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Photoautotrophic micropropagation- an advanced micropropagation technique

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Introduction

Micropropagation, an *in vitro* vegetative propagation method using pathogen-free propagules has been considered significant in agriculture, horticulture and forestry for producing pathogen-free stock plants or genetically superior clones that cannot be propagated by seeds or whose propagation efficiency is low in conventional vegetative propagation. However, the widespread use of micropropagated transplants is still limited by high production costs, mostly attributed to a low growth rate, a significant loss of plants *in vitro* due to microbial contamination, poor rooting, low percent survival at the *ex vitro* acclimatization stage and high labor costs.

In vitro aerial conditions in conventional micropropagation are affected by physical properties of the vessels, environmental conditions outside the vessel (inside the culture room) and plantlets (photosynthesis, transpiration, etc.) and generally characterized as having (1) low CO_2 concentration during the photoperiod, (2) high CO_2 concentration during the dark period, (3) low water vapor pressure deficit (high relative humidity), (4) low air current speed and (5) low photosynthetic photon flux (PPF). All these factors lead to non-functional stomata and limited epicuticular wax deposition on the leaves. Thus, immediately after transplantation, rapid water loss due to uncontrolled transpiration occurs, followed by wilting. Therefore to overcome the problems in conventional micropropagation a novel tissue culture technique of Photoautotrophic Micropropagation has been developed.

Concept of Photoautotrophic micropropagation

Recent research has revealed that most chlorophyllous explants/plants *in vitro* have the ability to grow photoautotrophically (without sugar in the culture medium) 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 and growth rates of plants *in vitro*. **Photoautotrophic micropropagation** refers to 'Micropropagation with no exogenous organic components (sugar, vitamins, etc.) added to the medium, and it has been developed along with the development of techniques of *in vitro* environmental control' (Kubota *et al*., 2001). Vitamins, growth regulators and gelling agent are not added to the medium, instead of gelling agents, porous substance like vermiculite should be employed as supporting materials. CO_2 concentration, photosynthetic photon flux, relative humidity, and air speed in the vessel are some of the most important environmental factors affecting plantlet growth and development; controlling these factors requires knowledge and techniques of greenhouse and horticultural engineering as well as the knowledge of physiology of *in vitro* plantlets. This technique for the 1st time was invented by **Dr. Toyoki Kozai** of Chiba University, Japan.

Photoautotrophic micropropagation of chlorophyllous plants can not only increase the growth of *in vitro* plantlets, but also minimize the risk of loss due to microbial contamination, reduce production costs, improve the physiological characteristics of the plantlets and enable better acclimatization of plantlets *ex vitro*. Photoautotrophic micropropagation has many advantages with respect to improvement of plantlet physiology (biological aspect) and operation/management in the production process (engineering aspect).

Advantages with biological aspects are

(1) Promotion of growth and photosynthesis

(2) High survival percentage / smooth transition to *ex-vitro* environment

(3) Elimination of morphological and physiological disorders

(4) Little loss of plantlets due to contamination.

Advantages of engineering aspects include

(1) Flexibility in the design of the vessel (larger vessels)

(2) Automation

(3) Simplification of the micropropagation system.

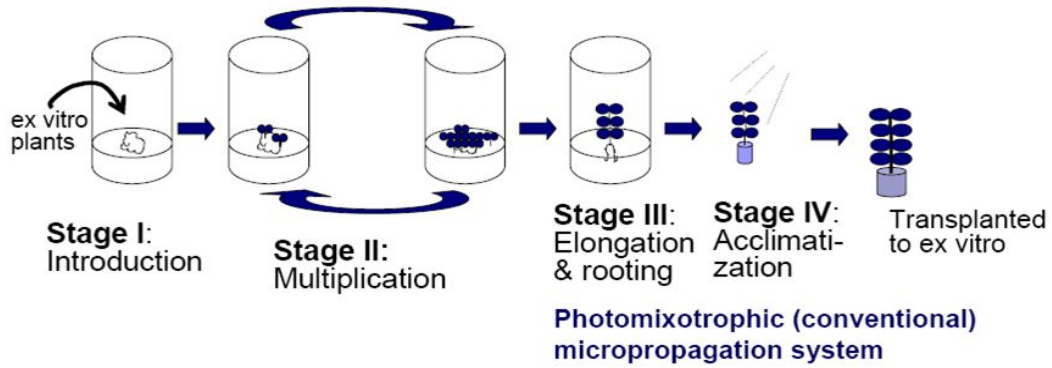
Constraints in photoautotrophic micropropagation

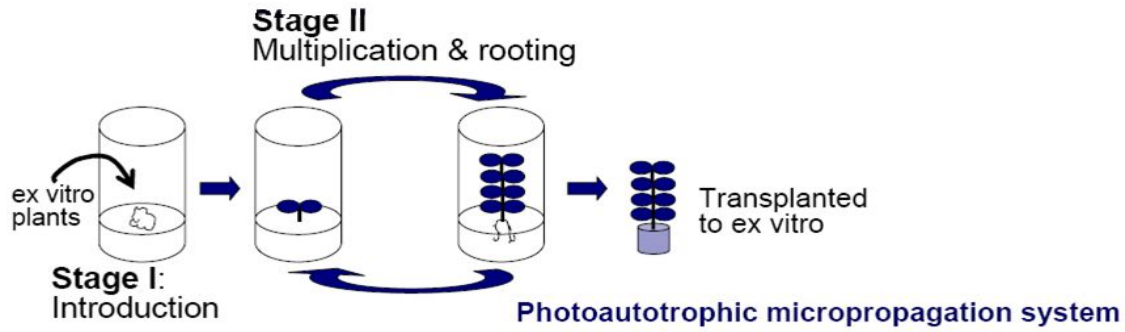
(1) Relative complexity of techniques and knowledge required for controlling the *in vitro* environment

(2) Expense for lighting, CO_2 enrichment, and cooling

(3) Limitation of application to multiplication systems using multiple buds/shoots.

Comparison between photoautotrophic and photomixotrophic micropropagation with respect to different stages





Successful photoautotrophic micropropagation also requires knowledge of when cultures should transit from photomixotrophic into photoautotrophical status. Developmental and operational stages for conventional micropropagation are generally classified into four for conventional micropropagation.

For photoautotrophical micropropagation, the number of classified stages may be less than in conventional photomixotrophic micropropagation, since multiplication and rooting stages are often combined as one stage in photoautotrophic micropropagation by reproducing photosynthetically, only the introduction /initiation stage of the culture must be under photomixotrophic conditions where pathogen free culture are established by culturing meristematic tissue. Once chlorophyllus are developed and are also to conduct photosynthesis, the culture is ready to move on to photoautotrophic micropropagation conditions. The acclimatization stage is often eliminated when plantlets are grown under optimal

photoautotrophic conditions. Thus, a photoautotrophic system could exclusively consist of two stages- Initiation (stage I) and Multiplication/rooting (stage II).

It has been reported by many workers that the *in vitro* and *ex vitro* growth and net photosynthetic rate of plantlets can be enhanced with the use of different film culture systems or larger vessels. The Vitron film culture system (in *Epidendrum*) and scaled-up vessel (in *Eucalyptus camaldulensis*) were better over the conventional culture vessels (Jaime *et al.*, 2005; Zobayed *et al*., 2000).

The survival percentage after transplanting the plants can be increased, as it was proved in threatened plant species, where by conventional micropropagation method gave 50 per cent survival and that in photoautotrophic micropropagation it was more than 80 per cent (Sarasan, 2010).

Conclusion

Photoautotrophic micropropagation would be necessary for the future development of transplant production. The outcomes of research and development in photoautotrophic micropropagation will contribute to improvement and trouble shooting in future agriculture, horticulture and forestry production system.

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