A new method to generate different CO_2 concentrations for developing a CO_2-response curve

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CO_2-response curves are finding increasing importance in photosynthesis research. Conventionally, these curves are generated using defined concentrations of CO_2 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 CO_2. We demonstrate that this inexpensive method can be used to generate CO_2-response curves with higher efficiency and as much accuracy.

THE CO_2-response curves refer to the pattern of increase in carbon assimilation rate (A) of leaves with increase in ambient CO_2 concentration[1-5]. These response curves most commonly follow a hyperbolic pattern[6,9]. The initial slope of this hyperbolic equation is often considered to represent the 'carboxylation efficiency' of the system[6]. Initially the increase in A is almost linear at low CO_2 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 CO_2 shifts the limitation from CO_2 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 response[9].

The CO_2-response curves have been widely used as an important experimental tool for arriving at various aspects that govern the photosynthetic processes[1,2,6-8]. For instance, by using the CO_2-response curves, it is possible to measure the CO_2 compensation point, to compare the plants for their maximum 'carboxylation efficiency' (g), to estimate the upper limit of CO_2 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 improvization of the procedure for the development of CO_2-response curves. Conventionally gas cylinders having various concentrations of CO_2 are used for generating CO_2-response curves. Here leaves are exposed to different concentrations of CO_2 for a fixed period of time under saturated light and the depletion of CO_2 is measured using an IRGA. Recently Heitholt *et al.*[2] mixed gases containing zero and 681 ppm of CO_2 in different proportions and arrived at different concentrations of CO_2. These techniques are costly and time-consuming.

With an objective to reduce the time and cost, we developed a simple device to obtain different CO_2 concentrations. In this paper we describe the methodology and show that it can be used to develop CO_2-response curves comparable to those obtained by conventional methods.

A diagram of the device to obtain different CO_2 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 CO_2 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 CO_2 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 CO_2. This CO_2-free air is proportionally mixed with air containing 330 ppm CO_2 (ambient air) to generate different concentrations of CO_2 lower than the ambient level.

The following procedure was adopted to obtain CO_2 concentrations higher than the atmospheric level. Carbon dioxide was generated in a Kipp's apparatus by reacting 10 g of $CaCO_3$ with 20 ml of 1N HCl. A known volume of this CO_2 is taken in a syringe and connected to the pumping system. CO_2 is pumped into the drum using an air pump that pushed atmospheric air (330 ppm). The pumping of atmospheric air dilutes

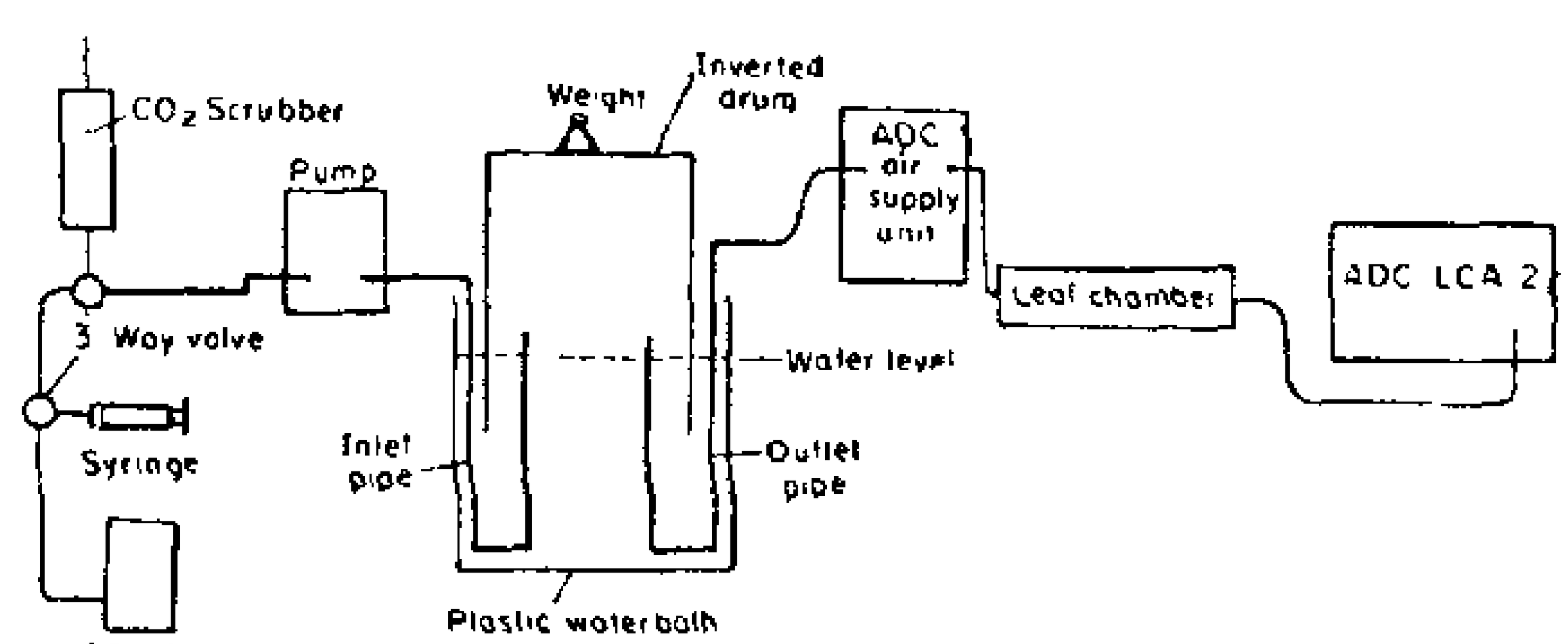

Figure 1. Experimental set-up to obtain different CO_2 concentrations for A/Ci curves using ADC 2CA-2.

the enriched air, resulting in a known concentration of CO_2 higher than the atmospheric concentration. For instance, 15 ml of CO_2 when diluted to 25 l in the aluminium drum, gives 600 ppm CO_2 gas mixture. By altering the volume of CO_2 taken for dilution, different concentrations of CO_2 were generated. In all, six concentrations, three lower and three higher than the atmospheric concentration, were generated.

The CO_2 concentration was continuously monitored by the IRGA which forms a component of the potable photosynthesis system (ADC LCA-2). The generated CO_2 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 CO_2 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 CO_2-response curves. The CO_2 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 (6.25cm^2) 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 CO_2 concentrations were recorded on the same exposed leaf area within 20 min of clamping.

Based on the measured A and gs, the intercellular CO_2 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 CO_2 transfer (mols m^{-2} s^{-1}), E the transpiration rate (mols m^{-2} s^{-1}), C_c the CO_2 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 CO_2 concentration (Ci).

From the data, the relative stomatal limitation was computed as proposed by Farquhar and Sharkey[6]. The CO_2 concentration at which A became positive was considered as the CO_2-compensation point.

The CO_2-response curves for both cowpea (Figure 2) and sunflower (Figure 3) resemble those reported by other workers[1,2,6,8–10]. *The initial increase in A to low*

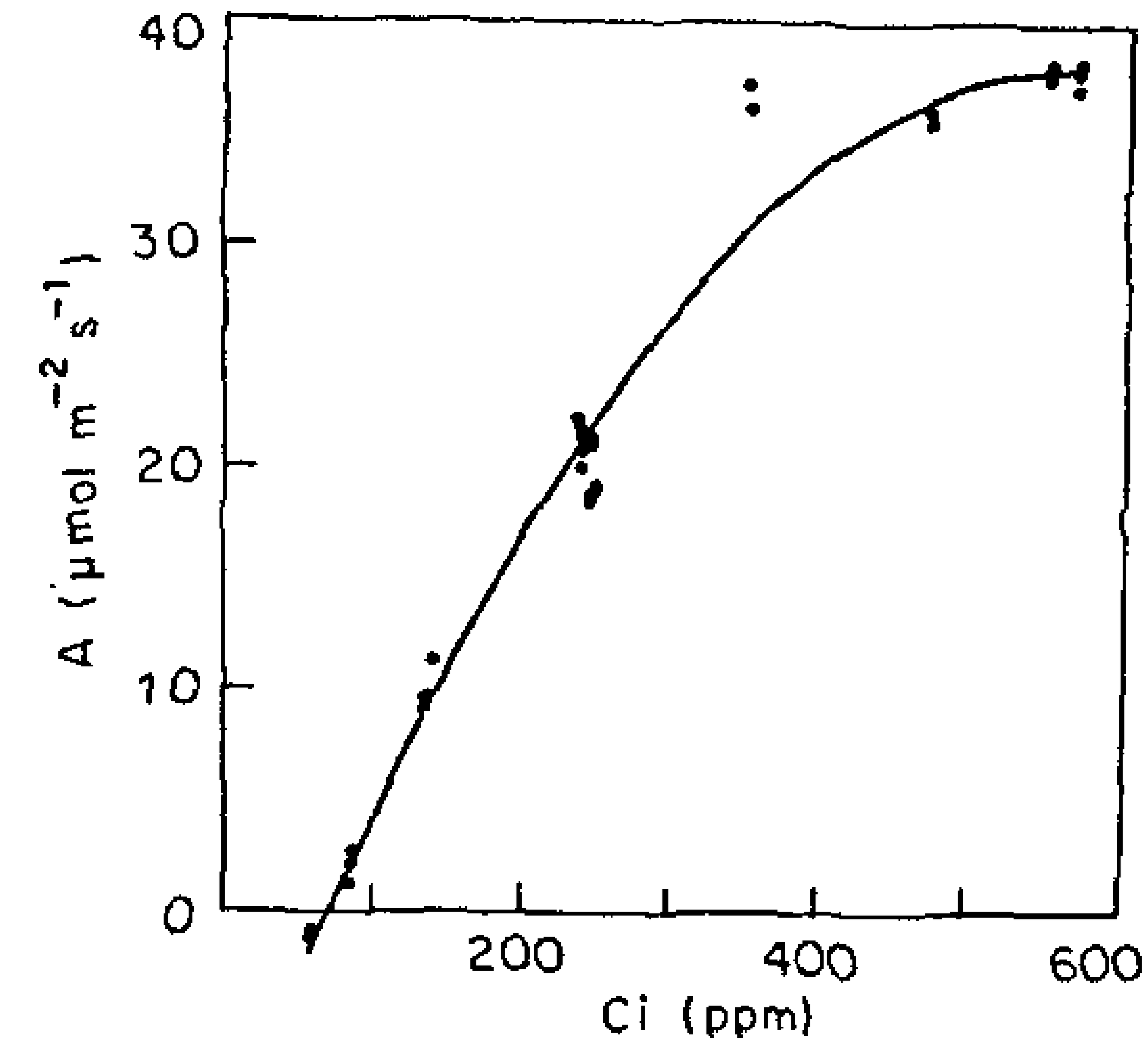

Figure 2. CO_2-response curve for sunflower ($R^2 = 0.99$). The initial slope (dA/dCi) was considered as the carboxylation efficiency of the leaf. $A = -11.614 + 0.174Ci - 0.000153Ci^2$.

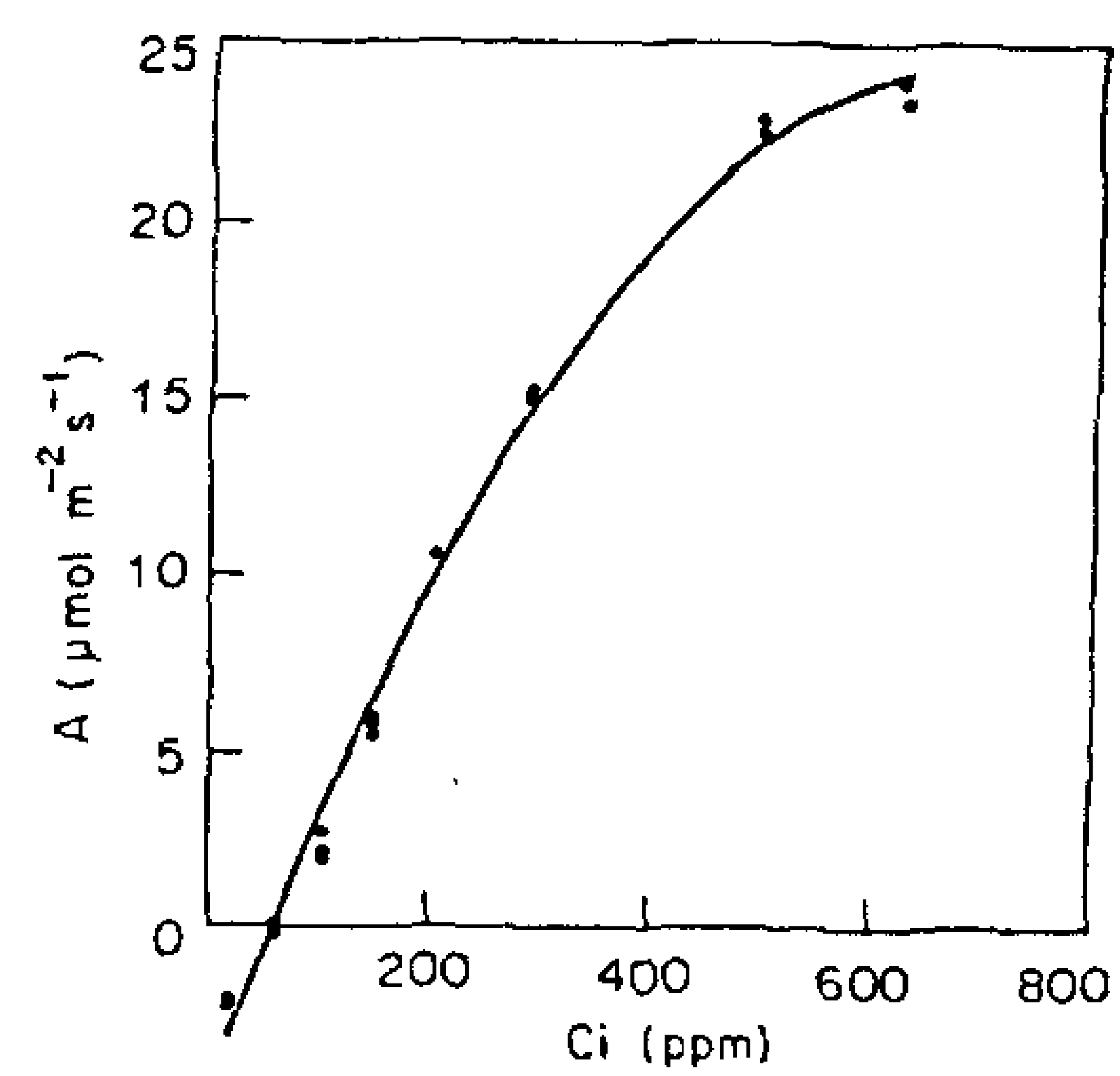

Figure 3. CO_2-response curve for cowpea ($R^2 = 0.98$). The initial slope (dA/dCi) was considered as the carboxylation efficiency of the leaf. $A = -4.779 + 0.0845Ci - 6.102 \times 10^{-6} Ci^2$.

Ci is often linear. This linear increase in A can be considered as the 'carboxylation efficiency' of the leaf[6,9]. This initial linear increase in A of sunflower and cowpea were 0.150 and 0.086 μmoles 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[2], sunflower[1] and amaranthus. For both the crops, CO_2 compensation point estimated was well within the range reported for C_3 plants.

We conclude that the method described here can be

Table 1. Gas exchange characteristics and relative stomatal limitation of cowpea and sunflower

	Cowpea	Sunflower
CO_2 compensation point (ppm)	60.0	70.0
A at 330 ppm CO_2	12.7	21.0
Ci (ppm)	220.0	245.0
Initial slope (dA/dCi)	0.086	0.150
Ci at A_{max}	500.0	473.0
A_{max}	22.75	36.00
Relative stomatal limitation (ls %)	36.36	30.00

used for developement of CO_2-response curves in plants with high precision. This method is inexpensive compared to the use of mixtures of gases from gas cylinders. Further the use of this technique becomes pronounced, especially when studies of a number of species or genotypes are undertaken, because each drum can supply an accurately known concentration of CO_2 for over 25 min.

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