

# Effect of CO<sub>2</sub> Concentration on Net Photosynthetic Rate of *Coffea arabusta* Somatic Embryos Cultured Photoautotrophically<sup>©</sup>

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## INTRODUCTION

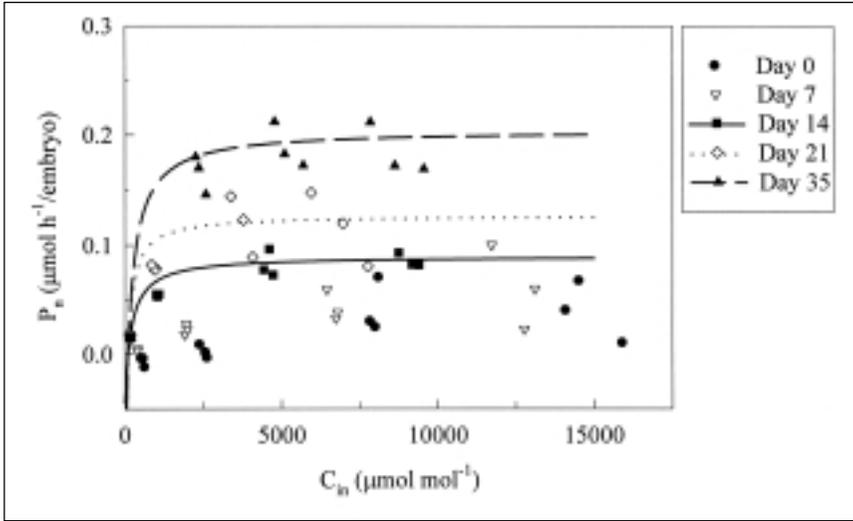
Somatic embryos have been used for woody plant micropropagation. In *Coffea*, Afreen et al. (2001) showed that *C. arabusta* cotyledonary embryos had photosynthetic ability and could be cultured photoautotrophically (sugar-free culture) (Kozai, 1991). Therefore, application of photoautotrophic culture from the cotyledonary embryo stage through the rest of the production stages potentially shortens the transplant production period and enhances the establishment of transplants *ex vitro* as compared with photomixotrophic culture (sugar-containing culture). In this study, effect of CO<sub>2</sub> concentration on the net photosynthetic rate of *C. arabusta* somatic embryos was investigated to determine the environmental conditions for the photoautotrophic culture of somatic embryos.

## MATERIALS AND METHODS

Ten cotyledonary embryos were cultured in each of three 22-ml vessels with MS medium and fibrous supporting materials (Florialite, Nisshinbo Ind. Inc., Tokyo). Cultures were placed for 36 d under 100 mmol m<sup>-2</sup>·s<sup>-1</sup> PPF, 16-h photoperiod, 26°C air temperature, 75% relative humidity, and 1500 mmol·mol<sup>-1</sup> CO<sub>2</sub> concentration. The number of air exchanges of the vessel was 3.0 h<sup>-1</sup>. On Days 0, 7, 14, 21, and 35 the vessels were sealed (0.05 h<sup>-1</sup> number of air exchanges) and changes in CO<sub>2</sub> concentration inside the vessel (C<sub>in</sub>) were recorded after it was adjusted to about 400, 3000, 8000, or 15,000 mmol·mol<sup>-1</sup>. The net photosynthetic rate (P<sub>n</sub>) and CO<sub>2</sub> compensation point (C<sub>c</sub>) were estimated based on C<sub>in</sub>, CO<sub>2</sub> concentration outside the vessels, vessel volume, CO<sub>2</sub> exchange rate of the medium (E<sub>M</sub>), and number of air exchanges of the vessel. A rectangular-hyperbolic model was fitted for P<sub>n</sub> to estimate the maximum P<sub>n</sub> (P<sub>m</sub>) and the CO<sub>2</sub> saturation point (C<sub>s</sub>). E<sub>M</sub> was estimated by measuring C<sub>in</sub> for vessels without somatic embryos. C<sub>s</sub> was estimated as the C<sub>in</sub> that gave 95% of simulated P<sub>m</sub>.

## RESULTS AND DISCUSSION

On Days 0 and 7, P<sub>n</sub> increased with increasing C<sub>in</sub> but it was not saturated at the C<sub>in</sub> examined. It was shown that effect of CO<sub>2</sub> concentration on P<sub>n</sub> were different between Days 0 to 7 and 14 to 35. On Days 14, 21, and 35, the P<sub>n</sub> was saturated and P<sub>m</sub> was estimated as 0.09±0.004, 0.13±0.03, and 0.2±0.02 mmol per h per embryo, and C<sub>s</sub> as about 5400, 3400, and 5000 mmol·mol<sup>-1</sup>, respectively (Fig. 1). The C<sub>c</sub> was about 100 mmol·mol<sup>-1</sup> on Days 14 to 35. Nguyen et al. (1999) also showed that photoautotrophic *C. arabusta* plantlets had high C<sub>s</sub> (>5000 mmol·mol<sup>-1</sup>). On Day 21, some somatic embryos had developed true leaves and 30% of somatic embryos had done so on Day 35. Increases in P<sub>n</sub> and P<sub>m</sub> with time observed in this experiment



**Figure 1.** Net photosynthetic rate per somatic embryo ( $P_n$ ) as affected by  $\text{CO}_2$  concentration inside the vessel ( $C_{in}$ ) on days 0, 7, 14, 21 and 35. All the somatic embryos on days 0, 7 and 14 were in the cotyledonary stages. Data on days 14, 21 and 35 were fitted with the model  $P_n = P_m \cdot (C - C_c) / (K + (C - C_c))$ .

were due to the growth and development of somatic embryos. Results suggest that photoautotrophic growth and development of the somatic embryos will be promoted by maintaining  $C_{in}$  at 3000 to 5000  $\text{mmol} \cdot \text{mol}^{-1}$ .

#### LITERATURE CITED

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