GETTING IN VITRO MICROTUBERS FROM LINES OF GENE-KNOCKOUT POTATOES (SOLANUM TUBEROSUM L.)

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Abstract

In order to create a system of seed production of potatoes in the short of period, it is necessary to improve the formation of *in vitro* microtubers. The formation of microtubers when 5 mg / l kinetin was added to the MS feed showed the best performance. Normal MS feed did not produce microtubers *in vitro*. The formation of microtubers in the dark state was better than in the light state. The combination of MS + 8% sucrose + 5 mg / l kinetin was the best indicator for the formation of *in vitro* microtubers studied.

Keywords: Solanum tuberosum, tuber formation, kinetine (KIN), sucrose, potato, microtuber, in vitro, gene-knockout.

Introduction

Potato (Solanum tuberosum L.), one of the most significant vegetable crops in Uzbekistan, is also a staple food for many countries around the world. The potato finishing process is a very complicated process and it can be changed in a variety of ways. Obtaining microtubers from potatoes *in vitro* includes several steps. Many researchers have used various growth regulators to produce potato microtubers *in vitro* (Hossain and Sultana, 1998). *In vitro* microtubers can be produced throughout the year. The potato plant is a widely recognized plant species in tissue culture. This technology is utilized in many countries to grow disease-free seeds (Wang and Hu, 1982). The technology of potato seed production has a number of advantages over the use of *in vitro* method for seedling replanting or production of microtubers. Microtubers are very convenient for processing, transportation and storage. Consequently, it is necessary to improve the production of *in vitro* microtubers. Extensive physiological studies have shown that endogenous formation is controlled by several factors such as hormonal combination, photoperiod ratio, nutrient content, etc. (Vreugdenhil & Struik, 1989; Coleman et al., 2001; Tuğrul & Samanci, 2001). Although research has been conducted on the production of microtubers in potatoes, little attention has been paid to the *in vitro* termination process with various explants to establish kinetin (KIN), sucrose concentration, and appropriate regeneration protocol. Thus, our experiment is designed to determine the best concentration of kinetin and sucrose for the production of microtubers.

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Materials and methods

The experiment was conducted in the Laboratory of Trans genomics and Tissue Culture of the Center for Genomics and Bioinformatics of the Academy of Sciences of Uzbekistan. For the experiment, clones of new potato lines grown *in vitro* using gene-knockout technology were selected in this laboratory.

These explants were cultured in a solid nutrient medium MS (pH 5.8). The MS culture medium was prepared at two different sucrose concentrations, including 4% and 8%. Kinetin (KIN) has been used as a hormone to induce microtubers production. Kinetin (KIN) at different concentrations (2, 3, 4, 5 mg / l) was added to the MS nutrient medium study. More than 100 explants were prepared for each concentration of hormones and sucrose. 50% of the cultures were incubated at 2500–3000 lux for a 16-hour photoperiod period, and the remainder were incubated at 17 ± 30 ° C in the dark. Microtubers were collected after 45–55 days of incubation. In the data formation, the duration of endogenous formation for explants, the processes involved in explants, the days of microtubers formation, the number of microtubers, and the average weight of microtubers were recorded.

Results and discussions

The *in vitro* tuberization process in potatoes was carried out on 5 different lines obtained on the basis of gene-knockout technology. Different explants, sucrose, and kinetin (KIN) concentrations were studied in detail. Experimental data were presented with different tables.

Formation of microtubers in vitro

MS nutrient media enriched with different concentrations of kinetin were utilized to obtain microtubers. The data are presented in Table 1. At the same time, the number of days required for the development of stolons in the MS nutrient medium was 13 days. MS + 5 mg / l kinetin, followed by MS + 4 mg / l kinetin and MS + 3 mg / l kinetin, MS + 2 mg / l kinetin in combination. In the combination of MS + 5 mg / l kinetin, stolon formation occurred faster on day 5 than the others. The experiment was then followed by combinations of 4 and 3 mg / l kinetin. MS + 3 mg / l kinetin along the length relative to plant height, followed by combinations with the addition of MS + 4 mg / l and MS + 5 mg / l kinetin (Fig. 1). In an interesting way, at high concentrations of kinetin, the formation of nodules was high, but the length of the stolons was low. On the other hand, in MS+3 mg / l kinetin nutrient media, stolon lengths were higher than others, but end genesis was lower. This demonstrates that high concentrations of kinetin were able to form more tubers.

Table 1. The effect of kinetin on the formation of potato microtubers

№	Media	Number of explant	Days of stolon formation	Length of tables (sm)	Days of microtuber formation	Number of microtuber explant	Microtuber weight (mg)

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1	MS	30	15,0	5,20	0	0	0
2	MS +2 mg/l KIN	30	10,0	3,04	46,30	3,18	185
3	MS +3 mg/l KIN	30	8,50	10,80	37,04	3,99	230
4	MS +4 mg/l KIN	30	8,00	9,63	32,72	5,01	320
5	MS +5 mg/l KIN	30	7,50	8,88	26,04	5,01	390

According to Table 1, no stocks were obtained in a normal MS nutrient medium. The longest day for the formation of microtubers was observed in the combination of MS + 2 mg / l kinetin (46.30 days) and the shortest day in the combination of MS + 5 mg / l kinetin (26.04 days). This suggests that the use of kinetin should be done gradually when working with high concentrations. The number of microtubers in the explants was statistically the same at the 4 and 5 mg / l kinetin combinations. The remaining combinations also had a similar trend to these parameters. The mean weight of the microtubers was 390 mg at the highest weight in the MS + 5 mg / l kinetin combination and 185 mg at the lowest weight in the MS + 2 mg / l kinetin combination. Scientists have found that higher concentrations of kinetin have the ability to produce heavier microtubers than lower concentrations. These conclusions are consistent with data identified by other scholars (Kotkas; Peter, 1998; Al-Momani and etc.., 2000; Shibli et al., 2001).

The effect of the photoperiod on the formation of microtubers

The completion process in the MS nutrient medium with the addition of 5 mg / l kinetin showed the best results. Thus, this combination was utilized to study the effect of the photoperiod on the formation of microtubers. The results are presented in Table 2. The effect of the photoperiod on the formation of gene-knockout potato lines *in vitro* was studied. He formed a short period (22.30 days) for the formation of microtubers, in particular, in transplants grown in the dark and long period (33.7 days) in light conditions. The number of tufts in the explants was high in light conditions (1.87) and the average weight was 118 mg, while in dark conditions the number of tufts was 3.75 and the average weight was 320 mg. Most of the microtubers cultured under light conditions were green. This alkaloid may be related to the synthesis of solanine. It should also be noted that the green microtubers began to grow, forming buds. Similar phenomena have been studied in detail by Wany I Hu (1982). In the dark incubation, the cultured microtubers turned brown but no buds were formed. The results of the experiment showed that the dark conditions were favorable for the finishing process. Mares et al. (1981) observed that termination at 16 or 24 photoperiods was better than at 8-hour photoperiod. Wattimena (1983) identified in his research that the longer the photoperiod, the better the finishing process.

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Effect of sucrose on in vitro tuberization

The outcome of sucrose on microtubers formation is shown in Table 3. Previous data showed that MS enriched with 5 mg / l kinetin showed the best results in the formation of nodules in the nutrient medium. Therefore, the same concentration was considered in this study as well. Sucrose at two different concentrations was studied in both hormone-free and hormonal combinations. Hormone-free 5% sucrose has been shown to be incapable of forming microtubers in vitro, whereas 8% sucrose has had very little influence on formation. Showed the lowest rate for all studies studied. Hormonal MS + 5 mg / l kinetin + 8% sucrose concentration showed the best results in the nutrient medium. Microtubers formed on average 27.13 days. The number of microtubers in the explants was also as high as 4.85 and the weight was as high as 398 mg. Gopal and etc. (1998) Pelacho and etc. (1994), Haque (1996), Amma and Maity (1998) obtained the maximum amount of various microtubers when enriched with 4-5 mg / l kinetin. These studies are evidence of previous work. Al-Sawy and etc. (2007) found that sucrose is a vital factor for microtubers formation.

Gene-knockout potato lines were researched in MS nutrient media at different concentrations of kinetin and sucrose during *in vitro* endogenous formation processes. It can be concluded that the MS nutrient medium was high when 8% sucrose and 5 mg / l kinetin were added. In the dark incubation mode, microtubers formation took place in a short day, and the weight of microtubers reached a maximum (398 mg).

Table 2. The effect of the photoperiod on the formation of microtubers

№		Media	Days of formation of microtubers	The number of microtubers	Weights of microtubers	Color of microtubers
1	Light condition	MS+ 8% sucrose + 5 mg/l KIN	33,07	1,87	118	Green
2	Dark condition	MS+ 8% sucrose+ 5 mg/l KIN	22,30	3,75	320	Brown

Table 3 Effect of sucrose on *in vitro* tuberization

№	Media	Sucrose concentration	Total number of explants	Days to tuberization	The number of microtuber explant	Weight of microtubers (mg)
1	MS	5%	30	53,67	0,00	0,00
2	MS	8%	30	43,29	2,29	120
3	MS+5 mg/l	5%	30	36,73	3,72	203

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	KIN					
4	MS+5 mg/l KIN	8%	30	27,13	4,85	398

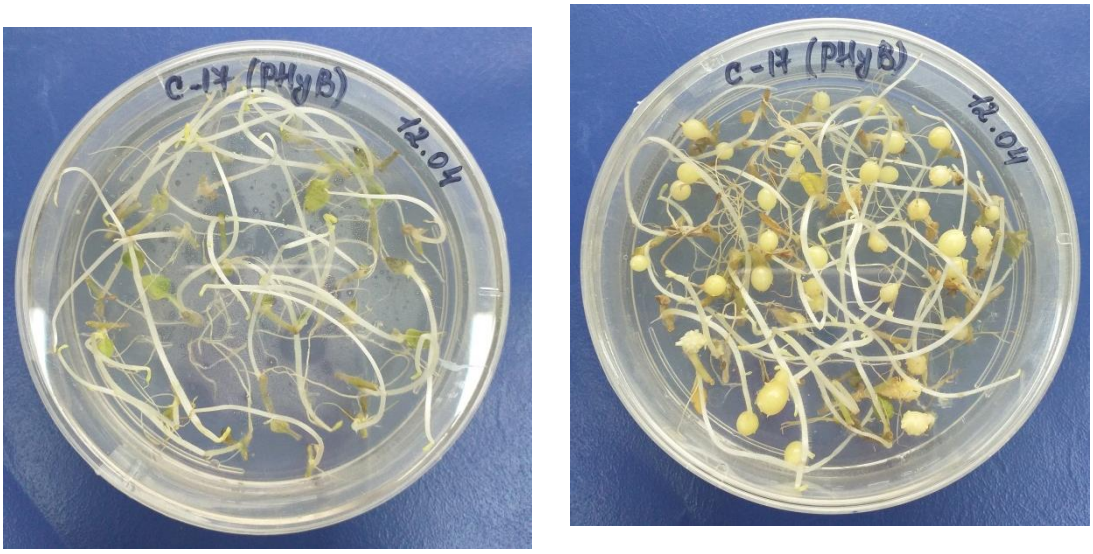

Figure 1: Sucrose effect on the formation microtubers
A - *MS + 5 %* sucrose concentrated nutrient environment; **V** - *MS + 5* mg / l kinetin + 8% sucrose concentrated nutrient environment

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