PHOTOAUTOTROPHIC (SUGAR-FREE MEDIUM) MICROPROPAGATION AS A NEW MICROPROPAGATION AND TRANSPLANT PRODUCTION SYSTEM

Photoautotrophic (sugar-free medium) Micropropagation as a New Micropropagation and Transplant Production System

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Preface

This book provides two basic concepts on plant propagation and value-added transplant production in a closed structure with artificial lighting: 1) photoautotrophic (sugar-free medium, photosynthetic or inorganic nutrition) micropropagation systems, and 2) closed transplant production systems with minimum resource consumption and environmental pollution. This book also describes the methodology, technology and practical techniques employed in both systems, which have been commercialized recently in some Asian countries such as China and Japan.

We often use a closed structure such as a tissue culture vessel, a culture room, a growth chamber, a plant factory with lamps, and a greenhouse to propagate plants and produce transplants. Main reasons why we use such a closed structure is: 1) higher controllability of the environment for desired plant growth, 2) easier protection of plants from damage by harsh physical environment, pathogens, insects, animals, etc, 3) easier reduction in resource consumption for environmental control and protection, and 4) higher quality and productivity of plants at a lower cost, compared with the plant propagation and transplant production under rain, wind and sunlight shelters and in the open fields.

Thus, there should be some knowledge, discipline, methodology, technology and problems to be solved on plant propagation and transplant production common to those closed structures, regardless of the types and sizes of the closed structure. Currently, however, there are not much discussion and consideration common to those closed structures, and there are few academic information and personnel exchanges among researchers in the fields of plant tissue culture, micropropagation, plant factory, greenhouse, etc. This is an ideal book that spans topics from physical state of culture environment to commercial application of photoautotrophic micropropagation. The book aims at providing the concepts, methodology, technology and practical techniques common to various kinds of plant propagation and transplant production.

Generally, in plant tissue culture and micropropagation, sugar, vitamins and amino acids are added to the culture medium, and explants and plants are grown *in vitro* heterotrophically or photomixotrophically. Fluorescent lamps are mostly used as the light source and maximum light intensity is around 100 μmol m^{-2} s^{-1}. Leafy part of an explant is often removed before transplanting on the culture medium, because it does not play an important role for its further growth and development. Most research papers on plant tissue culture deal with effects of different combinations of plant growth regulators and organic nutrients in the culture medium on plant growth and development *in vitro*.

On the other hand, in plant production using a greenhouse, a nursery and a plant factory, inorganic nutrient components only are supplied to the soil, substrate or water, and plants are grown photoautotrophically (or photosynthetically). Sunlight is mostly used as the sole light source and maximum light intensity under sunlight is about 1,000 μmol m^{-2} s^{-1}. A leaf is an essential part of seedlings and leafy cuttings for their photosynthetic growth. There is not much research on the effects of plant growth regulators supplied in the soil or substrate on the plant growth and development. Why

materials and methods in greenhouse crop research are so different from those in plant tissue culture and micropropagation research?

This is the first book which resolves these questions and also presents the unique combination of biological and engineering perspectives on photoautotrophic or sugar-free medium micropropagation system. The chapters involve the current innovative researches on control and optimization of the *in vitro* microenvironment, plant production in closed structure, and advances evidence on how some underrated aspects of the process actually determine the status of the final product. Chapter 2 provides information about the appropriate use of SI units, symbols and terminology for the environmental studies of plant tissue culture. Chapter 6 illustrates the physiological and anatomical features, which have a correlation with the physical factors of microenvironment and determines the quality of transplants. In chapter 14 a handful of plant propagation and cost efficiency related questions and answers are included to encourage readers to "have a go".

I have been a greenhouse researcher for the past 35 years, specializing in environmental control. Besides being so, I started a research project on measurement, analysis and control of physical environment in plant tissue culture for promoting plant growth *in vitro* 20 years ago. Recently, I have been working also on environmental control of plant factories using lamps as the sole light source.

When I started the research project on micropropagation, I wondered why all tissue culturists were using the culture medium containing sugar and other organic nutrients, even when they grew green-colored, leafy plants. Because, the plants were similar in size to the seedlings or transplants with unfolded cotyledonary leaves and leafy cuttings grown in a greenhouse and in the open fields. I was surprised that most plant tissue culturists were not interested in controlling the light intensity, relative humidity, CO_2 concentration and air movement in the culture vessel, which were the most important environmental factors in greenhouse environment control.

These ideas prompted us to find out the environmental factors restricting *in vitro* plants to grow on the sugar-free medium under pathogen free-condition. Then, we discovered that low CO_2 concentration in the vessel during the photoperiod was the main factor restricting the photosynthesis of plants *in vitro*. Step by step, we improved the relative humidity, light intensity, air movement, etc. in the vessel to promote the plant growth and enhance the plant vigor. Thus, I became confident that we could grow the green-colored, leafy plants *in vitro* on the sugar-free medium by properly controlling the *in vitro* environment for promoting photosynthesis. Finally, we developed a photoautotrophic micropropagation system using a large, forcedly ventilated and/or CO_2 enriched culture vessel containing sugar-free and inorganic medium, which looks like a miniature greenhouse, but under disease-free and artificial light conditions. These aspects are illustrated in Chapter 4 and 9.

During the research, we got an idea of a closed transplant production system. This system is a warehouse-like structure covered with opaque thermal insulators, in which ventilation is kept at a minimum, and artificial light is used as the sole light source for plant growth. The closed system is ideal for growing disease-free and vigorous transplants or small plants under pathogen-free and optimized environmental conditions, using minimized resource including fossil fuels, water, labor, time, and space. A detailed appraisal of which is given in Chapter 17.

I hope that this book will prove to be a useful one to researchers, graduate students and industry people in the fields of tissue culture, micropropagation, greenhouse horticulture, plant factory, controlled environment agriculture and forestry, readers who are interested in plant propagation and value-added transplant production for solving global and local problems on environmental conservation, food, feed and biomass production, and fossil-fuel resource saving by use of bio-resources.

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