization response under five levels of sucrose (40, 60, 80, 100 and 120gram litre^{-1}) in completely randomized design with 2×5 factorial combinations. The objective was to determine optimum concentration of sucrose for *in vitro* Tuberization. In both varieties, among the five concentrations of sucrose, Murashige and Skoog (MS) medium supplemented with 60 gram litter^{-1} sucrose exhibited a better response than the other concentrations in mean values of microtuber number, diameter, and weight and was found optimum. Accordingly, this medium gave an average value of (1.97 ± 0.02) microtuber number, (3.60 ± 0.04 mm) microtuber diameter, and (0.08 ± 0.002 gram) weight of microtuberin variety 'Ararsa' after 42.57 ± 0.58 days of culture. On the other hand, it gave mean value of (2.90 ± 0.031) microtuber number, (2.95 ± 0.01 mm) microtuber diameter, and (0.06 ± 0.001 gram) weight of microtuber in variety 'Hunde' after 35.67 ± 0.58 days of culture.

Keywords: *In vitro* tuberization; Microtuber; Potato; Sucrose

Introduction

Potato (*Solanum tuberosum* L.) is an important food and cash crop [1]. It ranks first in the world from none-grain crop to ensure food security [2]. It is a high biological value crop that gives an exceptionally high yield, more protein and calories, vitamins, minerals, carbohydrates and iron per unit area per unit time than any other major crops [3].

The conventional propagation of potato is characterized by low multiplication rate and susceptibility to pathogens. Susceptibility to pathogens often leads to poor quality and yields due to degeneration [3]. In Ethiopia, the production of potato is expanding steadily. However, its productivity has shown a decreasing trend [4,5] due to the unavailability of good quality clean seed tubers [6]. This can be avoided through *in vitro* tuberization, a better alternative to conventional propagation that can produce uniform and identical materials in large scale within a short time [7].

The most critical stimulus influencing *in vitro* Tuberization is sucrose at high concentration [8]. Sucrose is a cheap, safe and superior agent for *in vitro* Tuberization [9]. Hence, the present study was initiated with the objective to determine optimum concentration of sucrose for *in vitro* Tuberization.

Materials and Methods

Single nodal excision from one week old sprouts of the relatively clean tubers of 'Ararsa' and 'Hunde' potato varieties was used for *in vitro* Tuberization experiment at Tissue Culture Laboratory of Jimma University College of Agriculture and Veterinary Medicine. The two varieties were tested for Tuberization response under five levels of sucrose (40, 60, 80, 100 and 120 gram liter^{-1}) in completely randomized design with 2×5 factorial combinations.

The pH of the medium was adjusted at 5.8, agar (8 gram liter^{-1}) was added and then the medium was autoclaved at 121°C for 20 minutes at 15 psi. MS basal medium containing gibberellic acid (0.1 milligram liter^{-1}), naphthalene acetic acid (0.01 mg/l) and sucrose (30 g/l) was used for initiation. In the case of *in vitro* Tuberization, the Murashige and Skoog (MS) (1962) basal medium was prepared for each treatment combination.

All the surface sterilization procedures were carried out under aseptic condition of laminar flow chamber, following the procedure of Naik and Karihaloo [10]. One week old sprouts along buds were excised and used as initial explants. The excised explants were washed 3 times in running tap water with 0.25 millliliter of Tween-20, and then washed thoroughly three times with sterile distilled water and immersed in 70% ethyl alcohol for 10 seconds. The alcohol was removed by three times washing with sterile distilled water. Finally, the sprouts were sterilized with 10% sodium hypochlorite (NaOCl) for 20 minutes before dissection.

After removing the leaves, the excised explants were dissected into single nodes (2 cm long) on a sterile plate. Six explants were cultured into 40 milliliter of an initiation culture medium in culture jar and incubated under a 16 hour photoperiod at 24°C with a light intensity of 2500 lux. The sprouts were allowed to grow into plantlets having nodal segments for 3 to 4 weeks. The multiplication medium was decanted and the plantlets were kept in a conditioning medium before being used for *in vitro* Tuberization to avoid the carryover effects of hormones. Forty milliliters of *in vitro* Tuberization medium was dispensed into each culture jar before transferring the culture to the growth room. Finally, the culture was kept at a temperature of 18°C under dark condition.

Data collection and analysis

The first, second and third date of formation of microtuber was carefully followed and recorded. 50% days to set microtuber was recorded and used for analysis. The number of microtubers produced by each explant was counted before harvest. The diameter (in millimeter) of each microtuber was measured by Digital Caliper. Immediately after harvest, each microtuber was weighed on sensitive balance to get the mean microtuber weight in gram. After 15 days of light exposure, the microtubers were treated with gibberellic acid (GA3) and incubated in the dark before planting in the green house. The number of the microtubers germinated and established was counted to get their percent survival under in vivo. The data were subjected to the analysis of variance (ANOVA) at 5% level of significance using SAS

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Received September 19, 2013; **Accepted** September 23, 2013; **Published** September 26, 2013

Citation: Fufa M, Diro M (2013) The Effects of Sucrose on in vitro Tuberization of Potato Cultivars. Adv Crop Sci Tech 1: 114. doi:10.4172/2329-8863.1000e114

statistical software Version 9.2 [11]. The REGWQ multiple comparison procedure was used for separating significant means.

Results and Discussion

Analysis of variance (ANOVA) revealed that sucrose and variety interaction had very highly significant effect (α=5%) on days to Tuberization and on the average number, diameter (millimeter) and weight (gram) of microtubers (Table 1). This implied that there is interdependence of sucrose and genotype on *in vitro* Tuberization of potato. Thus, the response of genotypes to a given level of sucrose was not the same.

Effects of sucrose on days to Tuberization, mean microtuber number and diameter

At 40 gram liter^{-1} sucrose, both varieties did not produce microtubers. However, when 60 gram liter^{-1} sucrose was added to growth media, 'Hunde' variety produced microtubers in 36 days, which is significantly earlier than that of 'Ararsa' variety (43 days). Increasing concentration of sucrose from 60 to 80 gram liter^{-1} delayed microtuber formation in both varieties, but more pronounced on 'Ararsa' variety. This might be due to the marked variation in the responses of plant gene to changing sucrose status. Some genes are induced, some are repressed, and others are minimally affected [12].

Moreover, in both varieties, microtuber number and size get reduced as the concentration of sucrose increased from 60 gram liter^{-1} to 80 gram liter^{-1}. At 120 gram liter^{-1} of sucrose, both genotypes did not produce microtubers (Table 1). The absence of microtuber formation at high sucrose concentration might be due to the effect of super optimal level of sucrose that can result in an unfavorable osmotic condition for water uptake, and thus affecting microtuber formation of the seedlings.

Effects of sucrose on mean microtuber weight

In both varieties, a decreasing trend in mean weight (gram) of microtuber was observed as the level of sucrose increased (Figure 1). This might be again due to the effect of high sucrose level on osmotic condition of the culture that affect cell turgidity [12], and hence, microtuber weight.

In both varieties, Murashige and Skoog (MS) medium supplemented with 60 gram liter^{-1} sucrose exhibited a better response than the other concentrations in mean values of microtuber number, diameter and weight, and was found optimum. Accordingly, this medium gave an average value of (1.97 ± 0.02) microtuber number, (3.60 ± 0.04 mm) microtuber diameter, and (0.08 ± 0.002 g) weight of microtuber in the variety 'Ararsa' variety after 42.57 ± 0.58 days of culture. On the other hand, it gave mean value of (2.90 ± 0.031) microtuber number, (2.95 ± 0.01 mm) microtuber diameter, and (0.06 ± 0.001 g) weight of microtuber in variety 'Hunde', after 35.67 ± 0.58 days of culture (Table 1).

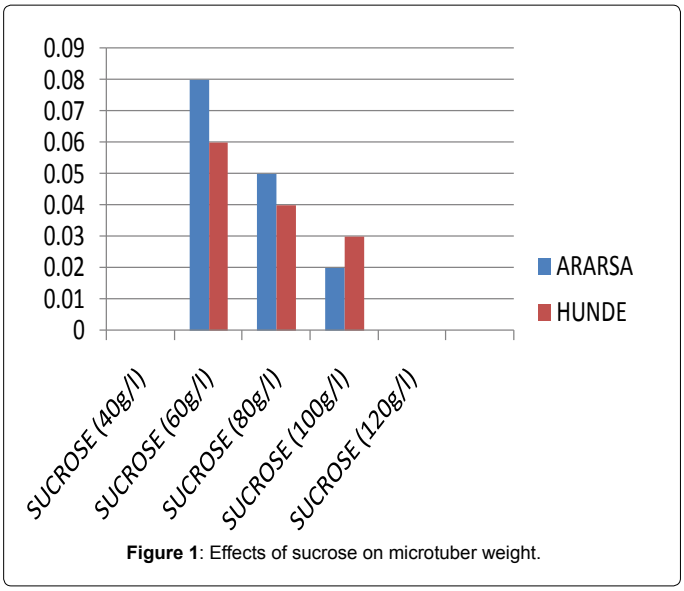

Figure 1: Effects of sucrose on microtuber weight.

The present result is in agreement with that of Aslam et al. [13], who found that a medium containing 6% sucrose was optimal in terms of minimum time of induction (34), mean tuber number (1.2) and weight (0.03 gram) of microtubers per single nodal explant in cultivar Desiree. Imani et al. [14] also reported that Murashige and Skoog (MS) medium supplemented with 60 gram liter^{-1} of sucrose as the best in producing the maximum number (4.20) and size (0.44 centimeter) of micro tubers. Iqbal et al. [9] also recorded similar results on mean numbers of tubers (4.8) on Murashige and Skoog (MS) medium treated with 60 gram liter^{-1} sucrose. Kanwal et al. [15], on the other hand, reported that Murashige and Skoog (MS) medium supplemented with 30 and 40gram liter^{-1} sucrose did not produce microtubers.

Conclusion

A protocol for *in vitro* Tuberization of potato varieties 'Ararsa' and 'Hunde' from single nodal explant has been developed. The result indicated that *in vitro* Tuberization of potato was highly dependent on sucrose and genotype interaction.

Murashige and Skoog (MS) medium supplemented with 60 gram liter^{-1} exhibited fewer days to microtuber formation (35.67 ± 0.58/42.67 ± 0.58), better mean number (2.90 ± 0.031/1.97 ± 0.02), diameter (2.81 ± 0.015/3.60 ± 0.04 millimeter) and fresh weight (0.06 ± 0.001/0.08 ± 0.002 gram) of microtubers in 'Hunde' and 'Ararsa' varieties, respectively. However, microtuber production needs further improvement since the size of microtubers produced was not large

	ARARSA				HUNDE			
Sucrose (g/l)	DT	MTN	MTD	MTWT	DT	MTN	MTD	MTWT
40	0.00 ± 0.00c	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}
60	42.67 ± 0.58^{b}	1.97 ± 0.02^{a}	3.60 ± 0.04^{a}	0.08 ± 0.002^{a}	35.67 ± 0.58^{c}	2.90 ± 0.031^{a}	2.95 ± 0.050^{a}	0.06 ± 0.001^{b}
80	45.00 ± 1.00^{a}	1.30 ± 0.08^{b}	3.07 ± 0.03^{b}	0.05 ± 0.001^{b}	40.00 ± 0.00^{b}	2.06 ± 0.081^{b}	2.81 ± 0.015^{b}	0.04 ± 0.001^{b}
100	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.02 ± 0.00^{d}	44.67 ± 0.58^{a}	1.30 ± 0.042^{c}	2.56 ± 0.044^{c}	0.03 ± 0.002^{c}
120	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}

Table 1: Effects of sucrose on days to tuberization, number, diameter and weight (g) of microtuber.
Means with the same letters in a column are not significantly different from each other using the Ryan-Einot-Gabriel-Welsch Multiple Range Test (REGWQ) at α= 0.05.
DT=Days to Tuberization, MTN=Microtuber Number, MTD=Microtuber diameter (mm), and MTWT=Microtuber Weight (g).

enough. Thus, trying different levels of Benzylaminopurine (BAP) in combination with sucrose and extending the time of harvesting may be helpful to improve the size of the microtuber.

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Citation: Fufa M, Diro M (2013) The Effects of Sucrose on in vitro Tuberization of Potato Cultivars. Adv Crop Sci Tech 1: 114. doi:10.4172/2329-8863.1000e114