A new method to generate different CO₂ concentrations for developing a CO₂-response curve


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CO₂-response curves are finding increasing importance in photosynthesis research. Conventionally, these curves are generated using defined concentrations of CO₂ from known mixtures of air from gas cylinders. We have developed an indigenous and novel method to generate a constant supply of varied concentrations of CO₂. We demonstrate that this inexpensive method can be used to generate CO₂-response curves with higher efficiency and as much accuracy.

The CO₂-response curves refer to the pattern of increase in carbon assimilation rate (A) of leaves with increase in ambient CO₂ concentration¹⁻⁵. These response curves most commonly follow a hyperbolic pattern⁶⁻⁹. The initial slope of this hyperbolic equation is often considered to represent the 'carboxylation efficiency' of the system⁶. Initially the increase in A is almost linear at low CO₂ concentrations. The slope of this initial linear increase (dA/dCi) can also be considered as a reflection of the mesophyll conductance (g). Subsequent increase in ambient CO₂ shifts the limitation from CO₂ to the regeneration of the other substrate, namely ribulose 1, 5-bisphosphate (RuBP). The poor regeneration of RuBP limits the further increase in A, leading ultimately to the saturation of the response⁶.

The CO₂-response curves have been widely used as an important experimental tool for arriving at various aspects that govern the photosynthetic processes¹⁻²⁻⁶⁻⁸⁻⁹. For instance, by using the CO₂-response curves, it is possible to measure the CO₂ compensation point, to compare the plants for their maximum 'carboxylation efficiency' (g), to estimate the upper limit of CO₂ concentration at which RuBP regeneration becomes limiting, and finally to calculate the relative stomatal and mesophyll limitation for photosynthesis in species under control conditions and when experiencing different abiotic stresses.

There has been a constant improvisation of the procedure for the development of CO₂-response curves. Conventionally gas cylinders having various concentrations of CO₂ are used for generating CO₂-response curves. Here leaves are exposed to different concentrations of CO₂ for a fixed period of time under saturated light and the depletion of CO₂ is measured using an IRGA. Recently Heitholt et al.² mixed gases containing zero and 681 ppm of CO₂ in different proportions and arrived at different concentrations of CO₂. These techniques are costly and time-consuming.

With an objective to reduce the time and cost, we developed a simple device to obtain different CO₂ concentrations. In this paper we describe the methodology and show that it can be used to develop CO₂-response curves comparable to those obtained by conventional methods.

A diagram of the device to obtain different CO₂ levels is shown in Figure 1. As shown in the figure, the aluminium drum of 25-litres capacity in its inverted position, is placed over water in a plastic bucket of 50 litres. When filled with air the drum floats over water in the inverted position and when air is removed the drum moves down gradually, thus proportionally decreasing the volume of air in the drum. An iron frame is used to support the drum over water and also to facilitate its easy up and down movement. An air pump was used to pump air into the drum. A copper tube is passed along the inner side of the bucket and brought up so that its tip is slightly above the level of water at the centre of the bucket. Through this tube air with known CO₂ concentration could be pumped into the inverted drum and when once it is full the air can be easily drawn out through the outlet tubing without any alteration in the composition of air maintained in the drum.

To obtain CO₂ levels lower than the atmospheric concentration (i.e.330 ppm), the ambient air is pumped through a KOH-soda lime trap. The air coming out of this trap will be free of CO₂. This CO₂-free air is proportionally mixed with air containing 330 ppm CO₂ (ambient air) to generate different concentrations of CO₂ lower than the ambient level.

The following procedure was adopted to obtain CO₂ concentrations higher than the atmospheric level. Carbon dioxide was generated in a Kipp's apparatus by reacting 10 g of CaCO₃ with 20 ml of 1N HCl. A known volume of this CO₂ is taken in a syringe and connected to the pumping system. CO₂ is pumped into the drum using an air pump that pushed atmospheric air (330 ppm). The pumping of atmospheric air dilutes

![Figure 1](image-url)
the enriched air, resulting in a known concentration of CO₂ higher than the atmospheric concentration. For instance, 15 ml of CO₂ when diluted to 25 l in the aluminium drum, gives 600 ppm CO₂ gas mixture. By altering the volume of CO₂ taken for dilution, different concentrations of CO₂ were generated. In all, six concentrations, three lower and three higher than the atmospheric concentration, were generated.

The CO₂ concentration was continuously monitored by the IRGA which forms a component of the potable photosynthesis system (ADC LCA-2). The generated CO₂ concentration remains fairly constant for a period over 20 min. Since the time of exposure of leaves to each concentration is not more than 5–7 min, the possible changes in the concentration of CO₂ do not impose any serious problems while measuring A.

The drums are sequentially connected to the air supply unit (ASU) of the portable IRGA, ADC LCA-2 in the open system.

Development of CO₂-response curves. The CO₂ assimilation rates were measured using the youngest fully expanded leaves of sunflower and cowpea at saturated light intensities. The experiments were conducted between 9 and 12 noon (local standard time). The selected leaf (6.25 cm²) was clamped to the leaf chamber and held perpendicular to the incident light. The RH in the leaf chamber was maintained at a steady-state level around the existing ambient RH by manipulating the rate of flow of dry air by the air supply unit. Gas-exchange rates were recorded after A and gs stabilized to a new steady-state condition. The assimilation rates at all the CO₂ concentrations were recorded on the same exposed leaf area within 20 min of clamping.

Based on the measured A and gs, the intercellular CO₂ concentration (Ci) was computed using the following formula.

\[ Ci = ((g_c - E/2) \times C_c - A) / (g_c + E/2) \]

where \( g_c \) is the total conductance to CO₂ transfer (mols m⁻² s⁻¹), \( E \) the transpiration rate (mols m⁻² s⁻¹), \( C_c \) the CO₂ concentration in the cuvette (ppm) and \( A \) the assimilation rate (μmols m⁻² s⁻¹).

The data logger of the portable photosynthesis system is equipped with these formulae and the instrument simultaneously calculates the Ci and is stored in the memory. The assimilation rates (A) were plotted against the intercellular CO₂ concentration (Ci).

From the data, the relative stomatal limitation was computed as proposed by Farquhar and Sharkey. The CO₂ concentration at which A became positive was considered as the CO₂-compensation point.

The CO₂-response curves for both cowpea (Figure 2) and sunflower (Figure 3) resemble those reported by other workers. The initial increase in A to low Ci is often linear. This linear increase in A can be considered as the 'carboxylation efficiency' of the leaf. This initial linear increase in A of sunflower and cowpea were 0.150 and 0.086 mmols m⁻² s⁻¹ ppm⁻¹ respectively (Table 1).

The relative limitation computed from the data showed a stomatal limitation of photosynthesis of 30 and 36.36% in sunflower and cowpea respectively. These values are in agreement with those previously reported in wheat, sunflower and amaranthus. For both the crops, CO₂ compensation point estimated was well within the range reported for C₄ plants.

We conclude that the method described here can be
Table 1: Gas exchange characteristics and relative stomatal limitation of cowpea and sunflower

<table>
<thead>
<tr>
<th></th>
<th>Cowpea</th>
<th>Sunflower</th>
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</thead>
<tbody>
<tr>
<td>CO₂ compensation point (ppm)</td>
<td>60.0</td>
<td>70.0</td>
</tr>
<tr>
<td>4 at 330 ppm CO₂</td>
<td>12.7</td>
<td>21.0</td>
</tr>
<tr>
<td>C (ppm)</td>
<td>220.0</td>
<td>245.0</td>
</tr>
<tr>
<td>Initial slope of &amp; d(G)</td>
<td>0.086</td>
<td>0.150</td>
</tr>
<tr>
<td>C at 4_{max}</td>
<td>500.0</td>
<td>473.0</td>
</tr>
<tr>
<td>4_{max}</td>
<td>22.75</td>
<td>36.00</td>
</tr>
<tr>
<td>Relative stomatal limitation (1 - &amp; a)</td>
<td>36.36</td>
<td>30.00</td>
</tr>
</tbody>
</table>

drum can supply an accurately known concentration of CO₂ for over 25 min.


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