1. Project title: Automated Hydroponic System for Potato Microplant Culture
2. Sanction No.: F.No.-8 (24)/97-Hort II
3. Date of Start: April 1, 1998
4. Date of termination: December 31, 2001
5. Institution's name: Central Potato Research Institute
   Place: Shimla
   District: Shimla
   State: Himachal Pradesh
   Deptt./Division name: Division of Crop Improvement
   Actual location (location of research scheme to be carried out): CPRI, Shimla (HP)-171 001
6. Principal Investigator
   Name: Dr Debabrata Sarkar
   Designation: Scientist
   Division/Section: Division of Crop Improvement
   Experience: 13 years and 9 months including Ph. D. work
   Address: Division of Crop Improvement, Central Potato Research Institute, Shimla-171 001, Himachal Pradesh, India
7. Co-investigators: Nil
8. Objectives:
   i) To automate potato micropropagation vis-a-vis microtuber production and in vitro microplant (germplasm) conservation by developing an Automated Hydroponic System (AHS).
   ii) To optimise the schedules and rate of medium flow (from medium) reservoir into and out of the hydroponic chamber for potato microplant cultures by computer-based programmable filling/evacuation system.
   iii) To study microplant growth and microtuber induction during potato micropropagation in the automated hydroponic chamber.
9. Duration of the scheme: 3 years 9 months (extension for nine months w.e.f. 01-04-2001 to 31-12-2001 accorded vide council approval F. No. 8-24/97-HORT.II of 11-04-2001)

10. Total cost of the scheme:
   Recurring: Rs. 12,43,240/-(revised)
   Pay of officers: Rs. 3,20,000/-
   Rs. 2,01,240/-

A] Budget head-wise including extension period:

<table>
<thead>
<tr>
<th>Description</th>
<th>No. of posts</th>
<th>1st year</th>
<th>2nd year</th>
<th>3rd year</th>
<th>Total</th>
<th>4th year (9 months extension period)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Establishment</td>
<td>One</td>
<td>Rs. 64,500/-</td>
<td>Rs. 64,500/-</td>
<td>Rs. 72,240/-</td>
<td>Rs. 2,01,240/-</td>
<td>Rs. 54,180/-</td>
</tr>
<tr>
<td>Senior Research Fellow</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Recurring contingencies including TA</td>
<td></td>
<td>Rs. 1,90,000/-</td>
<td>Rs. 70,000/-</td>
<td>Rs. 60,000/-</td>
<td>Rs. 3,20,000/-</td>
<td>Rs. 2,00,000/-</td>
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<tr>
<td>3. Non-recurring contingencies</td>
<td></td>
<td>Rs. 7,22,000/-</td>
<td>-</td>
<td>-</td>
<td>Rs. 7,22,000/-</td>
<td>Rs. 75,000/-</td>
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<td>Rs. 9,76,500/-</td>
<td>Rs. 1,34,500/-</td>
<td>Rs. 1,32,240/-</td>
<td>Rs. 12,43,240/-</td>
<td>Rs. 3,29,180/-</td>
</tr>
</tbody>
</table>

100% ICAR SHARE

* The expenditure for the extension period (01-04-2001 to 31-12-2001) and arrears of the SRF w.e.f. 26-05-1998 to 31-03-2001 to be met from the unutilized budget (balance) as in [B] below

B] Budget and expenditure position of the scheme up to 31-12-2001

Since the Institute's Audit and Accounts Section is yet to finalise the budget and expenditure positions of the scheme, these details will be sent separately by the institute after necessary completion accounting and other formalities

11. Total amount sanctioned: Rs. 12,43,240/-

12. Total amount spent up to March 31, 2001: To be sent separately by the institute after finalisation of Audit & Accounts' reports

13. Result of practical/scientific value: See Annexure I

Manuscript submitted: Nil
Papers presented at scientific meetings: Nil
Manuscript under preparation: Nil

15. Detailed progress report (to be annexed): See Annexure II

Signature
Name: Debabrata Sarkar
Designation: Scientist

Date: December 26, 2001

16. Comments of the Project Co-ordinator/Referee:

Significant research achievements have been obtained in the project despite the delays that occurred in the beginning. Not only the system has been fabricated and put to function successfully for quite some time, it has also led to identification of some important novel variations.

17. Remarks of the Council:
ANNEXURE I

There has always been a long-felt requirement for an integrated automated system for potato microplant culture *vis-a-vis* microtuber production. Initiation and multiplication of shoot cultures and microtuber induction in potato can be carried out sequentially in automated chamber. This will be less labour-intensive and time-consuming in terms of preparatory, inoculation and maintenance schedule. In the Automated Hydroponic System (AHS), potato microplants will be cultured in a hydroponic chamber connected to a medium reservoir. The medium from reservoir will be cycled in and out of the hydroponic culture chamber by a computer-based programmable filling and evacuation system. The growth and proliferation rate of microplants is expected to be high in large sized automated chamber. Besides, more number of propagules can be accommodated in the automated chamber thereby increasing the incubation space in growth room. As a result of these, microtuber production efficiency will be increased. Medium will bathe the cultures only for certain times daily and then will be recycled back to the medium reservoir. This will minimise nutrient depletion in the medium and the same medium or nutrient stock can be used for longer periods. An economised use of medium will reduce the cost of microtuber production *vis-a-vis* commercial potato micropropagation. A computer-based control of the automated system will facilitate precise control over culture schedules and promote better culture management in commercial environment.

Such an automated system for potato micropropagation and subsequent microtuber production has been developed. Since the system is based on growing of potato microplants and subsequent induction of microtubers in liquid nutrient media only, it has been designated as "Automated Hydroponic System for Potato Microtuber Production *in vitro*". In this automated hydroponic system, microplantlets of disease-free potato cultivars/clones are propagated and multiplied with subsequent microtuber production *in vitro* in a large hydroponic culture chamber on constantly recycled and/or stationary nutrient medium from a medium reservoir at the rates and schedules controlled precisely (automatically) by a computerized operating system. The merit of this integrated automated system lies in its simplicity and high production efficiency within a limited time and space to boot.
Summary

Conventional potato micropropagation is a highly time-consuming and labour-intensive process, which limits its successful integration in seed potato production programmes in developing countries. Therefore, a simplified automated hydroponic system (AHS) has been conceptualized and developed for potato micropropagation vis-a-vis microtuber production in vitro. In this automated hydroponic approach, potato microplants are micropropagated with subsequent induction of microtubers in a large hydroponic culture chamber on recycled and/or stationary liquid nutrient medium from a medium reservoir at the rates and schedules precisely controlled by an automated (computerized) operating drive. This integrated "Automated Hydroponic System for Potato Microtuber Production in vitro" essentially consists of a hydroponic (polycarbonate) culture chamber with a specially designed cover lid, a 4-lit polypropylene medium reservoir, a computer-compatible reversible variable-speed peristaltic pump, a computer with data interfaces and peripherals, a programmable timer and a single-head air pump. The polycarbonate autoclavable hydroponic culture chamber with a special cover lid for culture inoculation, medium dispensing, sterile aeration and sterile venting ports has been designed and fabricated. Appropriate in vitro culture protocol for potato shoot multiplication and subsequent microtuber production has been standardized in this automated hydroponic system. The hydroponic system fosters optimum potato microplant growth in vitro, and significantly increases the microtuber production efficiency within a limited time and space.

Introduction

Micropropagation technique has been successfully integrated into disease-free seed potato production programmes. This technique essentially involves virus elimination from systemically infected potato clones (cultivars) through meristem culture, maintenance and multiplication of disease-free clones through axillary shoot cultures, and production of microtubers under tuber-inducing conditions in vitro for direct and/or indirect utilization in healthy seed potato production programmes. Microtubers are miniature dormant tubers, which are particularly convenient for handling, storage and distribution. Unlike the micropropagated plantlets, they do not require time-consuming hardening period in greenhouses, and may be adapted easily to large-scale mechanized planting in the field. Therefore, in recent years, this micropropagation vis-a-vis microtuber technology has revolutionized potato production in developing countries.

Large-scale microtuber production in potato consists of three basic steps: 1. maintenance and multiplication of disease-free clones through axillary shoot cuttings in culture tubes on semisolid (agar-gelled) propagation medium; 2. initiation of axillary shoot cultures in 250 ml capacity Erlenmeyer flasks or Magenta boxes or other appropriate culture vessels on liquid
propagation medium; and 3. induction of microtubers in aforesaid diverse culture vessels under tuber-inducing conditions \textit{in vitro}. Each step of the above protocol has its own specific requirements in terms of nutrient medium, culture conditions, culture space, inoculation schedules and incubation intervals. Consequently, the production efficiency of microtubers is dependent upon the propagation efficiencies of individual culture stages. Therefore, the total system approach starting from initial multiplication through axillary shoot cuttings to final microtuber induction is not only highly time-consuming, but also labour-intensive. It has been estimated that about more than 60\% of microtuber production cost accounts for labour input towards time-consuming medium preparation, medium pouring in culture vessels and culture inoculation. Besides, the present schedule of microtuber production involving conventional culture tubes and 250 ml Erlenmeyer flasks/Magenta boxes/Melli jars are enormously space-intensive. For example, according to a modest estimate, production of 10,000 potato microtubers involves initial multiplication of 1000 culture tubes each containing three microplants followed by liquid propagation in 500 Erlenmeyer flasks/Magenta boxes/Melli jars with subsequent microtuber induction in these 500 culture vessels (after removing the liquid propagation medium from each vessel, and pouring in fresh microtuber induction medium under aseptic conditions). It can, therefore, be well assumed the extent of culture space and rigid culture sequence and/or schedule being required for large-scale potato micropropagation \textit{vis-a-vis} microtuber production in a commercial environment. In view of this, microtuber technology is yet to be realized in commercial seed potato production programmes as expected to be.

Therefore, there is a need for automation of potato micropropagation technology. Initiation and multiplication of shoot cultures and microtuber induction in potato can be carried out sequentially in an Automated Hydroponic System (AHS) where the medium from reservoir would be cycled in and out of the hydroponic culture chamber by a computer-based programmable filling and evacuation system. This will be less labour-intensive and time-consuming in terms of preparatory, inoculation and maintenance schedules. We have successfully developed an Automated Hydroponic System (AHS) for potato microplant cultures.

**The Automated Hydroponic System (AHS)**

The ‘Automated Hydroponic System Potato Microtuber Production \textit{in vitro}’ consists of:

1. Autoclavable polycarbonate hydroponic culture chamber with SS cover lid fabricated to accommodate culture inoculation, medium dispensing, sterile aeration and sterile
venting ports.

2. Autoclavable polypropylene 4 lit culture bottle as medium reservoir with autoclavable polypropylene filling/venting cap with thermoplastic elastomer (TPE) gasket and medium dispensing plus sterile venting ports.


5. Single-head air pump for aeration.

6. Programmable timer for controlling the air pump for aeration.

7. Silicone peroxide-cured autoclavable tubes for medium dispensing and sterile aeration.

8. Autoclavable polypropylene retrofittings with acetal nuts, silicone gaskets and silicone cap as port extensions.

9. Autoclavable hydrophobic 0.2 µ PTFE vent microfilters for sterile aeration and venting the hydroponic culture chamber and/or medium reservoir.

The total system is diagrammatically shown in Figure 1.

Design and fabrication of the hydroponic culture chamber

The design of the autoclavable polycarbonate hydroponic culture chamber is shown in Figure 2. The hydroponic culture chamber is made of autoclavable (121 °C) polycarbonate (transparent) material of 0.25 cm thickness. The chamber height is 30.5 cm with an internal diameter of 20.5 cm. The top SS cover lid has a diameter of 22.0 cm. At the centre of the SS cover lid, there is a culture inoculation port (with an SS screw cap) of 7.4 cm diameter with the top SS screw cap having a diameter of 7.9 cm. The SS screw cap has a height of 1.8 cm. The culture inoculation port (with the SS screw cap) is located on the SS cover lid at a distance of 7.3 cm from the periphery. Medium dispensing, sterile aeration and sterile venting ports are located on the SS cover lid at a distance of 2.5 cm from the periphery. Each port has a diameter of 1.0 cm. The distance between the periphery of the central culture inoculation port and other ports (medium dispensing, sterile aeration and sterile venting) is 3.5 cm. Each port excluding the inoculation port has a total height of 5.6 cm with internal and external extensions.

Assembly and operation of the Automated Hydroponic System (AHS)

The SS cover lid with its inoculation port sealed with a SS screw cap is used to close the
hydroponic culture chamber (Fig. 1). The sterile aeration and vent filtration ports in the culture chamber are fitted with 0.2 μ hydrophobic PTFE microfilters by silicone tubes in a way so that the microfilters stand vertically. A 20”-long silicone tube with a polypropylene barbed retrofit (at the end) is fitted to the inlet of the medium dispensing port extension so that it touches the bottom of the culture chamber. The outlet of the medium dispensing ports of the hydroponic culture chamber and the medium reservoir is connected by a 4’-long silicone tube. The inlet of the medium dispensing port of the medium reservoir is fitted with a 20’’-long silicone tube with a polypropylene barbed retrofit at its end touching the bottom. The sterile vent port of the medium reservoir is fitted with a hydrophobic 0.2 μ PTFE vent microfilter at the outlet, and with a 8”-long silicone tube at the inlet. The total system (i.e., hydroponic culture chamber plus medium reservoir containing liquid nutrient medium) is autoclaved at 121 °C for 20 min.

The explants (shoot cuttings/axillary shoots) are transplanted into the hydroponic culture chamber through the culture inoculation port by removing the SS screw cap on the cover lid under a laminar flow clean air work station. After inoculation, the system is placed in the tissue culture room with the medium dispensing tube (4’-long silicone tube connecting the chamber with the medium reservoir) connected to a peristaltic pump. The peristaltic pump is interfaced with a computer through RS-232 data interface cable. The outlet of the sterile aeration port (prefitted with a 0.2 μ vent microfilter) of the hydroponic culture chamber is connected to a air pump through a 6’-long silicone tube. The air pump is connected to a programmable timer. The power supply to the computer and programmable timer is provided through an UPS system. A computer programme has been customized for precise unattended scheduling of the medium flow rate, flow direction, flow duration, dispensing interval and culture bathing duration (to and fro the hydroponic culture chamber and medium reservoir) in this automated hydroponic system. It allows automatic dispensing of desired volume of liquid nutrient medium from the medium reservoir to the hydroponic culture chamber at a desired flow rate bathing the cultures for desired duration, and recycling back to the medium reservoir.

Automated hydroponic culture in vitro

The automated hydroponic culture system in potato consists of two stages: 1) shoot multiplication and 2) microtuber induction and development. The base cultures of disease-free potato cultivars are maintained and multiplied through single node cuttings (SNCs) on semisolid propagation medium in the culture tubes. The stock cultures are used for initiating hydroponic
culture in the automated system.

**Shoot multiplication stage:** Shoot cuttings with 6-8 nodes collected from twenty to thirty 28-day-old microplants are transferred into the hydroponic culture chamber, and cultured for 6 weeks under a 16-h photoperiod (approx. 70-80 \(\mu\)mol m\(^{-2}\) s\(^{-1}\) light intensity) at 24 °C. The cultures are periodically submerged in liquid propagation medium recycled in and out of the hydroponic chamber (from the medium reservoir) for a predetermined duration at 100 ml min\(^{-1}\) flow rate. The culture in hydroponic chamber is aerated with sterile air. After 6 weeks' incubation, the proliferating shoots attain an average height of 200 mm, and are ready for microtuber induction.

**Microtuber induction and development:** After shoot multiplication stage, the liquid propagation medium in the reservoir is replaced with microtuber induction medium. The cultures are incubated under dark at 20 °C for microtuber induction. The shoot cultures are initially submerged in 1 lit of induction medium for 2 weeks, and thereafter, the medium level is maximized periodically up to 2 lit at a predetermined rate and interval. The stoloniferous shoot growth continues up to 3 weeks reaching more than 300-400 mm in length. The induction medium in the reservoir is replaced with fresh stock after every five weeks. The microtuber cultures are aerated with sterile air. Microtubers start developing epigeally within two weeks of culture initiation. Microtubers are harvested after 10 weeks of incubation. On an average, 300-500 microtubers develop in the hydroponic culture chamber with an average fresh mass of 250 mg.

**Effect of medium level and refreshment on potato microplant culture in the AHS**

Studies were conducted to assess the efficacy of medium level and medium refreshment on microplant growth and microtuber initiation and development in the Automated Hydroponic System (AHS). During microplant culture *vis-a-vis* micropropagation in the AHS, shoot cultures were periodically submerged in 500 ml, 1000 ml, 1500 ml and 2000 ml of liquid propagation medium at a predetermined rate and interval. The results showed that optimum microplant growth and proliferation could be attained when the microplants were periodically submerged in 1000 ml of medium at a flow rate of 100 ml min\(^{-1}\) for 2 h followed by 6 h intermission. Submergence in more than 1000 ml of medium, although resulted in thicker and more stout stem growth, but was in general detrimental for overall microplant growth and proliferation per unit time. Medium refreshment during microplant culture/micropropagation did not have any
significant effect on microplant growth and proliferation.

During microtuber induction, initially the microplant cultures were submerged in 1000 ml of induction medium for 2 weeks, and thereafter, the medium level was raised up to 1500 ml, 2000 ml, 2500 ml, 3000 ml and 4000 ml at a flow rate of 100 ml min\(^{-1}\) at predetermined interval, and grown for the entire duration of microtuber production. It was observed that periodic submergence of microplant cultures with 3000-4000 ml induction medium resulted in a higher rate of contamination in the AHS. Optimum microtuber initiation and development occurred when the microplant cultures were periodically submerged in 2000-2500 ml of induction medium at a predetermined interval after initial 2-weeks submergence in 1000 ml of induction medium. In addition, refreshment of induction medium after initial 2-weeks of incubation did have a significant effect on microtuber growth and maturation.

**Conclusion**

The results showed that potato micropropagation *vis-a-vis* microtuber production could be effectively carried out in the "Automated Hydroponic System" developed. This automated system would not only save on labour and time, but also increase the multiplication and/or production efficiency of potato micropropagation within a limited time and space. In addition, the system offers adequate opportunities for future adaptation to changing requirements for potato micropropagation *vis-a-vis* microtuber production.
AUTOMATED HYDROPONIC SYSTEM FOR POTATO MICROTBUBER PRODUCTION IN VITRO

VENT FILTER
SILICONE TUBE
SILICONE TUBE
LU or LJJ
RS232
HYDROPONIC CULTURE CHAMBER
REVERSIBLE PERISTALTIC PUMP
MONITOR
COMPUTER
ELECTRIC CABLE
AERATION TUBE
MEDIUM RESERVOIR
SINGLE HEAD PUMP
UP SYSTEM
POWER
AUTOMATED HYDROPONIC SYSTEM FOR POTATO MICRO TUBER PRODUCTION IN VITRO

TOP PLAN

ELEVATION

Figure 2