Plant Cell, Tissue and Organ Culture (2005) 83: 251–257
DOI 10.1007/s11240-005-6839-2

Manipulation of the culture environment on *in vitro* air movement and its impact on plantlets photosynthesis

Yoshiaki Kitaya[1,2,*], Yoshitaka Ohmura[1], Chieri Kubota[3] & Toyoki Kozai[1]
[1]*Department of Bioproduction Science, Chiba University, 271-0092, Matsudo, Chiba, Japan;* [2]*Graduate School of Life and Environmental Sciences, Osaka Prefecture University, 599-8531, Gakuen-cho, Sakai, Osaka, Japan;* [3]*Department of Plant Sciences, The University of Arizona, Tucson, AZ 85721-0036, USA (*requests for offprints: E-mail: kitaya@envi.osakafu-u.ac.jp)*

Received 16 November 2004; accepted in revised form 2 May 2005

Key words: air movement, micropropagation, photosynthesis, plant tissue culture, potato

Abstract

Two-dimensional air current speeds in the culture vessel were measured using a tracer-based visualization technique and the effect of the air movement in the culture vessel on the photosynthesis of *in vitro* potato plantlets was assessed under a photoautotrophic culture condition. The air current speeds inside the vessel were varied by controlling free convection induced by spatial variations of temperatures in the culture vessel. For all conditions examined, upward air currents were observed around the plantlets in the central part of the culture vessel and downward air currents were observed near inside walls in the culture vessel. The upward and downward air currents were restricted by the presence of the plantlet. The upward air current speeds were affected by plantlet size inside the vessel and it was 24, 8 and 4 mm s^{-1} in culture vessels with no plantlets, a 10-mm-tall plantlet and a 60-mm-tall plantlet cultured inside the vessel, respectively. The upward air current speed was increased by 2 times by increasing wind velocity above the culture vessel from 0.1 to 1.0 m s^{-1}. Placing the black plate on the medium also increased the air current speeds by 1.5 times. The net photosynthetic rates of the plantlets increased from 2.0 to 2.5 μmol m^{-2} s^{-1} as the upward air current speed in the culture vessel increased from 2.4 to 8.0 mm s^{-1}. The air current speeds in the culture vessel were significantly slow. Enhancement of the air movement in the culture vessel is important to promote photosynthesis of the *in vitro* plantlets.

Introduction

Widespread commercial use of micropropagated transplants is still restricted due to relatively high production costs. Low growth rates of *in vitro* plantlets (called plantlets, hereafter) during multiplication and poor survival of the plantlets during acclimatization were the main reasons of the high costs (Kozai et al., 1992). Recently photoautotrophic micropropagation technique with sugar-free rooting medium has been developed (Kozai, 1991). The photoautotrophic micropropagation technique has advantages over the conventional one since it has less necessity for keeping aseptic environment, higher growth rates, less physiological disorders and higher survival of plantlets, and thus lower transplants production costs than conventional heterotrophic or photomixotrophic micropropagation techniques with rooting medium containing sugar (Kozai and Smith, 1994). The growth rates of plantlets are, however, still lower under the photoautotrophic *in vitro* condition than under growth chamber, greenhouse and field conditions. For example, Kozai et al. (1991) compared the growth of Chinese mustard seedlings *in vitro* and *ex vitro*, showing significantly smaller dry mass of seedlings cultured photoautotrophically in the vessel than that cultured under in

an open culture vessel in the same growth chamber. The low growth rate *in vitro* could be caused by limited photosynthesis, transpiration, water uptake and nutrient uptake of the plantlets *in vitro*.

Restricted air movement around plants generally limits their growth by decreasing the conductance of gases in the leaf boundary layer and thus by decreasing photosynthetic and transpiration rates (Yabuki and Miyagawa, 1970; Monteith and Unsworth, 1990; Kitaya et al., 2000, 2003a). The air movement is much slower in a plant tissue culture vessel (called the culture vessel, hereafter) than in a greenhouse or in a field because the culture vessel is almost airtight (Kitaya et al., 1997). The little air movement may retard gas exchange between plantlets and the ambient air in the culture vessel, and consequently limit plantlets growth. However, there is no research for manipulating air movement inside naturally ventilated culture vessels. The little air movement also retarded gas and heat exchanges between plant leaves and the ambient air under a microgravity condition (Kitaya et al., 2001, 2003b). Therefore enhancement of the air movement is important to promote photosynthesis and transpiration under *in vitro* conditions as well as the microgravity condition.

Photosynthesis and transpiration of the plantlets should be enhanced in order to promote growth of plantlets especially under photoautotrophic conditions. In a relatively small but long culture vessel like a test tube, the air movement due to free convection in the vessel is significantly restricted and thus a significant vertical variation of CO_2 concentration in the vessel is observed (Ohyama and Kozai, 1997). For example, the CO_2 concentration just below the plantlet height was 180–190 μmol mol^{-1} lower than that just below the lid of test tube (350 μmol mol^{-1}). Both CO_2 concentration outside the test tube and PPF affected the vertical profiles of CO_2 concentration inside the vessel. The CO_2 concentration profiles presented by Ohyama and Kozai (1997) showed that restricted air movement resulting in limited diffusion of CO_2 gas in the vessel reduces the flow of CO_2 from the air into the plantlet, thereby reducing net photosynthetic rate. The vertical gradient of CO_2 concentration is not observed in a box type vessel like the Magenta-type vessel in which air movement due to free convection is more enhanced than in the test tube type vessel. However little work has been done to identify and evaluate the effects of the air movement on photosynthesis and transpiration of the plantlets in culture vessels.

There are many reports showing the positive effect of forced ventilation on plantlet growth *in vitro* (Nakayama et al., 1991; Kubota and Kozai, 1992; De et al., 1993a, 1993b; Nguyen et al., 2001; Zobayed et al., 2000; Zobayed et al., 2001). Forced ventilation was considered to modify the environmental elements (e.g. CO_2 concentration and water vapor pressure) and also enhance the air current in the culture vessel.

In the present study, we tried to clarify the entity of the air current and to quantify the contribution of the air current to photosynthesis of plantlets in the culture vessel. The air current speed in the culture vessel was visualized under different conditions with and without plantlets, different colors of the medium surface, and outside wind velocity. The effect of the air current speed in the culture vessel on the net photosynthetic rate of potato plantlets was also investigated.

Materials and methods

Plantlet culture

Magenta-type polycarbonate vessels (370 ml in volume, 75×75 mm^2 in upper area, 61×61 mm^2 in bottom area, and 98 mm in height, each) commonly employed in the commercial micropropagation were used in the present study. Potato (*Solanum tuberosam* L. cv. Benimaru) plantlets were cultured on Murashige and Skoog (1962) sugar-free medium at an air temperature of 23 °C, a PPF of 140 μmol m^{-2} s^{-1} measured at a vessel lid surface with 16 h d^{-1} photoperiod and a CO_2 concentration of 1100 μmol mol^{-1} outside the culture vessel. A gas permeable membrane disc (100 mm^2 in area; Miliseal, Nihon Millipore Kogyo Co., Japan) was attached on the vessel cap. The air exchange rate of the culture vessel was 0.206 ml s^{-1}. Air velocity above the culture vessel was 1.0 m s^{-1}. Thickness of the medium containing agar (8 g l^{-1}) was approximately 10 mm.

Visualization of air movement inside the culture vessels

Fine particles of metaldehyde ($(CH_3CHO)_4$) were used as tracers (Torrance et al. 1969) for

visualization of air movements in culture vessels. The particles were light and highly reflective, and therefore commonly used for a tracer-based visualization of air movement. The bulk density of the particle is about 1.2 kg m^{-3} (Daws et al., 1965) and similar to the density of air at a temperature of 23 °C. Images of particle movements were captured using a video camera (VL-HX1-S, Sharp Co., Japan). An experimental apparatus was set up in a temperature-controlled chamber (Figure 1a). The light source was cool white fluorescent lamps (FPL27EX-N, Matsushita Electric Co., Japan) placed about 120 mm apart from the vessel caps. The measurement vessel containing metaldehyde particles was placed at the center of nine culture vessels. After a steady-state movement of particles was observed in the culture vessel in each measurement condition, a culture vessel that situated between the measurement culture vessel and the video camera was removed, and immediately, video images of the measurement culture vessel were taken from the front side for approximately 5 s (Figure 1b). During this 5s measurement, particles continued to move in a steady state, maintaining the same pattern of the air current inside the vessel.

Although the light source used before the image acquisition was fluorescent lamps, the video images were taken under an illumination from a tungsten lamp (1.25 W) for creating a better contrast to analyze the particle movement. The measurement vessel was spotlighted with the tungsten lamp under a reflector only during the time video images were captured. The tungsten lamp was placed about 150 mm apart from the vessel cap. The radiation flux attributable to the lamp was less than 1.0 W m^{-2} on the cap of the culture vessel, while the one from fluorescent lamps was 30 W m^{-2}. Together with the short visualization duration (5 s), the radiation from the tungsten lamp was considered to have a negligibly small effect on the inertial air movement in the culture vessel. Two-dimensional video images of the tracer movements inside the culture vessel were analyzed by tracing representing particles shown in a video screen. The air current speed was determined by measuring distances in which particles moved per second. The upward air current speeds were determined with 10–15 particles at a height ranging from 30 to 40 mm above the medium at the central region in the culture vessel.

Evaluation of effects of plantlet size, medium surface color and wind velocity above the culture vessel on air movement inside the culture vessel

The plantlet would be a resistance to the air movement in the culture vessel. The effect of the plantlet size on the air current speeds inside the culture vessel was assessed with 10 and 60 mm tall plantlets and compared with the culture vessel without plantlets. The medium contained activated charcoal (5 g l^{-1}) in this experiment.

The air current speeds inside the culture vessel would be varied, as a principle, by altering free convection induced by spatial variations of temperatures inside the culture vessel. The wind velocity would affect inner-surface temperature of the vessel cap and the medium surface color would affect surface temperature of the medium. Then air current speeds inside the culture vessel with a 60 mm tall plantlet were assessed under six conditions combined with three levels of the wind velocity above the culture vessel and presence/

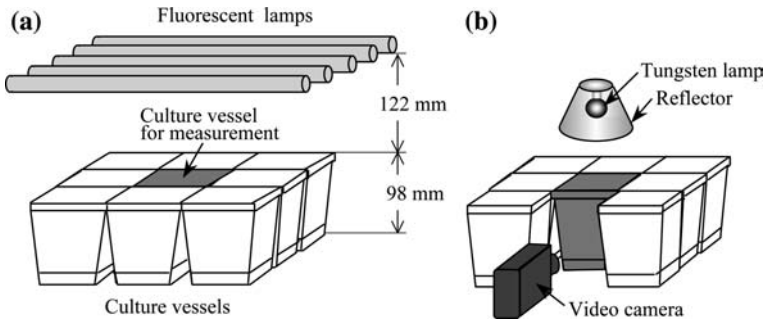

Figure 1. Schematic diagram of experimental apparatus before (*a*) and during (*b*) capturing images of tracer movement in the culture vessel with a video camera.

absence of a black plate (50×50 mm^2) on the medium. The medium contained no activated charcoal in this experiment. The wind velocity was measured with an anemometer (Model 6071, Nihon Kanomax, Japan) 40 mm above the culture vessel

The air current speeds in the vessel were determined with the same method mentioned above. The averaged upward air current speed (called air current speed, hereafter) was taken as the representative value around the plantlet. Measurements and analysis were repeated three times to confirm the results. A similar trend was observed in each replication.

The medium surface temperature and the air temperature 40 mm above the culture vessel were measured with copper–constantan thermocouples. The diameter of the thermocouple was 0.1 mm.

Measurement of the net photosynthetic rate of photoautotrophic potato plantlets under varied air current speeds in the culture vessel

The potato plantlets were cultured under the same culture conditions but without activated charcoal in the medium for 20 days as mentioned above and used for measurement of net photosynthetic rate under different air current speeds inside the vessel. Three plantlets cultured in three culture vessels were used and they had 60–63 mm of stem length and 1490–1830 mm^2 of leaf area at the time of analysis. The air current speeds in the vessel were controlled with the same combination of the wind velocity and the black plate as mentioned above (Figure 2).

The net photosynthetic rate on a leaf area basis, P (mol m^{-2} s^{-1}), of each plantlet was calculated by the following equation:

$$P = E(C_{out} - C_{in})/A$$

where E is the air exchange rate of the culture vessel, 2.06×10^{-7} m^3 s^{-1}, C_{in} and C_{out} are CO_2 concentrations (mol m^{-3}) of the inside and outside of the culture vessel, respectively, and A is the leaf area (m^2) of the plantlet. Air inside and outside the culture vessel were sampled with airtight microsyringes for measuring CO_2 concentration using a gas chromatograph (GC12A, Shimazu Co., Japan). Each sample volume was 0.5 ml. Measurements were replicated three times in every treatment.

Results and discussion

Upward air currents were observed around the plantlets in the central part of the culture vessel and downward air currents were observed near inside walls in the culture vessel (Figure 3) regardless of presence or size of the plantlet. Kitaya et al. (1997) reported the similar observation. Obviously the temperature distribution inside the vessel created such a pattern. Upward air current speeds were higher than downward air current speeds.

The air current speeds in culture vessels containing a single 10-mm-tall plantlet and a single 60-mm-tall plantlet were, respectively, 1/3 and 1/6 times that in the culture vessel containing no plantlet (Figure 4). The air movements were restricted by the presence of the plantlet in the culture vessel. The air current speed decreased more remarkably with the lager plantlet in the culture vessel. This is probably due to a resistance to the air movement created by the plantlet, and also due to reduction of the medium surface temperature by increasing shade of plantlets.

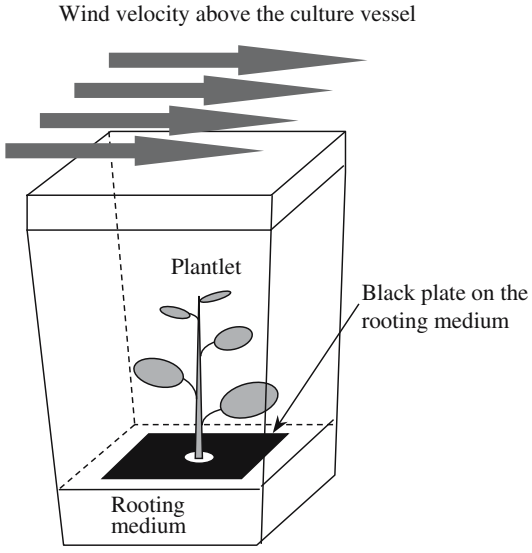

Figure 2. Apparatus for controlling the air current speed and measuring the net photosynthetic rate of plantlets as affected by different air current speeds in the culture vessel. The air current speeds inside the culture vessel were controlled by controlling free convection induced by the temperature distribution in the culture vessel. The temperature distribution in the culture vessel was varied with changing wind velocity above the culture vessel and placing a black plate on the medium.

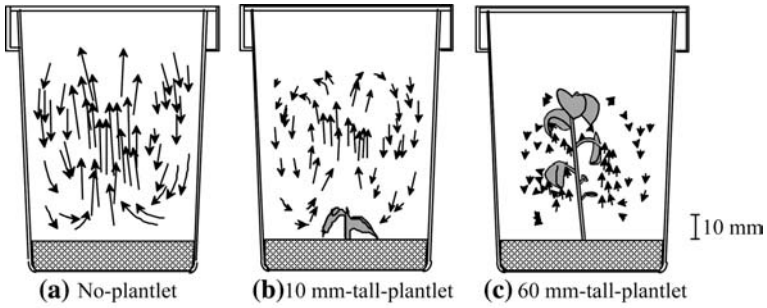

Figure 3. The tracer movement in the culture vessels containing no plantlet (*a*), a single 10-mm-tall plantlet (*b*) and a single 60-mm-tall plantlet (*c*). Each figure is a representative in three repetitions.

The air current speeds inside the culture vessel could be controlled in a range between 2.4 and 8.0 mm s^{-1} by controlling wind velocity above the culture vessel and placing a black plate on the medium (Figure 5). Air current speeds were increased by 2 times by increasing wind velocity above the culture vessel from 0.1 to 1.0 m s^{-1}. Placing the black plate on the medium also increased the air current speeds by 1.5 times under the same wind velocity above the culture vessel. In both cases, the major factor of enhancing air current speed inside the vessel was considered as reduction of surface temperatures inside the vessels. Increased wind velocity outside the vessel reduced the internal surface temperature of the lid and placing a black plate on the medium increased the surface temperature of the medium, both of which enhanced free convection of air.

The net photosynthetic rates of the plantlets increased linearly from 2.0 to 2.5 μmol m^{-2} s^{-1} as the air current speed in the culture vessel increased from 2.0 to 8.0 mm s^{-1} (Figure 6). The plots from the vessels with and without the black plate were approximated on the same line, showing that air current speed was the primary factor affecting the net photosynthetic rate under the present experimental conditions. Enhancement of air movement inside the culture vessel promoted the photosynthesis of the plantlets. Nakayama et al. (1991) reported that net photosynthetic rates of potato plantlets *in vitro* were greater in a forcedly ventilated culture vessel than in a naturally ventilated culture vessel even at the same CO_2 concentration inside culture vessels. The increase in the net photosynthetic rate would be due to the enhanced air movements in the culture vessel by the forced ventilation.

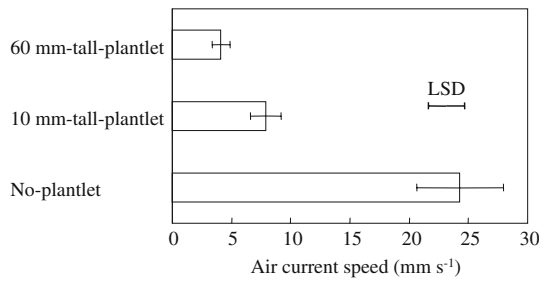

Figure 4. Effect of the plantlet size on the air current speed in the culture vessel. Least significant difference (LSD) at a significant level of 99% is shown.

The surface temperatures of the medium covered with and without the black plate were 27.0 and 27.9 °C, respectively, while the air temperature 40 mm above the culture vessel was 27.0 °C. The temperature difference, less than 1.0 °C, between the culture vessels with and without the black plate did not seem to affect directly the net photosynthetic rate, because the net photosynthetic rate at the same air current speed was almost the same regardless of presence or absence of the black plate (Figure 6).

Thickness of a boundary layer on the leaf surface and thus a leaf boundary layer resistance decreased with increasing the air current speed around the leaf (e.g., Yabuki and Miyagawa, 1970). Gas exchange between the plantlets and the ambient air are promoted more by the less leaf boundary layer resistance. Hence enhancement of the air movement would promote transpiration, which would contribute to promoting uptake of water and nutrients by the plantlets.

The air current speed in the culture vessel was higher with the black plate than without the black

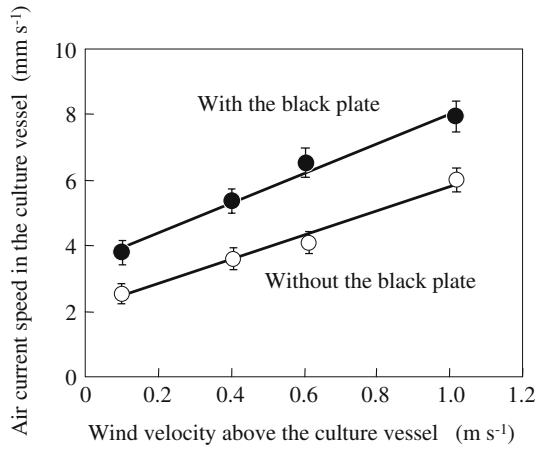

Figure 5. Effects of the wind velocity above the culture vessel and presence of the black plate on the medium surface on the air current speed in the culture vessel. The correlation coefficients (R^2) of the least square approximation lines obtained in the culture vessels with the black plate and without the black plate were 0.99 and 0.98, respectively.

plate on the medium. Activated charcoal is often added to plant tissue culture medium to absorb inhibitory substances from the medium and the headspace in the culture vessel. It appears from the present study that activated charcoal added to rooting medium has an additional role for enhancing air movements in the culture vessel as well as the black plate in this experiment.

In conclusion, the air current speeds observed in the culture vessel was significantly slow when compared with those in greenhouses and fields. Therefore diffusion processes in the culture vessel would be slow and limiting the photosynthesis and transpiration of plantlets. Enhancement of the air movement in the culture vessel is important to promote growth of plantlets through promoting their photosynthesis and transpiration. The air current speed could be controlled by controlling free convection induced by spatial variations of temperatures in the culture vessel. The highest air current speed under the present experimental condition was 8 mm s^{-1} in the culture vessel with a 60-mm-tall plantlet. It seems to promote more the photosynthesis, when the air current speed is raised. Other factors such as light intensity will affect indirectly the photosynthesis through affecting spatial temperature variation and thus air movement in the culture vessel, although the light intensity affects directly the plantlets growth (e.g., Kitaya et al., 1995). In order to establish the methods for controlling the air movements accurately, full understanding of patterns and speeds of the air current as affected by other factors that influence spatial variation of the temperature in culture vessels is required.

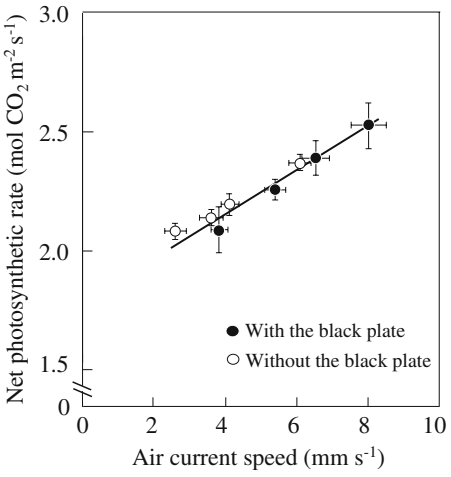

Figure 6. Effects of the air current speed on the net photosynthetic rate of plantlets in the culture vessel. The correlation coefficient (R^2) of the least square approximation line was 0.96.

References

Daws LF, Penwarden AD & Waters GT (1965) A visualization technique for the study of air movement in rooms. J. Instn. Heat. Vent. Engs. 33: 24–30

De Y, Desjardins Y & Gosselin A (1993a) Photosynthetic capacity and effects of forced ventilation on growth of *in vitro*-cultured strawberry plantlets. Acta Hortic. Sinica 20: 123–126

De Y, Gosselin A & Desjardins Y (1993b) Effects of forced ventilation at different relative humidities on growth, photosynthesis and transpiration of geranium plantlets *in vitro*. Can. J. Plant Sci. 73: 249–256

Kitaya Y, Fukuda T, Kozai T & Kirdmanee C (1995) Effects of light intensity and lighting direction on the photoautotrophic growth and morphology of potato plantlets *in vitro*. Scientia Hortic. 62: 15–24

Kitaya Y, Ohmura Y & Kozai T (1997) Method for visualization of air currents in plant tissue culture vessels. Environ. Control Biol. 35(2): 139–141 (in Japanese with English summary)

Kitaya Y, Tsuruyama J, Kawai M, Shibuya T & Kiyota M (2000) Effects of air current on transpiration and net photosynthetic rates of plants in a closed plant production. In: Kubota C & Chun C (eds) Transplant Production in the 21st Century (pp 83–90). Kluwer Academic Publishers

Kitaya Y, Kawai M, Tsuruyama J, Takahashi H, Tani A, Goto E, Saito T & Kiyota M (2001) The effect of gravity on surface temperature and net photosynthetic rate of plant leaves. Adv. Space Res. 28: 659–664

Kitaya Y, Kawai M, Tsuruyama J, Shibuya T & Kiyota M (2003a) Effects on air current speed on the transpiration rate and net photosynthetic rate of plant leaves. Adv. Space Res. 31(1): 177–182

Kitaya Y, Kawai M, Tsuruyama J, Takahashi H, Tani A, Goto E, Saito T & Kiyota M (2003b) The effects of gravity on surface temperatures of plant leaves. Plant, Cell and Environment 26: 497–503

Kozai T (1991) Autotrophic micropropagation. In: Bajaj YPS (ed) Biotechnology in Agriculture and Forestry (pp 313–343). Springer-Verlag

Kozai T, Ohde N & Kubota C (1991) Similarity of growth patterns between plantlets and seedlings of *Brassica campestris* L. under different *in vitro* environmental conditions. Plant Cell Tiss. Org. Cult. 24: 181–86

Kozai T, Fujiwara K, Hayashi M & Aitken-Christie J (1992) The *in vitro* environment and its control in micropropagation. In: Kurata K & Kozai T (eds) Transplant Production Systems (pp 247–282). Kluwer Academic Publishers

Kozai T & Smith MAL (1994) Environmental control in plant tissue culture. In: Aitken-Christie J, Kozai T & Smith MAL (eds) Automation and Environmental Control in Plant Tissue Culture (pp 301–318). Kluwer Academic Publishers

Kubota C & Kozai T (1992) Growth and net photosynthetic rate of *Solanum tuberosum in vitro* under forced and natural ventilation. HortScience 27: 1312–1314

Monteith JL & Unsworth MH (1990) Principles of Environmental Physics (pp. 101–120). Edward and Arnold Publishing Co, London

Murashige T & Skoog F (1962) A revised medium for rapid growth and bioassay with tabacco tissue cultures. Physiol. Plant. 15: 473–497

Nakayama M, Kozai T & Watanabe K (1991) Effect of the presence/absence of the sugar in the medium and natural forced ventilation on the net photosynthetic rates of potato explants *in vitro*. Plant Tiss. Cult. Lett. 8: 105–109 (in Japanese with English summary)

Nguyen QT, Kozai T, Heo J & Thai DX (2001) Photoautotrophic growth response of *in vitro* cultured coffee plantlets to ventilation methods and photosynthetic photon fluxes under carbon dioxide enriched conditions. Plant Cell Tiss. Org. Cult. 66: 217–225

Ohyama K & Kozai T (1997) CO_2 concentration profiles in a plant tissue culture vessel. Environ. Contr. Biol. 35(3): 197–202 (in Japanese with English summary)

Torrance KE, Orloff L & Rockett JA (1969) Experiments on natural convection enclosures with localized heating from below. J. Fluid Mech. 36: 21–31

Yabuki K & Miyagawa H (1970) Studies on the effect of wind speed upon the photosynthesis (2). Japan. J. Agric. Met. 26: 137–141 (in Japanese with English summary)

Zobayed SMA, Afreen F, Kubota C & Kozai T (2000) Water control and survival of Ipomoea batatas grown photoautotrophically under forced ventilation and photomixotrophically under natural ventilation. Ann. Bot. 86: 603–610

Zobayed SMA, Armstrong J & Armstrong W (2001) Leaf anatomy of *in vitro* tobacco and cauliflower plantlets as affected by different types of ventilation. Plant Sci. 161: 537–548