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Growth and Net Photosynthetic Rate of *Solanun tuberosum* in Vitro under Forced and Natural Ventilation

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Abstract. **Growth and net photosynthetic rate of potato *(Solanum tuberosum* L.) 'Benimaru' plantlet in vitro were studied under a conventional photomixotrophic condition [with 20 g sucrose/liter in the medium and under 70 μmol·m^{-2}·s^{-1} photosynthetic photon flux (PPF)] with minimal ventilation (MV) and under photoautotrophic conditions (without sugar in the medium and under 190 μmol·m^{-2}·s^{-1} PPF) with enhanced natural ventilation using an air diffusive filter (DV) or with forced ventilation (FV). Fresh weight of the plantlets cultured in the FV and DV treatments was 2.4 times that of the plantlets cultured in the MV treatment. Net photosynthetic rate and dry weight per plantlet were the highest in FV followed by DV. For photoautotrophic micropropagation, FV was superior to DV.**

A low net photosynthetic rate (NPR) and a low growth rate of chlorophyllous explants/plantlets in vitro are mainly due to low CO_2 concentrations inside the vessel during the photoperiod (Fujiwara et al., 1987) rather than to the low photosynthetic ability of the explants/plantlets (Pospisilova et al., 1987). Net photosynthetic rate and growth rate of the explants/plantlets were increased by increasing CO_2 concentrations inside the vessel (Kozai and Sekimoto, 1988; Mousseau, 1986) and photosynthetic photon flux (PPF) (Kozai et al., 1988).

The CO_2 concentration inside the conventional, small culture vessel during the photoperiod was increased by increasing the number of air exchanges of the vessel per hour (Kozai and Sekimoto, 1988) and/or by increasing the CO_2 concentration inside the culture room (Kozai and Iwanami, 1988). The number of air exchanges of the vessel is increased by employing an air diffusive filter on the vessel. Moreover, the CO_2 concentration inside large culture vessels would be increased and easily controlled with a forced ventilation system and, if necessary, CO_2 enrichment in the incoming air.

Growth and photosynthesis of strawberry *(Fragaria* × *ananassa* Duch.) (Fujiwara et al., 1988) and *Spathiphyllum* 'Merry' (Watanabe et al., 1990) plantlets were promoted under forced ventilation compared with the conventional ventilation system that had only a minima! number of air exchanges. In some cases, forced ventilation fails to promote the growth of plantlets (Walker et al., 1988). Photoautotrophic growth of plantlets in the vessels with forced and diffusive ventilation systems has not been studied. In the present study, growth and NPR of 'Benimaru' potato plantlets were compared under the photomixotrophic and photoautotrophic conditions with three ventilation treatments.

Cuttings with a single leaf, taken from 30-day-old plantlets subcultured on MS (Murashige and Skoog, 1962) medium containing 20 g sucrose/liter, were used as explants (mean fresh weight per explant, 56 ± 6 mg). The initial dry matter content of the explants was 7.8% of the fresh weight.

Table 1 describes the culture conditions of the three treatments. Liquid MS medium was used with rockwool cubes with forced ventilation (FV) and diffusive ventilation (DV). The minimal ventilation (MV) treatment, which was used as a conventional micropropagation system, had the same inorganic medium composition plus 8 g agar/

Table 1. General description of the treatments.

Treatment	Ventilation method	No. air exchanges/h	PPF[z] (μmol·m^{-2}·s^{-1})	Sucrose concn (g·liter^{-1})	Supporting material
FV	Forced	2.3–22[y]	190	0	Rockwool[x]
DV	Natural with air-diffusive filters	4.9	190	0	Rockwool
MV	Natural without air-diffusive filters	0.12	70	20	Agar[w]

[z]PPF: Photosynthetic photon flux on the surface of the empty culture shelf.
[y]Number of air exchanges in the FV treatment was increased with time.
[x]Rockwool cubes (15 × 15 × 25 mm; Micro plug, Grodania A/S, Denmark).
[w]Eight grams agar/liter (Kanto Chemical, Tokyo).

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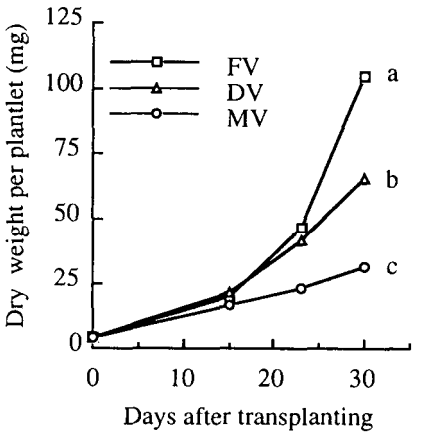

Fig. 1. Changes in dry weight per plantlet over time. Mean separation on day 30 by Duncan's multiple range test at $P = 0.05$. For description of treatments,see Table 1 and text.

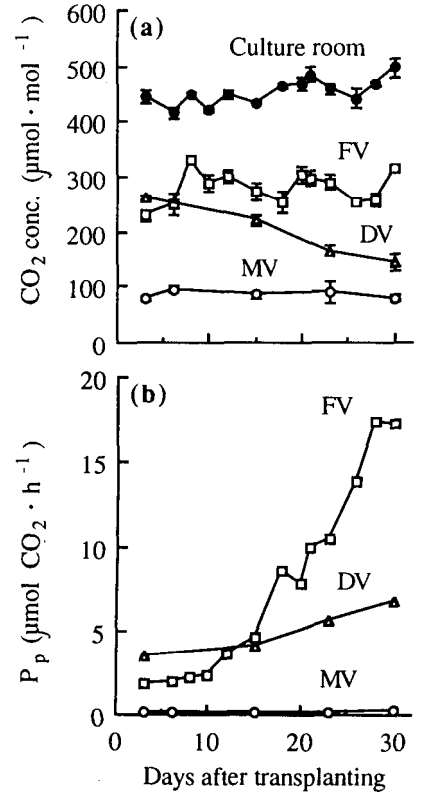

Fig. 2. Changes in (a) CO_2 concentration inside the culture room and the vessel containing the potato plantlets and (b) net photosynthetic rate per plantlet (P_p) under the steady-state conditions during the photoperiod over time. Vertical bars in Fig. 2b indicate the SE for mean CO_2 concentrations. For description of treatments, see Table 1 and text.

liter as a supporting material and also 20 g sucrose/liter. A preliminary experiment showed no significant growth differences between plantlets grown on agar medium and liquid medium with the rockwool cubes. With FV and DV, a multicell tray cut to fit in the culture vessels was used to hold the rockwool cubes in place. The pH of the medium was adjusted to 5.8 before autoclaving. Glass jars (480 ml) with polycarbonate screw caps

Table 2. Mean fresh weight per plantlet (W_f), number of leaves per plantlet, shoot : root fresh weight ratio on day 30, and net photosynthetic rate per leaf dry weight during the photoperiod (P_d, μmol CO_2/gram dry weight per hour) on days 15, 23, and 30. For description of treatments. see Table 1 and text.

		Treatment		
	Day	FV	DV	MV
W_f (g)	30	1.18 a[z]	1.16 a	0.49 b
Leaves (no.)	30	13 a	13 a	13 a
S : R ratio	30	3.3 a	2.8 a	3.3 a
P_d	15	450	340	23
	23	430	250	27
	30	340	180	19

[z]Mean separation by Duncan's multiple range test, $P = 0.05$.

were used for DV and MV. For FV, large polycarbonate vessels (2600 ml) were connected to a FV system. The culture room air was bubbled through 500 ml distilled water for humdification and was pumped through a filter (pore size, 0.2 μm; Millex-FG50, Millipore, Molsheim, France) into the FV vessels. The caps in the DV treatment were modified: three holes (10 mm in diameter) were drilled in each cap and each hole was covered with a disk (14 mm in diameter) of an autoclavable air diffusive filter (pore size, 0.5 μm; Milli-seal, Millipore, Tokyo). The number of air exchanges of the DV and the MV vessels was estimated according to Kozai et al. (1986). The number of air exchanges of the FV vessels was gradually increased to maintain the CO_2 concentration inside the vessel in a range between 250 and 300 μmol·mol^{-1}. Culture vessels of all three treatments were placed in the same culture room. The air temperature and relative humidity (RH) inside the culture room were, respectively, 22 ± 1C and 50% ± 10% during the photoperiod (16 h·day^{-1}), and 20 ± 1C and 85% ± 10% during the dark period (8 h·day^{-1}).

The medium and air volumes per plantlet in all three treatments were similar. Twenty-eight explants per vessel were cultured in the FV treatment with 280 ml medium and five explants per vessel in the DV and the MV treatments with 50 ml medium. Planting density was 1.3×10^3 plantlets/m^2 in all the treatments, and the volume of the vessel per plantlet was 96 ml for MV and DV and 93 ml for FV.

To avoid possible damage to explants, especially by water stress during transplanting, vessels in the DV and the FV treatments were placed under 70 μmol·m^{-2}·s^{-1} PPF without ventilation for the first 2 days. The ventilation rate per vessel in the FV treatment was initially 100 ml·min^{-1} and was increased by 50 to 100 ml·min^{-1} every 2 or 3 days up to 950 ml·min^{-1} on day 30.

Plantlets in three vessels in the DV and the MV treatment and in one vessel in the FV treatment were taken for destructive measurements of fresh and dry weights and number of unfolded leaves on days 15, 23, and 30 of culture. Mean relative growth rate (RGR) were calculated as (per day): RGR = (In W_2 - 1n W_1)/(T_2 - T_1), where W_1 and W_2 are mean dry weight per plantlet on day T_1 and T_2, respectively.

The CO_2 concentrations inside the vessel (C_v) and the culture room (C_r) were measured over the culture period using a gas chromatograph (GC-12A; Shimazu, Kyoto, Japan). Air samples for CO_2 concentration measurement was taken 4 h after the initiation of the photoperiod, when the C_v and C_r were considered to have reached steady-state conditions (Fujiwara et al., 1987).

Net photosynthetic rate per plantlet (P_p and per leaf dry weight (P_d) were estimated from mean C_v and mean C_r using the following equations (Fujiwara et al., 1987): $P_p = k \cdot R \cdot (C_r - C_v)/n$ (micromoles CO_2 per hour) and $P_d = P_p/W_1$, (micromoles CO_2 per gram dry weight per hour), where k is a conversion factor (4.1×10^{-2} mol CO_2/liter); R is the ventilation rate per vessel (liters per hour); n is the number of plantlets per vessel; and W_1 is the mean leaf dry weight per plantlet (grams).

Dry weight per plantlet in the FV and the DV treatments on day 30 was 3.3 and 2.1 times, respectively, that in the MV treatment (Fig. 1). Fresh weights per plantlet in the FV and DV treatments were 2.4 times that in the MV treatment (Table 2). Dry matter content of the plantlets on day 30 was 8.9% for FV, 6.2% for DV, and 6.5% for MV. Plantlet growth promotion due to high PPF and increased number of air exchanges by using an air diffusive filter was in agreement with Kozai et al. (1988). However, there were no significant differences among the treatments in the number of unfolded leaves and shoot : root fresh weight ratio on day 30.

Increasing the number of air exchanges of the vessel might have affected CO_2 and ethylene concentrations, RH, and air velocity in the vessel. Growth promotion of the plantlets in the FV and the DV treatments are probably attributable to some of these factors, especially CO_2 concentration.

The C_v in the MV vessels during the photoperiod remained unchanged throughout the culture period and was at ≈ 100 μmol·mol^{-1}, which is near the CO_2 compensation point. The C_v in the DV vessels at the beginning of the experiment (270 μmol·mol^{-1}) was similar to that in the FV vessels. However, the C_v in the DV vessels at the end of the experiment (150 μmol·mol^{-1}) was about half of that in the FV vessel (Fig. 2a). This might have caused lower dry weight in the DV than the FV treatment.

The P_p increased dramatically in the FV but slowly in the DV treatment. It remained unchanged at about zero in the MV treatment (Fig. 2b). The plantlets in the FV treatment had higher P_d than in the DV and the MV treatments on each of the three measurement days (Table 2).

Relative growth rate of the plantlet, estimated during the period of days 0 to 15, 15 to 23, and 23 to 30, remained constant throughout the culture period in the FV treatment. Relative growth rate of the plantlet during the period of days 23 to 30 was 0.11 for FV, 0.07 for DV, and 0.04 for MV. The lower RGR in the DV treatment than in the

FV treatment may have resulted from the lower C_v in the last part of the culture period.

Effects of ethylene on the fresh and dry weights of the plantlet were not examined in this study, since ethylene was found to have no significant effects on fresh and dry weights of potato plantlets (Jackson et al., 1991). However, research is needed on the effects of accumulated ethylene on plantlet morphology under a photoautotrophic culture condition.

Air velocity influences photosynthesis and transpiration of plants. Nakayama et al. (1991) reported that the NPR of potato plantlets cultured photoautotrophically under forced ventilation was 1.5 times that under enhanced natural ventilation at 500 µmol CO_2/mol and 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPF. The average air velocity (the ventilation rate divided by the sectional area of the vessel) in this study was 0.17 $cm \cdot s^{-1}$ in the FV vessels on day 30, when the number of air exchanges was 22/h. More variation in growth of the plantlets was observed in the FV vessels than in the DV and the MV vessels. This might have been caused by a large spatial variation in air velocity in the FV vessels. To obtain uniform growth of the plantlets, air distribution in the vessel needs to be improved.

This study demonstrates the value of forced and diffusive ventilation on growth promotion of potato plantlets in vitro. Carbon dioxide enrichment of the incoming air also is worthy of consideration for further growth promotion.

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