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Review

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A Closed Transplant Production System, A Hybrid of Scaled-up Micropropagation System and Plant Factory

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Abstract

Photoautotrophic micropropagation systems do not include sugar in the culture media. This characteristic provides advantages to scale up the micropropagation systems comparing photomixotrophic micropropagation systems. A closed, large-scale photoautotrophic micropropagation for transplant production system has been developed at Chiba University, Japan. New concepts and technologies were adapted to produce high quality transplants at minimum usage of resources, and as scheduled. Newly developed software for production management was used to enhance the efficiency of the transplant production system. Currently, virus-free transplants of sweetpotato (*Ipomoea batatas* (L.) Lam.) are vegetatively propagated and produced under sterilized conditions in this system. This system can also be used for production of transplants of any other species including horticultural and woody plants with a minimum of modification.

Introduction

Micropropagation systems have advantages over other transplant production systems that use seeds or cuttings, with respect to genetic and phenotypic uniformity and scheduled year-round production of disease-free or pathogen-free transplants (Aitken-Christie et al., 1995). Kozai (1991) and, Nguyen and Kozai (1998) reported systems developed to produce a large number of quality transplants at low cost, based upon the idea of photoautotrophic (no sugar in the culture medium) or photosynthetic micropropagation under high CO_2 concentration or CO_2 enrichment. With photoautotrophic micropropagation, it is possible to use large culture vessels with minimum risk of microbial contamination. In the large vessels used for the photoautotrophic micropropagation, forced ventilation has been found to have several advantages over natural ventilation (Jeong et al., 1995). Environmental control techniques have been developed to exclude any problems caused from scaling-up the micropropagation systems and the efficiency of the systems can be maximized as a result.

A closed system, a scaled-up micropropagation system was developed at the Matsudo campus of Chiba University, Japan (Chun and Kozai, 2000). This system is a research facility to study biological and engineering aspects of transplant production in closed systems and to test the feasibility of closed systems for production of transplants. Various technologies for scaling-up the micropropagation systems were applied to produce high quality transplants with the highest efficiency. In this system, the transplants are vegetatively propagated and produced under sterilized conditions. This system can be thought of as scaled-up culture vessels of a photoautotrophic micropropagation system. High quality transplants, defined as transplants that are physiologically and morphologically superior and show vigorous growth after being transplanted into fields and greenhouses, can be produced regardless of weather conditions.

This system is presently being used to produce virus-

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free transplants of sweetpotato (*Ipomoea batatas* (L.) Lam.), although it can produce transplants of any species. Sweetpotato is used as a fresh vegetable, a feed for animals and a raw material for producing biodegradable plastics and hydrogen gas, and vast amounts of quality transplants will be needed in the near future (Kozai et al., 1999a).

Conventionally vine cuttings are used as transplants for sweetpotato production. Because the vine-cutting transplants do not have roots, almost 100% of them wilt after transplanting. Even though large portions of the transplants recover from the wilting in a few weeks, the delay of the early growth caused by the wilting after transplanting may decrease the final yield and quality of sweetpotato. In the newly developed system, however, sweetpotato transplants with root balls are produced in multi-cell trays for direct transplanting into fields (Islam et al., 2000). In this case, transplants keep growing without wilting and a higher yield can be expected. Machines can be used for transplanting the sweetpotato transplants with root balls, which is difficult with vine-cutting transplants.

Scaled-up photoautotrophic micropropagation system

Recent studies (Jeong et al., 1995; Kozai, 1991; Kozai et al., 1999b; Nguyen et al., 1998) revealed that most chlorophyllous plants in vitro have the ability to grow photoautotrophically, and that the low CO_2 concentration in the air-tight culture vessel during the photoperiod is the main cause of the low net photosynthetic rate of plants in vitro. Also, the net photosynthetic rate of plants in vitro is considerably lower when cultured on sugar-containing medium than when cultured on sugar-free medium. Furthermore, we have shown that the photoautotrophic growth of chlorophyllous plants in vitro can be significantly promoted by increasing the CO_2 concentration and light intensity or photosynthetic photon flux (PPF), by decreasing the relative humidity in the culture vessel, and by the use of fibrous and/or porous supporting materials with high air porosity. By using a culture medium containing no sugar, the loss of plants in vitro due to microbial contamination can be significantly reduced. When a culture vessel with a high ventilation rate or high number of air exchanges is used, the relative humidity in the vessel is reduced. This reduction in relative humidity results in enhanced rooting and a high percent survival at the ex-vitro acclimatization stage, especially when porous supporting materials are used in vitro.

Gas-permeable filters are attached to the lid or sides of the culture vessels to enhance the natural ventilation of culture vessels in many photoautotrophic micropropagation systems, and thus to maintain higher CO_2 concentration in the vessels during the photoperiod. This increases the net photosynthetic rate and suppresses the relative humidity, which in turn increases the transpiration rate. On the other hand, in forced ventilation, air pumps or air compressors are used to flush a particular gas mixture directly through the culture vessel. In photoautotrophic micropropagation using large culture vessels, forced ventilation has several advantages over natural ventilation.

Fujiwara et al. (1988) developed a large culture vessel (58 cm × 28 cm × 12 cm high) with a forced ventilation system for enhancing the photoautotrophic growth of strawberry (*Fragaria × ananassa* Duch.) explants and/or plantlets during the rooting and acclimatization stages. Kubota and Kozai (1992) showed that the net photosynthetic rate and photoautotrophic growth of potato (*Solanum tuberosum* L.) plants cultured using a large culture vessel with forced ventilation, containing a multi-cell tray with rock-wool cubes, were significantly greater than those cultured using a conventional (small) culture vessel with natural ventilation. Heo and Kozai (1998) developed a forced ventilation micropropagation system with a culture vessel containing a multi-cell tray widely used for plug seedling production. The cells were filled with sterilized vermiculite or cellulose plugs. The photoautotrophic growth of sweetpotato plants cultured with this system were several times greater than the photomixotrophic growth of plants cultured with conventional or small culture vessels containing sugar and with natural ventilation. However, the growth in the culture vessel was not uniform, with larger plants near the air inlet and comparatively smaller plants near the air outlet. Zobayed et al. (1999) developed large culture vessels with air distribution pipes for forced ventilation to provide an air current pattern, which enables uniform distributions of CO_2 concentration and relative humidity as well as those of air current speeds, and thus uniform plant growth.

The idea of a forced ventilation micropropagation system can be further extended to an aseptic culture room. In this case, each aseptic culture room is considered as a large culture vessel that contains sterilized trays and plants. The closed transplant production system at Chiba University can be considered as a scaled-up culture vessel that adapts forced ventilation.

A closed transplant production system

A closed transplant production system at Chiba University (Figure 1) was developed in 2000 to study bio-

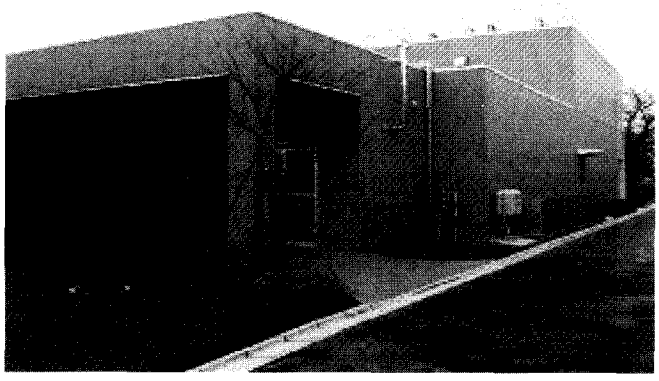

Figure 1. The closed transplant production system developed at chiba University, Japan.

logical and engineering aspects of transplant production in closed systems and to test the feasibility of closed systems for production of transplants (Chun and Kozai, 2000). In this system, which has a floor area of 500 square meters, sweetpotato transplants are vegetatively propagated and produced under sterilized conditions. The system consists of several spaces in a sterilized area and a non-sterilized area.

Sterilized area

The sterilized area consists of a transfer room, three production rooms (micropropagation room and production rooms 1 and 2) and a low-temperature storage room 2. To enter the sterilized area, workers must pass through air showers and materials must be sterilized and transferred though double-door systems that have UV lamps. To maintain a positive pressure in the sterilized area, air is supplied from outside through a ventilation system that has filters and air heaters. The amount of air supplied to the production rooms is kept at a minimum by maintaining the production rooms as closed as possible.

A. Transfer room

Plantlets grown in the production rooms, each with 4-6 unfolded leaves, are "transferred" into the transfer room. In this room, workers prepare explants (single-node leafy cuttings) from the plantlets and place them on multi-cell trays. The newly prepared trays with explants are placed on conveyers and "transferred" back to the production rooms. Plantlets that have grown in the production rooms can also be "transferred" into low-temperature storage room 2 through the transfer room to slow down their growth for a few days or a few weeks in order to meet production schedules.

Two transplant growth measuring systems (Figure 2) are located in the transfer room. Each system consists of two digital cameras and a computer. By locating individual explants or a multi-cell tray with transplants in the system, the length, the width and the area of leaves of individual explants, and the maximum and height and volume of the transplant canopy can be estimated. These systems also determine the change in leaf color of explants and transplants. The quality of explants that are used for propagation and the quality of transplants that are consigned to consumers can be evaluated with these systems.

B. Production rooms

The production rooms are where the sweetpotato plantlets are grown to be propagated and/or to be consigned as transplants. There is no difference in the facilities or operational methods among these rooms. The only differences among the rooms are in the degree of cleanness or sterilization and the numbers of basic modules that they contain. The degree of cleanness is highest in the micropropagation room and lowest in the production room 2. Plantlets can be moved from the rooms with a higher degree of cleanness to one with a lower degree of cleanness, but not in the opposite direction. Two basic modules are installed in the micropropagation room and four are installed in each of production rooms 1 and 2. All of the rooms have a tray transporting system and an irrigation system.

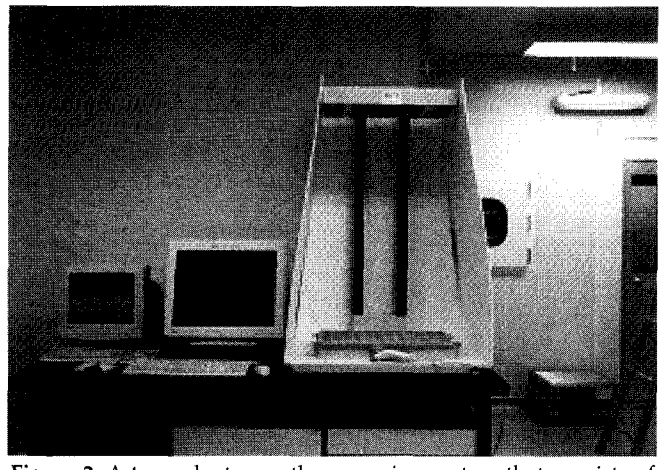

Figure 2. A transplant growth measuring system that consists of a stereo-eyed digital camera system, a bar-code scanner and a computer.

C. Basic modules

The basic module is a bookshelf-like structure located in each production room in which plantlets in multi-cell trays are placed and cultured (Figure 3). Each basic module has a lighting unit, an air conditioning unit, a control unit and seven shelves (2670 mm × 685 mm). The vertical spacing between shelves is 520 mm. Eight multi-cell trays (approx. 300 mm × 600 mm) can be accommodated on each shelf. Each shelf is lighted with sixteen 32-W fluorescent lamps and three 16-W fluorescent lamps. The output of each lamp (excluding the 16-W lamps) can be controlled with pre-decided lighting patterns to achieve the desired PPF uniformly distributed at the surface level of empty trays. The PPF of each shelf is automatically checked once a day to find any malfunctioning lamps using a PPF sensor installed on a tray-transporting unit (described later). The PPF on the empty tray surface of each shelf can be controlled between 140 to 300 μmol m^{-2} s^{-1} when the lamps are turned on.

The air conditioning unit consists of three home-use air conditioners, three mixing fans and a humidifier that are installed on the top of each module. These devices are operated with pre-decided operating patterns designed to minimize energy consumption for eliminating the heat produced by the basic module. The conditioned air is distributed by the mixing fans to each shelf through an air duct and through holes in the backside panel of the shelves. Air temperature of the basic module can be controlled in the range between 22 and 30°C, relative humidity can be controlled up to 80%, and air current speed can be controlled in the range between 0 and 1 m s^{-1}.

Figure 3. A tray transporter installed between the basic modules in each production room.

A Local Operating Network (LON) unit, which is a remote control/monitoring system, independently operates the devices of each basic module. The devices of each basic module such as air conditioners, fans, lamps, a humidifier and environmental measuring devices are operated by selecting an operation pattern from ten patterns memorized in the LON unit. Maximum, minimum and averaged values of environmental parameters of each basic module and important incidents such as errors and warnings are reported to the gateway computer. These decentralized functions of each module give a great amount of freedom for future scale-ups of this closed system.

During the dark period, if necessary, the air of each room circulates through a filtering system to remove dust, pathogens, insects and debris. An air sampler is installed in the air circulation line for monitoring microbes. The air pressure of the air circulation line is monitored to know when to change the filters.

D. Tray transporting systems

Since workers are not permitted to enter the production area for handling the trays with plantlets, the transportation of trays needs to be automated. A tray transporting system is installed in each room of the production area. This system consists of a tray transporter, conveyers, and a double-door system.

The tray transporter (Figure 3) was developed by modifying a transporter that is used for inventory management in warehouses. It is mainly for transporting trays with plantlets from one location to another in the same room and/or from a room of the production area to the transfer room. The tray transporter has another function. It carries sensors and a digital camera for measuring environmental factors at any location in the basic module and for capturing continuous images during its operation.

A conveying system is installed between the transfer room and each room of the production area. It is used for transferring trays both ways. A double-door system is installed on the conveyer to separate each room of the production area from the transfer room. A door opens when trays are passed on the conveyer, but two of the doors never open at the same time to maximize the independence of each room.

E. Irrigation systems

An automatic irrigation system (Figure 4) is installed on each tray transporter. The irrigation system was developed based upon the concepts of microprecision irrigation (Murase, 2000). Only the proper amount of nutrient solution for a particular plant (or for a particular cell of a tray) is delivered from this irrigation system. Therefore no draining or recycling process is needed and a very high efficiency of water usage can be expected. It is also possible to adjust the amount of water for a certain plant (or cell).

When a command for irrigating a particular tray is given, the transporting/irrigation system moves to the designated tray. It lifts the tray, weighs it, and moves it to the irrigation device. Then, the irrigation device with 72 needle-type nozzles moves up and down for injecting nutrient solution. An irrigation pattern including injection positions and amounts of nutrient solution for each injection can be chosen out of 64 patterns. After injection, the tray is weighed again and then returned to the original location.

A device that automatically prepares nutrient solutions is located in the machine room (a non-sterilized area). This device dilutes stock solutions to pre-set concentrations and supplies them to the nutrient tank (1 L) of each irrigation system. The nutrient solution is delivered to each cell by gas pressure supplied by a tank of compressed nitrogen gas. Solenoid valves are installed in each of the 72 lines. The amount of nutrient solution injected into a cell is controlled by opening a valve for a specified time. A pan for catching nutrient solution and/or media that drip from a tray is installed at each irrigation system.

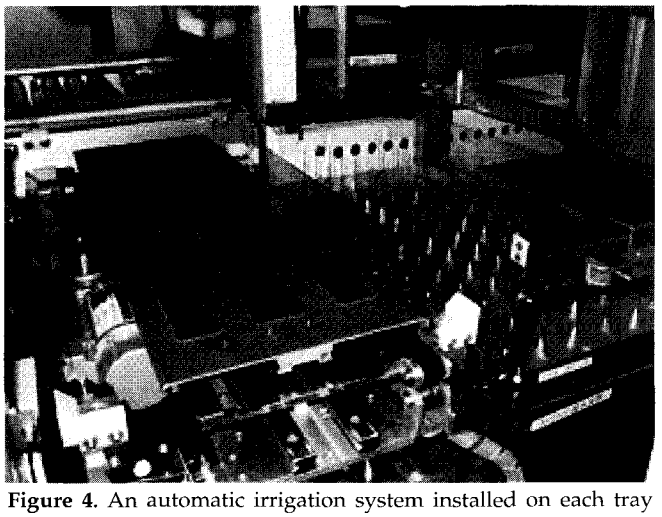

Figure 4. An automatic irrigation system installed on each tray transporter.

F. Low temperature storage room 2

Low temperature storage room 2 (2942×1592×2230 mm(H)) is located in the sterilized area. It is used for storing plantlets for a few days or a few weeks when it is desired to maintain their growth at a minimum. This room is dimly lighted and kept at a temperature in the range between 3 and 15℃. An air conditioner (1.5 kW) and a humidifier (1.0 l h^{-1}) are installed. Two movable racks, each with four shelves, are placed in this storage room. Four trays with plantlets can be accommodated on each shelf. A specially designed lighting panel (32 W) is installed in each shelf, and the PPF of each shelf can be controlled with a light controller.

Non-sterilized area

The areas for transplant production in the non-sterilized area include a cleaning room, a preparation room, low temperature storage room 1 and a control room.

A. Cleaning room

Reused trays, supporting materials (media) and other materials are first cleaned in a washing area in this room. Then, they are packed in plastic bags and sterilized by dipping in hot water (about 70℃) for a few hours. The water is heated mainly by a solar heater installed on the roof of this system, and a boiler is used as an auxiliary heater. Materials that have been cleaned and sterilized are transferred to the preparation room. This room has a door of the low temperature storage room 1 and the transplants are transferred to this room to be packed and shipped.

B. Preparation room

People and materials must pass through this area to enter the transfer room. It has a door to the air shower room and a pass-box. There is an autoclave in this room to sterilize the tools and materials that are used in the sterilized area.

C. Low temperature storage room 1

All transplants to be shipped must pass this area through a pass box between the transfer room. Transplants can be directly shipped out or stored in this storage room. The transplants can be stored here for anywhere from a few hours to a few weeks to adjust to shipping schedules. It is important to have a buffering capacity to adjust to

sudden changes in the amounts of orders and/or scheduled or nonscheduled incidents in the production process. To provide more degrees of freedom to the production facility, it is important to have not only a greater capacity in terms of space but also the ability to hold transplants for a longer duration. Currently, sweetpotato transplants can be stored for about two weeks without any decrease in their economic value.

This room has six movable racks with lamps for dim lighting, similar to those used in low temperature storage 2. Four air conditioners (1.1 kW) and two humidifiers (1.5 l h^{-1}) are installed for controlling air temperature and relative humidity of this storage room.

D. Control room

Computer systems for the production managing system, a plant operating system and a database system are located in the control room (Figure 5).

Production managing system

The production managing system is the system that manages the whole process of this facility. It consists of four subsystems: a production planning subsystem, a production simulating subsystem, a process managing subsystem and a research supporting subsystem (Hoshi et al., 2000).

The production planning subsystem finds the best plan to produce and ship out the transplants as the orders are received. The plan takes into consideration the resources, such as space, labor, plant materials and time that are available. This subsystem develops a production schedule that specifies how many explants should be prepared on a given day, if a particular order is found to be within the production capacity of the system. The production simulating subsystem is a kind of virtual factory that simulates the production with several models. The growth of trays of plantlets is simulated based upon the environmental conditions where the trays are located. The concepts of decentralized and object-oriented systems (Hoshi, 1992) are used for simulating the actual production activities in this system. If the production plan is found to be feasible by the production simulating subsystem, the process managing subsystem gives various instructions related to tray transporting, irrigation, manual operations by workers and others. Each worker carries a handheld terminal (a small communicating device) to receive instructions from the subsystem. The workers only need to follow the instructions that are shown on the small monitor of the handheld terminal.

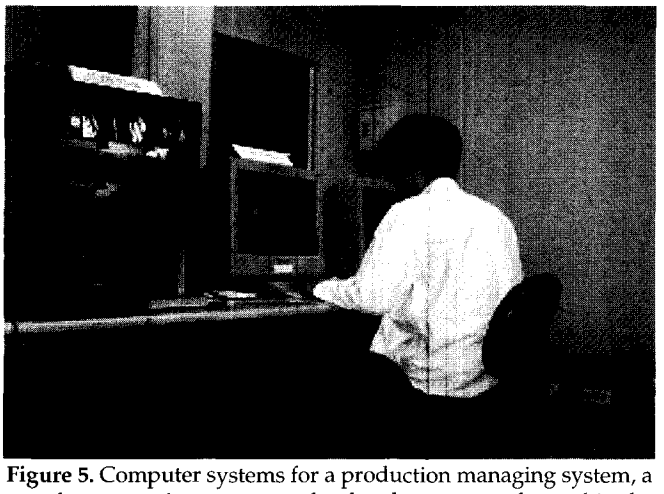

Figure 5. Computer systems for a production managing system, a plant operating system and a data base system located in the control room.

The research supporting subsystem is used to assist researchers with their experiments. The researchers can manage their plantlets independently from the regular production processes with the support of this subsystem.

Plant operating system

The plant operating system is the system that actually operates the facilities and monitors the actions of the facilities. It consists of a few subsystems: a facility operating subsystem, a plant monitoring subsystem and an alarm subsystem. The facility operating subsystem gives orders of particular actions to the facilities with the commands from the process managing subsystem of the production managing system. The operation of each facility and the results by the action are monitored by the plant monitoring subsystem. They can be monitored on the computer monitors. If problems are detected by the plant monitoring subsystem, the alarm subsystem takes several steps to protect the facilities. For major alarms, this subsystem sends a message to the cellular phones of the management staff, and thus the staff can check the facilities and reset them. The history of alarms and warnings are stored in a database system.

Database system

Data related to the growth of transplants and the plant operation (which are produced and/or retrieved by the production managing system, the plant operating system and the plant growth measuring system) are saved and managed in the computer of the database system. The data saved in this system is searchable and compatible with other software. Not only numerical data but also text and image can be saved in this database.

E. Research area

The research area has three laboratories (a biological lab, a physical lab, and a basic module lab) for research and other work that is done to support production. The biological lab is used to analyze plant samples and microorganisms in the air on a regular basis. The physical lab is used to examine samples of the nutrient solutions and supporting materials. The basic module lab is used for examining environmental details and conducting other experiments in the basic module.

Concluding Remarks

The authors and their research group conduct various studies related to transplant production using the closed system at Chiba University. Energy and mass analyses in the production area are important for improving system performance. These analyses can help to decrease energy consumption and production costs. From preliminary experiments with similar closed systems, the electricity consumed for producing a transplant for 14 days is about 0.35 MJ (= 0.1 kWh), which costs 1.5 Japanese yen (Ohyama and Kozai, 1998). Both the initial and operational costs of the closed system can be decreased by selecting facilities that have better performances and proper capacities and by optimizing their operation.

This closed system introduces many new concepts and technologies. Some of the pre-existing technologies and concepts are applied to this system, and several concepts and technologies have been newly developed for this system. Each technique or concept can be applied to other industries in agriculture, while this new concept of a closed system for transplant production itself can be utilized as a new agricultural business.

The research results from the closed system at Chiba University will create opportunities for new agricultural businesses and research areas. And these results can be utilized for further improvement of the systems and for development of small-sized systems that farmers can use for production of their own transplants. The closed transplant production system, a scaled-up system of photoautotrophic micropropagation system, can be used for producing high quality transplants of any species including horticultural woody plants. This system will maximize the production efficiency and will make it possible to produce transplants according to pre-decided schedules, which is presently difficult to achieve in agricultural production systems.

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