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Effects of CO_2 concentration and moisture content of sugar-free media on the tissue-cultured plantlets in a large growth chamber

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Abstract

The dynamic fluctuations of CO_2 concentration in the tissue culture growth chamber after transplantation of petunia, chrysanthemum and tomato plantlets were recorded with a real-time control system to determine the critical CO_2 concentration levels of 35 $\mu l\, l^{-1}$ at which CO_2 enrichment is needed. The experimental data showed that the tissue-cultured plantlets of petunia, chrysanthemum and tomato had the same CO_2 concentration dynamics. The results indicated that CO_2 enrichment was proper on the second day after transplantation. Petunia plantlets were used to conduct experiments under PPFD of 80 $\mu mol\, m^{-2}\, s^{-1}$, and CO_2 concentrations of $350 \pm 50\ \mu l\, l^{-1}$, $650 \pm 50\ \mu l\, l^{-1}$ and $950 \pm 50\ \mu l\, l^{-1}$ as well as medium moisture contents of 60%, 70% and 80%, with the result that plantlets grew better under CO_2 concentration of $650 \pm 50\ \mu l\, l^{-1}$ than under the other two concentrations with all the different media water contents. Three media water contents under the same CO_2 concentration produced plantlets with the same quality. The impacts of CO_2 concentrations on plantlets are more important than those of the media water contents. Sugar-free tissue culture, as compared with the conventional culture, showed that CO_2 enrichment to $350 \pm 50\ \mu l\, l^{-1}$ can promote the growth of the cultured plantlets. Sugar-free tissue culture produced healthy plantlets with thick roots, almost equivalent to the common plantlets.

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Keywords: Sugar-free tissue culture; Petunia; Chrysanthemum; Tomato; CO_2 enrichment

Abbreviations: CO_2, carbon dioxide; PPFD, photosynthetic photon flux density; MS, Murashige and Skoog medium.

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1. Introduction

Tissue culture is featured with many advantages such as rapid propagation of plants, massive production of disease- and virus-free seedlings and preservation of plant species resources [1,2]. However, the conventionally and commonly used tissue culture practices are typically based on sugar-added mediums, which are in favor of the rapid development and growth of bacteria in the culture medium to eventually contaminate the growth environments of plants. To reduce and/or avoid the intrusion and propagation of disease-inducing bacteria into the growth medium, the tissue culture containers have to be put into air-tight and closed chambers, or antimicrobial has to be put into the containers, which slow down the growth rate of plantlets, physiological disorders and informalities. Furthermore, excessive usage and random disposure of the antimicrobial and disinfectant will pollute the environment, and even devastate the ecosystem. All these disadvantages have been affecting the rapid development and popularization of the tissue culture production [3]. Fujiwara et al. [4] indicated that a too low illumination intensity and a too low CO_2 concentration in the tissue culture chambers are the main reasons for the incapability of seedlings' photosynthesis and auto-supply of nutrients. In this study, a new tissue culture concept, sugar-free tissue culture, was proposed by integrating it with environmental control techniques. The new method replaces sugar with CO_2 to meet the demands of plant growth on carbine resources, to make full use of engineering measures to regulate the cultural environmental factors such as gases, illumination intensity, humidity, etc., so as to promote plant growth under auto-supplied nutrients and improve the quality and survival rate of seedlings, thus reducing the turnover period [4–8]. In addition, there is no sugar in the culture medium so that the tissue is hardly is contaminated. Because of the reduced usage of the antimicrobial, disinfectant and hormone, sugar-free tissue culture will be a environmentally friendly method.

Later, researchers have conducted various experiments related to the sugar-free tissue culture, involving the illumination intensification, CO_2 concentration enrichment, introduction of large-scale culture vessels with gas-permeable film, and recipes of sugar-free cultural mediums. Many different types of growth vessels were designed and developed [9–11], in which CO_2 were enriched indirectly. Tissue cultural containers covered with gas-permeable sheet were put into large-scale growth chambers, where CO_2 is added to indirectly increase the CO_2 concentration in the tissue cultural containers in the chambers. It is imaginable that to increase the CO_2 concentration in the containers, the CO_2 concentration in the chambers where the containers are put must be much higher than that desired in the containers. Kozai noted that when the CO_2 concentration in the growth chamber reached 1890 $\mu l\, l^{-1}$, the CO_2 concentration in the tissue cultural containers was only 363 $\mu l\, l^{-1}$, equivalent only to the atmospheric CO_2 concentration [12]. Though it is possible to use forced ventilation to regulate the micro-environment for gas enrichment in the tissue cultural containers, the limited volume, normally less than 20 L, and the expensive electronic components associated with high operational costs limit the wide application of container ventilation [13].

If it is possible to tissue culture plantlets in the open growth chamber without the tissue cultural container, plantlets are grown in the open chamber where CO_2 of high purity and high pressure is added and regulated to the desired levels. This surely would make operational managements easy and lower the production cost, which will promote the popularization of the sugar-free propagation technique.

The objectives of this study were: (1) to monitoring the CO_2 consumption dynamics and the critical CO_2 concentration level at which CO_2 has to be added before photosynthesis of plantlets is maintained during the initial stage of tissue-cultured plantlets; (2) to quantify the influences of the enriched CO_2 concentration and the media moisture content on sugar-free tissue-cultured seedlings in a large-scaled open growth chamber; and (3) to demonstrate the possibility of tissue culture with sugar-free media plantlets grown in an open growth chamber under CO_2 enriched environments.

2. Materials and methods

The materials used in the experiments were tomato, petunia and chrysanthemum plantlets. The conventionally tissue-culturally micro-propagated tomato, chrysanthemum and petunia steams were cut into pieces of about 1 cm long with 1 leaf (tomato), 2 leaves (chrysanthemum) and 2–3 leaves (petunia) before they were transplanted to medicine trays of $15 \times 22 \times 4$ cm filled with vermiculite as the culture media. The weight of