Photoautotrophic Micropropagation: Importance of Controlled Environment in Plant Tissue Culture©

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Micropropagation is a method to produce genetically identical plantlets by using tissue culture techniques. 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. 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. Photoautotrophic micropropagation has many advantages with respect to improvement of plantlet physiology (biological aspect) and operation/management in the production process (engineering aspect), and it results in reduction of production costs and improvement in quality of plantlets.

INTRODUCTION

Micropropagation, an in vitro vegetative propagation method using pathogen-free propagules, has been considered significant in agriculture 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. Recent research, however, 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.

ENVIRONMENTAL CONTROL IN MICROPROPAGATION

Most of the factors that bring about the high production costs are directly or indirectly related to the heterotrophic or photomixotrophic characteristics of plant growth in vitro in conventional, heterotrophic or photomixotrophic micropropagation. In conventional micropropagation, sugar in the culture medium is the main or sole source of carbon and energy for plant growth in vitro. The supply of sugar to the culture medium to promote plant growth in vitro has been considered to compensate for the low or negative net photosynthetic rate of plants in vitro, and the poor photosynthetic ability of plants in vitro is a main reason for the low or negative net photosynthetic rate.

For successful photoautotrophic micropropagation, understanding the in vitro environment and basics of environmental control is critical. For plants growing pho-

toautotrophically, promotion of photosynthesis is the primary way to enhance the growth rate of the plantlets. To promote in vitro photosynthetic rates, it is necessary to know the status of environmental conditions (for example, air temperature and CO_2 concentration) in the vessels and how to maintain them in optimum ranges for maximizing net photosynthetic rates of the plantlets. Lack of understanding of the in vitro environment or of the interaction of plantlets and the in vitro and ex vitro environments, makes it more difficult to improve the micropropagation system by applying the photoautotrophic micropropagation method.

CHARACTERISTICS OF IN VITRO ENVIRONMENTAL CONDITIONS IN THE CONVENTIONAL PHOTOMIXOTROPHIC MICROPROPAGATION

In vitro aerial conditions 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 a: (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). As shown in Fujiwara et al. (1987) and other reports, the typical diurnal change in CO_2 concentration in a conventional culture vessel containing chlorophyllous plantlets is characterized with a linear increase during dark period followed by a sharp decrease within a few hours after onset of photoperiod to reach as low as the CO_2 compensation point. This decrease in CO_2 concentration clearly shows that the chlorophyll-containing plantlets in vitro retain high photosynthetic ability, but the low CO_2 concentration, caused by the limited ventilation of the vessel, forces the plantlets to grow photomixotrophically (using sugar as a supplemental source for energy and carbon). Enhancing ventilation is therefore the first important key to success in photoautotrophic micropropagation. Increasing the CO_2 concentration inside the growth chamber (CO_2 enrichment), high photosynthetic photon flux (PPF), and use of porous supporting materials have been recognized as significant factors, in addition to enhanced ventilation, for success in photoautotrophic micropropagation.

ADVANTAGES AND DISADVANTAGES OF PHOTOAUTOTROPHIC MICROPROPAGATION

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, and (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, and (3) simplification of the micropropagation system. Disadvantages of photoautotrophic micropropagation often considered when introducing the technique in commercial operations are: (1) relative complexity of techniques and knowledge required for controlling the in vitro environment; (2) expense for lighting, CO_2 enrichment, and cooling; and (3) limitation of application to multiplication systems using multiple buds/shoots. Kozai (1991) and Kubota (2001) summarize more details of the advantages/disadvantages of photoautotrophic micropropagation.

Figure 1. Tomato plantlets cultured (left) photoautotrophically under high PPF, vessel ventilation rate and CO_2 concentration without explant (nodal cuttings) leaf removal, and photomixotrophically under low PPF, vessel ventilation rate and CO_2 concentration with (right, the conventional method) and without (center) explant leaf removal.

APPLICATIONS OF PHOTOAUTOTROPHIC MICROPROPAGATION

For the past 20 years, photoautotrophic micropropagation was successfully applied to many plant species including acacia (*Acacia mangium*), cauliflower (*Brassica oleracea*), chrysanthemum (*Chrysanthemum morifolium*), citrus (*Citrus macrophylla*), coffee (*Coffea arabusta*), eucalyptus (*Eucalyptus camaldulensis*), grapevine (*Vitis vinifera*), potato (*Solanum tuberosum*), red raspberry (*Rubus idaeus*), sugarcane (*Saccharum* spp.), sweetpotato (*Ipomoea batatas*), tobacco (*Nicotiana tabacum*), and tomato (*Lycopersicon esculentum*). Figure 1 shows tomato plantlets cultured under photoautotrophic conditions, compared with those under photomixotrophic conditions for the same culture period (Kubota et al., 2001). The culture conditions were combinations of high ventilation of the vessels with/without CO_2 enrichment and high PPF. Use of porous supporting materials is more significant for woody plant species that are generally slow and difficult to root during in vitro culture (Kozai and Kubota, 2002). Figure 2 shows a scale-up system for photoautotrophic micropropagation of eucalyptus (Zobayed et al., 2000).

A relatively new application of the photoautotrophic micropropagation method is somatic embryogenesis, which is a key technology for mass production of elite clones and has been introduced commercially, producing transplants for planting in clonal forestry. One of the challenges preventing wider application of somatic embryos is low percent germination of somatic embryos and conversion to plantlets. *Coffea* somatic embryos were shown to have photosynthesis ability at the cotyledonary stage (Afreen, 2001) and both growth and development (conversion/germination) of somatic embryos were enhanced under photoautotrophic conditions when CO_2 concentration was properly controlled (Uno and Kubota, unpublished) (Fig. 3).

CONCLUSION

Photoautotrophic micropropagation is an advanced plant production technique that emerged as an integration of biology and engineering for practical applications. Such integration will be necessary for the future development of transplant production systems. The outcomes of research and development in photoautotrophic micropropagation will contribute to improvement and problem-solving in future agriculture, forestry and horticultural production systems.

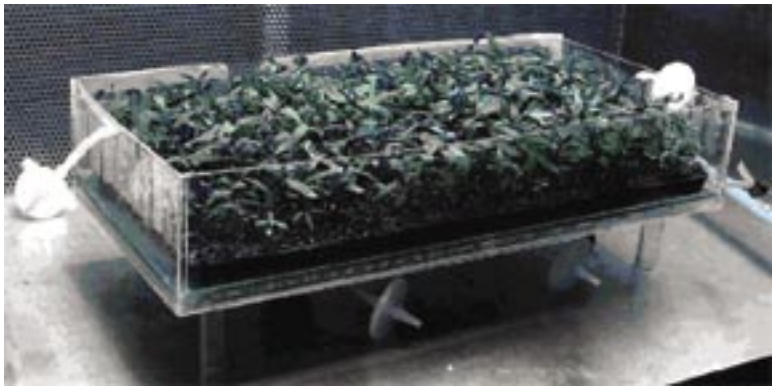

Figure 2. Scaled-up photoautotrophic culture vessel (picture taken after removal of the lid). The vessel was 610 mm long, 310 mm wide, and 105 mm high (volume approx. 20 liters) (By courtesy of Zobayed; for descriptions refer to Zobayed et al., 2000). The CO_2-enriched air is pumped into the vessel through the inlets into the air distribution chamber beneath the plug tray. A nutrient solution in the reservoir (not shown in the picture) is sent to the root zone to soak the vermiculite-based supporting material in the plug tray and returned to the reservoir as scheduled with a timer.

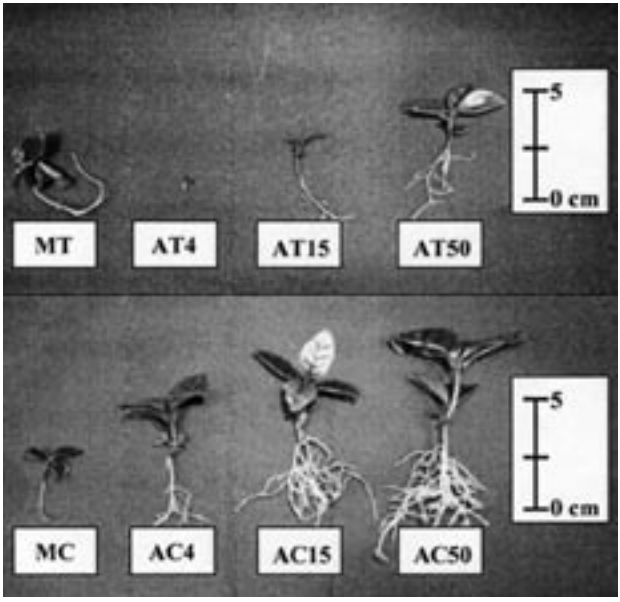

Figure 3. Plantlets or cotyledonary embryos obtained from torpedo-shaped (upper picture) and cotyledonary embryos (lower picture) cultured for 61 days under photomixotrophic [indicated as "MT" and "MC"; sucrose in an agar-gelled medium, low CO_2 concentration and low photosynthetic photon flux (PPF)] and photoautotrophic ("AT" and "AC"; no sugar in the medium, porous supporting materials, high CO_2 and high PPF) conditions (Uno and Kubota, unpublished). Numbers in treatment codes indicate the CO_2 concentration (400, 1500, or 5000 $\mu mol \cdot mol^{-1}$).

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Biogeography of Mycorrhizal Fungi and Their Use in Ornamental Container Production©

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Mycorrhizae – from the Latin words myco and rhizi, meaning fungus and roots – refers to a partnership association between plants and soil-dwelling fungi. Horticulturists consider mycorrhizae as beneficial when both partners realize a net gain from the association. Sometimes colonization of plant roots by mycorrhizal fungi results in an enhancement of plant growth. Mycorrhizae play an important role in plant nutrition and the stability of plant communities. Three types of mycorrhizae are most common: ectomycorrhizae, ectendomycorrhizae, and endomycorrhizae. Our research focuses exclusively on a class of endomycorrhizae called arbuscular mycorrhizal or AM fungi.

Arbuscular mycorrhizal fungi have unique vegetative and reproductive life stages. During their vegetative stage, they develop a hyphal matrix (or mycelium) comprised of runner, penetration, or absorbing hyphae that extend out from the surface of colonized fine lateral roots. Runner hyphae follow growth of fine lateral roots as they grow in the soil. Sometimes runner hyphae grow out into the soil probing for more roots. Penetrating hyphae branch off runner hyphae and infect fleshy young roots usually within a short distance of the root growing tip. Once inside the root, these hyphae pierce cortical cell walls and form arbuscules between the root cell wall and cell membrane, called the plasmalemma. Arbuscules are organs for exchanging nutrients with root cells. In addition to arbuscules, penetrating hyphae also produce storage organs called vesicles in spaces between root cells. Finally, absorbing hyphae branch off of runner hyphae in a fan-like pattern. They grow into soil surrounding the infected root and absorb water and nutrients. The absorbed water and nutrients subsequently move to the penetrating hyphae and into the infected root.