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A simple technique to expose tree seedlings to elevated CO_2 for increased initial growth rates

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Initial growth rates of most tree species that are used in afforestation programmes are very low. Therefore, polybag planted seedlings have to be maintained in the nurseries for a long period of time. Growing plants in an elevated CO_2 atmosphere increases the growth rates as well as biomass production in many annual crop and tree species. Higher temperature and relative humidity in association with elevated CO_2 concentration helps to boost the biomass and leaf area production. We demonstrate here an easy and cost-effective method for obtaining elevated CO_2 concentrations for better growth of tree seedlings in the nursery.

APART from maintaining a balanced ecosystem, forests are major sinks of CO_2. Deforestation and burning of fossil fuel (due to population pressure) are the two major reasons for accumulation of CO_2 in the atmosphere leading to global climate change. Currently the CO_2 concentration in the atmosphere is around 360 ppm and it is increasing at the rate of 1.8 ppm per year[1]. Hence afforestation is a practically feasible way to address the global climate change, especially in tropical countries where forest felling is occurring at a faster rate.

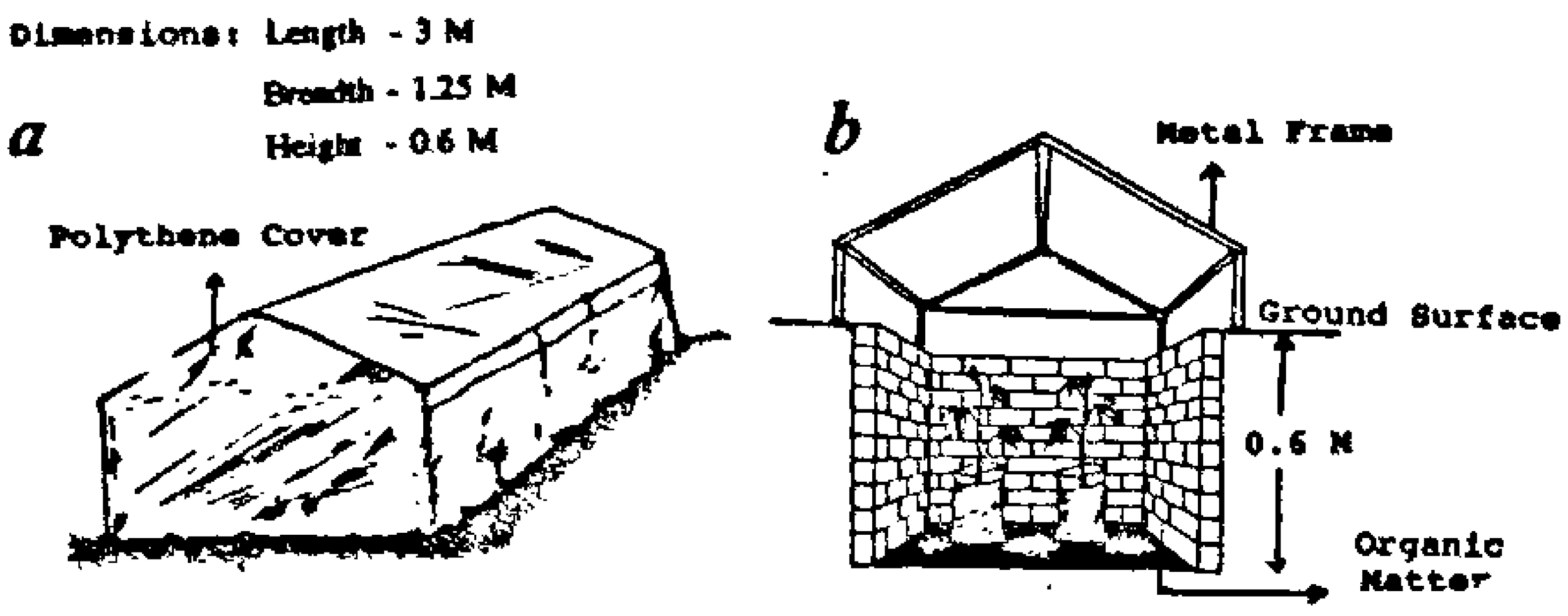

Figure 1*a*, *b*. Set up of the CO_2 enrichment system used for growing seedlings under elevated CO_2 concentration. ***a***, Depicts the polythene cover enclosing the seedlings inside the trench; ***b***, Cross-sectional view of the trench in which seedlings are kept and enclosed to expose to elevated CO_2 concentration. Floor of the trench is spread with decomposed organic matter. A metal frame is placed on the trench that supports the polythene cover to enclose the seedlings.

For any successful afforestation programme it is necessary to produce robust and healthy seedlings. Growing plants under elevated CO_2 concentrations has proved to enhance the biomass production[2]. For obtaining elevated CO_2 concentrations, there are different systems available. Depending upon the need and purpose, one can select the desirable type of CO_2 enrichment system. These include, closed systems in controlled environment cabinets, 'Solardome' glass house, open top chambers, FACE (free air CO_2 enrichment), etc[3].

There are advantages and disadvantages associated with these methods. However, one major constraint in most of these techniques is the high cost involved to expose the plants to elevated CO_2. In the present study we have developed a simple and economic technique to grow polybag seedlings under elevated CO_2 concentrations obtained from decomposition of organic matter. We demonstrate its successful use in obtaining increased growth rates in thirteen tree species at seedling stage.

Rectangular trenches of 3 m length, 1.25 m width and 0.6 m depth, were made in a place exposed to open sunlight. All the four sides of the cut ends of the trench were provided with a single layer of brick lining to avoid sliding of the edges. Inside the trench a layer of well decomposed organic matter was spread uniformly all along the floor. 16 kg of organic matter was required for the trench of above dimension to get a CO_2 concentration of 700–750 ppm (Figure 1).

A rectangular metal frame was placed over the trench completely enclosing it. The frame was fabricated using hollow galvanized iron tube of 1.5 cm diameter. Height of the frame was 1.6 m with a gable roof. Using high density polythene sheet of 125 μ gauge, a cover was tailored to suit the size of the frame. Length of the polythene cover was 12 inches longer than the frame so as to allow a free and flat fall on the ground after enclosing the metal frame. On this free lying polythene, a thin layer of sand was poured to keep the complete system air tight.

In this system we made use of the CO_2 produced by decomposition of organic matter spread on the floor of the trench. CO_2 thus released was trapped in the polythene chamber and a concentration of 700–750 ppm was obtained. Seedlings were exposed to higher concentrations of CO_2. Polybag seedlings were arranged in the trench so that the leaves of adjacent plants did not overlap and cause mutual shading (Figure 1 *b*). Seedlings were exposed to elevated CO_2 between 3.30 pm and 11.00 am. Before closing the trenches water was sprinkled on the organic matter to stimulate soil respiration. Polybag seedlings were also watered before closing the chamber. In this system, apart from CO_2, relative humidity and temperature also built up inside the chamber.

Photosynthetic photon flux (PPF) over a wavelength of 400–700 nm, CO_2 concentration and relative humidity were measured using LI-6000 Portable Photosynthesis system (LI-Cor Inc, Nebraska, USA). PPF measurement inside the trench was made at 15 cm below the roof of the polythene structure. Temperature inside the trench and ambient air were measured simultaneously. Leaf area was measured using portable leaf area meter (LI-3000, LI-Cor Inc, Nebraska, USA). The thirteen species studied were – *Annona squamosa, Zizyphus jujuba, Tamarindus indica, Acacia auriculiformis, Derris indica, Spathodia campanulata, Feronia elephantum, Artocarpus integrifolia, Swietenia microphylla, Eucalyptus citriodora, Tectona grandis, Dalbergia latifolia* and *Dalbergia sissoo*.

Carbon dioxide concentration inside the trench started to build after enclosing the trenches at 350 ppm and reached 700–750 ppm in less than an hour. It remained high all through the night and started decreasing after sunrise in the morning (Figure 2). There was no difference in the concentration of CO_2 thirty minutes after closing the trenches in the air samples drawn from different heights inside the trench. In the morning, photosynthetically active radiation started increasing (Figure 3 *a*) corresponding with the decrease in the CO_2 concentration inside the trench (Figure 2 *b*), suggesting the utilization of higher concentration of CO_2 by the plants in the presence of sunlight for photosynthesis. Simultaneously, rise in the temperature inside the trenches was also more after sunrise (Figure 3 *b*). An average of 1.21°C rise was seen inside the chamber compared to ambient air up to 8.00 am, while this difference from 8.00 am to 11.00 am was 4.69°C. Therefore, it was not possible to expose the seedlings for long periods after 11.00 am. Along with CO_2 and temperature, build up of relative humidity was also noticed inside the trench (Figure 3 *c*). RH built up inside the chamber within thirty minutes after closing and reached the saturation level. It remained so till the trenches were exposed to open air, next day.

Using this system, seedlings of thirteen tree species were exposed to elevated CO_2, relative humidity and

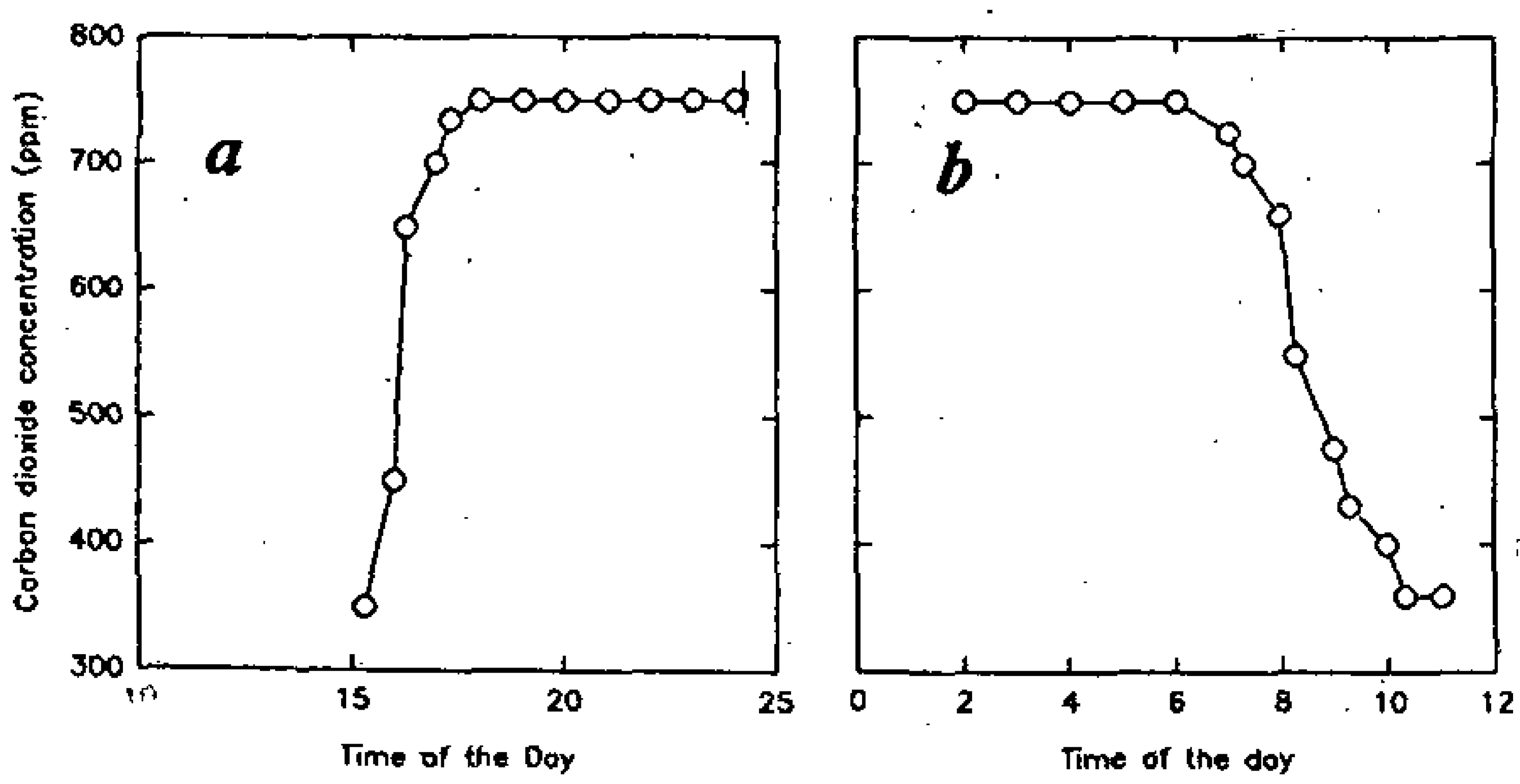

Figure 2*a*, *b*. Changes in the CO_2 concentration inside the chamber. *a*, Build-up of the CO_2 concentration inside after enclosing the trench (1530 h to 0600 h). *b*, Depletion of CO_2 concentration after 0700 h up to 1100 h.

Table 1. Dry matter (g/plant) production after ninety days of growth of seedlings under ambient air and elevated CO_2 concentrations

Species	Dry matter produced per plant (g/plant)		Increase in dry matter production in elevated CO_2 plants over ambient air grown plants (%)
	Grown under ambient air	Grown under elevated CO_2	
Annona squamosa	6.14	8.32	35.5
Zizyphus jujuba	4.24	9.54	125.0
Tamarindus indica	3.96	4.45	12.3
Acacia auriculiformis	9.30	13.60	44.0
Dalbergia sissoo	4.40	12.20	193.0
Spathodia campanulata	7.90	14.80	88.0
Derris indica	4.04	9.50	135.0
Feronia elephantum	4.75	5.58	17.0
Artocarpus integrifolia	3.40	8.70	155.0
Swietenia microphylla	7.88	11.03	101.0
Eucalyptus citriodora	5.54	8.38	51.0
Tectona grandis	0.70	3.92	460.0
Dalbergia latifolia	6.14	8.38	36.0
Mean	5.26	9.10	111
C.D. at 5%			
Treatment 0.60			
Species 0.63			

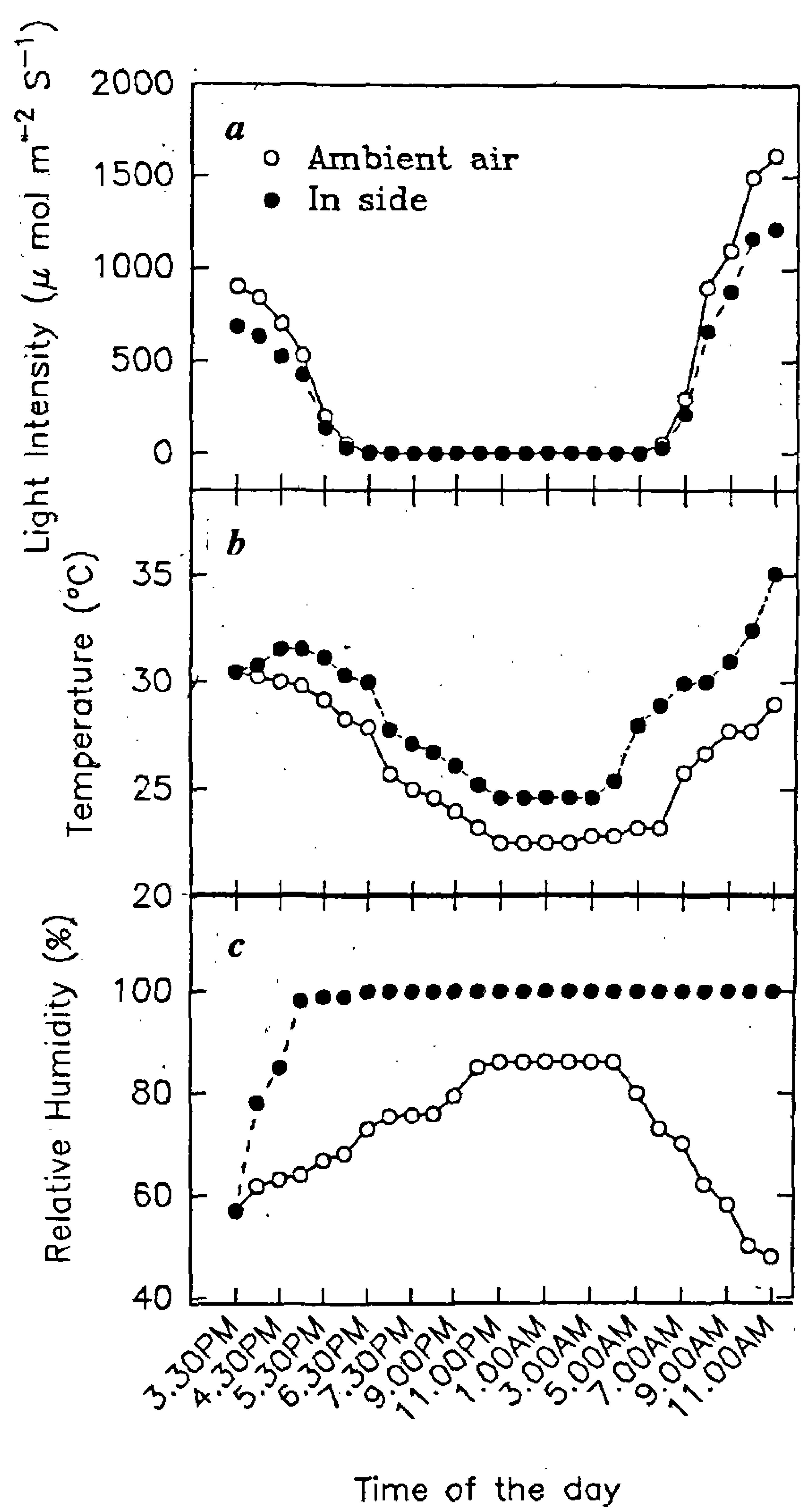

Figure 3*a–c*. Changes in (*a*) light intensity, (*b*) temperature and (*c*) relative humidity in the ambient air (O) and inside the chamber (●) for the period during which the seedlings were exposed to elevated CO_2 concentration.

temperature. Six-month-old seedlings were subjected to this treatment for ninety days and their responses were assessed to study the effectiveness of the system described.

There was a positive response to elevated CO_2 in biomass production, though the extent of response varied with the species. From this data it is evident that growth rates were better under elevated CO_2 concentration (Table 1). Among the various species, *Tectona grandis* showed the highest response to elevated CO_2 followed by *Dalbergia sissoo* and *Artocarpus integrifolia* in terms of biomass production. The total dry matter of *T. grandis* was 460 per cent more under elevated CO_2, compared to that of ambient air grown seedlings. The least responsive was *Tamarindus indica* recording only 12% increase (Table 1).

In this system, plants are making use of higher concentrations of CO_2 for carbon assimilation in the presence of light in the morning hours between 7.00 and 11.00 am. Evidences from a large body of literature show that it is possible to increase the biomass production by growing plants under elevated CO_2 (refs 2, 4, 5). In the present method of CO_2 fertilization, plants were

Table 2. Leaf area (cm) produced after ninety days growth under elevated CO_2 and ambient air grown seedlings

Species	Leaf area per plant (cm^2)		Increase in leaf area of elevated CO_2 plants over ambient air grown plants (%)
	Grown under ambient air	Grown under elevated CO_2	
Annona squamosa	45	189	320
Zizyphus jujuba	73	260	256
Tamarindus indica	61	78	27
Acacia auriculiformis	224	415	85
Dalbergia sissoo	173	641	270
Spathodia campanulata	260	824	216
Derris indica	155	520	235
Feronia elephantum	113	164	45
Artocarpus integrifolia	74	326	340
Swietenia microphylla	278	426	53
Eucalyptus citriodora	334	454	35
Tectona grandis	32	356	1012
Dalbergia latifolia	115	197	71
Mean	149	373	228
C.D. at 5%			
Treatment 20.12			
Species 21.21			

also experiencing relatively higher temperatures compared to ambient air grown plants. Imai and Murata[6], noticed enhancement of leaf area and whole plant dry weight at higher CO_2 associated with higher temperature. Long[7] has shown that proportionate increase in photosynthesis resulting from elevated CO_2 will rise with temperature. He also suggested that elevated CO_2 alters the magnitude of response of leaf canopy carbon gain to rising temperature and relative humidity which apparently enhances growth. It is likely that temperature and humidity associated with higher concentration of CO_2 must have helped in higher carbon assimilation and leaf expansion rates respectively.

Another important plant character that showed a remarkable increase under elevated CO_2 was leaf area per plant. A significant increase in leaf area was noticed among the species under elevated CO_2 when compared to ambient air grown plants (Table 2). All the species responded positively to elevated CO_2 concentration. An average of 228% increase in leaf area production was recorded under elevated CO_2 (mean of all the thirteen species studied). Similar type of studies conducted under controlled conditions have reported increase in leaf area in plants grown under high concentrations of CO_2 (refs 8–10). When higher temperature is associated with elevated CO_2 concentrations, leaf area is further enhanced[11]. It is due to higher leaf initiation rate, leaf expansion and individuals leaf area. Saman *et al.*[12] have shown that leaf elongation rates were more under elevated CO_2 concentrations and were associated with higher sucrose phosphate synthase activity during the early vegetative stage when growing blades were strong carbohydrate sinks. Leaf elongation rate is one of the ways by which leaf area development can be stimulated. Relative humidity is another important weather parameter that determines leaf growth. Elevated CO_2 concentrations, associated with higher temperature and RH might have influenced the leaf expansion rates. Relative humidity can reduce the vapour pressure deficits and thus help in maintaining high turgidity of the cells providing congenial conditions for cell division, elongation and expansion. Another possible reason for higher growth seen under this system could be due to lower dark respiration rates. According to Tanaka[13], efficiency of respiration increases as a result of whole plant CO_2 enrichment. Decreased rates of respiration at higher concentrations of CO_2 was noticed[14,15]. Even a small change in the respiration rate can have a considerable influence on assimilate retention and therefore its utilization for growth processes.

In the above method described for growing polybag seedlings of forest tree species, the main advantage is that there is no need of adding CO_2 from an external source, thereby reducing total cost considerably. Construction of the system is within the scope of a small workshop and material required is easily available. This system can be effectively used to enhance the initial growth rates of the seedlings specially forest tree species which have very poor growth rates. This can help in getting robust seedlings that may perform better when field planted and also helps in reducing the casualty.

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