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Internal Air Current Patterns Depend on the Ventilation Method in a Scaled-Up Culture Vessel for Micropropagation

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Air current patterns were visualized inside a scaled-up culture vessel under natural or forced ventilation. Metaldehyde particles were used as tracers, and their patterns were recorded as video images by a high-resolution-and-contrast camera. Under natural conditions, the air currents were mainly influenced by natural convection that developed due to the lighting scheme, which caused differences in temperature among various articles in the chamber, including a sweet potato plantlet, supporting material, a multi-cell tray, and the culture vessel. Under forced ventilation, the air current pattern and air speed were affected by ventilation rates and by air-supply methods that were either parallel downward or circular upward. Uniformity of air movement could be achieved with air distribution pipes inside a modified vessel. Under forced ventilation, growth, photosynthetic rate, and transpiration of the micropropagated plantlets were enhanced around the air outlet as well as the inlet in the large-scale vessel. Those plant responses were probably induced by uniform spatial distribution of air current and gas concentrations.

Keywords: air current pattern, diffusion coefficient, forced ventilation, particle image velocity, plantlet, uniform growth

In conventional micropropagation systems, airtight, small-scale culture vessels (several hundred mL in air volume) are commonly used to reduce the microbial contaminations that result from sugar being added to the culture medium (Sharma et al., 2005). However, the air currents within such vessels are slow and, thus, the diffusion coefficients are small compared with the growing conditions available in the greenhouse or field (Kozai et al., 1992). Diffusion coefficients for gas and water vapor also depend on the speed of the air currents. When the air speed is high, the thickness of the boundary layer surrounding the plantlet leaves is reduced, which increases the diffusion coefficient and, therefore, the photosynthetic rate. In contrast, the smaller diffusion coefficients in standard culture vessels, as well as the lower air current speed, inhibit photosynthesis and transpiration, and retard the growth of *in vitro* plantlets (Yabuki and Miyagawa, 1970). Nakayama et al. (1991) have reported that the net photosynthetic rate (NPR) and growth of potato plantlets improves when forced ventilation is used to increase the air speed within the culture vessel. Likewise, by raising that speed, the boundary layer around the leaves of cucumber (*Cucumis sativus* L.) becomes thinner, and the NPR is enhanced (Yabuki and Miyagawa, 1970). All these results demonstrate that air currents in culture vessels do affect growth and NPR of *in vitro* and/or *ex vitro* plantlets.

Under conditions of no or simply natural ventilation, air currents develop mainly because of normal by natural convection, which causes differences in temperature among the plantlets, vessel side walls, and the headspace of the culture medium. Walker et al. (1989) and Kitaya et al. (1997) have visualized the air current in hermetically sealed culture vessels to understand these microclimate conditions.

A novel technique of particle image velocimetry (PIV) has been suggested as an attractive tool for conducting non-intrusive measurements (Keane and Adrian, 1991; Lee et al., 2003). PIV is used for studying particle velocities for certain flow types, including gas within a wind tunnel or liquid movements for viscous fluids. This effective tool is probably adaptable for analyses of air current patterns both inside sealed culture vessels as well as on the scale of a greenhouse. However, visualization via PIV has not been extensively investigated with regard to a particular vessel design for micropropagation, in which the goal is uniform air distribution within a forced ventilation system

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in order to enhance plantlet growth.

In a scaled-up culture vessel (12.4 L air volume), photoautotrophic growth (i.e., without any sugar in the culture medium) and the NPR of sweet potato (*Ipomoea batatas* (L.) Lam., cv. Beniazuma) plug plantlets is greater with forced ventilation than under natural conditions (Heo and Kozai, 1999). However, growth is not uniform in forcedly ventilated vessel, and is greater near the air inlet than at the air outlet. These spatial variations are due to a lower CO_2 concentration near the air outlet.

The objectives of this study were to analyze the air current patterns in scaled-up culture vessels under natural and forced ventilation conditions, using a high-resolution-and-contrast camera. In addition, we investigated various means for achieving an even distribution of air currents in culture vessel used for micropropagation that would promote uniform growth of *in vitro* sweet potato plantlets.

MATERIALS AND METHODS

Culture Vessel Used for Visualization

We modified an autoclavable, box-type polycarbonate vessel (368 × 178 × 170 mm; Bio-Safe Carrier, Nalgene, USA) for use in visualizing air currents under natural or forced ventilation. Our visualization system consisted of a line-light guide and light source control unit for illumination, a camera and camera control unit for image filming, and a computer monitor and VTR for recording (Fig. 1). Air current patterns and speeds under various ventilation rates were investigated inside the culture vessels. Three types of vessels were designed with different air-supply methods: 1) circular, upward supply with an inlet and outlet inside the vessel, 2) downward supply of air inside the vessel from an acrylic panel with 40 holes in the vessel lid, and 3) uniform, upward supply from three air distribution pipes (1 mm diameter holes) on a cell tray inside the vessel. For this third version, the number of holes for maintaining stable air resistance around the outlet was always twice as many as for the inlet, regardless of the distance from the air inlet. For the control, one vessel was used that lacked both inlet and outlet; this represented conditions of no or natural ventilation. For the forced-ventilation designs, the inlet and outlet were located 40 and 168 mm from the bottom of the vessel for upward and downward air-supply conditions, respectively. Ventilation rates were set at 4, 6, or 8 mL s^{-1}, as determined with an air flow-rate meter (Purge type No. 5S; Kofloc, Japan) and a flow controller (KT-6; Iuchi Sangyo, Japan).

Plant Material and Environmental Conditions for Visualization in a Growth Chamber

Single-node stem cuttings (leaf area of 1.8 cm^2; Fig. 2) were taken from plantlet of sweet potato (*Ipomoea batatas* (L.) Lam.). Twenty cuttings each were placed in multi-cell trays (Santomi Sangyo, Japan) that were filled with cellulose plugs (Sorbarods, Baumgartner Papiers SA, Switzerland) as supporting material. The

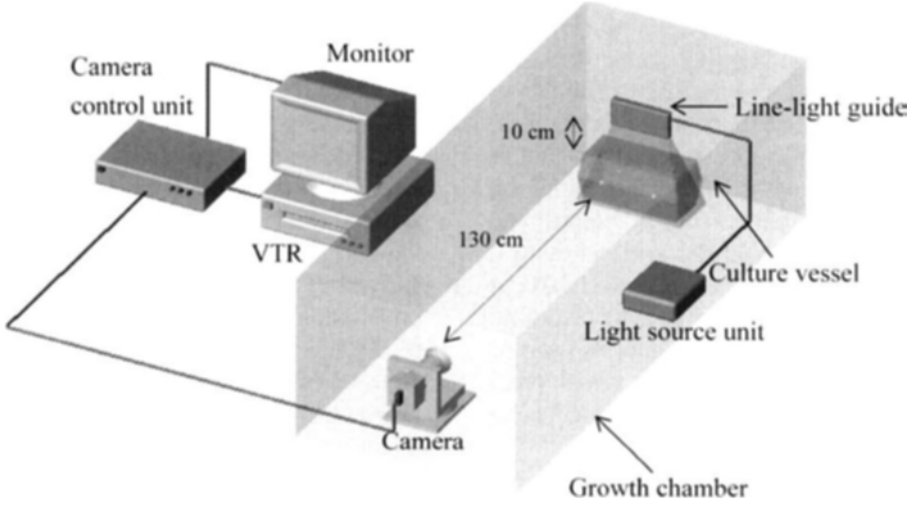

Figure 1. Schematic diagram of visualization process in scaled-up culture vessel using super-eye system.

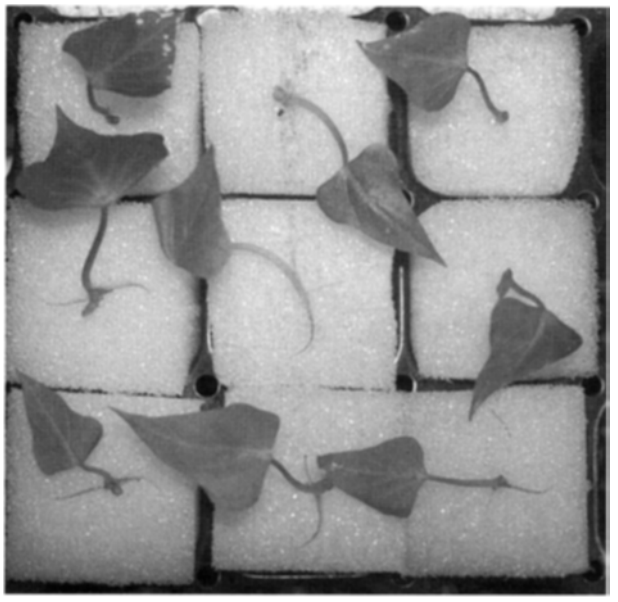

Figure 2. Single-node cuttings of sweet potato used as plant material.

culture vessels were placed in a growth chamber with an air temperature of 25 ± 1°C and a relative humidity of 70 ± 10%. The CO_2 concentration was maintained at an ambient condition of 350 to 400 μmol mol^{-1}. To stabilize the light intensity, a halogen lamp with line-light guide (MORITEX, Japan) was used as the lighting source for visualization. A short-wave radiation absorbability of 92% was measured on the multi-cell trays, using a solar meter (MS-100; Hidehiro, Japan) and a voltmeter (TR6846; Advantest, Japan).

Visualization of Air Current Patterns in Culture Vessels

Air currents were visualized in vessels, with or without plantlets, following a tracer method that used feathery metaldehyde (Daws et al., 1965; Torrance et al., 1969; Kitaya et al., 1997). Powdery metaldehyde ($C_2H_4O_4$; Sigma, USA) has high trackability. After being sublimed by heating at 120°C, the fine particles were air-cooled, then re-crystallized to a needle shape to make them visible. The movements of these tracers could be tracked in the air because of their remarkably small, relative density. After inducing the tracers, the vessels were placed in the growth chamber for 5 min to establish and maintain a steady-state temperature. Tracer movements at the two-dimensional surface of the vessel were recorded for 2 min with a high-resolution-and-contrast camera (Super-eye C2874; Hamamatsu Photonics, Japan). The recorded images were placed on a monitor for estimating the pattern and speed (distance per second) of the air currents. This super-eye system can produce high-quality images in the dynamic range by combining analogue-image processing with a camera tube. The camera images are then analyzed to understand the uniformity of the air current patterns and speeds.

RESULTS AND DISCUSSION

Air Current Patterns and Speed under Downward-Forced Ventilation

Tracer movement was analyzed for a downward-

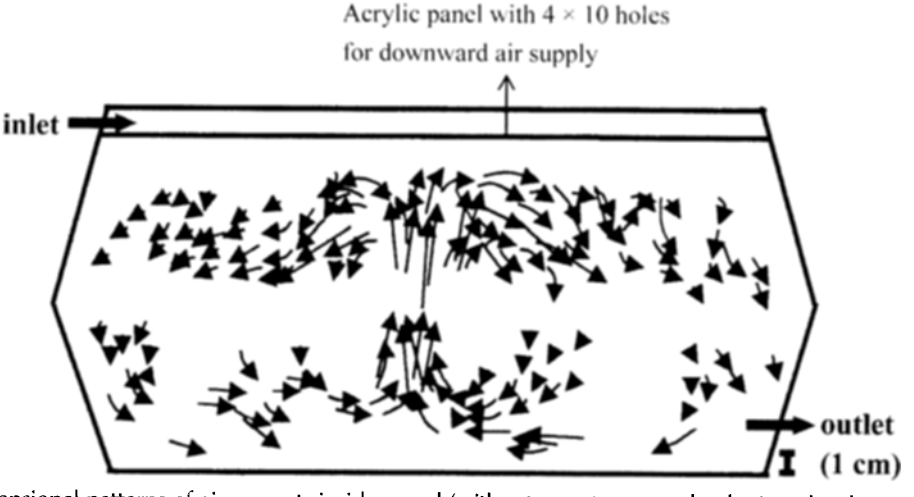

Figure 3. Two-dimensional patterns of air currents inside vessel (without sweet potato plantlets) under downward-forced ventilation. Arrow lengths and directions represent air current and direction, respectively. Forced ventilation rate was 33 mL s^{-1}.

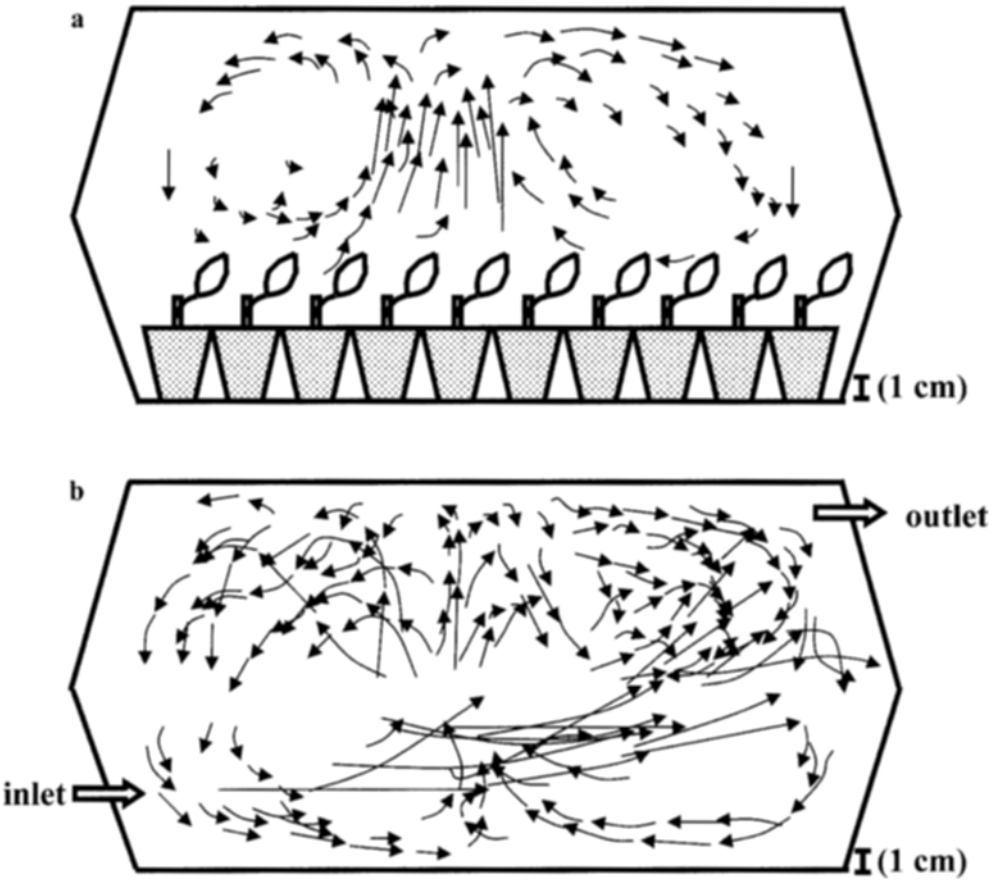

Figure 4. Two-dimensional patterns of air currents in naturally ventilated vessel with sweet potato plantlets (**a**) and in forced-ventilation vessel (8 mL s^{-1}) without plantlets (**b**). Arrow lengths and directions represent air current and direction, respectively.

forced ventilation rate of 33 mL s^{-1} in empty vessels (Fig. 3). Arrows were used to indicate the direction and of the air current and the distance the tracers moved. Air current was vigorous in the headspace of the vessel, while dead zones were observed around the ventilation panel, sidewalls, and four lower corners of the vessel, all areas where the ventilation rate was reduced. However, these dead zones disappeared when that rate was increased to more than 8 mL s^{-1}. Because plant growth also increased with the ventilation rate, these data suggest that downward air currents may influence plant height. Spatial distribution of the air currents was uneven near the air inlet and outlet, with the former (in the subordinate position) being relatively lower than that measured in the headspace.

Air Current Pattern and Speed under Natural and Upward-Forced Ventilation

Under no or natural ventilation conditions, i.e., without a forced air supply, upward currents were observed in the central region and along the inside wall of the vessel, regardless of any plantlet presence (Fig. 4a). In the naturally ventilated vessel, a more extensive dead zone was detected at the sidewalls and in the plant canopy, compared with conditions noted for the downward ventilation test. Upward air movement occurred in the central region of the vessel because of a temperature difference between the bottom surface of the vessel and the surrounding air, which was caused, directly or indirectly, by incident light from the lamp as well as heat exchange between the air of the outer and inner vessel surfaces. Air also moved downward between the inside wall and the surrounding air. These upward and downward internal air currents were attributed mainly to natural convection due to temperature differences between the surface of the multi-cell tray and its headspace. Average upward air speed in the central portion of vessels containing cuttings was 33 mm s^{-1}, which was lower than the 37 mm s^{-1} recorded from empty vessels with no or only natural ventilation. Air speed in vessels with cuttings was partially affected by short-

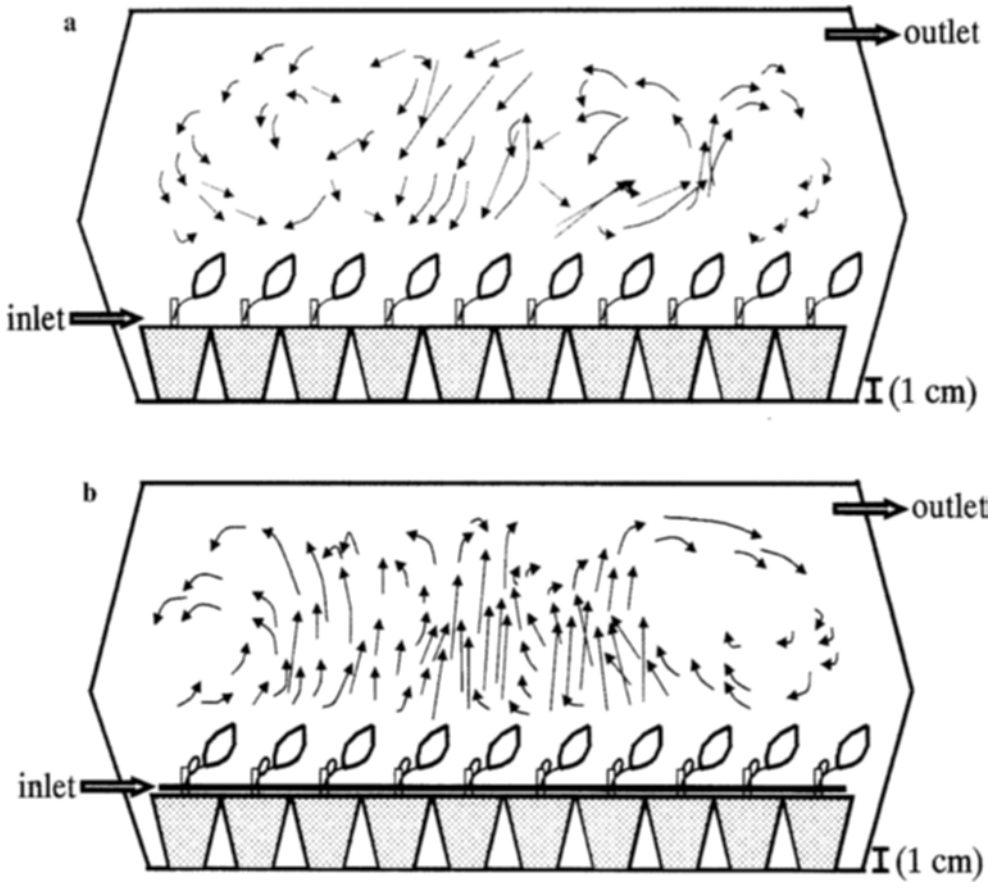

Figure 5. Two-dimensional patterns of air currents in culture vessel with sweet potato plantlets under upward-forced ventilation (8 mL s^{-1}): (**a**) without air distribution pipes, or (**b**) with three air distribution pipes. Arrow lengths and directions represent air current and direction, respectively.

wave radiation absorption by those plantlets.

In contrast, for empty vessels with an upward-forced ventilation rate of >8 mL s^{-1} (Fig. 4b), air movement was vigorous in all parts of the vessel, compared with less uniform distribution observed with the downward or natural ventilation conditions. Moreover, an air current speed of 124 mm s^{-1} in the horizontal direction along the surface of the empty vessel was four times higher than the upward speed. By increasing the forced ventilation rate, horizontal air movement was remarkably increased near the air inlet and in the central region. Movement in the headspace of empty vessels was improved by increasing the forced ventilation rate, but was relatively inactive near the air outlet compared with that measured near the inlet.

For upward ventilation conditions, air current patterns were uneven when plantlets were present, compared with a more uniform distribution seen with empty vessels (Fig. 5a). Air movement via forced convection was vigorous around the air inlet, but upward air currents were not active compared with ventilated, empty vessels, even though the ventilation rates were the same regardless of the presence of plantlets. The air current speed in the horizontal direction significantly decreased when plantlets were included, probably because they created a resistance body near the inlet. Again, decreased air speed inside plantlet-filled vessels was due to increased short-wave radiation by the cuttings. Raising the forced ventilation rate resulted in greater upward air speed inside the vessel. At a rate of 33 mL s^{-1}, the upward air current speed in vessels containing only supporting material was 70 mm s^{-1}, which was about 2 times higher than that of the empty vessel (data not shown). Likewise, the short-wave radiation absorption rate inside vessels filled only with supporting material (no plantlets) was higher than in the empty vessels.

In the forced-ventilation vessels that had been modified with air distribution pipes, upward air currents in the center were active and uniform in the horizontal direction compared with pipeless vessels,

even though the forced ventilation rates were the same (cf., Fig. 5a, 5b). Those pipes enabled uniform air distribution throughout the vessels, thus promoting plantlet growth near the outlets. Kitaya et al. (1997) have reported that the upward and downward air current speeds are 7 to 12 mm s^{-1}, respectively, for tissue culture vessels that contain potato plantlets in an air volume of 370 mL. However, those speeds were remarkably lower than those measured in the culture vessels designed for this experiment, but were still less than those from the greenhouse or field. Clearly, these low air speeds inhibited plantlet growth because of limited photosynthesis and transpiration. Kitaya et al. (2003) also have reported that increased air speeds can enhance the net photosynthetic and transpiration rates of sweet potato leaves and tomato seedling canopies grown in a closed system, the latter species showing a doubling in its net photosynthetic rate when air speed is increased from 0.1 to 1.0 m s^{-1}. In addition, Heo and Kozai (1999) have reported that *in vitro* growth of sweet potato cuttings under upwardly forced ventilation is not uniform, but is greater near the air inlet than the outlet. Those reports support our findings that variations in growth and net photosynthetic rates between inlets and outlets were caused by the spatial distribution of air currents in the culture vessels. We also showed that these diminished growth and photosynthetic rates resulted from diffusion coefficients that were lower near the outlet. Therefore, the increase in net photosynthetic rates for *in vitro* and *ex vitro* sweet potatoes was due to decreased resistance to gas diffusion in the boundary layer.

In general, low air diffusivity results in an uneven spatial distribution of gas concentrations in the headspace of culture vessels, with the diffusion coefficient depending on the air current speed. Moreover, when air speed is high, the boundary layer thickness is reduced, and a larger diffusion coefficient means that photosynthetic capacity is enhanced. Gas distribution in the headspace can be affected by the presence of plantlets and a culture medium, as well as by the physical characteristics of the culture vessel. Growth and plant morphology are then not uniform because of the uneven spatial distribution of gases such as CO_2.

Under forced ventilation, eddy diffusion in the headspace resulted from induced convection. This rise in the eddy diffusion coefficient of gases, and in air flow, enhances CO_2 uptake over a photoperiod to a greater extent than when only CO_2 concentration is increased (Fujiwara et al., 1988; Walker et al., 1989; Nakayama et al., 1991). In our study, greater eddy diffusivity near the air inlet improved the exchange of substances on the plantlet surfaces as the boundary layer became thinner.

Air current speeds in the vessel were also estimated from images obtained by our visualization system. Here, spatial distribution of air currents was not uniform under either upward- or downward-forced ventilation, regardless of the ventilation rate, but current patterns and speed were affected by the forced ventilation rate, air-supply method, and the presence or absence of plantlets. Air movement and speed in the vessel headspace also could be affected by air speed around the vessel, as well by the shape and size of the container. Several other custom-designed culture vessels for micropropagation have been tested for large-scale, automated, and mechanized micropropagation (Monette, 1983; Tisserat and Vandercook, 1985; Simonton et al., 1991; Young et al., 1991). Thus, the next step should be to develop forced ventilation or photoautotrophic commercial micropropagation systems that feature larger vessels outfitted with a gas supply for CO_2 enrichment.

Based on our results, we can conclude that air currents are an essential factor in designing culture vessel systems that incorporate forced ventilation. Such physical characteristics can be effectively analyzed three-dimensionally using a method such as PIV technology. Adopting large-scale vessels for commercial purposes will enable producers to enhance air movement, thereby improving photosynthesis, transpiration, and growth of *in vitro* plant species.

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