PHYSIOLOGICAL STUDIES OF CARBON DIOXIDE ENRICHMENT EFFECTS ON SOME CROP PLANTS

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PHYSIOLOGICAL STUDIES OF CARBON DIOXIDE ENRICHMENT EFFECTS ON SOME CROP PLANTS

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CERTIFICATE

This is to certify that the thesis entitled **"Physiological Studies of Carbon Dioxide Enrichment Effects on Some Crop Plants"** submitted to the Post Graduate School, Indian Agricultural Research Institute, New Delhi, by **Mr. P. Raghuveer Rao**, in partial fulfilment of the requirements for the award of the degree of **DOCTOR OF PHILOSOPHY IN PLANT PHYSIOLOGY**, is a record of *bonafide* research carried out by him under my guidance and supervision and that no part of the thesis has been submitted for any other degree or diploma.

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ABSTRACT

The increase in CO_2 concentration in the atmosphere has generated a worldwide concern. Apart from environmental effect (determining the temperature of the planet), CO_2 also affects plants directly as it is the primary substrate for photosynthesis (biological effect). Therefore, it was felt that experiments involving long term exposure of crop plants to high CO_2 concentration under our agro-climatic conditions are necessary.

For this purpose, the experiments were designed in a way such that plants receive higher levels of CO_2 during the entire period of growth and development. Two methods were developed to grow the plants in enriched CO_2 conditions. In the first method, the night-respired CO_2 in the field conditions was trapped using transparent polythene cover during night time. This raised the CO_2 level inside the cover which allowed the plants to have CO_2 enriched conditions for few morning hours. The second method used an open top chamber lined with transparent polythene sheet and commercially available pure CO_2, to elevate the level of CO_2 to 600 ppm in outdoor conditions.

The method using polycover was found to be inappropriate for CO_2 enrichment studies because of increased temperature and humidity inside the polycover along with increased CO_2 levels. Open-top chamber method proved to be successful as the desired level of CO_2 could be obtained keeping the temperature and humidity similar to ambient air. Enriching the air with CO_2 either using polycover or open-top chambers resulted in increased growth and biomass production in all the crops studied. Open top chamber studies showed that growing crops at 600 ppm CO_2 increased photosynthesis which was sustained throughout the growth period. High photosynthesis was accompanied by decreased respiration, and plants matured early under CO_2 enriched conditions. CO_2 enriched plants showed increased accumulation of starch in the leaves, higher economic yield and harvest index.

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CHAPTER-I

INTRODUCTION

Atmospheric carbon dioxide (CO_2) concentration has increased over the past century from about 290 ppm in the late 1800s to the current level of about 350 ppm. The rise in atmospheric CO_2 became steep after the industrial revolution and increased man-made activities including industrial waste disposal, fossil fuel combustion and accelerated land clearing, causing an increase of about 100 million tons of CO_2 a year. The CO_2 monitoring station at MaunaLoa in Hawaii has shown the rise in CO_2 to be about 0.8 to 1.0 per cent annually (Trabalka *et al.*, 1985; Strain, 1987) and it is projected that the CO_2 levels will double or increase several fold during the next century (Allen, 1989). This increase in CO_2 level has generated a worldwide concern as it will affect global climate. This is because of the fact that inspite of its relatively small concentration, CO_2 plays a vital role in determining the temperature of the planet (environmental effect).

The level of atmospheric CO_2 also affects plants directly as it is the primary substrate for photosynthesis and carbohydrate production (biological effect). Therefore any change in the level of CO_2 concentration will have a direct effect on growth and productivity of crop plants (Lemon, 1983; Bazzaz *et al.*, 1985; Cure and Acock, 1986). This effect of CO_2 has been shown as early as 1804 by de Sausere. Since then a number of studies have been

conducted, more so in the past decade. Many global models have been developed for predicting the possible impact of climate change, but the magnitude and rate of change are much disputed (Plass, 1959; MacCracken and Luther, 1985; Mitchell, 1989). There is a large body of literature on several aspects of plant response to CO_2, examining the effects on many species, but most are with a strong bias towards the crops of temperate latitude, and under very different agro-climatic conditions. Relatively very less work has been done on crop species of tropical agroclimatic zones.

Most studies on CO_2 enrichment effects on crop plants have been conducted under controlled environments with low irradiance and only a few under field conditions. However, there seems to be a general agreement that increasing CO_2 increases photosynthesis which may lead to increased dry matter production varying from 20 to 40 percent with a mean of 33 percent crop yield (Kimball, 1983). The magnitude depends, however, on species studied and environmental conditions. Many short-term experiments, have shown a large increase in the rate of photosynthesis (Ho, 1977; Huber *et al.*, 1984; Campbell and Young, 1986; Sengupta, 1988; Sharma and Sengupta, 1990). However, measurements made over a short period of time do not necessarily provide reliable information regarding what occurs when plants are grown under high CO_2 concentration for a longer period. This is because several reports showed in case of photosynthesis that though the initial rate was high, it decreased later (Claugh *et al,*. 1981; Campbell *et al.*, 1988). There are some reports where CER (carbon dioxide exchange rate)

measured at high CO_2 concentrations was similar to or less than that in plants grown under ambient CO_2 (Havelka *et al.* 1984; Peet *et al.*, 1986). Decline in photosynthesis in later stages has been reported to be caused due to high starch accumulation causing feedback inhibition/or physical damage at chloroplast level (Huber *et al.* 1984; Madsen, 1975; Wulff and Strain, 1982).

In some field studies, in open-top chambers, there was no decrease in photosynthetic efficiency in spite of great increase in starch. This was due to high sink demand because of early maturity of fruits in the field resulting in non-acclimation of CER (Radin *et al.*, 1987). Most of the studies are far from conclusive due to variability in the crop species and experimental conditions. In addition to the effect on photosynthesis, increased CO_2 level also affects stomatal conductance. Morrison and Gifford (1984) reported that a doubling of CO_2 decreased stomatal conductance. However, stomatal sensitivity is not a limitation to CO_2 transport. Reduction of stomatal conductance coupled with enhanced photosynthesis results in increased water use efficiency. Thus, overall effect of increased photosynthesis is stimulatory on growth resulting in increased dry matter production.

Earlier in this laboratory, it has been observed that short-term exposure of crops to high CO_2 concentration resulted in increased CER with increased partitioning of assimilates to various sinks. Therefore, it was felt that experiments involving long term exposure of crop plants to high CO_2 concentration under our agro-climatic condition are necessary. Techniques for long-term exposure using

open-top chamber are not available in this country. Therefore an indigenous technique was developed using open-top chamber for growing plants outdoor at the desired level of CO_2 concentration. The present study was undertaken with the following objectives :

1) To develop and assess methodologies for growing crop plants in high CO_2 conditions, in open top chambers,

2) to determine the response of crop species to elevated levels of CO_2 concentration in terms of growth, biomass and yield,

3) to elucidate interactions between photosynthesis vis-a-vis growth and yield in response to elevated CO_2 levels.

CHATPER-II

REVIEW OF LITERATURE

As global population increases and industrialization takes place, with a concomitant increasing use of fossil fuels, carbon dioxide is injected into the atmosphere at increasing rates. The preindustrial atmospheric CO_2 concentration has been estimated to be 280 ppm (Gammon *et al.*, 1985) and it has increased to 353 $\pm$ 5 ppm today. This rise is an increase of 21 per cent in 170 years, with the most rapid increase occuring since 1950, when the concentration was 310 ppm. There is little dispute that this increasing CO_2 in the atmosphere will get further accelerated and reach a mean of 600 ppm by the year 2030 A.D. (Idso, 1980). The controversy lies in the likely impact of this increased CO_2 on agriculture and the uncertainty of timing and scale of changes being brought by increasing CO_2 in the atmosphere. According to Idso (1980) the increase in carbon dioxide is measurable and global warming may occur sometime in the more distant future. There is no evidence that the earth has already begun to warm. There is cause to be concerned, but the changes will not be as rapid or as severe as some sensational news reports indicate. Some changes will even be for the betterment of earthy human population - like increased food production. Reports are pouring in from various parts of the world, particularly from Duke phytotron (USA) and open top chamber studies, showing varied response of crops when grown

under elevated CO_2 conditions. In India, little or no effort is being made to study the changing levels of CO_2 in the atmosphere and its likely impact on Indian agriculture. In this chapter, the available and relevant literature is reviewed, in order to understand and summarise physiological changes in crops as effected by increased carbon dioxide. The literature review is divided into major subheads and dealt with accordingly.

2.1 Carbon dioxide enrichment effects on photosynthesis, respiration and stomatal conductance.

2.2 Carbon dioxide enrichment effects on chlorophyll, starch and sugars.

2.3 Carbon dioxide enrichment effects on growth and phenology.

2.4 Carbon dioxide enrichment effects on yield and yield components.

2.5 Interaction of enriched carbon dioxide, temperature, light, water and nutrients on crop growth.

2.1 CARBON DIOXIDE ENRICHMENT EFFECTS ON PHOTOSYNTHESIS, STOMATAL CONDUCTANCE AND RESPIRATION

PHOTOSYNTHESIS

Carbon dioxide is an essential component of the vital process of photosynthesis. Plants growing in higher atmospheric CO_2 concentration exhibit increased rate of photosynthesis, because more CO_2 enters the leaves due to increased CO_2 gradient between the external atmosphere and the air space inside the leaves (Acock and Allen, 1985).

Brun and Cooper (1967) have found that photosynthetic rate of individual soybean leaves was four times more at 1670 ppm CO_2 as compared to normal atmosphere. Sengupta (1983) has reported that wheat leaf, under short term CO_2 exposure, showed increasing photosynthetic rate with CO_2 increasing from 100 to 600 ppm. With further increase in the level of CO_2, the increase in the rate of photosynthesis was marginal till 3,400 ppm. At 10,000 ppm the rate of photosynthesis started declining.

Sharma (1986) observed an increase of net carbon exchange rate (NCER) at 600 ul L^{-1} in mungbean leaves at three different growth stages. The time course study of NCER at hourly intervals for 5 hours showed doubled NCER at 600 ul L^{-1} and more than doubled NCER at 900 ul L^{-1} as compared to 300 ul L^{-1}. It rose to a peak after 3-4 hours and declined a little at the end of the fifth hour.

Mortensen and Sandrik (1984) have shown that short term (a few hours exposure) enrichment with CO_2, increased net photosynthesis irrespective of light flux density in the light regime applied. The CO_2 optimum was about 900 ppm and the light compensation point was decreased in CO_2 enriched plants when compared to normal plants of popular clones.

Uday Kumar and Prasad (1991) have reviewed and found that, in all short term experiments, elevated CO_2 levels caused increased photosynthetic rate. In long term experiments with continuous CO_2 enrichment the results have been contradictory and they believe,

the absolute reduction in photosynthesis is caused by the following factors:

1. End product inhibition - Accumulation of end products - starch and sucrose.
2. Limitation due to inadequate inorganic phosphate (pi) recycling.
3. Decrease in content and activation of RUBISCO.

Short term exposure to high CO_2 in soybean showed an increased rate of photosynthesis and growth, but with prolonged high CO_2 exposure, these high rates are sometimes not maintained (Clough and Peet, 1981). Sasek *et al.* (1985) found photosynthetic rates lower than the expected rates on instantaneous exposure to high CO_2 in cotton grown at 350 and 1000 ppm.

Long term CO_2 enrichment up to 1000 ppm produced a decline in net photosynthesis in cotton plants, the decline in photosynthesis in the high CO_2 plants being a result of non stomatal limitations that contributed to higher interval resistances (Delucia *et al.*, 1985).

In contrast, Ziska and Teramura (1992) have shown that when two rice cultivars of contrasting morphologies, IR-36 and fuziyama-5 were exposed to ambient (360 ppm) and ambient plus 300 ppm (660 ppm) CO_2 from the time of emergence until 50 per cent grain fill, exposure to increased CO_2 resulted in about a 50 per cent increase in the photosynthetic rate for both cultivars and photosynthetic enhancement was still evident after 3 months of exposure to high CO_2 environment.

Tolbert (personal communication) observes, in long term experiments in which plants were grown with full sunlight, the rate of photosynthesis and growth of a C_3 plant increased with increasing CO_2. At 1000 ppm the C_3 plant may grow twice as fast as with 330 ppm. Because C_4 plants contain a concentrating mechanism, increasing atmospheric CO_2 has less effect upon them, and rate increases of only 10 to 20 per cent have been reported.

Allen *et al.* (1988) have shown that, under more favourable conditions of high light intensity and high temperature, the net photosynthetic rates of *Azolla* sp. in the high CO_2 (640 ppm) treatment were as much as 70 per cent greater than for those in the low CO_2 treatment (340 ppm). They also observed that net photosynthesis in 340 ppm air was influenced more by air temperature than by solar radiation, while solar radiation had the greater effect on net photosynthesis in 640 ppm air.

Idso (1991) has reported that photosynthetic response to atmospheric CO_2 enrichment is inversely proportional to the degree of CO_2 induced stomatal closure in sour orange, cotton, soybeans and water hyacinth. Cure (1985) reviewed the CO_2 enrichment effects on C_3 agronomic plant species and estimated that a doubling of the current atmospheric CO_2 concentration would lead to a 28 per cent increase, on an average, in net photosynthesis.

STOMATAL CONDUCTANCE

Stomatal conductance has been observed to decrease with increasing CO_2 concentration in most of the studies of over 50

species, though a few cases of no response or very large response have also been reported (Morison, 1985, 1987).

Oberbauer *et al.* (1985) have shown that stomatal conductance decreased in two tree species from Atlantic lowlands of Costa rica, when CO_2 concentration was raised from 350 to 675 ppm. Stomatal response to CO_2 varies greatly among species and may be influenced by other environmental factors such as soil moisture and light (Tolley and Strain, 1985). Although strong evidence suggests that stomata respond more to internal CO_2 concentration than to external concentrations (Mott, 1988), the mechanism by which CO_2 controls stomatal activities is not known (Pearey and Bjorkman, 1983).

Woodward (1987) has shown that stomatal density and stomatal index increased markedly as the CO_2 partial pressure was reduced below 340 ppm. Above 340 ppm there is a slight decrease in stomatal density in several species studied (Woodward and Bazzaz, 1988).

In soybean, a 25 per cent decrease in stomatal conductance from 150 to 112 S m^{-1} with a doubling of ambient CO_2 was observed (Valle *et al.*, 1985).

Idso *et al.* (1987) have shown a midday increase in foliage temperature of 1.1°C, with increasing CO_2 from 340 to 640 ppm in cotton.

Plants of 16 agricultural and horticultural species were grown from seed in spaced pots in the glasshouses, one with normal and

the other with twice the present atmospheric CO_2 concentration. Averaging across all species and soil moisture contents, transpiration rate was less reduced by high CO_2 (21 per cent) than was stomatal conductance (36 per cent) and this is attributed to the increased leaf temperature caused by reduced stomatal conductance (Morison and Gifford, 1984).

RESPIRATION

Little information is available on the effects of elevated CO_2 concentration on dark respiration rates. Enhancement of photosynthesis may lead to increased respiration because of the increased availability of substrate for respiration. Several arctic tundra species showed a substantial increase in dark respiration with increased CO_2 (Oechel and Strain, 1985). Wulff and Strain (1982) showed no influence of elevated CO_2 on dark respiration or on light compensation in *Desmodium paniculatum*. Amthor (1989) has shown a decline in dark respiration with CO_2 enrichment in crops. It is not yet clear whether growth and maintenance respiration change/respond to the same degree, to elevated CO_2.

Madsen (1976) has shown decreased dark respiration in tomato leaves with CO_2 concentration over the range of 300 to 5000 ppm.

Gifford *et al.* (1985) studied respiratory characteristics of wheat and sunflower under CO_2 enriched (600 $\pm$ 50 ppm) conditions. Each species responded differently. In wheat, a 45 per cent reduction in respiration was recorded by both roots and whole

plants. Results suggest that reduced respiration rates for wheat plants growing in high CO_2 concentration may also contribute to the increased growth rate. Hrubec *et al.* (1985) measured dark respiration in individual leaves of soybean. Respiration was enhanced in young leaves, but not in mature ones. However, they believe that respiration rate was similar in CO_2 enriched and control atmospheres when all leaves are taken into account.

Reuveni and Gale (1985) showed that the effect of enriching CO_2 (950 ppm) for a short term on respiration was lower during night time. There was no significant difference between the rates of respiration measured at 50 and 350 ppm CO_2 nor was there any significant difference in response to CO_2 at 19 versus 25°C temperature. The effect of high CO_2 was generally greater for roots than tops, although this was not entirely consistent. The data suggest that the rise in the CO_2 level of the atmosphere will affect plants not only during the day (mainly by suppressing photorespiration and increasing net photosynthesis) but also at night by suppressing respiration.

Lawlor (1991) while reviewing an article on the effects of elevated CO_2 concentration observes that, dark respiration of plants grown in elevated CO_2 is generally depressed compared to that of plants grown in normal conditions and the absolute difference between the two is larger at high temperatures. The causes of depressed respiration are not known but are not related simply to the accumulation of non-metabolic dry matter, which might alter the basis of expression. It may involve direct effects of CO_2 on

mitochondrial electron transport. The contribution of respiration to dry matter accumulation and the effects of environment on the process has yet to be fully understood.

2.2 CARBON DIOXIDE ENRICHMENT EFFECTS ON CHLOROPHYLL,STARCH AND SUGARS

Chlorophyll content, measured on a dry weight or leaf area basis, and the chl a/b ratio are also affected by CO_2 enrichment. The decline in chlorophyll concentration in the plants grown in 675 and 1000 ppm CO_2, when measured on a dry weight basis, was largely a result of the increase in specific leaf weight. However, a decrease in chlorophyll content in high CO_2 grown plants was also apparent when measured on a surface area basis (Delucia *et al.*, 1985).

Cave *et al.* (1981) showed in their studies that a relationship exists between chlorophyll and starch accumulation in CO_2 enriched plants. In immature leaves, the total chlorophyll content per unit dry weight and the chlorophyll a:b ratio are significantly lower in plants grown at 1000 ppm CO_2. Results indicate that the chlorotic appearance of leaves in high CO_2 grown clover plants is due to a decrease in chlorophyll content per unit dry weight possibly resulting from large starch grains and starch accumulation altering normal chloroplast structure and function. Within each CO_2 treatment, there was a significant increase in the starch content of the leaves in the late afternoon as compared to the early morning. Starch grains in CO_2 enriched conditions occupy a greater

chloroplast volume, which in turn disturbs the configuration of grana stacks.

Yelle *et al.* (1989) found tomato plants to contain high starch and more sugars when grown at 900 ppm than the control plants.

Madsen (1968, 1975, 1976) observed that CO_2 enrichment caused a high level of starch accumulation in tomato leaves with CO_2 concentration in the range of 300 to 5000 ppm. Relative amounts of starch, glucose and sucrose increased while the amino acid content decreased.

Many plants, such as cotton, tomato, clover,desmodium and soybean showed visible chlorosis and subtle changes in leaf texture when grown at elevated levels of CO_2. These effects have been interpreted as being symptomatic of excessive starch accumulation, and it has been suggested that starch accumulation results in feedback inhibition of photosynthesis at the enzyme level (Delucia, 1985).

2.3 CARBON DIOXIDE ENRICHMENT EFFECTS ON GROWTH AND PHENOLOGY

It is important to understand how crops behave with regardto growth and development under enriched CO_2. The critical question to be answered/understood is, whether increased photosynthesis leads to increased growth which may in turn bring changes in crop phenology. A comparative study at the Duke University Phytotron of whole plant responses of wheat, maize, soybean, radish, sugarbeet, okra and some C_4 grasses showed that CO_2 enrichment

lead to increased growth and yield in most of the crops studied, especially the C_3 plants as compared to C_4 plants (Sionit *et al.*, 1982, 1985).

Carbon dioxide enrichment studies have shown that CO_2-induced increases in leaf area are largely due to more extensive branching in dicotyledonous plants (Rogers et al., 1984) and tillering in grasses (Sionit *et al.*, 1980).

Specific leaf weight (SLW) has been observed to increase in response to elevated CO_2 in soybean (Leadley and Reynolds 1988; Rogers *et al.*, 1983) and sweet potato (Biswas and Hilman, 1985), but not in maize (Rogers *et al.*, 1983). The increases in SLW are presumably due to the increase in starch content (Huber *et al.*, 1984), but the leaves of soybean plants grown in high CO_2 are also thicker due to an increase in the number of palisade cells, an effect which does not occur in maize (Thomas and Harvey, 1983).

There is strong support from literature that specific leaf area (SLA) decreases with increasing CO_2, primarily because of increased starch and cell number (Garbutt *et al.*, 1990).

Carbon dioxide enrichment in case of rice has been shown to increase dry weight, plant height and tillering. The increased tillering reported was due to the production of tillers at lower nodes in plants grown in high CO_2 where ambient control plants failed to produce a tiller (Imai and Murata, 1979).

Bhattacharya *et al.* (1985) have grown sweet potato in controlled environment chambers at 350, 675 and 1000 ppm. The

length of main stem, total branch length, number of branches, and leaf area were higher for plants grown at 675 or 1000 ppm. The production of total dry matter of plants increased at each harvest interval in response to CO_2 enrichment but it was greatest in 1000 ppm CO_2. Specific leaf weight also increased with increased CO_2 concentration.

Cassava plants were grown for three months in CO_2 enriched glasshouses (700 ppm) at two different temperatures 28/21°C and 33/26°C. Cassava grew vigorously under the conditions of high CO_2 and high temperature and, dry matter increased by 150 per cent (Imai *et al.*, 1984).

Idso *et al.* (1987) have shown that stimulatory effect of atmospheric CO_2 enrichment is strongly temperature-dependent. For a 3°C increase in mean surface air temperature, the growth enhancement factor for a CO_2 increase of rises from 1.30 to 1.56. On the other hand, results also indicate that atmospheric CO_2 tends to reduce plant growth at relatively cold air temperatures i.e. below a daily mean air temperature of approximately 18.5°C, in case of two floating aquatic plants and three terrestrial species (carrot, radish, water hyacinth). Farrar and Williams (1991) also showed that cold adapted plants had little response to elevated levels of CO_2, with some species showing a decline in biomass accumulation.

Peet (1986) has reported a contrasting picture in case of cucumber under CO_2 enriched conditions. Except in the first 16 days after seeding, there was no increase in biomass, leaf area, or relative growth rates nor was there any increase in fruit number or

weight at 1000 ppm in controlled environment chambers. However, flower production was earlier in plants grown in 1000 ppm.

Bhattacharya *et al.* (1985) have reported that the appearance of flowers was 10-12 days earlier in high CO_2 atmosphere than in ambient CO_2 atmosphere in case of cowpea (*Vigna unguiculata* L.). In contrast to this finding, Hasketh and Hellmen (1973) found that floral initiation was delayed greatly at 1000 ppm in four cultivars of sorghum, but only slightly in maize, sunflower, and cotton. Flowerbud initiation and flowering were hastened in Alaska pea (Paez *et al.*, 1980).

Reekie and Bazzaz (1989) examined the relation between CO_2 level and reproduction in four species. In *Gallardia pulchela*, doubling of CO_2 reduced the time required for flowering by six days, in *Gaura brochycarpa* also, doubling CO_2 reduced the time taken to flowering. However, these reductions do not appear to be related to increased growth at elevated CO_2. The response of *Lupinus lexenis* was the reverse - elevated CO_2 increased rather than decreasing the time taken for flowering except when the plants were given much underground space. No clear trends were found in *Oenothera laniculata*.

2.4 CARBON DIOXIDE ENRICHMENT EFFECTS ON YIELD AND YIELD COMPONENTS

Enriched CO_2 in the atmosphere can also affect other reproductive parameters, such as seed number, seed size and seed nutrient content. It is commonly found that the increase in yield is due to an increase in the number of reproductive structures rather

than their mean size. In soybean, these yield increases were found to be almost entirely due to greater number of seeds, which in turn was due to an increase in the number of pods, rather than in number of seeds per pod, which decreased (Ackerson *et al.*, 1984; Havalka *et al.*, 1984; Rogers *et al.*, 1983).

Bhattacharya *et al.* (1985) exposed cowpea to 350, 675 and 1000 ppm CO_2 concentration and found that seed weight (yield) and number of seeds per pod, were significantly greater in CO_2 enriched atmosphere than ambient CO_2 atmosphere. Although CO_2 enrichment caused a significant increase in the total number and weight of seeds as well as pods, it did not affect the ratio of seed dry weight to the total dry weight of the above ground plant parts (harvest index). In case of bean, elevated CO_2 increased the number of pods, decreased the seeds per pod and did not affect the mean seed weight (Gustafson, 1984).

Ziska and Teramura (1992) showed an intraspecific variation in rice to increased CO_2. Two rice cultivars of contrasting morphologies, IR-36 and fugiyama-5 were exposed to 360 ppm and 660 ppm CO_2 from the time of emergence until 50 per cent grain fill. There was a significant increase in harvest index (HI) for IR-36 but not for fujiyama-5.

Kimball's (1983) popular assemblage and analysis of 430 prior observations during the past 64 years shows that most of the studies were performed in greenhouses or growth chambers. Open fields might respond less than greenhouses or growth chambers to increased CO_2 because nutrient levels in general world-wide

agriculture are lower than those in the indoor studies or, open fields might respond more because light levels are generally higher outside. The analysis showed that yields probably will increase by 33 per cent (with a 99.9 per cent confidence interval from 24 to 43 per cent) when the present atmospheric CO_2 concentration is doubled.

Cock and Yoshida (1973) subjected rice to CO_2 levels of about 900 ppm in field for 30 days before and after anthesis. The pre-anthesis treatment increased grain number per unit land area and grain weight, whereas the post anthesis treatment increased percentage of filled grains and grain weight, leaving grain number per unit land area unaffected. Both the pre- and post-anthesis CO_2 treatments increased yield levels above that of control levels. In case of whole season CO_2 enrichment, Baker *et al.* (1988) found that the higher number of panicles per plant was primarily responsible for high grain yields in higher CO_2 atmosphere. Imai *et al.* (1985) have grown rice plants in glasshouses under conditions of normal (350 ppm) and elevated (700 ppm) CO_2 at 28/21 and/or 33/26°C. The yield increased by 23-72 per cent at high CO_2 because of an increase in grain number per panicle rather than in grain weight. The harvest index of rice increased at high CO_2 due to an increase in yield per plant. Rice showed a higher response to high CO_2 at 33/26°C than at 28/21°C.

Bhattacharya *et al.* (1985) grew stem cuttings of sweet potato in controlled environment chambers at 350, 675 and 1000 ppm CO_2. At the final harvest, the number and diameter of tubers were

greater at high CO_2 concentration and the dry weight of roots and tubers increased 1.8 and 2.6 times in plants grown at 675 and 1000 ppm CO_2 respectively.

In wheat, increase in yield with CO_2 enrichment was due to an increase in the number of grains rather than in the grain weight (Fisher and Aguilar, 1976; Havalka *et al.*, 1984b). In case of wheat (Havalka *et al.*, 1984) exposed to 1200 ppm, it was found that the greatest influence occurred during the period from jointing to anthesis, while CO_2 enrichment increased yields of spring wheat the most, during tillering (Fischer and Aguilar, 1976).

2.5 CARBON DIOXIDE ENRICHMENT AND ENVIRONMENTAL INTERACTION EFFECTS

It is of importance to understand how other environmental factors influence plant growth under CO_2 enriched conditions. Environmental factors such as light, water and temperature have a significant effect on plant growth. Soil nutrients have both quantitative and qualitative impact on growth and development of crop plants, hence how they would respond at doubled CO_2 concentration in the atmosphere is of great curiosity.

Wheeler *et al.* (1991) had grown four cultivars of potato at 350 and 1000 ppm CO_2 in combination with 12 hours or 24 hours photo periods at 400 or 800 u mol $M^{-2}S^{-1}$ photosynthetic photon flux (PPF). Air temperature and relative humidity (RH) were held constant at 16°C and 70 per cent respectively. CO_2 enrichment increased tuber yield and total plant dry weight by 39 per cent and 34 per cent respectively, under a 12 hour photoperiod at 400 u mol $m^{-2}S^{-1}$,

27 and 19 per cent under 12 hour at 800 u mol $m^{-2}S^{-1}$. There was varietal difference in response to treatments. The results show a pattern of greater plant growth from CO_2 enrichment under lower PPF and a short photoperiod.

Bowman and Strain (1987) have shown that increasing atmospheric CO_2 may result in alleviation of salinity stress in salt-sensitive plants. It was found that these plants when grown in CO_2 enriched atmosphere had higher biomass and decreased stomatal conductance, resulting in higher water use efficiency. Paez et al. (1984) have shown that increased CO_2 in case of tomato plants, ameliorated the detrimental effects of drought on plant growth.

Several investigators have shown that the growth and yield enhancing effects of CO_2 disappear under nitrogen and phosphorus limitations (Brown and Higginbotham 1986; Goudria and Reuiter 1983). Light saturation is usually higher under elevated CO_2 than under ambient CO_2 (Valle *et al.*, 1985), and high CO_2 may compensate for low light (Acock and Allen, 1985).

Idso *et al.* (1987) reported that growth modification factor (i.e. the biomass growth increase ratio) for a 300 ppm elevation of CO_2 increased somewhat linearly (0.087 per°C) with increasing temperature over the range of 19 to 34°C for five C_3 species of carrot, radish, cotton, water hyacinth and water fern. In fact, they reported a negative effect of elevated CO_2 for temperature less than 19°C.

CHAPTER III

MATERIALS AND METHODS

All the data utilised in this report were obtained from investigations conducted at Plant Physiology Division, Indian Agricultural Research Institute, New Delhi. The aim of this investigation was to observe and study the effect of elevated level of CO_2 above the present ambient concentration on growth of crop plants. For this purpose, the experiments were designed in a way such that plants receive higher levels of CO_2 during their entire period of growth and development. Two methods were employed to expose the plants to elevated levels of CO_2.

UTILIZATION OF NIGHT TRAPPED CO_2

This is based on the principle that plants release respired CO_2 during night time, when no photosynthesis takes place under normal field situations. The respired CO_2 during night time gets mixed in the atmosphere for further use during the day. In this technique, the night-released CO_2 was trapped by a special structure erected above the plant canopy. This trapped CO_2 is then utilised during early hours of the day (details are given in the following pages). This may help the plants to grow under higher CO_2 level without using any external, artificial source of CO_2. The CO_2 trapped during night time would increase the CO_2 level and the effect of this increased CO_2 on plant growth may be studied.

USE OF EXTERNAL SOURCE OF CO_2

This method involves the use of commercially available CO_2 to enrich its level on plants' environment. This type of treatment was applied using specialized structure and a new method of mixing air and CO_2 to obtain the desired level of CO_2 (details are given in the following pages) above the plant canopy in an open top chamber.

3.1 CO_2 ENRICHMENT STUDIES BASED ON NIGHT TRAPPED CO_2 USING POLYCOVER

The experiment was conducted under field conditions in two sets of plots of size 1x5 meters. Seeds of individual crops were sown in well prepared seed beds of these plots. The crops were allowed to germinate and grow with recommended irrigation and cultural practices. In one set, plants were allowed to grow under normal/ natural environment, while in the other a special structure was erected above it (Plate 1a and 1b).

The structure consists of an oval shaped frame made of iron which was covered by a transparent polyethylene sheet (85 per cent transmittance). Height of the structure at the centre was one meter above the soil as seen in plates 1a and 1b. Polyethylene sheet was erected in such a way that there can be no exchange of air from inside to outside. The plants were covered with polyethylene sheet at 5 PM every day and removed between 9 AM and 10 AM depending upon the season. Thereafter the plants were allowed to grow under natural environment for the rest of the day. This led to the night trapped CO_2 under the polyethylene cover being utilized during the first few hours of morning sunlight.

Plate 1 a,b. Design of polycover experiments for conducting CO_2 enrichment studies by trapping night respired CO_2

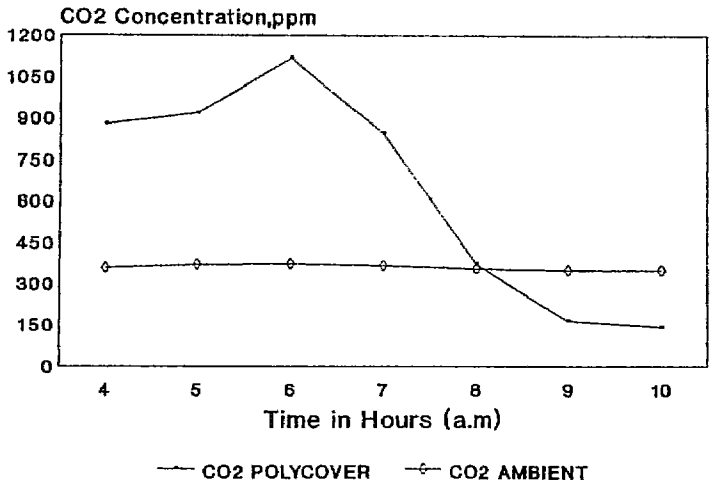
METHODS OF CO_2, TEMPERATURE AND RELATIVE HUMIDITY MEASUREMENT

Carbon dioxide, temperature and relative humidity inside the polycover was measured using IRGA (LICOR-6200) portable photosynthesis system. Observations were recorded during the period of crop growth by drawing air samples from the covered / area through a tube. When samples were drawn from the structure using IRGA, it was found that CO_2 increased gradually and reached a peak at midnight, as shown in Fig. 1 and 2. During early hours of the day the CO_2 concentration in the polyethylene trap went on decreasing and became less than ambient between 9 AM and 10 AM depending on the season. For this reason, the trap was removed and plants were allowed to grow under natural environment for the rest of the day. This means that plants were allowed to photosynthesize under high CO_2 only for approximately 3-4 hours. This technique was followed throughout the crop growth period. As seen from Fig.1 and 2 there was a rise in temperature and relative humidity (RH), due to polyethylene cover. Both the sets of plots had similar soil fertility status, equal number of plants and cultural practices except for the above treatment.

3.1.1 CROPS UNDER STUDY

The following crops were grown for this study. For each crop two cultivars, one dwarf and a tall one were used. In the table below, the crops grown, cultivars used, dates of sowing and dates of harvest are shown.

Fig.1 MEAN OF POOLED DATA FOR CO2, TEMP AND RH INSIDE POLYCOVER AND AMBIENT CONDITIONS FOR GROUNDNUT (SUMMER)



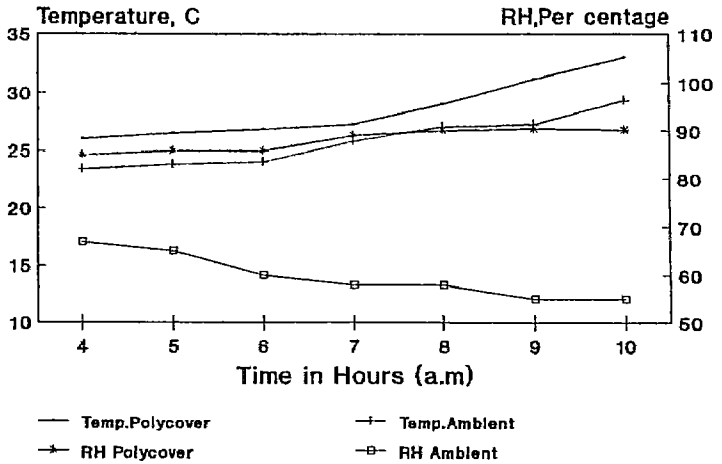
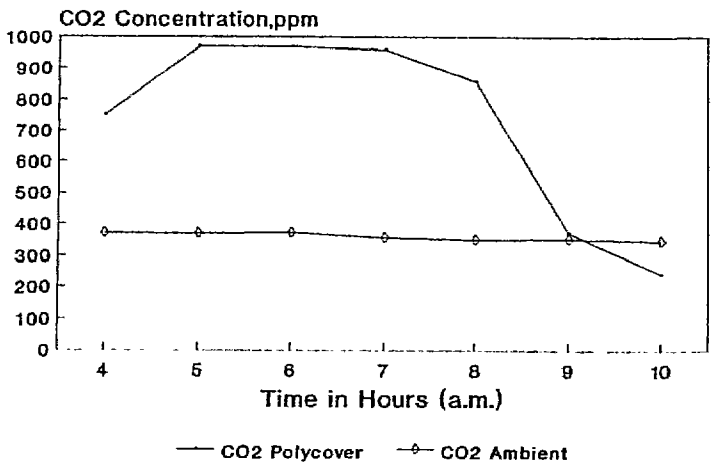
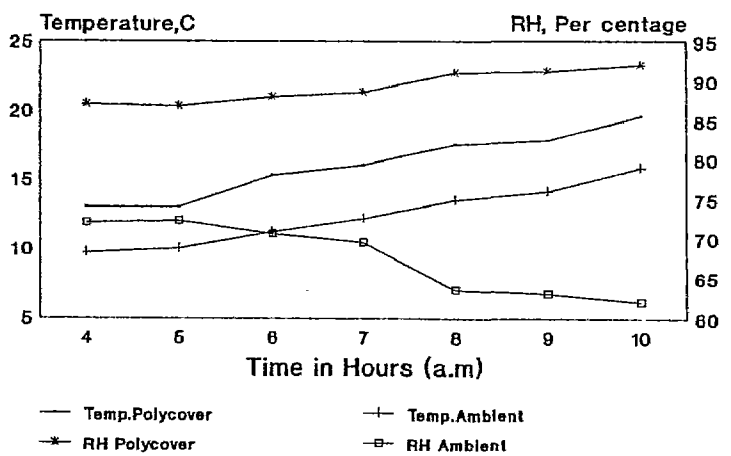


Fig.2 MEAN OF POOLED DATA FOR CO2, TEMP AND RH INSIDE POLYCOVER AND AMBIENT CONDITIONS FOR PEA (WINTER)





CROP	CULTIVARS	DATE OF SOWING	DATE OF HARVEST
Groundnut (*Arachis hypogoea* L.)	J-11 (erect)	19.7.89	5.12.89
	M-13 (spreading)	19.7.89	19.12.89
Pea (*Pisum sativum* L.)	DMR-10 (dwarf)	11.12.89	16.4.90
	L-116 (tall)	11.12.89	16.4.90

3.1.2 OBSERVATIONS

Groundnut

At 125 days after sowing (DAS) the vegetative parameters viz. main shoot length, branch number and branch length were recorded. At maturity, the stem dry weight and leaf dry weight were obtained by oven drying at 80°C for six hours and thereafter at 60°C until uniform or constant weight was obtained. Pod number and pod weight per plant were recorded.

Pea

In pea, the growth and yield data in the form of plant height, leaf dry weight, stem dry weight, pod number per plant, seed number per plant and seed weight per plant were recorded at maturity.

3.2 CO_2 ENRICHMENT IN OPEN TOP CHAMBERS

In this experiment, a modified, cheap and indigenous technique for the study of crop response to elevated CO_2 using open top chamber as described earlier by Rogers *et al.* (1983) was developed. The open top chamber design as seen from plates 2a and 2b and figure 3 are described here.

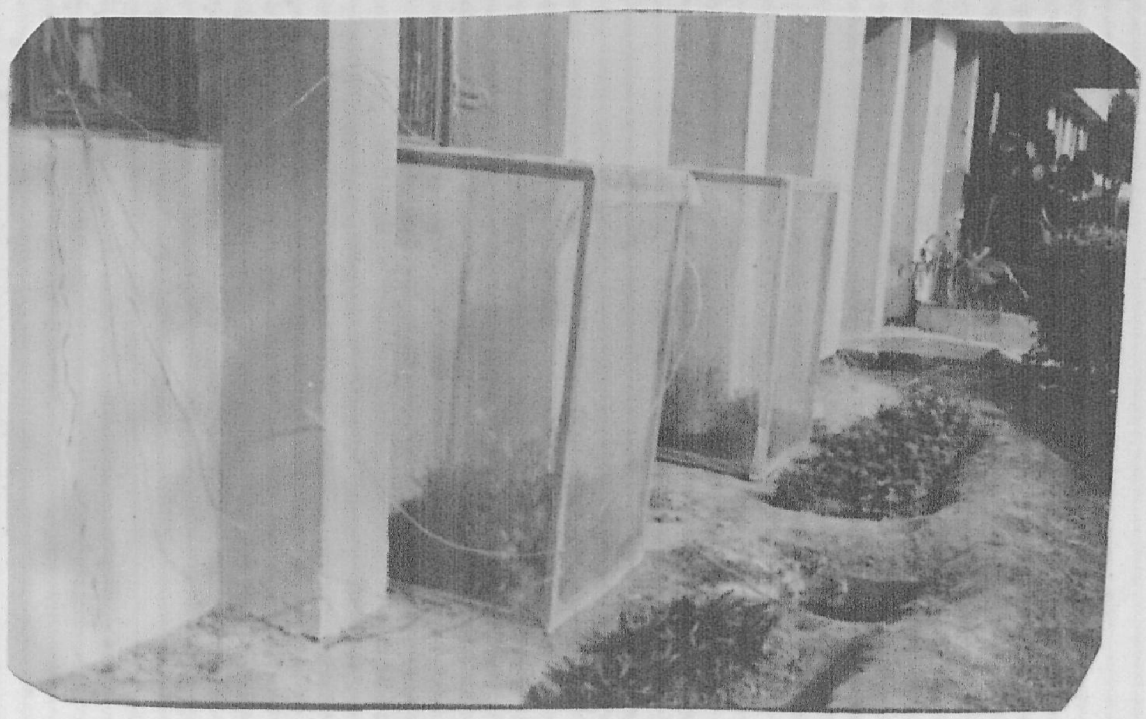


Plate 2 a,b. Operation and design of open top chambers for conducting CO_2 enrichment studies

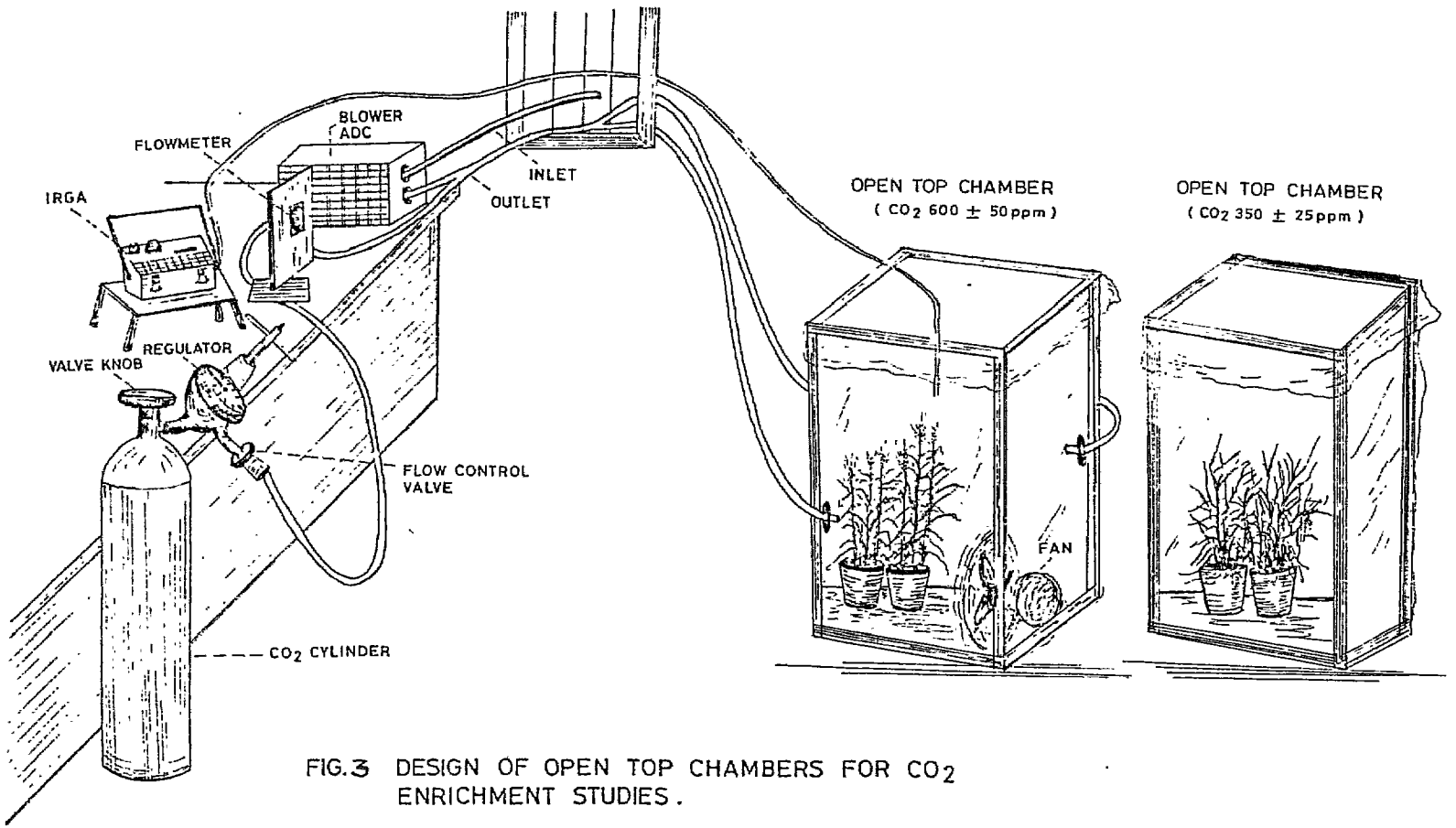


FIG.3 DESIGN OF OPEN TOP CHAMBERS FOR CO_2 ENRICHMENT STUDIES.

Structure of open top chamber

A chamber made of wood was erected on a plot size of 0.9x0.9 meter. The height of the chamber was 1.45 meter and the total volume was 0.928 m^3. The walls of the chamber were covered with clear polyethylene film. The top of the chamber was kept open for free movement of air and to maintain ambient temperature and humidity. The chamber had two entry points for the release of CO_2 air mixture. A small fan was fitted inside the chamber for thorough mixing of gases to obtain a uniform CO_2 concentration inside the chamber.

Preparation of CO_2-air mixture

Carbon dioxide (CO_2) (99.97 per cent v/v CO_2, less than 10 ppm CO) was purchased from a commercial source (Universal Gas Suppliers Pvt. Ltd., Kirti Nagar, Delhi) and stored on site in a cylinder. As the commercial CO_2 in the cylinder was 99.97 per cent pure, it was required to be diluted to obtain 600 ppm, which was to be supplied to the chamber. The CO_2 from the cylinder was released through a regulator @ 4 kgs/cm^2 and was allowed to pass through a flowmeter to maintain the required flow. The gas was further regulated by passing through another flowmeter @ 0.3 litre per minute. The ambient air with ambient level of CO_2 (352 ppm) was sucked by a circulatory pump (ADC) through a nylon tubing. Into this air, the CO_2 gas was injected using a needle. This CO_2-air mixture was then pumped inside the open top chamber through two entry points, one at the top and another at the base. The CO_2 air mixture at the point of entry into the chamber had a

concentration more than 2000 ppm. This mixture was further mixed inside the chamber with the help of a circulating fan. This along with the air turbulence from the open top reduced the concentration of CO_2 in the chamber to 600 ± 50 ppm.

The concentration of CO_2 in the chamber was monitored through the suction pump (inlet) of an IRGA, by drawing air through another tubing from the chamber to the IRGA. To maintain the level of CO_2 (600 ppm), the flow of CO_2 was regulated as and when necessary. In general, when wind velocity was not very high the level of CO_2 was maintained at 600 ± 50 ppm.

In these open top chambers as monitored through IRGA, it was seen that temperature and relative humidity were the same as in ambient atmosphere. In one open top chamber, the CO_2 enriched conditions (600 ± 50 ppm) were maintained while in another open top chamber of the same dimensions, normal air was allowed to move freely and this chamber served as control. The CO_2 enrichment was done only during the day from 9 AM to 5 PM, from germination to maturity.

3.2.1 CROPS UNDER STUDY

The type of crop, cultivar and their date of sowing are given below :

CROP	VARIETY	DATE OF SOWING
Wheat (*Triticum aestivum* L.)	Kalyan Sona	10.10.91
Sunflower (*Helianthus annus* L.)	Modern	15.12.91
Mungbean (*Vigna radiata* L.Wilezek)	PS-16	13.3.92

The crops were grown in pots of 3.44 litre capacity. The soil prepared for pot culture was of fine quality and thoroughly mixed with sufficient farm yard manure. It was ensured that all the pots had similar kind of soil-farm yard manure mixture, free from any pests and diseases (treated with BHC 10%).

Sowing

Initially a large number of seeds were sown and after seedling emergence, equal number of plants were kept in each pot. In case of wheat six plants per pot, sunflower three and, mungbean three seedlings per pot.

3.2.2 OBSERVATIONS

The following physiological, biochemical, growth and yield data were recorded.

3.2.2.1 PHYSIOLOGICAL OBSERVATIONS

Observations on photosynthesis, repiration and stomatal conductance, were obtained using Infra Red Gas Analyser (IRGA-LICOR-6200 model).

LI-6200 Portable Photosynthesis System

IRGA (LICOR-6200) consists of a CO_2 analyser, a system console and a sensor housing with interchangeable leaf chambers. The LI-6200 CO_2 analyser is a non dispersive, infrared type (NDIR) calibrated for measurements of 0-2000 ppm. A pump in the analyser circulates air from the measurement chamber to the analyser, where CO_2 concentration is measured, and returns it to the chamber.

The system console is a micro computer that handles the data logging, calculations, data storage and communications. The sensor housing contains the connectors and electronics for the leaf chamber sensors and fans and, the intake and exhaust parts from the analyser.

PHOTOSYNTHESIS (Pn)

The net exchange of CO_2 between a leaf and the atmosphere was measured by enclosing the top most, fully expanded leaf in the assimilation chamber and the rate at which the CO_2 concentration changes over a short time interval (typically 10-20 seconds) was monitored. The net photosynthesis was then calculated using this rate of change and other factors such as the leaf area enclosed, the volume of the enclosure, and temperature. A fully expanded, top most leaf was taken for all observations which were recorded between 10 AM and 12.30 PM under a clear sky with light intensity around 1500-1600 u E sec^{-1}. Results are calculated from the observation means (3 replications) and are presented in user-

specified units as u mol M^{-2} sec^{-1}. In wheat, photosynthesis was measured at 45 DAS, in mungbean at 30, 40, 50 and 60 DAS and in sunflower at 25 and 50 days after sowing.

RESPIRATION (Rn)

The methodology involved in the measurement of respiration was the same as photosynthesis, except that the leaf chamber was covered with a black cloth to cut off light. The purpose of covering the chamber with a black cloth was to eliminate photorespiration. The change in CO_2 in the chamber would indicate the respiratory loss (dark) of CO_2 only. In this case, change in CO_2 concentration in the chamber was due to respiration and not photosynthesis, since leaves were not exposed to light. The same leaf as used in photosynthesis was also used here and the units for respiration are same as in photosynthesis. Respiration rates were measured at 30, 40, 50 and 60 DAS in mungbean and sunflower.

STOMATAL CONDUCTANCE (Sc)

If a leaf is enclosed in a chamber, the humidity within the chamber normally rises as the leaf transpires. In the LICOR system, the increase in humidity is balanced by the flow of partially dried air returning to the chamber from the CO_2 analyser. The transpiration rate is calculated from the change in leaf chamber humidity with time (if any) and the flow rate of that portion of the total flow which passes through the desicant. The transpiration rate is then used with the leaf and air temperatures to calculate total leaf resistance, from which the boundary layer resistance is subtracted yielding the stomatal resistance and conductance.

The same leaf as used in photosynthesis was used for stomatal conductance measurement and the units are expressed as mol M^{-2} sec^{-1}. Whenever photosynthesis was measured, stomatal conductance was automatically measured by the instrument.

3.2.2.2 BIOCHEMICAL OBSERVATIONS

Biochemical observations in the form of chlorophyll (a, b and total), sugars (reducing, non reducing and total), starch and total free amino acids (TFAA) content were recorded in the leaves of CO_2 enriched and control plants.

CHLOROPHYLL ESTIMATION

DMSO (dimethyl sulfoxide) method was used. Fully expanded, top most leaf was taken, cleaned with water, dried and cut into small pieces leaving midribs. The cut pieces were thoroughly mixed, out of which 50 mg (0.05 g) leaf sample was taken in triplicate for analysis. 10 ml DMSO was added to each test tube, thereafter the samples were incubated in dark for four hours at 60°C. Samples were colled, filtered and O.D. was taken at 663 and 645 nM using spectronic-20 (Bausch and Lomb, USA). The amount of chlorophyll a, b and total chlorophyll were calculated using the following formulae :

mg of chlorophyll a/g leaf

$$= [12.7 \times (1)\ 663\ D - 2.69 \times (1)\ 645\ D] \times \frac{V}{1000 \times W}$$

mg of chlorophyll b/g leaf

$$= [22.9 \times (1)\ 645\ D - 4.68 \times (1)\ 663\ D] \times \frac{V}{1000 \times W}$$

mg of total chlorophyll/g leaf

$$= [22.2 \times (1)\ 645\ D - 8.02 \times (1)\ 663\ D] \times \frac{V}{1000 \times W}$$

Where

V = Volume of DMSO

W = Weight of leaf sample

D = Optical density

Chlorophyll was measured at 45 DAS in wheat and mungbean.

ESTIMATION OF SUGARS, AMINO ACIDS AND STARCH

SAMPLING

Leaves were plucked from the plants and cleaned properly under running water and then water was removed using tissue paper. The leaves were cut into small pieces of about 1-2 mm size. 1 gram of thoroughly mixed leaf bits were weighed accurately and taken for estimation of sugars, amino acids and starch.

REAGENTS

Arsenomolybdate reagent : 25 grams of ammonium molybdate was dissolved in 450 ml of distilled water and subsequently 21 ml of concentrated sulphuric acid was added to it. 3 grams of sodium arsenate heptahydrate dissolved in 25 ml of distilled water was

added to the above solution and mixed. The solution was incubated for 48 hours at 37°C before it was ready for use.

Copper-carbonate tartarate reagent

(Somogyi's) : 24 grams of anhydrous sodium carbonate and 12 grams of sodium potassium tartarate were dissolved in 25 ml of distilled water. Cupric sulphate pentahydrate solution made from 4 grams was added while stirring continuously, followed by 16 grams of sodium hydrogen carbonate.

180 grams of sodium sulphate was added to 500 ml of hot water and later boiled to expel air. After cooling, the above two solutions were mixed and made to one litre.

EXTRACTION

1 gram of leaf tissue was taken and plunged into 90 per cent ethanol, the supernatant was collected into a beaker after boiling for few seconds. Extraction was repeated four times with 20 ml of 80 per cent (v/v) ethanol in water. After boiling the samples for 4-5 minutes and decanting the supernatant, the combined extract was made to 100 ml (McCready *et al.* 1950). The pale leaf tissue waskept for starch analysis. Two replications were taken for each determination. This extract was also used for amino acids and sugars estimation.

REDUCING SUGARS

Clarification : Of the above extract, 50-70 ml was taken in a conical flask and evaporated on a water bath, taking care not to let the liquid dry out completely. Subsequently, the sample was treated with one

ml saturated solution of leaf acetate to precipitate the colloidal substances. It was then filtered and leaf was precipitated with 3 ml of saturated disodium hydrogen phosphate solution. After 2-3 washings the volume was made to 50 ml. An aliquot of this was used for determining the reducing sugars by Nelson's arseno molybdate method (Nelson, 1944) using improved copper reagent of Somogyi (1952).

Assay

An aliquot (0.1-0.2 ml) from the above clarified extract was taken for the assay of reducing sugars. The volume was made to 2 ml with distilled water, one ml of Somogyi's copper reagent and heated in a boiling water bath for 12 minutes. After cooling the samples in running tap water, one ml of arsenomolybdate reagent was added and the final volume was made upto 10 ml. Absorbance was measured at 530 nM in Spectronic-20 (Bausch and Lamb, USA). Standard curve was made using glucose in the range of 10-100 ug.

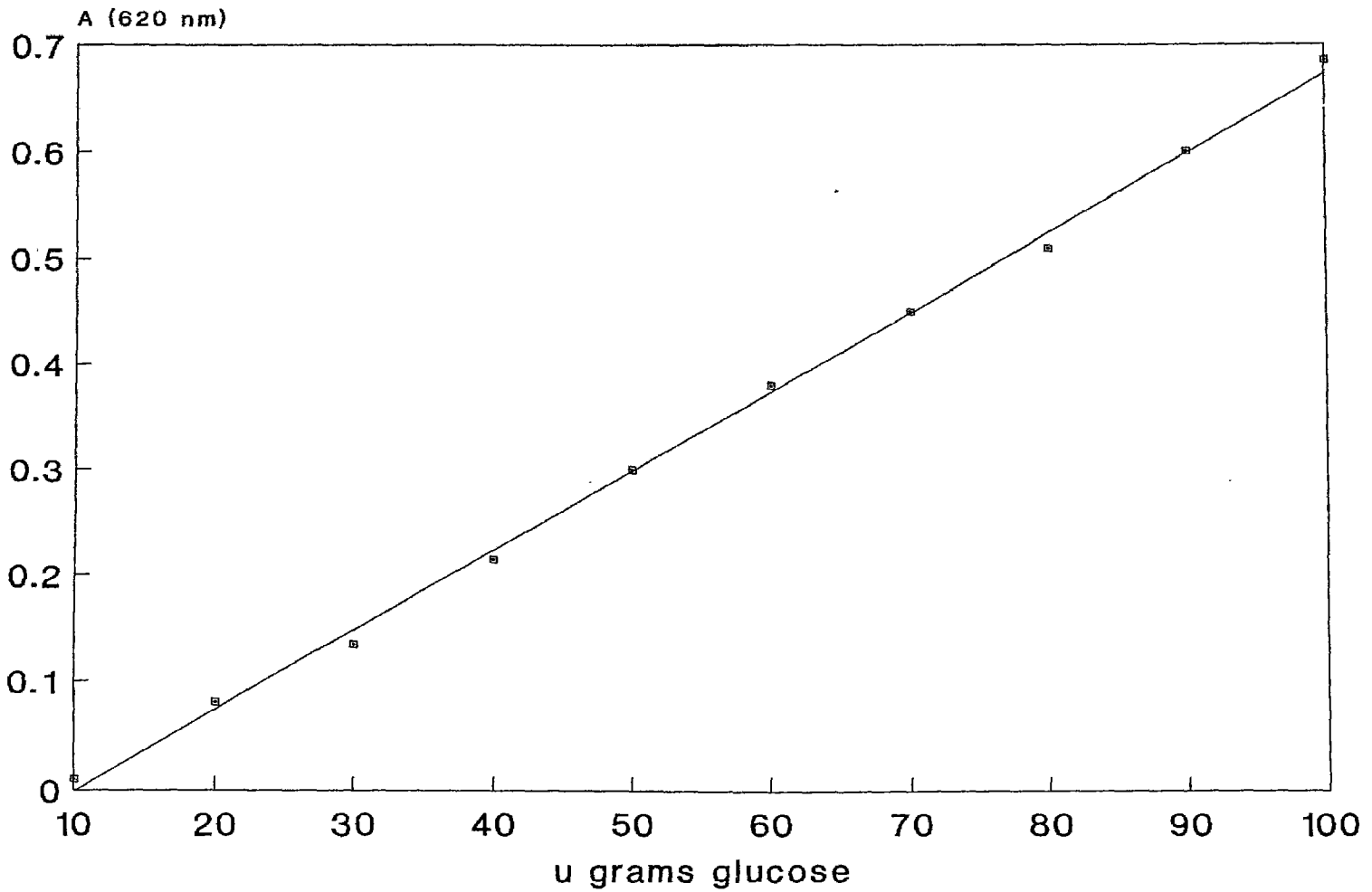
TOTAL SUGARS

Five ml of the clarified extract made for reducing sugar estimation was hydrolysed by boiling with 2.5 ml of 0.5N HCl for 30 minutes in a water bath and later neutralised to slightly acidic side with NaOH and made to 10 ml volume. This solution was used for the determination of total sugars. An aliquot of this (0.5 to 1 ml) was analysed for sugars as described above in two replicates.

NON-REDUCING SUGARS

Non-reducing sugars were calculated by subtracting the reducing sugars content from the total sugar content.

Fig.4 STANDARD CURVE OF SUGARS



TOTAL FREE AMINO ACIDS (TFAA)

Free soluble amino acid content was estimated following the method of Rosen (1957). The extract prepared above for soluble sugar estimation was used here for amino acid estimation. To 0.5 ml of extract, 0.5 ml of distilled water, 0.5 ml cyanide acetate buffer and 0.5 ml of Ninhydrin (3% in methyl cellusolve) were added. The tubes were kept for 10 minutes in boiling water for colour development and then 5 ml of 1:1 isopropyl alchohol : water mixture was added. Absorbance was recorded at 570 nM against a reagent blank, in Spectronic-20 (Bausch and Lamb, USA). A standard curve was prepared using glycine in the range of 100-700 n moles. The amino acid content was expressed as uM of glycine for gram fresh weight.

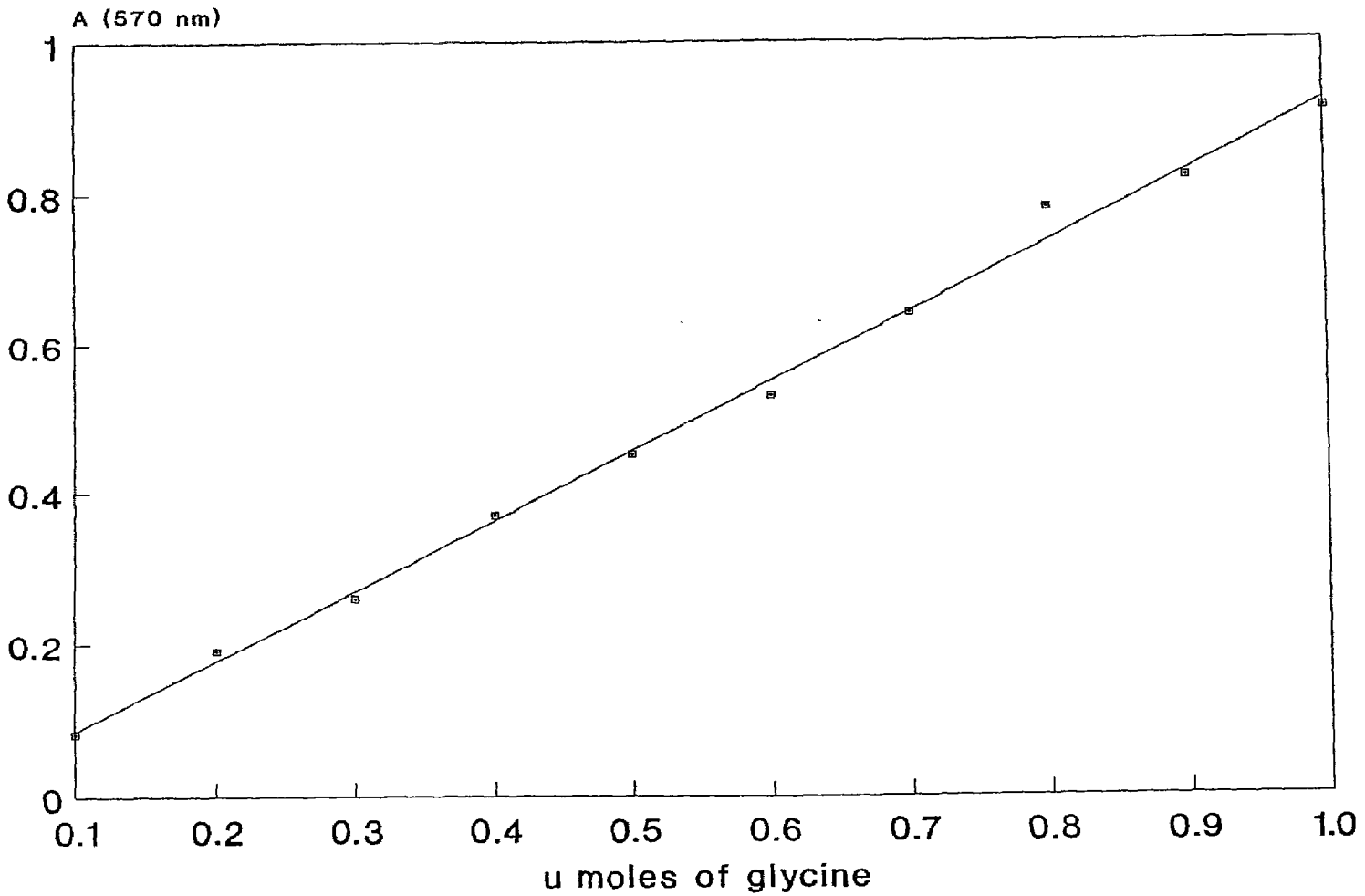
STARCH CONTENT

The residue left after extraction for reducing sugars was used for starch estimation. The residue is dried until it becomes free from moisture and then grinded in a pestle and mortar to a fine powder.

50 mg of dried sample is hydrolysed by boiling with 10 ml of 1N HCl for 30 minutes in a glycerine bath at 112-115°C. After cooling, the samples were transferred into a 100 ml volumetric flask. The residue was repeatedly washed with distilled water until a negative test (iodine test) was obtained.

The extract so collected was finally made to 100 ml. Starch was determined by the anthrone method (McCready *et al.* 1950). An aliquot (0.5 ml) of the above extract was made to 2.5 ml with distilled

Fig.5 STANDARD CURVE OF AMINO ACIDS



water. This was then mixed thoroughly with 10 ml of freshly prepared anthrone reagent (100 mg anthrone in 100 ml of chilled, concentrated sulphuric acid), in a cooled water bath. Subsequently, the tubes containing this mixture were kept in a boiling water bath for 15 minutes and then rapidly cooled in running tap water. Absorbance was measured at 620 nm in Bausch and Lamb Spectronic-20. A reference standard curve was prepared using glucose in the range of 10-100 ug. Starch content was calculated by multiplying the glucose values with 0.9 (Pucher *et al.* 1948).

3.2.2.3 GROWTH AND YIELD OBSERVATIONS

LEAF AREA

Leaf area was measured on a per plant basis using a standard leaf area meter (Model LICOR 3100). It was expressed as cm^2.

LEAF AND STEM DRY WEIGHT

Dry weight data of leaves and stem was obtained using standard procedures. Initially they were dried at 80°C for 6 hours and later kept at 60°C until two successive dry weights were similar. The mean values are expressed in gms per plant.

DAYS TO FLOWERING

Days to flowering was recorded when there was 50 per cent flowering in the sample.

YIELD

Yield data was expressed in grams per plant basis. Yield was measured in the form of grain weight and grain number.

HARVEST INDEX (HI)

It was calculated using the formula :

$$HI = \frac{\text{Seed yield plant}^{-1}}{\text{Total biomass plant}^{-1}} \times 100$$

3.3 STATISTICAL ANALYSIS

Standard error : Standard errors were calculated to measure the sampling variability due to chance or random forces. The following formula was used :

$$\text{Standard error} = \frac{\text{Standard deviation}}{\sqrt{n}}$$

where,

n = Number of observations.

Test of Significance

To test the difference between means of two samples which are independent, the Student's "t" test was applied. The hypothesis tested was that the sample came from normal population. To carry out the test, the "t" statistic was calculated as follows :

$$t = \frac{\bar{X}_1 - \bar{X}_2}{S \sqrt{\frac{n_1 n_2}{n_1 + n_2}}}$$ at (n_1+n_2-2) degrees of freedom

where, $\bar{X}_1$ = Mean of first sample

X_2 = Mean of second sample

S = Combined standard deviation of two samples

n_1 = Number of observations in first sample

n_2 = Number of observations in second sample

"S" is obtained as :

$$S = \sqrt{\frac{(n_1-1)\,S_1^2 + (n_2-1)\,S_2^2}{n_1 + n_2 - 2}}$$

where,

S_1 = Standard deviation of first sample

S_2 = Standard deviation of second sample.

If the computed value of 't' is greater than it's table value at 5% level of significance, with $(n_1 + n_2 - 2)$ degrees of freedom, the two sample means are said to be statistically different.

The table values of 't' at 5% level of significance are :

1.734 when n = 10

1.860 when n = 5

2.132 when n = 3

CHAPTER IV

EXPERIMENTAL FINDINGS

This chapter summarises the results of experiments conducted to study the changes on physiological, biochemical, growth and yield characteristics of various crops when grown in an atmosphere enriched with carbon dioxide (CO_2). The findings are presented mainly under two heads:

a) Carbondioxide enrichment studies based on night trapped CO_2 using polycover, and

b) Carbondioxide enrichment studies conducted in open top chambers.

Tables 1 and 2 represent the weather data for the entire period of study.

4.1 CO_2 ENRICHMENT STUDIES BASED ON NIGHT-TRAPPED CO_2 USING POLYCOVER

In this experiment, the plant-respired CO_2 released during night time was trapped, using a specialised structure (details of the structure are given in Materials and Methods). This enriched CO_2 was utilised by the crop plants during early sunshine hours for photosynthesis. In this experiment, groundnut and peas were studied. The results will be discussed in the following scheme :

Table 1. Weather data for crops grown under polycover

Period	Monthly Mean Relative Humidity (%)	Monthly Mean Sunshine (hrs)	Temperature°C		
			Monthly Mean of Maximum	Monthly Mean of Minimum	Monthly Mean
July 89	63	7.3	36.4	27.1	31.8
Aug 89	71	5.7	34.3	26.0	30.2
Sept 89	67	8.4	34.4	23.4	28.9
Oct 89	52	8.7	34.3	17.0	25.7
Nov 89	54	8.1	28.4	11.7	20.1
Dec 89	75	4.6	21.0	7.5	14.2
Jan 90	65	6.8	21.7	9.0	15.7
Feb 90	72	5.5	22.2	11.1	16.6
Mar 90	57	7.7	26.8	13.4	20.1
April 90	59	8.7	35.8	19.1	27.2
May 90	63	7.2	38.2	24.5	31.2

Table 2. Weather data for crops grown in open top chambers

Period	Monthly Mean Relative Humidity (%)	Monthly Mean Sunshine (hrs)	Temperature°C		
			Monthly Mean of Maximum	Monthly Mean of Minimum	Monthly Mean
Oct 91	54.03	4.75	33.23	15.90	24.56
Nov 91	62.70	6.41	27.28	10.76	19.02
Dec 91	70.87	4.10	22.48	9.95	16.22
Jan 92	66.84	5.28	21.13	7.41	14.27
Feb 92	65.83	6.29	21.72	8.73	15.22
Mar 92	51.35	6.04	28.75	13.81	21.28
April 92	38.57	7.53	35.35	19.06	27.20
May 92	33.48	7.83	38.54	23.98	31.26

4.1.1 Groundnut

4.1.2 Peas

4.1.1 GROUNDNUT

Two cultivars of groundnut, M-13 (spreading type) and J-11 (erect type) were taken for the study. Growth and yield data are presented in Tables 3 and 4.

Effect of Night Trapped CO_2 on Vegetative Parameters of Groundnut at 125 DAS

Table 3 shows the growth data at 125 days after sowing (DAS). The values are the means of 10 observations. Main shoot length of M-13, grown in polycover was 20.4 cm while that of plants sown in open air was only 16.1 cms. This shows that plants sown in polycover had 26.71 per cent increase over control in main shoot length. The length of all the branches of plants grown in polycover was significantly higher than those of open air grown plants. The per cent increase in branch length of M-13, polycover grown plants was 24.23, 21.93, 24.02, 31.98 and 42.93 in branches 1, 2, 3, 4 and 5 respectively.

In case of J-11, the difference in the length of main shoot and branches of polycover grown plants and control plants did not differ significantly. Length of main shoot of J-11 in polycover was 29.8 cm, while in control it was 29.2 cm i.e. only a 2.05 per cent increase was seen. Branch number in J-11 was only four. Except branch number three of J-11 polycover plants, the increase in branch length was not found to be significant. The increase in shoot length of

Table 3. Effect of night trapped CO_2 by polycover on main shoot length and their branches in two cultivars of groundnut at 125 days after planting, n = 10 ± S.E.

	M-13			J-11		
	Open air	Polyhouse	% Change	Open air	Polyhouse	% Change
Main shoot	16.10 ± 1.03[a]	20.40 ± 1.23[b]	26.71	29.20 ± 1.41[a]	29.80 ± 0.98[b]	2.05
Branch No. 1	27.45 ± 1.32[a]	34.10 ± 2.09[b]	24.23	35.40 ± 2.50[a]	37.70 ± 2.06[a]	6.50
2	26.90 ± 1.31[a]	32.80 ± 1.84[b]	21.93	33.00 ± 2.60[a]	36.00 ± 1.22[a]	9.10
3	25.40 ± 1.85[a]	31.50 ± 1.97[b]	24.02	27.80 ± 1.76[a]	34.40 ± 1.91[b]	23.70
4	22.20 ± 1.89[a]	29.30 ± 0.68[b]	31.98	29.90 ± 2.86[a]	31.25 ± 1.87[a]	4.50
5	20.50 ± 1.54[a]	29.30 ± 1.24[b]	42.93			

Values designated by the same letter are not significantly different at 0.05 level according to Students' "t-test"

branches 1, 2 and 4 was 6.5, 9.1 and 4.5 per cent respectively while branch number three of J-11 polycover grown plants showed a 23.7 per cent increase over control.

As seen from Table 3 (Effect of night trapped CO_2 on main shoot and branch length of groundnut), the length of main shoot of M-13 (spreading type) showed an increase of 26.71 per cent under polycover over control, while J-11 showed only a 2.05 per cent increase. The increase in branch length of M-13 in polycover was statistically significant over that of control. In case of J-11 there was a marginal and non-significant increase in branch lengh in polycover grown plants over the control plants.

Effect of Night Trapped CO_2 on Reproductive Characters of Groundnut at Harvest

Table 4 shows an increase in growth and yield when grown under protected cultivation. In M-13, there was 65.1 per cent increase in stem dry weight, 74.4 per cent increase in leaf dry weight, 69.1 per cent increase in total dry weight of plants grown under polycover. There was 56.7 per cent increase in pod number and 64.4 per cent increase in pod weight per plant, in polycover grown plants over control. M-13 plants under polycover showed a statistically significant increase over control plants in vegetative as well as yield characters.

J-11 plants (Table 4) under polycover showed a 19.3 per cent increase in stem dry weight, 38 per cent increase in leaf dry weight, 27.6 per cent increase in total dry weight, 17.0 per cent in pod number and 11.8 per cent in pod weight per plant over control

Table 4. Effect of night trapped CO_2 by polycover on vegetative and reproductive characters at harvest in two cultivars of groundnut, n = 10 ± S.E.

Treatment	Stem dry wt (g)	Leaf dry wt (g)	Total dry wt (g)	Pod number	Pod weight per plant
			J-11 (Erect type)		
Open air	9.87 ± 0.63[a]	7.94 ± 0.85[a]	17.81 ± 1.34[a]	30.50 ± 3.26[a]	7.58 ± 0.64[a]
Polycover	11.78 ± 1.24[a]	10.96 ± 1.08[b]	22.74 ± 1.77[b]	35.70 ± 3.64[a]	8.59 ± 0.84[a]
% Change	19.3	38.0	27.6	17.0	11.8
			M-13 (Spreading type)		
Open air	10.17 ± 0.77[a]	8.48 ± 0.87[a]	18.68 ± 0.95[a]	42.1 ± 3.64[a]	13.66 ± 0.79[a]
Polycover	16.80 ± 1.20[b]	14.79 ± 0.95[b]	31.59 ± 1.22[b]	66.0 ± 4.94[b]	22.47 ± 1.53[b]
% Change	65.1	74.4	69.1	56.7	64.4

Values designated by the same letter are not significantly different at 0.05 level according to Students' "t-test"

All values are per plant basis.

plants. Except for increases in leaf dry weight and total dry weight, the others were not statistically significant.

As seen from Table 4, a comparison of M-13 and J-11 shows a 65.1 per cent increase in stem dry weight of M-13, while J-11 polycover plants show 19.3 per cent increase over control which was not significant. Pod number (56.7 per cent) and pod weight (64.4 per cent) of M-13 polycover plants showed a significant increase over control plants. J-11 polycover plants showed a 17.0 per cent increase in pod number and 11.8 per cent increase in pod weight per plant over control, which was not significant.

4.1.2 PEA

Growth parameters of DMR-10 at harvest

As seen from Table 5, DMR-10 (dwarf cultivar) plants grown under polycover show 110.4 cms plant height, while in open air it was only 84 cms i.e. there was a significant increase of 72.5 per cent in height of plants grown under polycover over control plants. Leaf dry weight of plants grown under polycover showed a 44.7 per cent increase, while stem dry weight showed an increase of 47.5 per cent which was significant.

Yield parameters of DMR-10

DMR-10 (Table 5) under polycover showed a decrease in seed weight per plant (8.22 per cent), pod number per plant (17.2 per cent) and seed number per plant (26.3 per cent) over control plants. However, the decrease in the above parameters was not significant.

Table 5. Effect of night trapped CO_2 by polycover in two cultivars of pea, n = 5 ± S.E.

	DMR-10 (Dwarf)			L-116 (Tall)		
	Open air	Polycover	% Change	Open air	Polycover	% Change
Plant height (cm)	64.00 ± 6.36[a]	110.40± 5.89[b]	72.50	139.40± 5.83[a]	140.80 ± 3.66[a]	1.00
Leaf dry weight (g)	2.10 ± 0.37[a]	3.04 ± 0.21[b]	44.70	1.14 ±0.26[a]	1.81 ± 0.27[a]	58.77
Stem dry weight (g)	2.60 ± 0.44[a]	3.84 ± 0.49[b]	47.60	3.17 ± 0.63[a]	3.12 ± 0.23[a]	- 1.50
Seed weight (g)	4.74 ± 0.81[a]	4.35 ± 0.59[a]	- 8.22	3.56 ± 1.48[a]	2.61 ± 0.47[a]	-26.60
Pod number	11.60 ± 1.83[a]	9.60 ± 1.96[a]	-17.20	9.20± 1.66[a]	5.80 ± 0.66[b]	-36.90
Seed number	25.80 ± 4.49[a]	19.00 ± 3.66[a]	-26.30	25.40 ± 4.51[a]	10.80 ± 2.87[b]	-57.00

Values designated by the same letter are not significantly different at 0.05 level according to Students' "t-test"

All values are per plant basis.

Growth parameters of L-116 (tall cultivar) at harvest

In L-116 (Table 5) variety of pea, the plant height was almost the same in polycover (140.8 cms) and control (139.4 cms), with a marginal increase of about 1 per cent. Leaf dry weight of L-116 under polycover showed an increase of 58.77 per cent, which was not significant. Stem dry weight of L-116 polycover plants (3.12 gms) decreased as compared to control plants (3.17 gms) by 1.5 per cent which was not significant.

Yield parameters of L-116 (tall cultivar)

L-116 (Table 5) plants under polycover showed a decrease in yield over control plants in the form of a decrease in seed weight per plant (26.60 per cent), pod number per plant (36.9 per cent) and seed number per plant (57 per cent). The decrease in seed weight per plant was not significant, while the decrease in pod number and seed number per plant were significant.

As seen from Table 5, pea cultivar, DMR-10 (dwarf) showed an increase in plant height by 72.5 per cent, while there was marginal or no increase in L-116 (tall) cultivar when grown under polycover over the control plants. However, both the cultivars showed a lower yield when grown under protected cultivation.

4.2 CARBON DIOXIDE ENRICHMENT STUDIES CONDUCTED IN OPEN TOP CHAMBERS

In this study, open top chambers were used for conducting CO_2 enrichment experiments. The design and operation of these open top chambers are described in detail in the chapter Materials

and Methods. In one of them a CO_2 concentration of 600 ± 50 ppm was maintained, while in another it was maintained at ambient CO_2 levels (350 ± 25 ppm). Nature of crops grown, their sowing dates and cultural practices were also dealt with in the previous chapter.

For brevity and convenience the results of this experiment are dealt with under the following heads :

4.2.1 PHYSIOLOGICAL PARAMETERS

a) Photosynthesis

b) Stomatal conductance and

c) Respiration

4.2.2 BIOCHEMICAL PARAMETERS

1) Chlorophyll

2) Sugars, amino acids and starch

4.2.3 GROWTH AND PHENOLOGY

1) Leaf area

2) Leaf, stem and total dry weight

3) Flowering

4.2.4 YIELD PARAMETERS

4.2.1 PHYSIOLOGICAL PARAMETERS

Under this head, results obtained using a portable photosynthesis system (LICOR-6200) are described. The rate of photosynthesis was measured in the top most, fully expanded leaf at the stage indicated in each crop plant. The same leaf was used to measure stomatal conductance and respiration.

PHOTOSYNTHESIS (Pn)

WHEAT

As seen from Table 6, Pn in ambient CO_2 grown plants was 11.72 at 45 DAS, while plants grown at 600 ppm CO_2 showed a Pn of 14.74 u mol M^{-2} sec^{-1}. But plants grown in ambient CO_2 when momentarily exposed to 600 ppm CO_2 showed a higher Pn than plants grown in 600 ppm CO_2. Plants grown in 600 ppm CO_2 and momentarily brought to ambient levels of CO_2 showed a Pn much lower (8.91) than plants grown at ambient CO_2 (11.72) levels. All the differences were statistically significant.

MUNGBEAN

Pn in mungbean was measured from 30 days onwards upto 60 DAS at an interval of 10 days (Table 7). At 30 DAS there was an increase of 87.0 per cent in plants grown at 600 ppm CO_2 for long time. Even under short term exposure to high CO_2 the increase was same as found in long term at 30 DAS. At 40 DAS the Pn in ambient CO_2 grown plants was 16.82, while in plants grown at 600 ppm CO_2 it was 24.17. At all stages from 30-60 DAS, Pn was higher in CO_2 enriched plants than in ambient CO_2 grown plants. At 50 DAS the Pn in general was less than what it was at 40 DAS in both ambient as well as CO_2 enriched grown plants. As seen from the table, at 60 DAS, there was a drop in Pn in ambient (13.56) CO_2 grown plants, while plants under CO_2 enriched conditions (23.43) still had a high Pn. Interestingly plants grown at ambient CO_2 levels when instantly exposed to 600 ppm CO_2 (short term enrichment)

Table 6. Physiological parameters of wheat at 45 DAS under different CO_2 concentrations in open top chambers, n = 3 ± S.E.

	Plants grown in ambient CO_2 (350 ± 25 ppm)			Plants grown in CO2 enriched atmosphere (600 ± 50 ppm)		
	Measured at 350 ± 25 ppm	Measured at 600 ± 50 ppm	% Change	Measured at 350 ± 25 ppm	Measured at 600 ± 50 ppm	% Change
Photosynthesis (u mol M^{-2} sec^{-1})	11.72 ± 0.29[a]	21.4 ± 0.29[b]	83.28	8.91 ± 0.30[a]	14.74 ± 0.19[b]	65.43
Stomatal conductance (mol M^{-2} sec^{-1})	0.053 ± 0.01[a]	0.027 ± 0.01[b]	-96.30	0.15 ± 0.01[a]	0.07 ± 0.006[a]	-114.24
Leaf temperature (°C)	30.84 ± 0.17[a]	32.95 ± 0.09[b]	2.11*	33.63 ± 0.13[a]	33.09 ± 0.06[b]	- 0.54*

Values designated by the same letter are not significantly different at 0.05 level according to Students' "t-test"

*Figures represent absolute change in temperature.

Table 7. Physiological parameters of mungbean grown under CO_2 enriched conditions in open top chambers, n = 3 ± S.E.

	Plants grown in ambient CO_2 (350 ± 25 ppm)			Plants grown in CO2 enriched atmosphere (600 ± 50 ppm)		
	Measured at 350 ± 25 ppm	Measured at 600 ± 50 ppm	% Change	Measured at 350 ± 25 ppm	Measured at 600 ± 50 ppm	% Change
			30 DAS			
Photosynthesis (u mol $M^{-2}.sec^{-1}$)	11.27 ±10.89[a]	15.45 ± 0.58[b]	37.09	11.09 ± 0.58[a]	15.12 ± 0.25[b]	36.34
Stomatal conductance (mol $M^{-2}.sec^{-1}$)	1.44 ± 0.05[a]	0.69 ± 0.007[b]	-108.70	1.27 ± 0.005[a]	1.08 ± 0.02[b]	- 17.59
Respiration (umol $M^{-2}.sec^{-1}$)	9.84 ± 0.38[a]	7.25 ± 0.44[b]	- 35.72	5.94 ± 0.18[a]	3.59 ± 0.15[b]	- 65.46
Leaf Temperature (°C)	38.29 ± 0.18[a]	40.77 ± 0.05[b]	2.48*	39.87 ± 0.06[a]	41.53 ± 0.01[b]	1.66*
			40 DAS			
Photosynthesis (u mol $M^{-2}.sec^{-1}$)	16.82 ± 0.40[a]	23.02 ± 0.20[b]	36.86	10.86 ± 0.23[a]	24.17 ± 0.18[b]	122.56
Stomatal conductance (mol $M^{-2}.sec^{-1}$)	2.30 ± 0.02[a]	1.67 ± 0.04[b]	- 37.72	3.62 ± 0.06[a]	3.35 ± 0.06[b]	- 8.06
Respiration (umol $M^{-2}.sec^{-1}$)	3.94 ± 0.01[a]	1.15 ± 0.17[b]	-242.61	5.07 ± 0.23[a]	3.49 ± 0.28[b]	- 45.27
Leaf Temperature (°C)	35.89 ± 3.53[a]	36.48 ± 0.12[a]	0.59*	39.74 ± 0.05[a]	37.49 ± 0.05[b]	- 2.30*

Table 7 contd.

	Plants grown in ambient CO_2 (350 ± 25 ppm)			Plants grown in CO2 enriched atmosphere (600 ± 50 ppm)		
	Measured at 350 ± 25 ppm	Measured at 600 ± 50 ppm	% Change	Measured at 350 ± 25 ppm	Measured at 600 ± 50 ppm	% Change
			50 DAS			
Photosynthesis (u mol $M^{-2}.sec^{-1}$)	16.85 ± 0.21[a]	22.64 ± 0.04[b]	34.36	10.21 ± 0.43[a]	22.39 ± 0.73[b]	119.29
Stomatal conductance (mol $M^{-2}.sec^{-1}$)	4.73 ± 0.01[a]	4.19 ± 0.04[b]	- 12.89	2.57 ± 0.17[a]	1.64 ± 0.006[b]	- 56.71
Respiration (umol $M^{-2}.sec^{-1}$)	4.44 ± 0.18[a]	1.16 ± 0.08[b]	-282.70	7.85 ± 0.16[a]	3.86 ± 0.08[b]	-103.36
Leaf Temperature (°C)	32.94 ± 0.08[a]	34.60 ± 2.28[b]	1.66*	35.85 ± 0.05[a]	41.09 ± 0.08[b]	- 5.24*
			60 DAS			
Photosynthesis (u mol $M^{-2}.sec^{-1}$)	13.56 ± 0.352[a]	24.72 ± 0.70[b]	82.30	13.57 ± 0.45[a]	23.43 ± 0.79[b]	72.60
Stomatal conductance (mol $M^{-2}.sec^{-1}$)	0.91 ± 0.01[a]	0.87 ± 0.02[a]	- 4.60	0.79 ± 0.01[a]	0.53 ± 0.01[b]	- 49.06
Respiration (umol $M^{-2}.sec^{-1}$)	4.65 ± 0.16[a]	2.47 ± 0.23[b]	- 88.20	5.60 ± 0.13[a]	1.68 ± 0.25[b]	-233.30
Leaf Temperature (°C)	29.56 ± 0.08[a]	29.95 ± 0.05[b]	0.39*	32.76 ± 0.04[a]	33.87 ± 0.10[b]	1.11*

Values designated by the same letter are not significantly different at 0.05 level according to Students' "t-test"

*Figures represent absolute change in temperature.

showed a Pn that was similar or a little higher than that of plants grown continuously at 600 ppm CO_2 (long term enrichment), except at 40 DAS where short term treatment showed a higher value than long term. All the values in the table 7 were statistically significant.

SUNFLOWER

Pn was measured at 25 and 50 DAS (Table 8). The Pn value of plants grown at ambient CO_2 was 11.34, whereas plants grown at high CO_2 concentration of 600 ppm (long term enrichment) had 35.82 i.e.there was more than 200 per cent increase in Pn rate. In short term CO_2 enrichment at 25 DAS there was an increase of only 173 per cent.

At 50 DAS, the per cent increase in Pn that was found at 25 DAS was not noticeable in CO_2 enriched plants. Both under long term and short term CO_2 enrichment the per cent increase was around 50 as compared to ambient CO_2 grown plants. It was found that at 50 DAS, plants grown at 350 ppm CO_2 (ambient grown plants) showed an increase in Pn from 25 DAS but in long term CO_2 enrichment the Pn was lower (29.56) at 50 DAS, than what it was at 25 DAS (35.82).

PHOTOSYNTHESIS (Pn) AT DIFFERENT CO_2 LEVELS

In this experiment, plants grown under ambient CO_2 concentration were momentarily exposed to higher CO_2 levels of 600, 1000 and 1600 ppm. The same leaf (top most, fully expanded) was exposed to different CO_2 levels and Pn was measured. The crops in which Pn was recorded were wheat, mustard, pea and mungbean

Table 8. Physiological parameters of sunflower grown under CO_2 enriched conditions in open top chambers n = 3 ± S.E.

Days after sowing	Plants grown in ambient CO_2 (350 ± 25 ppm)			Plants grown in CO2 enriched atmosphere (600 ± 50 ppm)		
	Measured at 350±25 ppm	Measured at 600±50 ppm	% Change	Measured at 350±25 ppm	Measured at 600±50 ppm	% Change
	Photosynthesis (u mol CO_2 M^{-2} sec^{-1})					
25	11.34 ± 0.41[a]	31.05 ± 0.46[b]	173.81	21.56 ± 0.31[a]	35.82 ± 0.64[b]	66.14
50	14.43 ± 0.88[a]	22.51 ± 0.46[b]	55.99	19.67 ± 0.49[a]	29.56 ± 0.95[b]	50.28
	Stomatal conductance (mol M^{-2} sec^{-1})					
25	0.71 ± 0.02[a]	0.46 ± 0.002[b]	- 54.35	1.39 ± 0.02[a]	1.2 ± 0.024[b]	- 15.83
50	1.57 ± 0.04[a]	0.62 ± 0.002[b]	-153.23	1.73 ± 0.04[a]	1.73 ± 0.04[a]	0
	Respiration (u.mol M^{-2} sec^{-1})					
25	7.02 ± 0.05[a]	4.81 ± 0.06[b]	- 46.25	6.57 ± 0.32[a]	4.85 ± 0.06[b]	- 35.46
50	8.05 ± 0.07[a]	4.33 ± 0.36[b]	- 85.91	12.32 ± 0.08[a]	4.80 ± 0.48[b]	- 156.67
	Leaf Temperature (°C)					
25	34.38 ± 0.16[a]	36.45 ± 0.06[b]	2.13*	33.69 ± 0.05[a]	34.52 ± 0.17[b]	0.93*
50	33.64 ± 0.18[a]	36.81 ± 0.02[b]	3.17*	32.66 ± 0.31[a]	33.58 ± 0.06[b]	0.92*

Values designated by the same letter are not significantly different at 0.05 level according to Students' "t-test"
*Figures represent absolute change in temperature.

(Table 9). At that point of time mungbean had a Pn of 15.96 which was higher than wheat (15.58), mustard (11.62) and pea (11.09) in ambient CO_2 grown plants (350 ppm). When a single leaf was exposed to 600 ppm CO_2, there was 143 per cent increase in Pn of mustard, while an increase in Pn of wheat by 37.36 per cent. Pea had a 65 per cent increase and mungbean had only 41.8 per cent increase. At 1000 ppm CO_2, wheat showed 3.9 times increase in Pn, while mustard had 6.3 times increase, pea 2.3 times and mungbean showed only 0.6 times increase as compared to ambient CO_2 grown plants.

Pn of the crops under study had still higher rates at 1600 ppm CO_2 concentration. However, in mungbean Pn at 1000 ppm (26.46) was higher than what it was at 1600 ppm CO_2 (25.73) i.e. there was a slight decrease in Pn at 1600 ppm CO_2 as compared to 1000 ppm.

STOMATAL CONDUCTANCE (SC)

WHEAT

Stomatal conductance (SC) was measured at 45 DAS in wheat as seen from Table 6. Plants grown at ambient CO_2 levels showed a SC of 0.053 mol M^{-2} sec^{-1}, while plants grown at 600 ppm CO_2 showed an SC of 0.07 i.e. SC of CO_2 enriched plants was 32 per cent more than ambient CO_2 grown plants. However, when ambient CO_2 grown plants were momentarily exposed to 600 ppm CO_2, SC was reduced upto 96 per cent, likewise plants grown in CO_2 enriched atmosphere when brought to ambient CO_2 levels, showed (0.15) maximum SC.

Table 9. Effect of short-term exposure of ambient grown plants to different CO_2 enriched conditions on photosynthesis and stomatal conductance, n = 3 ± S.E.

	Ambient CO (350± 25 ppm)	600 ± 50 ppm	1000 ± 50 ppm	1600 ± 50 ppm
		Photosynthesis (u mol M^{-2} sec^{-1})*		
Wheat	15.58 ± 0.25[a]	21.40 ± 0.29[b] (37.36)	76.45 ± 0.78[c] (390.70)	138.80 ± 4.04[d] (790.89)
Mustard	11.62 ± 0.04[a]	28.30 ± 0.10[b] (143.55)	85.59 ± 4.66[c] (636.57)	99.90 ± 1.71[d] (759.72)
Pea	11.09 ± 0.84[a]	18.30 ± 0.20[b] (65.01)	36.87 ± 2.91[c] (232.46)	66.17 ± 1.46[d] (496.66)
Mungbean	15.96 ± 0.04[a]	22.64 ± 0.44[b] (41.85)	26.46 ± 0.41[c] (65.79)	25.73 ± 0.86[c] (61.22)
		Stomatal conductance (mol M^2 sec^{-1})*		
Wheat	0.65 ± 0.01[a]	0.17 ± 0.01[b] (-58.46)	0.17 ± 0.00003[c] (-73.85)	0.17 ± 0.005[c] (-73.85)
Mustard	0.23 ± 0.003[a]	0.20 ± 0.02[a] (-13.04)	0.18 ± 0.007[b] (-23.91)	0.13 ± 0.005[c] (-42.61)
Pea	0.33 ± 0.007[a]	0.31 ± 0.001[a] (-6.06)	0.28 ± 0.004[b] (-15.30)	0.27 ± 0.004[b] (-18.18)

Table 9 contd.

	Ambient CO (350± 25 ppm)	600 ± 50 ppm	1000 ± 50 ppm	1600 ± 50 ppm
Mungbean	1.27 ± 0.01[a]	1.13 ± 0.02[b] (-11.02)	1.02 ± 0.01[c] (-19.69)	0.83 ± 0.006[d] (-34.65)
		Leaf Temperature (°C)**		
Wheat	20.05 ± 0.06[a]	21.02 ± 0.20[b] (1.15)	23.14 ± 0.06[c] (3.09)	23.37 ± 0.003[d] (3.32)
Mustard	21.99 ± 0.07[a]	22.00 ± 0.06[b] (0.01)	22.50 ± 0.06[c] (0.51)	24.03 ± 0.04[d] (2.04)
Pea	22.83 ± 0.04[a]	23.12 ± 0.05[b] (0.29)	24.29 ± 0.05[c] (1.46)	23.01 ± 0.04[d] (0.18)
Mungbean	22.50 ± 0.06[a]	22.70 ± 0.10[b] (0.20)	23.70 ± 0.03[c] (1.20)	24.60 ± 0.01[d] (2.10)

Values designated by the same letter are not significantly different at 0.05 level according to Students' "t-test"

* Figures in parentheses indicate percentage change over plants grown in ambient CO_2

** Figures in parentheses indicate absolute change over plants grown in ambient CO_2

MUNGBEAN

As seen from Table 7, it was found that SC at 30 DAS which was 1.44, increased to 2.3 at 40 DAS and further increased to 4.73 at 50 DAS and later reached a minimum of 0.91 at 60 DAS in ambient CO_2 grown plants. Plants grown in CO_2 enriched conditions also showed increasing values from 30 to 40 DAS which later started declining at 50 DAS and became minimum at 60 DAS (0.53). When comparison was made between plants grown in ambient and CO_2 enriched conditions, SC in ambient CO_2 grown plants at 40 and 50 DAS was more than that of plants grown in CO_2 enriched conditions and at 30 and 60 DAS, SC was greater in CO_2 enriched plants as compared to control plants. However, in short term CO_2 enrichment, SC was always less than that of ambient grown plants at 30, 40, 50 and 60 DAS.

SUNFLOWER

In sunflower SC was measured at 25 and 50 DAS (Table 8). SC increased from 25 DAS to 50 DAS under both the conditions. It was also found that plants grown at 600 ppm CO_2 had higher SC at 25 and 50 DAS as compared to ambient CO_2 grown plants as seen from Table 8. From the table we can see that short term CO_2 enrichment of control plants showed a decrease in SC of 54 per cent at 25 DAS and 153 per cent at 50 DAS when exposed to 600 ppm CO_2.

STOMATAL CONDUCTANCE (SC) AT DIFFERENT CO_2 LEVELS

As in case of Pn at different CO_2 levels, SC was also measured in different crops at different CO_2 levels. As seen from Table 9 at ambient CO_2 levels (350 ppm) mungbean had the highest SC of 1.27. When CO_2 concentration was raised from 350 ppm to 1000 ppm, SC of wheat, pea and mungbean decreased and the maximum decrease was seen in wheat (73.8 per cent) and minimum in pea (15.3 per cent). At 1600 ppm CO_2 level, except wheat other two crops showed a further decline in SC.

RESPIRATION (Rn)

Respiration rate (Rn) was measured on the same leaf on which photosynthesis was measured. Rn was observed in mungbean and sunflower. The units of Rn are the same as Pn units (u mol M^{-2} sec^{-1}).

MUNGBEAN

As seen from Table 7, Rn was maximum at 30 DAS (9.84) and then decreased at 40 (3.94) and 50 DAS (4.44) and it was 4.65 at 60 DAS. In plants grown in CO_2 enriched atmosphere, Rn was maximum at 50 DAS (5.86) and minimum at 60 DAS (1.68). At all growth stages, Rn of plants grown in CO_2 enriched conditions was less than that of ambient CO_2 grown plants. Dark respiration generally decreased under high CO_2 conditions. At 30 DAS, control plants had more than double the Rn of CO_2 enriched plants and at 60 DAS the trend was even higher by 2 times in control plants. Under short term CO_2 enrichment of ambient grown plants, Rn decreased at 30, 40, 50 and 60 DAS.

SUNFLOWER

Rn in sunflower was measured at 25 and 50 days after sowing (DAS) as seen from Table 8. Plants grown in CO_2 enriched atmosphere (600 ppm CO_2) had almost similar Rn at 25 and 50 DAS. In case of control (ambient CO_2 grown plants) plants, there was a marginal increase (from 7.02 to 8.05) from 25 to 50 DAS. At these two stages of 25 and 50 DAS, the Rn of control plants was higher than that of CO_2 enriched grown plants. At 25 DAS, the Rn of control plants was 7.02, while in CO_2 enriched conditions, it was 4.85, i.e. there was a decrease of 45 per cent in Rn. At 50 DAS the Rn of ambient CO_2 grown plants was 8.05 and CO_2 enriched plants had 4.80 Rn i.e. there was a decrease of 80 per cent in Rn when plants were grown at higher CO_2 levels. Under short term CO_2 enrichment, Rn invariably decreased at 25 and 50 DAS and the extent of decrease was 85 per cent at 50 DAS, while it was only 46 per cent at 25 DAS.

4.2.2 BIOCHEMICAL PARAMETERS

CHLOROPHYIL

Chlorophyll content was measured in ambient and CO_2 enriched conditions in wheat and mungbean. Chlorophyll a, b and total were estimated using DMSO and were expressed in mg/gram f.wt.

WHEAT

Chlorophyll content decreased when plants were grown in high CO_2 environment (at 600 ppm CO_2). As seen from Table 10,

Table 10. Chlorophyll content, amino acids, sugars and starch content in wheat at 45 DAS grown at two different CO_2 concentrations in open top chambers, n = 3 ± S.E.

	Plants grown in ambient CO_2 (350 ± 25 ppm)	Plants grown in CO_2 enriched atmosphere (600 ± 50 ppm)	% Change
Chlorophyll (mg/g f.wt.)			
Total	2.18 ± 0.015[a]	1.254 ± 0.032[b]	- 42.48
A	1.24 ± 0.06[a]	0.578 ± 0.193[b]	- 53.39
B	0.74 ± 0.026[a]	0.575 ± 0.008[b]	- 22.30
Total Free Amino Acids (TFAA, u mols/g f.wt)	54.67 ± 2.33[a]	25.77 ± 1.67[b]	- 52.86
Total sugars (mg/g f.wt)	4.1 ± 0.115[a]	5.60 ± 0.155[b]	36.59
Reducing	3.23 ± 0.088[a]	2.13 ± 0.088[b]	- 34.06
Non-reducing	0.87 ± 0.033[a]	3.30 ± 0.06[b]	279.31
Starch (mg/g f.wt)	61.9 ± 1.69[a]	79.0 ± 2.31[b]	27.63

Values designated by the same letter are not significantly differentat 0.05 level according to Students' "t-test".

total chlorophyll decreased by 42.4 per cent, while chlorophyll a and b contents decreased by 53.3 and 22.3 per cent respectively when plants were grown in high CO_2 media as compared to ambient CO_2 grown plants.

MUNGBEAN

In mungbean, chlorophyll content was measured 45 days after sowing (Table 11). Chlorophyll content of CO_2 enriched plants (total) was 1.85, while that of ambient plants was 2.0 mg/gm f.wt

Table 11. Chlorophyll (45 DAS) and starch (60 DAS) content of mungbean grown at two different CO_2 concentrations in open top chambers, n = 3 ± S.E.

	Plants grown in ambient CO_2 (350 ± 25 ppm)	Plants grown in CO_2 enriched atmosphere (600 ± 50 ppm)	% Change
Chlorophyll (mg/gm F.wt)			
Total	2.023 ± 0.054[a]	1.851 ± 0.051[b]	- 8.5
A	1.175 ± 0.005[a]	1.170 ± 0.006[a]	- 0.43
B	0.738 ± 0.025[a]	0.588 ± 0.003[b]	- 20.33
Starch (mg/g F.wt)	91.13 ± 0.65[a]	152.6 ± 1.45[b]	67.14

Values designated by the same letter are not significantly different at 0.05 level according to Students' "t-test"

i.e. CO_2 enriched plants showed 8.5 per cent decrease in chlorophyll content.

Chlorophyll a and b of plants grown in CO_2 enriched conditions decreased by 0.43 and 20.3 per cent respectively. However, decrease in chlorophyll was negligible and insignificant.

SUGARS, AMINO ACIDS AND STARCH

In case of wheat, sugars, amino acids and starch content were estimted at 45 DAS, while in case of mungbean only starch was estimated at 60 DAS.

WHEAT

As seen from Table 10, total free amino acids (TFAA) decreased by 52.8 per cent when plants were grown in CO_2 enriched

atmosphere as compared to ambient CO_2 grown plants. In case of sugars, total sugar increased from 4.1 to 5.6 mg/gm/f.wt in plants grown under 600 ppm CO_2 as compared to plants grown in ambient CO_2. However, plants under high CO_2 environment showed increase in non-reducing sugars (279 per cent) and a decrease in reducing sugars (34 per cent) as compared to ambient CO_2 grown plants.

Starch content of CO_2 enriched plants was 27.6 per cent more than that of ambient CO_2 grown plants (Table 10).

MUNGBEAN

In mungbean, starch content was measured 60 days after sowing (Table 11). Ambient CO_2 grown plants had 91.3 mg/gram f.wt whereas plants grown in 600 ppm CO_2 had 152.6. There was an increase of 67.1 per cent starch content when plants were grown under high CO_2 environment.

4.2.3 GROWTH AND PHENOLOGY

In this subhead, results of wheat, mungbean and sunflower are discussed in relation to leaf area, leaf/stem dry weight and days taken to flowering under various subheads.

LEAF AREA

Leaf area of wheat and mungbean were measured using leaf area meter and expressed in cm^2.

WHEAT

In wheat, leaf area was recorded at 25 and 45 DAS (Table 12). When plants were grown in high CO_2 environment the leaf area

Table 12. Growth parameters of wheat grown at two different CO_2 concentrations in open top chambers, n = 5 ± S.E.

25 DAS			50 DAS		
Plants grown in ambient CO_2 (350 ± 25 ppm)	Plants grown in CO_2 enriched atmosphere 600 ± 50 ppm	% Change	Plants grown in ambient CO_2 (350 ± 25 ppm)	Plants grown in CO_2 enriched atmosphere 600 ± 50 ppm	% Change
Leaf Area (cm^2)					
11.10± 0.74[a]	18.88± 1.20[b]	70.09	14.28± 0.59[a]	31.32 ± 0.90[b]	119.33
Leaf Dry Weight (g)					
0.028± 0.002[a]	0.088 ± 0.011[b]	214.29	0.063± 0.006[a]	0.114 ± 0.016[b]	80.95
Stem Dry Weight (g)					
0.019± 0.001[a]	0.028 ± 0.001[a]	41.41	0.051± 0.002[a]	0.119 ± 0.009[b]	133.33
Biomass (g)					
0.048± 0.002[a]	0.115± 0.009[b]	139.58	0.115± 0.007[a]	0.233± 0.019[b]	102.61

Values designated by the same letter are not significantly different at 0.05 level of significance to Students' "t-test"

All values are per plant basis.

increased by 70 per cent at 25 DAS and the per cent increase was 119.6 when measured at 45 DAS. In both the stages there was an increase of leaf area of plants grown in CO_2 enriched conditions as compared to ambient CO_2 grown plants.

MUNGBEAN

In mungbean also leaf area was measured at 25 and 45 DAS. As seen from Table 13, at 25 DAS CO_2 enriched plants had 70.5 per cent more leaf area as compared to leaf area of ambient CO_2 grown plants. At 45 DAS, there was 77.5 per cent increase in leaf area of plants grown in CO_2 enriched (600 ppm CO_2) environment, as compared to ambient grown plants.

LEAF/STEM AND TOTAL DRY WEIGHT

Oven dry weight of leaf, stem and total plant were recorded, which will be presented below. The crops for which these parameters were recorded are wheat, mungbean and sunflower. The values are expressed in gms/plant.

MUNGBEAN

There was an increase of 230 per cent in leaf dry weight at 25 DAS in CO_2 enriched plants (34.8) as compared to ambient (20.4) CO_2 grown plants. However, at 45 DAS the per cent increase in leaf dry weight of CO_2 enriched plants was only 39.5.

As seen from Table 12, total stem dry weight increased both at 25 DAS (75 per cent) and 45 DAS (52.2 per cent) when plants were grown in CO_2 enriched atmosphere as compared to plants grown under ambient CO_2 (350 ppm). Total biomass of ambient CO_2

Table 13. Growth paameters of mungbean grown at two different CO_2 concentrations in open top chambers, n = 5 ± S.E.

25 DAS			50 DAS		
Plants grown in ambient CO_2 (350 ± 25 ppm)	Plants grown in CO_2 enriched atmosphere 600 ± 50 ppm	% Change	Plants grown in ambient CO_2 (350 ± 25 ppm)	Plants grown in CO_2 enriched atmosphere 600 ± 50 ppm	% Change
Leaf Area (cm^2)					
20.4 ± 0.76[a]	34.8 ± 1.26[b]	70.59	58.4 ± 4.26[a]	103.66 ± 5.37[b]	77.50
Leaf Dry Weight (g)					
0.06 ± 0.006[a]	0.198 ± 0.01[b]	230.00	0.44 ± 0.03[a]	0.614 ± 0.03[b]	39.55
Stem Dry Weight (g)					
0.04 ± 0.005[a]	0.07 ± 0.01[b]	75.00	0.41 ± 0.02[a]	0.624 ± 0.021[b]	52.20
Biomass (g)					
0.11 ± 0.004[a]	0.27 ± 0.01[b]	145.45	0.85 ± 0.03[a]	1.24 ± 0.05[b]	45.88

Values designated by the same letter are not significantly different at 0.05 level of significance to Students' "t-test"

grown plants was 0.11 gms per plant, whereas that of CO_2 enriched plants was 0.27 gms, i.e. there was an increase of 145.4 per cent in terms of biomass when plants were grown under high CO_2 of 600 ppm at 25 DAS. However, at 45 DAS there was only a 45.5 per cent increase in total biomass of plants grown under high CO_2 atmosphere.

WHEAT

As seen from Table 13, at 25 DAS leaf dry weight of CO_2 enriched plants was 0.088 as compared to that of control plants which was only 0.028 i.e. CO_2 enriched plants had 214.2 per cent increase in leaf dry weight which was significant. At 45 DAS the increase in leaf dry weight was only 80.9 per cent in plants grown in CO_2 enriched conditions as compared to control plants.

At 30 DAS stem dry weight of CO_2 enriched plants showed an increase of 41.4 per cent over ambient CO_2 grown plants. However, the values were not statistically significant. At 45 DAS, the per cent increase was 133.3 which was statistically significant (Table 13). Total biomass at 25 DAS in CO_2 enriched plants as compared to ambient CO_2 grown plants was 139.5 per cent more while at 45 DAS it was 102.6 per cent more.

SUNFLOWER

In sunflower, total biomass was measured at 25 DAS and 75 DAS and expressed in gms per plant.

Plants under CO_2 enriched atmosphere (600 ppm CO_2) at 25 DAS showed a 63.0 percent increase in total biomass as compared

to ambient CO_2 grown plants. At 75 DAS (Table 14) the per cent increase was 40.5 in total biomass of plants grown in CO_2 enriched atmosphere as compared to ambient CO_2 grown plants.

Table 14. Total plant dry weight (in gms) of individual sunflowerplants grown at two different CO_2 concentrations in open top chambers, n = 5 ± S.E.

Days after sowing	Plants grown in ambient CO_2 (350 ± 25 ppm)	Plants grown in CO_2 enriched atmosphere (600 ± 50 ppm)	% Change
25	1.575 ± 0.750[a]	2.568 ± 0.102[b]	63.05
75	10.584 ± 0.513[a]	14.88 ± 1.065[b]	40.59

Values designated by the same letter are not significantly different at 0.05 level according to Students' "t-test"

DAYS TAKEN TO FLOWERING/SPIKE EMERGENCE

Flowering time or appearance of flowers (spike in wheat) have been recorded in both CO_2 enriched and ambient CO_2 grown plants. Whenever there was flowering in 50 per cent of the population, that particular date as recorded.

As seen from Table 15, in case of wheat spike emergence was 10 days earlier in CO_2 enriched plants. In case of mungbean, flower appearance was earlier by only 2 days and in case of sunflower, CO_2 enriched plants flowered earlier by 12 days as compared to control plants. All the above crops in Table 15 show an early flowering in plants grown under open top chamber in which CO_2 concentration was 600 ± 50 ppm and the values were statistically significant.

Table 15. Number of days to flowering/spike emergence when grown at two different CO_2 concentrations in open top chambers, n = 5 ± S.E.

	Plants grown in ambient CO_2 (350 ± 25 ppm)	Plants grown in CO_2 enriched atmosphere (600 ± 50 ppm CO_2)
Mungbean	41.8 ± 0.80[a]	38.2 ± 0.86[b]
Sunflower	61.8 ± 0.80[a]	49.2 ± 0.05[b]
Wheat	82.0 ± 1.24[a]	72.0 ± 0.81[b]

Values designated by the same letter are not significantly differentat 0.05 level according to Students' "t-test"

4.2.4 YIELD PARAMETERS

Yield data at harvest was recorded and expressed on per plant basis. Both wheat and mungbean were grown in pots and equal number of plants were maintained per pot.

WHEAT

At harvest (Table 16), wheat plants in CO_2 enriched atmosphere showed an increase in total grain weight (7.81 per cent), grain number/ear (5.11 per cent), 1000 grain weight (2.08 per cent) and harvest index (9.70 per cent), as compared to ambient CO_2 grown plants. However, except increase in grain number/ear, the increases were not significant as seen from Table 16.

MUNGBEAN

Table 16 shows data related to mungbean at harvest. Yield in the form of total grain weight increased by 75.6 per cent in CO_2

Table 16. Changes in yield attributes of wheat and mungbean grown at ambient and CO_2 enriched conditions in open top chambers, n = 5 ± S.E.

	Ambient grown plants (350±25 ppm CO_2)	Plants grown in enriched CO_2 (600±50 ppm)	% Change
	WHEAT		
Total grain weight (g/plant)	2.56 ± 1.62[a]	2.76 ± 0.16[a]	7.81
Grain number/ear	70.40 ± 0.74[a]	74.00 ± 0.88[b]	5.11
1000 Grain weight (g)	36.50 ± 2.99[a]	37.36 ± 2.49[a]	2.08
Harvest Index (%)	39.98 ± 1.69[a]	43.86 ± 1.57[a]	9.70
	MUNGBEAN		
Total grain weight (g/plant)	2.46 ± 0.09[a]	4.32 ± 0.09[b]	75.61
Grains/pod	5.40 ± 0.50[a]	5.60 ± 0.40[a]	3.70
Pods/plant	16.80 ± 1.50[a]	24.00 ± 1.50[b]	42.86
Harvest Index (%)	27.10 ± 0.67[a]	33.80 ± 0.45[b]	24.72

Values designated by the same letter are not significantly different at 0.05 level of significance to Students' "t-test"

enriched plants as compared to control plants. There was an increase in grains per pod (3.7 per cent) and pods per plant (42.85 per cent). The harvest index was 33.8 in CO_2 enriched plants as compared to 27.10 in ambient CO_2 grown plants. All the values in the Table were statistically significant except for grains per pod.

CHAPTER-V

DISCUSSION

The present study on the effects of carbon dioxide enrichment on crop plants was undertaken based on the current concern over anthropogenic climatic change. In spite of several studies, conducted in the past decade, there is no consensus regarding the effects of CO_2 doubling on environment as well as on the crops. Very few studies on CO_2 enrichment have been conducted with cultivars adapted to the agroclimatic condition of this region, primarily due to lack of suitable techniques and infrastructure required. Primary requirement was therefore to develop suitable methodology for CO_2 enrichment and two such methods, one using polycover (by trapping night respired CO_2) and the other using open top chamber were developed and tried for studying the effects of high CO_2 on crop plants.

CARBON DIOXIDE ENRICHMENT USING POLYCOVER

In this method, level of CO_2 above the crop canopy was increased by trapping the CO_2 released in the night due to plant respiration. This was achieved by covering the plants, after sunset with airtight transparent polythene cover erected above the plants. The accumulated CO_2 was used by the plants in the first few hours of morning (sunshine) after which, the cover was removed. Thus the plants were subjected to few hours of CO_2 enrichment at early sunshine hours. This method is an adaptation of protected

cultivation used primarily to elevate the temperature, which is not popular in India because of high solar radiation and heat transport by convection from the leaf causing plant injury. In protected cultivation, the microclimate is modified sufficiently to produce more (Enoch, 1984).

One of the reasons for high production in protected cultivation is increased CO_2 concentration, because CO_2 released due to dark respiration is unable to escape. This accumulated CO_2 is used rapidly by the plants during early sunshine hours is clear from the fact that, after few sunshine hours the level of CO_2 in the enclosure declined below the ambient (Fig. 1 & 2). After exposure to sunshine for a couple of hours the enclosure was removed and the plants were then exposed to natural environmental condition. This method is different from the protected cultivation as the plants are covered only during the dark hours and early sunshine hours, while during most of the day, they are exposed to natural environmental conditions. The data on CO_2 concentration within the polycover (Fig.1 and 2) show that CO_2 level within the polycover increased gradually reaching a maximum of 1000-1200 ppm by 1-2 A.M. The level went down after sunrise and reached below ambient level by 8-9 A.M. Thus plants grown under polycover were exposed to effective high CO_2 levels for approximately 2-3 hours each day. Two points which need to be noted regarding this method are that firstly, effective CO_2 enrichment occurs only for few hours and secondly, for most of the day plants remain subjected to natural environmental condition. One disadvantage in this type of experiment is that the

temperature and relative humidity recorded when the plants were covered with polythene, was higher than those of ambient condition. Since these factors could not be eliminated, they might have contributed to the differences observed between plants grown under polycover and those raised in ambient conditions.

Two cultivars of Groundnut, J-11 (erect type) and M-13 (spreading type) were used in this experiment. Both showed increased length of main shoot as well as of branches under this treatment. Spreading type (M-13) showed more increase in main shoot (26.05 per cent) and branch length (20 per cent), as compared to the erect type (2.05 and less than 15 per cent) cultivar. At harvest, M-13 and J-11 both showed increase in dry matter and yield under polycover as compared to plants grown under natural conditions. M-13 showed 64.4 per cent increase in pod weight per plant while J-11 showed only 11.8 per cent increase under polycover as compared to ambient grown plants. M-13 cultivar values were statistically significant while J-11 values were not statistically significant in all cases. In general, both the cultivars showed higher growth and yield under polycover. M-13 (spreading type) showed better response. Changing the microclimate of the plants by using polycover resulted in enhanced growth and yield in groundnut. This increase was in terms of both in size and dry weight of the plants. Polycover treatment not only enhanced the growth but was effective in partitioning assimilates to pods and ultimately leading to high seed yield.

Growth parameters showed appreciable increase when pea was grown under polycover. Plant height of dwarf variety (DMR-10) increased under polycover, while that of L-116 (tall) did not show significant response. There was increase in growth in terms of dry matter production in both the cultivars under polycover. However, DMR-10 (dwarf) showed higher response, i.e. leaf dry weight increased by 44.7 per cent, stem dry weight increased by 47.6 per cent. In case of L-116, leaf dry weight increased by 58.77 per cent and stem dry weight decreased by 1.5 per cent. Yield data showed a decrease in seed weight, seed number and pod number in both the cultivars under polycover as compared to ambient grown plants. The increased temperature within the polycover might have differentially affected vegetative and reproductive parameters in peas. Although there was rise in CO_2 within the polycover, concomitant increase in temperature might have had adverse effect on seed development as in other crops. This might be the reason why inspite of increased dry matter production, the yield did not increase. Although humidity was also high within the polycover, the effect of high humidity and changes in yield is poorly understood.

Mixed response of crop plants grown under polycover suggest several possibilities. Although plants were exposed to high CO_2 for a short period in the early hours of sunshine when photosynthetic rates are at their peak. But along with high CO_2, there was rise in temperature (3.7 to 2.7°C) as well as relative humidity (28.3 to 17.4 per cent). If plants utilised greater amount of CO_2, it is reflected

mainly in biomass production as reported for other crops (Imai and Murata 1979; Bhattacharya *et al.*, 1985). This increase in plant growth and biomass production was observed in both the summer (groundnut) and winter (pea) crops. But the response differed among the cultivars. In general, dwarf cultivars showed better response than the tall ones specially in peas, indicating variability within same crop species.

The increase in temperature appears to have varied effect on crop species. The appreciable increase in growth and yield in groundnut under high CO_2, high temperature and high humidity conditions appear to be characteristic of the plant species. In general, groundnut requires higher temperature for its growth. Thus even under increased temperature, enrichment of CO_2 caused both increase in growth and yield. On the other hand, peas like wheat appears to be more sensitive to temperature specially during the time of vegetative growth (Abrol *et al.*, 1991). Thus, under increased level of CO_2 and high temperature, increase in vegetative growth occured but yield was reduced. However, further studies are required to confirm these observations.

Since the methodology employed for CO_2 enrichment using polycover had certain shortcomings like concomitant increase in temperature and Relative Humidity, in the following pages another method using open top chamber shall be discussed.

CARBON DIOXIDE ENRICHMENT STUDIES USING OPEN TOP CHAMBERS

In the night trapped CO_2 experiment the level of CO_2 concentration could not be controlled as it depended on the respiratory release of CO_2 of the plants. Moreover, plants were totally covered and maintained at high CO_2 level only for a short period with high humidity within the chamber. It was therefore felt necessary to expose the plants to high CO_2 keeping all other environmental factors similar to ambient condition. The concept of open top chamber was developed by Rogers *et al.* (1983). Open top chamber which was sufficiently modified to suit our needs was fabricated and standardized to grow plants at high CO_2 conditions (600 $\pm$ 50 ppm).

The main feature of open top chamber is to grow plants under high CO_2 in a chamber with the top open, so as to maintain the temperature and humidity as those of ambient conditions. Pure CO_2 gas was purchased from the local market for use in this experiment. Flow of this gas was regulated using a regulator and a flow meter. The gas was then passed into a tube through which ambient air was drawn for a CO_2-air mixture. This CO_2-air mixture was then pumped into the open top chamber where it was mixed with the ambient air using a circulatory pump. The open top chamber was of 0.9 x 0.9 x 2.0 meter size having an air volume of 0.928 m^3. The four walls of the chamber were lined with transparent polyethylene sheet but keeping the top open for free mixing with ambient air. The flow of CO_2 was regulated in such a way that the level of CO_2 within the chamber was maintained at about 600$\pm$50 ppm. This was

monitored regularly by drawing air from the chamber and was measured by IRGA (LICOR 6200 Model). This method of CO_2 enrichment was found to be very steady except during windy hours.

In order to expose crop plants to enriched CO_2 conditions, seeds were sown in pots. After germination, pots were placed within the chamber and CO_2 treatment was given only during the day time. The plants were allowed to grow within the chamber throughout the growth and development phases. The temperature and relative humidity in the chamber were monitored regularly and it was observed to be similar to ambient air. Thus crops within the open top chamber had only one environmental factor (CO_2) different from ambient air. Any change in the growth and other physiological parameters was thus assumed to be due to the increase in CO_2 concentration. The changes that occurred due to the increased CO_2 level in open top chamber in different crop plants are discussed in the following pages.

Wheat, mungbean and sunflower were grown in open top chamber in an environment of 600 ± 50 ppm CO_2 and compared with the plants grown under ambient level of CO_2 (350 ppm). The differences in growth of crops grown in the two levels of CO_2 were quite remarkable. In general, the growth of the plants was much better under 600 ppm CO_2 (Plates 3 & 4). In wheat, growth parameters were recorded at two stages. Under 600 ppm CO_2, leaf area of wheat plants was significantly higher at 25 DAS (70 per cent) and 45 DAS (119 per cent) compared to ambient grown plants (Table 12). Increased leaf growth in terms of dry weight was much

Plate 3. Wheat crop at 45 DAS in CO_2 enriched conditions (600 ±50 ppm) and control (350 ±25 ppm)

Plate 4. **Mungbean plants showing extra growth in CO_2 enriched (600 ±50 ppm) conditions as compared to control (350 ±25 ppm)**

higher at 25 DAS (214 per cent) compared to 45 DAS (81 per cent). The increase in stem dry weight in CO_2 enriched plants was greater at 45 DAS compared to 25 DAS, but total biomass showed greater increase at 25 DAS. These data indicate that under conditions of 600 ppm CO_2 the growth of leaf and stem increased due to accumulation of dry matter in both leaf and stem. At early stages, greater accumulation of dry matter takes place in the leaves while at later stages it takes place in stems, under CO_2 enriched conditions. In mungbean, almost similar effect was observed in plants grown in 600 ppm CO_2. Leaf area, leaf and stem dry weight increased in plants grown under enriched CO_2 conditions. Greater increase in leaf and stem dry matter accumulation was observed at 25 DAS compared to 45 DAS. It reflected greater biomass production at early stage of crop growth due to CO_2 enrichment. In sunflower, only total dry matter accumulation at 25 and 75 DAS was recorded. In this crop also greater increase in total biomass was recorded in early stage.

These findings clearly indicate that under conditions of 600 ppm CO_2, these three C_3 crop species responded positively in terms of crop growth. The increase in crop growth was due to increase in both size and dry weights of leaf and stem. At early stages increase in leaf area and dry weight suggests that increase in CO_2 favours early leaf growth. Increased dry weight of stems at later stage, indicates the tendency of plants to store reserves in stem. There might be differences in response of plant species to increased CO_2 concentration. Increase in leaf size as a result of CO_2

enrichment has been reported in several C_3 species (Ford and Thorne, 1967; Hardy and Havelka, 1977; Ho, 1977; Richer and Strain, 1988). Response of leaves to CO_2 enrichment also depends on the stages of growth. Newton (1985) has reported that in cucumber, when seedlings were exposed to high CO_2, expansion rate and size of leaves increased in first few days only. The increase in leaf area is also accompanied by increases in leaf area index (LAI) and leaf weight (Sasek and Strain, 1989). The increase in leaf area and leaf dry weight in CO_2 enriched plants appeared to be due to increased availability of photosynthates as well as improved turgor due to changes in water status caused by increased stomatal conductance (Peacy and Bojorkman, 1983; Sasek and Strain, 1989).

The present study showed that in addition to increased leaf growth there is also increased growth of the stem and thereby increased total biomass in CO_2 enriched plants. These changes in the pattern of growth indicate a significant modification in the partitioning of photosynthates to different organs. Increase in dry weight and biomass due to CO_2 enrichment have been reported in several plant species. Mauney *et al.* (1978) found that soybean, cotton and sunflower plants grown in 660 ppm CO_2 showed an increase in dry weight of 382, 110 and 60 per cent, respectively. Idso (1980) reported 82 per cent increase in biomass in cotton when CO_2 was doubled in open top chambers. Many of the dry matter increase values differ in various studies from the mean response of dry matter to CO_2 doubling (Cure and Acock, 1986). This is because of greater variability of conditions of experiments conducted and

plant species used. However, in most of the studies like the present one, the increase in CO_2 above ambient level caused increase in plant biomass, indicating greater production of carbohydrates under CO_2 enriched conditions.

As increase in CO_2 concentration increased plants' vegetative growth, it is expected that it may also affect the reproductive characters of CO_2 enriched plants. In general, it was expected that CO_2 enrichment might delay the flowering of plants. However, in all the three crops, flowering time was reduced. Mungbean plants flowered 3 days earlier, sunflower by 12 days and in wheat, spikelet emergence took place 10 days earlier in CO_2 enriched plants (Plate 5) as compared to ambient grown plants. Similar findings were reported in cowpea at 675 and 1000 ppm by Bhattacharya *et al.* (1985). In contrast, some reports show delayed flower initiation in sorghum (Hasketh and Hellmers, 1973) and a slightly delay in maize, sunflower and cotton. Thus, it appears that the time of flowering is affected differently by high CO_2 in various kinds of plants. It may be noted here that the conditions of experiment are not similar in all these studies and thus results obtained do not confirm each other. However, the present study clearly indicates that earliness in flowering occurs under CO_2 enriched condition in all the three crops under study.

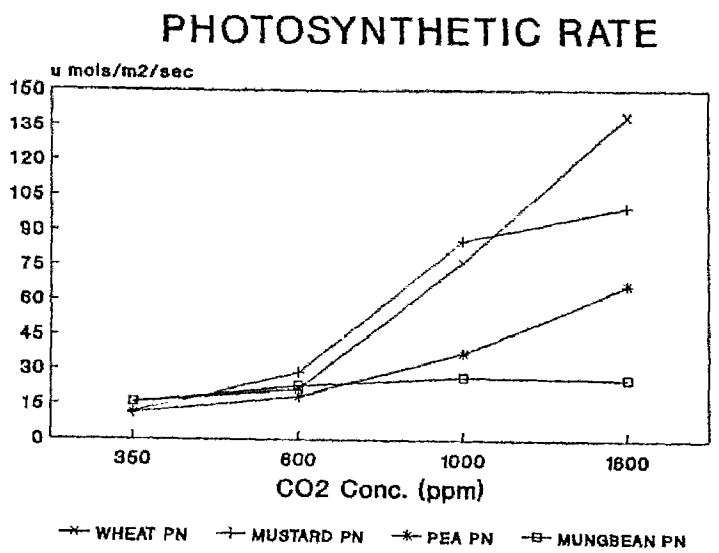
The rate of photosynthesis depends on many environmental factors including the concentration of CO_2 prevailing around the plant canopy. Thus, it is expected that the increase in CO_2 concentration would lead to higher rate of photosynthesis. It has

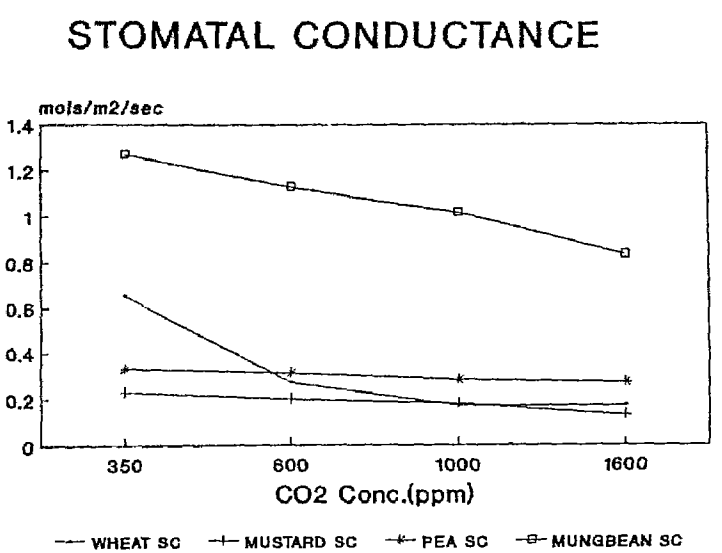
Plate 5. Wheat crop showing early spike emergence in CO_2 enriched conditions (600 ±50 ppm) as compared to ambient CO_2 levels (350 ±25 ppm)

been shown as early as 1904 by Blackman that CO_2 is a limiting factor for photosynthesis. The scientific communities are now very much concerned that global climate is changing with a faster rate of change of atmospheric CO_2 especially due to industrial development. The level of CO_2 has risen from 270 ppm to the present level of 350 ppm during the past 100 years or so. Now the question arises whether the present level of CO_2 is still limiting or not? If it is still limiting any increase above the present level of CO_2 could increase the rate of photosynthesis. Earlier in this laboratory, short-term experiment in mungbean has shown that at 600 and 900 ppm CO_2, the rate of photosynthesis increases 2 to 3 fold (Aruna Sharma, 1986). In wheat, the rate of photosynthesis increases linearly up to 10000 ppm when leaves were exposed for a short period (Sengupta, 1988).

In the present investigation four crops (wheat, peas, mustard and mungbean) were used to study the short-term response of photosynthesis to high CO_2. In this system, plants were grown under ambient conditions. Then individual leaves were exposed to varying levels of CO_2 concentrations ranging from 350 ppm to 1600 ppm of CO_2. All the crop plants showed increase in the rate of photosynthesis with the increase in CO_2. The rate of increase in Pn was almost linear up to 1000 ppm, but was comparatively less at 1600 ppm CO_2. Wheat and mustard showed greater response than pea and mungbean. Minimum response was noticed in mungbean crop (Fig. 6). These findings clearly indicate that short term exposure of plants to higher levels of CO_2 would cause

Fig.6 SINGLE LEAF PHOTOSYNTHESIS AND STOMATAL CONDUCTANCE OF WHEAT,MUSTARD,PEA AND MUNGBEAN PLANTS GROWN AT 350 PPM CO2 AND EXPOSED TO SHORT TERM CO2 ENRICHMENT





increased photosynthesis in most of the crop species. However, the response to increased CO_2 may vary depending upon age and nature of crop species. Reports from literature also show similar findings as ours. In tomato, 70 per cent increase in photosynthetic rate was reported at 750-1000 ppm CO_2 (Ho, 1977), in soybean it doubled at 720 ppm (Huber *et al.*, 1984), and was almost four times that of ambient at 670 ppm (Brun and Cooper, 1967), and about 2.5 times in mungbean at 900 ppm (Sharma and Sengupta, 1990). Under conditions of moderate to high light intensities and temperature, the rate of CO_2 fixation in C_3 plants roughly doubles with doubling of present atmospheric conditions (Campbell *et al.*, 1988). This suggests that the C_3 pathway, found in most plants, is generally strongly limited by the low CO_2 concentration in the present day atmosphere (Sengupta and Sharma, 1993). However, it may be noted that short period exposure may not necessarily provide the true information regarding what occurs when plants are grown at high CO_2 concentrations for a longer duration. Uday Kumar and Prasad (1991) postulated that under short term there will be increase in photosynthates and under long term there will be absolute reduction in photosynthesis caused by end product inhibition and/or inadequate inorganic phosphate recycling. In our present study with 600 ppm CO_2 there was no inhibition of photosynthesis in wheat and mungbean at different growth phases even under long term exposure. Increased photosynthetic rate has in fact led to increased starch and subsequently to higher dry matter accumulation. This may possibly be due to the differences in crop response to CO_2 enrichment or the level of CO_2 concentration. At

CO_2 concentration higher than 600 ppm, excessive starch formation may take place causing feedback inhibition or affecting short-distance transport mechanism. In earlier studies of short term exposure of mungbean, it has been shown that at 900 ppm CO_2, sucrose formation reaches a saturation level and excess amount of CO_2 fixed is partitioned to starch (Sharma and Sengupta, 1991). The present study thus showed that in these C_3 crop species no such saturation of photosynthetic rate takes place at 600 ppm CO_2 and that is why higher level of photosynthetic rate was maintained even after long term exposure. Starch and sugar content measured in wheat plants showed that about 29 per cent increase in starch content occurred in CO_2 enriched plants (Table 10). On the other hand, CO_2 enriched plants had 282 per cent increase in non-reducing sugars while reducing sugars were low in CO_2 enriched grown plants as compared to control. This suggests that increased amount of starch due to exposure to high CO_2 is not sufficient to inhibit the rate of photosynthesis while large increase in non-reducing sugars may be utilised for export. Reduction in reducing sugars resulted in reduction in amino acid content (Table 10). When the response of crop species to high CO_2 was compared, it was observed that as compared to wheat, high CO_2 grown mungbean plants showed lower photosynthetic rate at early stage of crop growth. Sunflower plants showed much greater response to high CO_2 as compared to wheat and mungbean. This clearly showed that species do differ in their respnse to CO_2 enrichment. Mauney *et al.* (1978) have shown in their glasshouse experiment that the rate of photosynthesis when measured at frequent intervals on single leaf

at 600 ppm compared to 330 ppm CO_2, increases 41 per cent for soybean, 15 per cent for cotton, 7 per cent in sunflower and 2 per cent for sorghum.

Plants grown either under ambient conditions or in elevated CO_2 conditions for a long duration, gradually adapt and acclimatise to the prevailing environment and respond accordingly. When there is any abrupt change in the environmental conditions, plants respond differently to such changes. In this experiment, photosynthetic rates were measured by brief exposure of ambient grown plants to 600 ppm CO_2 and the plants grown in 600 ppm CO_2 to ambient level of CO_2 (350 ppm). The results showed that photosynthetic rate of ambient grown plants in 600 ppm CO_2 was higher than photosynthetic rate of plants grown and measured at 600 ppm CO_2. On the other hand, photosynthetic rate of 600 ppm CO_2 grown plants and measured at 350 ppm CO_2 was less than the photosynthetic rate of ambient grown plants. In sunflower plants, changes in photosynthetic rate from ambient to high CO_2 was less than that of high CO_2 grown plants. Moreover, changes from high CO_2 to ambient conditions showed greater photosynthetic rate than ambient grown plants. In mungbean slight reduction in photosynthetic rate occured when high CO_2 plants were shifted to ambient conditions as compared to ambient grown plants. It has been shown in some C_3 crop species, that plants grown in high CO_2 show low photosynthetic rate values when measured in ambient air (Ho, 1977; Peet *et al.*, 1986; Sasek *et al.*, 1985). This may be due to decrease in RUBP carboxylase activity or amount of the enzyme

protein formed under high CO_2. The greater increase in photosynthetic rate when plants were transferred from ambient to 600 ppm CO_2 is due to availability of greater substrate (CO_2) for RUBP as the ambient level of CO_2 is limiting for photosynthesis. However, in the present experiment, similar response was not observed in all the three crops.

From the foregoing discussion, it is clear that under CO_2 enriched conditions, the photosynthetic rates increased accompanied by an increase in plant growth.

All the plant species grown under elevated CO_2 concentrations showed lower dark respiration per unit leaf area. In mungbean, reduction of respiration to the extent of 63 per cent was observed in long term high CO_2 grown plants while it was 30-40 per cent in sunflower. Short term exposure to high CO_2 resulted in greater reduction in respiration in mungbean plants compared to sunflower. Thus, the present experiment showed that increase in CO_2 concentration increased plant growth in terms of dry weight, increased photosynthesis and decreased dark respiration. Reports in literature have shown that growth increased in most plant species due to increase in CO_2 level, and respiration rate also increased in some cases, but decreased in others (Amthor, 1991). Gifford et al. (1985) reported about 45 per cent reduction in respiration in wheat but in sunflower root respiration increased. In some tree species, lower rates of dark respiration per unit mass were reported when grown and measured at elevated CO_2 concentration (Bunce, 1992). Thus, it appears that response of respiration to increased CO_2

depends on crop species because of indirect and direct effects of CO_2 on respiration. Both increases and decreases in respiration rate have been reported in high CO_2 and effects of CO_2 on respiration vary among species (Gifford *et al.*, 1985; Brunce, 1990; Amthor, 1991). The change in respiration has been attributed to several factors. These are related to changes in 1) levels of non-structural carbohydrates, 2) growth rate and structural phytomass accumulation, 3) composition of phytomass, 4) direct chemical interaction between CO_2 and respiratory enzymes, 5) direct chemical interactions between CO_2 and other cellular components, 6) dark CO_2 fixation rate, and 7) ethylene biosynthesis. However, most of these are speculative and little work has been done on these aspects. There are several evidences to show that CO_2 may directly affect the respiration by acting on the respiratory enzymes. For example, carbonate formation could modify mitochondrial nucleotide translocation (ADP-ATP antiport), representing direct effect on respiratory control (Amthor, 1991). Membranes may be readily affected by CO_2 (Mitz, 1979), which could result in altered rates of transport and gradient maintenance processes, leading to altered respiration rate through respiratory control process.

From this study, it is apparent that when CO_2 level is increased, growth and photosynthesis in leaves of plants increased but respiration rate decreased. This indicates that some respiration is unnecessary and could profitably be eliminated in current environment (Gifford *et al.*, 1985; Reuveni and Gale, 1985; Bunce and Caufield, 1991). Thus, substantial effect of reduced plant

carbon dioxide efflux on global carbon balances would be expected if reduction occurs in plant species which are capable of sequestering carbon from year to year.

Although enrichment of CO_2 caused increased rate of photosynthesis in most of the crop plants, chlorophyll content of leaves decreased in CO_2 enriched condition in wheat and mungbean. The reduction in both chlorophyll a and b as well as total chlorophyll was noticed in these crop plants (Tables 10 & 11). However, no such relationship of chlorophyll with CO_2 enrichment was noticed as the amount of reduction of chlorophyll a and b was not similar in wheat and mungbean crops. Thus the cause of reduction in the amount of chlorophyll could not be accounted for, although some evidences showed that greater amount of chlorophyll destruction was due to heavy starch accumulation (Cave *et al.*, 1981). But in this study the amount of starch accumulation due to CO_2 enrichment does not appear to be large enough to cause destruction of chlorophyll. These earlier studies used much higher levels of CO_2 where starch accumulation in the chloroplast was high enough to destroy chloroplast structure.

In this study, stomatal conductance and leaf temperature were also recorded (Tables 6-9). It was observed that increase or decrease of photosynthesis due to CO_2 enrichment has no direct correlation with stomatal conductance. Leaf temeprature by and large increased under high CO_2 conditions.

Wheat and mungbean under CO_2 enriched conditions at 600

ppm showed an increase in yield and harvest index. Among the two, mungbean showed a greater response (Table 16). In both the crops, the increase in yield was primarily because of increase in number of reproductive structures as well as increased grain weight. Since the experiments were conducted in pot culture, it is too early to extrapolate these results to field conditions. However, our results are in agreement with several other workers from different laboratories (Ackerson *et al.*, 1984; Havelka *et al.*, 1984 and Kimball, 1983).

In conclusion the study can be summarised as, enriching the air with high CO_2 either using polycover or open top chamber resulted in increase in growth and biomass in all the crops studied. Increase in growth might be influenced by high temperature and high humidity existing in the polycover. In the open top chamber, temperature and humidity was comparative with those found in ambient conditions. In open top chamber studies, wheat and mungbean showed appreciable increase in photosynthesis which was sustained throughout the growth period. High photosynthesis accompanied by decreased dark respiration resulted in increased biomass production. Effective partitioning to the reproductive sinks, as indicated by starch and sugar content, might have resulted in increase in economic yield and harvest index of crops in open top chamber with high CO_2 concentrations.

CHAPTER-VI

SUMMARY

Recent reports on climate change have clearly indicated that the level of CO_2 in the atmosphere is increasing and would probably be double the present level about 100 years from now. As CO_2 is the substrate for photosynthesis, any change in its concentration would greatly influence the growth and productivity of crop plants. Earlier in this laboratory it has been shown that short term exposure of crop plants to enriched CO_2 conditions increased the rate of photosynthesis and partitioning of assimilates to various sinks. In the present study an effort is made to understand the physiological implications of long-term exposure of crop plants to enriched CO_2 conditions. For this purpose, two methodologies were tried for exposing crops to elevated levels of CO_2. In the first method, plants were exposed for a couple of hours during early morning to high CO_2 (respired by the plants), trapped using polythene cover during night time. In the second method, commercial CO_2 and air mixture was supplied in an open top chamber to increase CO_2 concentration.

In the first method, plants were covered under polythene during night time which increased CO_2 concentration to 1000-1200 ppm which was utilized via photosynthesis during early sunshine hours. The polycover was removed later and plants were exposed to ambient conditions. Groundnut and pea were grown under

polycover and compared with ambient grown plants. The major findings are :

1) Exposure of plants to high night trapped CO_2 resulted in an increase in growth and yield of groundnut. Increase in plant height, leaf area, total biomass and number of pods per plant was observed for both the cultivars, but the spreading type (M-13) was found to be more responsive than erect type (J-11).

2) In peas, vegetative growth and biomass increased but there was no increase in seed yield. Dwarf variety (DMR-10) showed better response than the tall variety (L-116).

In the second method, plants were grown in pots and exposed to 600 $\pm$ 50 ppm of CO_2 in an open top chamber (0.9 x 0.9 x 2.0 meter), lined with transparent polythene keeping the top open. In this experiment, the air within the chamber was supplemented with CO_2 and calibrated in such a way as to maintain the level of CO_2 at 600 $\pm$ 50 ppm without changing other environmental factors such as light, relative humidity and temperature. The enrichment treatment was given from 9 A.M. to 5 P.M. daily throughout the period of crop growth. Wheat, mungbean and sunflower crops were used for this experiment. The plants grown in an environment of 600 $\pm$ 50 ppm were compared with plants grown in a similar chamber but without an elevated level of CO_2. The salient findings of the experiment are :

1) Difference in response in the form of increased rate of photosynthesis was observed in plants which were exposed to high CO_2 for a brief period (short-term) compared to those which were grown continuously from germination to maturity in ambient condition. Greater increase in photosynthetic rate was observed in short-term exposure. The rate of photosynthesis increased almost linearly up to 1600 ppm CO_2 in short-term exposure.

2) Under long-term exposure, plants maintained higher rates of photosynthesis throughout the growth period compared to ambient CO_2 grown plants. No acclimation in photosynthetic rate was observed in any of the three crops.

3) Although all the crop species showed an increase in photosynthesis, the response was different in different crop species.

4) Plants grown in ambient conditions and exposed to 600 $\pm$ 50 ppm CO_2 showed greater enhancement of photosynthesis as compared to plants grown and measured at 600 $\pm$ 50 ppm CO_2.

5) Chlorophyll content of CO_2 enriched plants was less than ambient grown plants. There was a visible symptom of yellowness in CO_2 enriched plants. Chlorophyll a, b and total chlorophyll decreased both

in wheat and mungbean, but the magnitude of decrease was not the same.

6) Under high CO_2 dark respiration decreased and starch content was high in wheat and mungbean. Total free amino acids (TFAA) decreased, while total sugars increased in wheat under CO_2 enriched conditions.

7) Growth in the form of leaf area and dry weight increased in all crops under CO_2 enrichment. However, this increased growth was not the same at different stages of crop growth and there was also difference between species.

8) Days taken for flowering and maturity were less in CO_2 enriched crops as compared to ambient grown plants. That is, crop plants under CO_2 enriched atmosphere completed their life cycle earlier.

9) Under CO_2 enrichment both wheat and mungbean showed high yield in pot culture as compared to ambient CO_2 grown plants.

The present investigation has given us a preliminary insight into the effects of CO_2 enrichment on some crop plants. This study supports the generalized view that plants under CO_2 enriched atmosphere grow more and yield more primarily because of increased photosynthesis. However, the increase in growth and yield is species-dependent and even cultivars respond differently. Out of the two methods, one using polycover was found to be

inappropriate for CO_2 enrichment studies because of high temperature and humidity existing inside the polycover. Open top chamber method proved to be successful and consistent data were obtained by this method.

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*Originals not seen

Appendix I

Mean of pooled data for CO_2, temperature and relative humidity inside polycover and ambient conditions for groundnut (summer)

Time (A.M.)	Inside polycover			Ambient		
	CO_2 (ppm)	Temp. (°C)	RH (%)	CO_2 (ppm)	Temp. (°C)	RH (%)
4	878	26.0	85.0	357	23.4	67
5	920	26.5	86.0	368	23.8	65
6	1116	26.8	86.0	370	24.0	60
7	846	27.2	89.0	363	25.8	58
8	370	29.0	90.1	353	27.0	58
9	163	31.2	90.5	348	27.2	55
10	142	33.0	90.2	348	29.3	55

Appendix II

Mean of pooled data for CO_2, temperature and relative humidity inside polycover and ambient conditions for pea (winter)

Time (A.M.)	Inside polycover			Ambient		
	CO_2 (ppm)	Temp. (°C)	RH (%)	CO_2 (ppm)	Temp. (°C)	RH (%)
4	750	13.0	87.0	370	9.7	72.0
5	970	13.0	86.8	369	10.0	72.2
6	970	15.3	88.0	370	11.2	70.6
7	960	16.0	88.5	355	12.1	69.5
8	857	17.5	91.0	348	13.5	63.5
9	370	17.8	91.2	350	14.1	63.0
10	240	19.6	92.0	345	15.8	62.0

Appendix III

Standard curve of sugars

µ grams glucose	A (620 nm)
10	0.01
20	0.08
30	0.135
40	0.215
50	0.300
60	0.380
70	0.450
80	0.510
90	0.600
100	0.685

Appendix IV

Standard curve of amino acids

μ grams of glycine	A (570 nm)
0.1	0.08
0.2	0.19
0.3	0.26
0.4	0.37
0.5	0.45
0.6	0.53
0.7	0.64
0.8	0.78
0.9	0.82
1.0	0.91

Appendix V

Single leaf photosynthesis and stomatal conductance of wheat, mustard, pea and mungbean plants grown at 350 ppm CO_2 and exposed to short term CO_2 enrichment

	A. Photosynthetic Rate ($u\ mol.M^{-2}.sec^{-1}$)				B. Stomatal Conductance ($mols.M^{-2}.sec^{-1}$)			
	350	600	1000	1600	350	600	1000	1600
	(CO_2 ppm)				(CO_2 ppm)			
Wheat	15.58	21.40	76.45	138.80	0.65	0.27	0.17	0.17
Mustard	11.62	28.30	85.59	99.90	0.23	0.20	0.18	0.13
Pea	11.09	18.30	36.87	66.17	0.33	0.31	0.28	0.27
Mungbean	15.96	22.64	26.46	25.73	1.27	1.13	1.02	0.83