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Effect of Different Sugar Concentrations on Invitro Micro-Propagation of Potato (*Solanum Tuberosum L.*) using MS Medium without Hormonal Supplementation

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Abstract
In the present study sprouts of potato (*Solanum tuberosum*) were cultured on Murashige and Skoog (MS medium with out Hormonal supplementation) with table sugar as a cheap carbon source with different concentrations of 40%, 60% and 60% which affected *in vitro* micropropagation of potato after spraying the potato with GA3 for initiating sprouting. After 40-50 days of culture, growth of rhizogenesis and callogenesis with 40%, 60% and 80%, and color change in the sprouts was observed. Rhizogenesis and callogenesis growth of the sprouts in MS medium showed better growth with 40% sugar as a carbon source as compared to 60% and 80%. It was also observed that the sprouts supplemented with 40% sucrose turned grassy green along with 2 ± 1cm and 6 ± 1cm growth in rhizogenesis and callogenesis. The sprouts supplemented with 60% sucrose turned light green after inoculation while showing 1.8 ± 1cm and 5 ± 1cm growth in rhizogenesis and callogenesis. The potato sprouts supplemented with 80% sucrose turned hyaline after culture and showed a growth of 1.5 ± 1cm and 4.7 ± 1 cm in rhizogenesis and callogenesis. In this way the results showed that 40% was excellent for the micropropagation of potato.

Keywords: *in vitro, micropropagation, rhizogenesis, callogenesis sprouts, Solanum tuberosum*

Introduction

Plant tissue culture is a practice which involves the *in vitro* growth of plant cells, tissues, organs or a plantlet in artificially prepared medium under sterile conditions [1]. Haberlandt (1902) originated the concept of cell culture and was the first to attempt to cultivate isolated plant cells in vitro on an artificial medium [2]. Plant tissue culture relies on the fact that many plant cells can regenerate into an entire plant, this ability of cells is called totipotency [3]. Single cells, protoplasts, pieces of leaves, stem or roots also termed as explants can be used to produce a new plant on specific plant medium which contains essential nutrients and plant growth regulators (PGRs) depending upon need[4]. Moreover environmental conditions are also monitored which includes, light intensity, temperature, humidity and vice versa [5].

Potato is widely used all around the world and is the favorite food for many as it is used in cooking and for making French fries as well . In order to meet the requirement of the potato, we need to increase its production and for that we need to utilize our lab resources other than just relying on the potato of the fields . Potato could be cultured *in vitro* using MS or MS + basal medium [6]. Basal medium usually refers to the plant growth hormones that are being used additionally in the culture medium[7]. Our study was only focused on MS medium and the growth was measured in term of the growth (in cm) of rhizogensis (root formation) and callogenesis (shoot formation) and color change. Potato sprouts sprayed with GA3 were used as explants in the study for micropropagation of potato *in vitro*.

Materials and Methods

The present study was conducted at the Plant Tissue Culture Laboratory of the Forman Christian College Lahore Pakistan from December 2012 till June 2012. My study was based on the in vitro micro propagation of potato tubers using sucrose at three different concentration 40%, 60% and 80%. Potato was sprayed with GA3 from all sides and was placed in the dark room at 25^{o}C for around 2-3 days to initiate sprouting

and then these sprouts were used in this study [8]. The *in vitro* sprouts were then routinely cultured every week on normal propagation media in test-tubes (25 × 150 mm) using sprout cuttings in order to attain sufficient quantities for the experiments. Growth conditions during the in vitro micro propagation were: 16-h photoperiod; 3,000 lux light intensity and 24 ± 1°C. Treatments consisted of carbon sources (table sugar) and. Brown and white sugars differ in their sucrose contents and color density.

Preparation of Maintenance and Conservation medium

The maintenance/propagation medium was solid agar (1%) MS (Murashige and Skoog, 1962) supplemented with micro and macronutrients, iron EDTA and vitamins according to the appendix A and dissolved in distilled water and are kept in brown bottles at 4-6°C to avoid oxidation and regeneration. For preparation 250ml, 500ml etc volume of media distilled water was added according to our need. Same way according to appendix A sucrose as a source of carbohydrate was added for the required volume. Depending upon the need various sucrose concentrations of media were used i.e., 40%, 60% and 80%. Adjusted the pH 5.8+-0.1 by electric pH meter also stirred the solution with magnetic stirrer to homogenize the solution.[9] Weighed agar on weighing balance, and added 1% agar into the solution to solidify the medium. Boiled it to homogenize. Prepared media was poured into the culture tubes. These tubes were covered with polypropylene sheets and sealed with rubber bands. Culture tubs were kept in rack and autoclaved for sterilization at 121°C temp and 15 psi pressure for 15 minutes [10].

Sterilization Methods and Conditions

Glass ware was first washed with detergent and washed with water and dried in an oven at 95°C. Then it is wrapped in a brown cover and autoclaved at 121°C temp and 15 psi pressure for 15 minutes. Sprouts were removed from the potato which were used as explants.[11] For the sterilization of explants, liquid detergent of vim brand was added in a autoclaved containing autoclaved distilled water and 2 drops of tween 20 (a surfactant). It was stirred so that all the dust gets removed from the explant. After 1-2 min, rinsed it 2-3 times with distilled water. One drop of mercuric chloride (0.1%-1%) was added for one minute in the distilled water containing explants. [12]Rinsed it again with distilled water. Further sterilization of it was done in laminar flow cabinet, where it was rinsed 2-3 times with distilled water. Instruments used for inoculation e.g. forceps, scalpels, spatula, needles and blades were sterilized with 70 % ethanol. The instruments were placed in beaker containing ethanol and were flamed over spirit lamp before every use. Laminar flow cabinet was irradiated with UV light for 30-40 minutes before inoculation. Then sprayed and scrubbed thoroughly with ethanol. Ultra filter air was used in laminar flow cabinet during inoculation [13]. Hands were washed first with soap and then clean your hands with absolute ethanol (70%).

Inoculation and incubation

Before the inoculation, the laminar flow was irradiated with UV. light for 30 minutes. The ultra filtered air was turned on. The spirit lamp was placed in the centre on the laminar flow. Explants were rinsed thoroughly with autoclaved water 3-4 times in laminar flow. On right corner of the laminar flow hood, the beaker having half filled alcohol was placed which contained scalpels and forceps. Then one of the culture tubes was taken and its polypropylene sheet was removed. The mouth of the test tube was immediately placed near the flame of the spirit lamp to avoid contamination. One of the forceps was taken from the beaker and placed over the flame for few seconds. With the help of it explant was put in the culture tubes. Sealed the culture tube again, with polypropylene sheet and the rubber band. Same protocol was followed for inoculation of all of the explants in the culture tubes.

Culture conditions

The inoculated culture tubes rack were taken to the Tissue culture lab and put in the growing room where the temperature was about 25±2 °C and a cool white fluorescent light was used. Explants were incubated in low light intensity at 10-15 µmol m^{-2} $s^{-1.}$ For sub-culturing[14]. same protocol was repeated for inoculation as previously explained.

Growth indicators

To measure the outcome of the research, two growth indicators in order to evaluate whether which percentage was success for the experimentation were used which were

1. Color change
2. Growth (in cm) of the rhizogenesis and callogenesis

Results and Discussion

After 7 days of inoculation all explants turned green with different shades. For different sucrose concentrations, different growth was observed. In the MS basal medium containing 40% sucrose, the explant

started showing growth after 8 days of inoculation, whereas the rhizogenesis and callogensis started after 12 days of inoculation. (Fig 1 and 2). It was observed that this concentration provided fast growth as compared to the other two concentrations. For the media containing 60% and 80% sucrose the rhizogenesis and callogenesis were also observed. (Fig 3). The plant that contained longer roots had more amount of root hair on it.

All our cultures tubes in the culture room were carefully and regularly checked. The ones which got contaminated were immediately removed. Red and white fur like structure on the top of the culture indicated the contamination due to poor handling during inoculation.

Figure 1

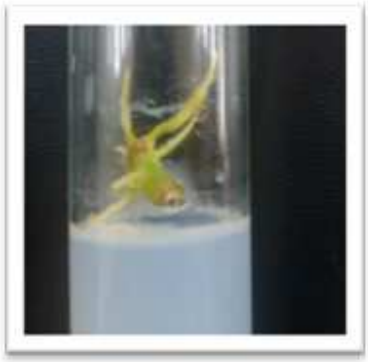

Figure 2

Figure 3

Figure 1: Growth of potato explants observed with grassy green color after 8 days of incubation on MS medium supplemented with 40% sucrose

Figure 2: Growth of potato explants observed with light green after 8 days of incubation on MS medium supplemented with 40% sucrose. Brown coloured explants turned green when grown on MS medium.

Figure 3: Callogenesis and rhizogenesis from single sprout were observed with hyaline color after 12 days of inoculation. MS medium was supplemented with 60% sucrose.

The growth of the rhizogenesis and callogenesis in cm measured after 14 days of inoculation with different sucrose concentrations is given in Table 1.

Table 1: Effect of different sucrose concentrations on the growth of rhizogenesis and callogenesis

Species	40%	60%	80%
Rhizogenesis	2 cm	1.8 cm	1.5cm
Callogenesis	6cm	5cm	4.7cm

It was observed that the best growth was shown by the plants which were supplemented wit 40% sucrose.

Conclusion

We have argued about how using different concentrations of sugar which is considered to be a cheap source of carbon results in effective micro-propagation of potato. As our work was based on sprouting and inoculation in MS medium, using sugar was economical and easy as if helped saving time and laboratory resources consumed in making combinations of plant hormones (basal medium). Later success of the process was determined by measurements of growth in roots and shoots both in cm. During inoculation, safety and sterilization were the key points kept in mind to avoid bacterial contamination which results in loss of both medium and explants. This article can help others who want to do work in this respective field as they can try MS+Basal medium and see the ratio of growth in MS+sugar Vs MS+Basal. As we see world today is facing severe hunger problems due to increase in population and low availability of fertile land, using such techniques can help increasing food and

combating hunger and mortality related to unavailability of food in countries like Ethiopia. Tissue culturing technique helps in in vitro micropropagation of many plants like wheat, gram, potatoes, bananas and tulip plants too in relatively less time. After inoculation, the cultured plant can then be hardened (with specific protocol) before transferring to the soil where is gives its vegetative parts like fruit, flower etc.

"Cite this article"

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