AUTOMIZATION OF TISSUE CULTURE SYSTEM
A SUMMARY OF SELECTED DEVELOPMENT

J G Moon, Ph.D
Center for Agrimation
Korea Institute of Machinery and Materials
P.O. Box 101, Yusong, Taejon 305-600, KOREA

ABSTRACT

Tissue culture, or micropropagation, is being used for the vegetative multiplication of several hundred millions of superior plants annually for horticulture and forestry. It is often more expensive than other forms of propagation using cuttings or seeds, because it is labor intensive and more specialized. The aim of automation is to reduce the cost per plantlet by reducing labor input, and finally, to yield profit, as business activity. Labor usually accounts for 70 - 80% of the *in vitro* and *ex vitro* cost.

This paper shows aspects of tissue culture automization, such as technical and economical approaches in view of automization.

keywords : tissue culture, micropropagation, automation, in vitro

INTRODUCTION

Since de Bry's report in 1986, "What would happen if we used robots in micropropagation laboratories? ", the study of automated micropropagation has been an important subject seeking technical and economic solutions. This study was mainly derived from Hänning's unembryo and the production of plantlets under virus-free conditions. It was the first such attempt during the last 40 years following recognition and development of 'plant tissue culture'

The advantages of tissue cultures are as follows: (also see fig. 1)

- Rapid clonal multiplication
- Provision of disease indexed plants
- Preferential method for international distribution
- Cost reduction
- Year round production
- Germplasm storage.

Compared with a general propagation method:

1) The procedure can be carried out in a relatively small place, and with certain level of quality.
2) *In vitro* conditions such as nutrient, hormone and other physical environmental factors are appropriate.
3) Microorganisms such as fungi, virus and other insects inhabiting higher plants can be removed.
4) Botanical and biological characteristics make experiments using protoplast and independent cell growing possible, which was almost impossible before.

Labor cost in tissue culture constitutes about 70 - 80% of the whole production cost. The labor-intensive nature of this work requires extensive manpower. These factors pose an important problem that must be solved. In theory, 70% of all plants on the earth could be propagated by the tissue culture method. This method is the technical solution needed to solve the food and environmental problems we now face. Automated tissue culture is one of the most important means to provide plants or seeds consistently.

The purpose of this paper is to describe the specific techniques needed for automated tissue culture, directing attention to this field by reviewing the status world wide.

WORLD POTENTIAL

World wide about 460 million plants are produced per year, and a half of world production take place in the Western Europe. The Netherlands produces about 61.5 million plants, as about 15% of world production. There is an increasing trend to use pots in Europe, while crop methods are popular in Asia and other countries.

A production of more than 1 million plants per year is the objective of automation. We can guess that there are about 200 laboratories in existence. The mean value of yearly increases for the last 10-year period was significant; 30% every year in the Netherlands, especially. Recently, due to amazing demand for flowering plants, as well as vegetables other than ordinary crops, the new market requirement is getting bigger and bigger.

North America is the secondary micropropagation production area. This area is producing more than 30% of the world's production. Starting about 1965, orchids were the first crop commercially produced by micropropagation in North America. About 47 million ornamental plants were produced in 1985. Fruits were produced from about 1.2 million plants in 1984, even though the fruits were small.

Until recently, the production of plant tissue culture was not important in Asia, but more and more the importance of production by micropropagation is increasing. Currently about 105 commercial laboratories are operating, producing about 74 million plants per year. Much of the 44 million plants produced, or, about 60%, is orchids. (see table 1)

Though areas of South and Central America, Eastern Europe, Eastern Asia and Africa have significant labor, production by micropropagation is feeble. In these areas large scale complexes are operated. In 1988, more than 120 commercial micropropagation laboratories were operating in Poland, with a total production of 15 million plants. The most popular plants produced are Spathiphyllum, Philodendron, Dieffenbachia and Cordyline. Until now, because micropropagation as a new technique is not well developed the National Complex is used for the production of special crops in laboratories.

The development of an automated system was initiated by European countries and was aimed at producing good quality crops and flowering plants. Geographically, European markets, international airports, harbors and highways are well situated. The weather is mild, an important condition for agriculture, producers competent and the farmland is developed for micropropagation.

Europeans have set up commercial firms as a practical solution, i.e.;

- Phyto Nova (Netherlands): automated tissue culture process
- V.C.I. (Netherlands): automated manufacturing liquid medium in tissue culture
- Hawe (Netherlands): automated culturing of greenhouse tomato
- Agri Systems (Netherlands): plant factory system of tomato production
- Dummen (Germany): automated prune-technique
- Evtrac Ltd (Taiwan): automated manufacturing of tissue culture in liquid medium
- Fides B.V. (Netherlands): cutter for transplant, automated prune-technique
- A.B.O. (Israel): automation of greenhouse, transfer, seeding, harvest

These techniques have not been completely developed yet. Another 10 years may be needed for their completion.

Other notable research being conducted on automated tissue culture systems includes the following:

- Automated feeding transfer (Suggs, C.W. et U.S.A)
- Air-pruned transplant production system for fully automated transplanting (Huang, B.K. U.S.A, Ai.f. Japan)
- Optimization of containerized transplant production: The hybrid-float system (Suggs, C.W. et U.S.A.)
- Development of automatic transplanter using chain pots for vegetable crops (Namba, T. ; Tanimura, M. Japan)

TECHNICAL CONSIDERATIONS IN AUTOMATION

The system requires full automation with stream lined flowing, except for batch style.

- Media preparation, sterilization and dispensing of recognized system and integration of a controlled cutting system

- Cutting system
- Collection of required cut pieces of explants
- Recognition of the explants
- Pick and place new cuttings into new containers
- Controlled position for containers in, and their segmental recovery from, the growth rooms
- Mechanized plant system for transfer of cultures to *in vivo*
- Environment control system
- Total system integration
- Container coding system

Sterilization

Sterilization in the system consists of two parts, the explant itself and the operational parts. This sterilization is critical for successful culturing. Some chemicals, such as Alcide, NaOCL, hypochlorites and mercuric chloride have been used widely, not only in the past but also in recent days, because of ease of handling and cost effectiveness.

One of the most recent developments is the hot-air system which used a specific high temperature with compressed air. In some situations where mechanical operations are used, such as cutting plants by mechanical knives, the hot air system has strong advantage over any other method. An automated system needs a fully automated sterilization system not only for surface sterilization of explants but also for the whole system.

This may be a significant limitation of automization itself.

Media preparation

The batch system has been used widely since the beginning of micropropagation. Fari (1987), PPS (1992), Hitach (1987) made significant guess towards automation, most of which was focused on productivity increase through sterilization. Major consideration should be focused on:

- Container: size, shape, material
- Medium: temperature, concentration, viscosity
- Other methods: autoclave
- Contamination in process
- System requirement
- Recycling

Liquid nutrient is most suitable for automation except for use with self standing structured explants. Many researchers have reported widely on the use of this medium; however, results of long-term effects are not yet revealed. Effects of aeration, contamination, survivability, vitrification and cycling, for example, may be amongst subjects to be explored. The system basically requires mixing, heating, cooling, dispensing, feeding, weighing, sterilizing and storing.

Robotization

In de Bry (1986), the possibility of using robots was first explored. Following this, many contributions have been made in this field as outlined in Miles (1989), Miwa (1987) and Fujita (1989).

The cost of robots is considerably high, ranging from hundreds of thousands of dollars to several million dollars. Despite their cost, robots can minimize unpleasant, boring or unsafe work. They can effect reduced labor costs, increased productivity, reduced contamination and can last a long time.

With the help of computer systems, robotization can be more easily adopted *in vitro* as well as *ex vitro* as a future possibility. A robot hand (or gripper, or effector) is essential and the most complicated tool to be developed. American researchers seem to be closer to middle type finger, while Japanese to SMA (shape memory alloy). Recent developments have been reported by PPS with soft-air-touch technique and a laser cutting system.

Future prospects for robots in tissue culture will depend on cost effectiveness. There may be alternatives for using simple manipulators instead of full robotization in tissue culturing.

Laboratory design

The needs of tissue culture laboratories differ from the requirements of ordinary buildings. Therefore, careful design is necessary to eliminate problems associated with plants. Something like the 'clean room', well known in semiconductor industries, is an example, to avoid biological contamination of sensitive materials. Buildings need enough space for various types of work, workers, preparation, storage, growing, washing, etc. Also, additional support facilities are needed; e.g., electricity, water, gas, air-conditioning and compressed air. Details relating to setting up a tissue culture laboratory have been published in many sources. One of the most recent developments is that described by Hartmann et al., (1990).

IN KOREA

In Korea, rice, the staple food has supported itself since about the 1980's. The importance of vegetable plants is now secondary in importance. Cereals, which rank next to rice, are easily available to local markets.

With regard to the general status of Korea, micropropagation is being studied as a small scale constituent technique in Labs. Automation or mechanisation of tissue culture is not yet at a practical level economically, and technologically.

Recently a national plan 'BIOTECH 2000' was established, indicating the nation's efforts in plant production. This plan is similar to that of the Biotech Research Initiative of U.S.A and the Mobilization Program of France.

There have been some micropropagation achievements in potato microtuber prduction techniques. One project is a totally automated operating system of micropropagation for potato

microtuber production. The research of agricutural robots is another new subject of interest to researchers exploring a robot system for plantlet production. Other automization project at beginning stages, seem to have potential to be more active in the near future. (see table 2)

RESULT AND DISCUSSION

Automation of micropropagation will reduce labour costs, making plants more price competive. Feasibility evaluation for up to 1 million plants per year will be the first step, and production capacity of more than 5 million plants should be a breakthrough benchmark for serious automation.

More technological aspects in the future, concerning mechanical approaches must include consideration of the following:

- Gas permeable container
- Shape and size of container
- Sterilization method
- Effective cutting method
- Liquid or liquified media
- System integration with a streamlined process
- Computer capacity and its adaptation

At this moment, there is no perfect system in the world for the automation of plant tissue cultures. Future prospects will depend on a thorough understanding amongst interested scientists in the matching of automation to plant growth and development.

Only standardized and automized plants can be objects for automation.

REFERENCES

1. Levin R., Gaba V., Tal B., Hirsch S., Nola D., Vasil IK.. 1988. Automated plant tissue culture for mass propagation. Bio/Technology 6:1035-1040
2. Deleplanque H., Bonnet P., Postaire JPG.. 1985. An intelligent robotic system for in vitro plantlet production. Rovises 5:305-314
3. Maurice V., Vandercook CE., Tisserae B.. 1985. Automated plant surface sterilization system. Physiol Veg 23:127-133
4. Vandercook CE., Tisserae B.. 1985. Development of an automated plant culture system. Plant Cell Tissue Organ Culture 5:107-117
5. Wolf L.J., Hartney V.J., 1986. Computer system to assist with management of a tissue

culture laboratory. N Z J For Sci 16:392-402
6. Cooper P. A., Giant J. E. etc. 1992. Development of a prototype automated cutting and placing system for tissue culture multiplication. Combined Proceedings International Plant Propagators' Society, Volume 42. 1992
7. J. Aitken-christie. 1991. Automation. Micropropagation. Kluwer Academic Publishers: 363-388
8. D. P. Holdgate. etc. 1992. Automated micropropagation and the application of a laser beam for cutting. Transplant Production System. 297-311
9. Lins Kerr. etc. RACHEL. An automated tissue culture system. Lincoln University
10. Pierik R.L.M. 1991. Commercial micropropagation in Israel. Micropropagation, Kluwer Academic Publishers: 155-165
11. Hempel M. etc . 1991. Commercial production in Poland and other Eastern Europe countries. Micropropagation, Kluwer Academic Publishers: 167-171
12. Zimmerman R.H. etc. 1991. Commercial micropropagation in North America. Micropropagation, Kluwer Academic Publishers: 173-179
13. Gavinlertvatana P. etc. 1991. Commercial micropropagation in Asia. Micropropagation, Kluwer Academic Publishers: 181-189
14. Handro W. etc. 1991. Commercial micropropagation in South and Central America. Micropropagation, Kluwer Academic Publishers: 199-203
15. Novak . 1991. Micropropagation and plant tissue culture in developing countries of Africa. Micropropagation, Kluwer Academic Publishers: 205-213
16. Miles GE. 1989. Robotic transplanting for tissue culture In Vitro Cell Dey Biol 25: 22A, 46
17. Miwa Y. 1987. Plant tissue culture robot operated by a shape memory alloy actuator and a new type of sensing, In: Moet Hennessy (ed) Electronics and Management of Plants, Paris,7
18. Fujita M. 1989. Application of robotics to mass propagation system, In Vitro Cell Dev Biol 2:22A, 47
19. Fari M. 1987. Development of technical and biological basis of micropropagation toward automation and industrialization. Isn: Moet Hennessy (ed) Electronics and Management of Plants. Paris: 55-56
20. Hitachi. 1987. Sterilization of culture vessel and culture medium by an electrical method-as an alternative to autoclaving. Japanese Parent No.J62122-579
21. Research proposal, Automization of micropropagation, prepared by Korea Institute of Machinery and Materials. May. 1996
22. Master plan, "Biotech 2000". The Ministry of Science and Technology.
23. Lue De Bry. 1988. Prospects in the mating of robotics to plan culture and research. Jacobs Suchard R & D.

Table 1. Total number of plants produced by micropropagation method in 1988

Western Europe (15)	212.5	248
Eastern Europe (5)	15.0	120
North America (2)	150.0	250
Asia (12)	74.0	145
South and Central America (20)	50.0*	100*
Africa (23)	2.0	50*
Total = 460		

( ) number of countries located in

* author's estimate

Table 2. Micropropagation research institution in Korea

Institutions	Name	research fields
Universities	Baeje	culture of Chinese cabbage medicine
	Chungnam	transformation, robot
	Chungbuk	flower, vegetables
	Korea	gene search
	Kyungbuk	flower, gene transformation gene regeneration, robot
	Seoul	transformation
	Sunchunhyang	production of secondary metabolism pesticide resistant of gene
	Gaengsang	flower, gene transformation gene regeneration
Research Institute (semi-government)	KIMM	total system
	KGTRI	ginseng and tobacco
	KRIBB	potato microtuber
Research Institute (government)	Provincial RDA	vegetable
	HRI, RDA	flower, vegetable
	FGRI	tree
	AES, RDA	popato, vegetable
Companies	Dongyang moolsan	garlic
	Hungnong seed	vegetable
	Seoul seed	vegetable

KIMM - Korea Institute of Machinery and Materials
KGTRI - Korea Ginseng and Tobacco Research Institute
KRIBB - Korea Research Institute of Bioscience and Biotechnology
FGRI - Forest Genetics Research Institute
Provincial RDA - Provincial Rural Development Administration
HRI, RDA - Horticultural Research Institute, RDA
AES, RDA - Alpine Experiment Station, RDA

Figure. 1. Basic proces of micropropagation